European Union Risk Assessment Report

4,4'-ISOPROPYLIDENEDIPHENOL

(BISPHENOL-A)

CAS No: 80-05-7

EINECS No: 201-245-8

RISK ASSESSMENT

Complete risk assessment in one document

(February 2010)

The risk assessment report of Bisphenol-A from 2003 has been merged with the addendum from 2008 to have all information available in one document.
Within this document you will find first the Addendum from 2008 for both Environment and Human Health and then the complete RAR from 2003.

The Addendum includes the summaries of all the endpoints for exposure and hazard from the 2003 RAR to which new relevant information had been added. Based on this the risk characterisation had been revised.

By combining these reports, the document has become quite big.
Please consider this, before printing the report!

Updated European Risk Assessment Report

4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A)

CAS Number: 80-05-7

EINECS Number: 201-245-8

FINAL APPROVED VERSION AWAITING PUBLICATION

GENERAL NOTE

This document contains:

- **Part I Environment** (pages 218)
- **Part II Human Health** (pages 173)

Updated European Risk Assessment Report

4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A)

CAS Number: 80-05-7

EINECS Number: 201-245-8

FINAL APPROVED VERSION AWAITING PUBLICATION

Environment Addendum of February 2008

(to be read in conjunction with published EU RAR of BPA, 2003 for full details)

LEGAL NOTICE

Cataloguing data can be found at the end of this publication

Luxembourg: Office for Official Publications of the European Communities, 2008

Printed in Italy

4,4'-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A)

CAS Number: 80-05-7

EINECS Number: 201-245-8

UPDATED RISK ASSESSMENT

Environment Addendum of February 2008

Rapporteur for the risk assessment is the United Kingdom.

Contact point: (environment)
Environment Agency
Chemicals Assessment Unit
Red Kite House, Howbery Park, Wallingford,
Oxfordshire, OX10 8BD
Fax: +44 (0)1491 828 556
E-mail: ukesrenv@environment-agency.gov.uk

The scientific work on the environmental sections was carried out by the Building Research Establishment Ltd (BRE) (with input on the aquatic effects assessment by Watts & Crane Associates), under contract to the rapporteur.

Date of Last Literature Search :	**2007**
Review of report by MS Technical Experts finalised:	**2007**
Final report:	**2008**

Foreword

This risk assessment of the priority substance covered by this Draft Risk Assessment Report is carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and if necessary, recommending a strategy to limit the risks of exposure to the substance.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2] which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented to the Competent Group of Member State experts for endorsement. Observers from Industry, Consumer Organisations, Trade Unions, Environmental Organisations and certain International Organisations are also invited to attend the meetings. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

This Draft Risk Assessment Report is currently under discussion in the Competent Group of Member State experts with the aim of reaching consensus. During the course of these discussions, the scientific interpretation of the underlying scientific information may change, more information may be included and even the conclusions reached in this draft may change. The Competent Group of Member State experts seek as wide a distribution of these drafts as possible, in order to assure as complete and accurate an information basis as possible. The information contained in this Draft Risk Assessment Report does not, therefore, necessarily provide a sufficient basis for decision making regarding the hazards, exposures or the risks associated with the priority substance under consideration herein.

This Draft Risk Assessment Report is the responsibility of the Member State rapporteurs. In order to avoid possible misinterpretations or misuse of the findings in this draft, anyone wishing to cite or quote this report is advised contact the Member State rapporteurs beforehand.

[1] O.J. No L 084, 05/04/1993 p. 0001 - 0075
[2] O.J. No. L 161, 29/06/1994 p. 0003 – 0011
[3] Technical Guidance Document, Part I-V, ISBN 92-827-801[1234]

Introduction

A risk assessment of bisphenol-A produced in accordance with Council Regulation (EEC) 793/93 has already been published (EC, 2003). The conclusion was that further information was needed about toxic effects in fish and aquatic snails (and potentially terrestrial organisms), and environmental risks were also identified for certain PVC applications and thermal paper recycling (for the aquatic, sediment and terrestrial compartments). The test requirements were published in two Commission Regulations (EC No. 642/2005[3] and 506/2007[4]) with a delivery deadline of November 2006 and November 2007 respectively.

The UK rapporteur began work on a risk reduction strategy for the environment shortly afterwards, and an interim report was prepared (Defra, 2003). Industry was able to provide more detailed information on use pattern and releases for a number of the applications being considered, including measured emissions data. Based on this evidence, the rapporteur considered that the emissions had been over-estimated in the published report, and revised PECs were agreed at EU Technical Meetings in 2003 and 2005. In addition, bisphenol-A may be formed via the degradation of tetrabromobisphenol-A (CAS no. 79-94-7), which is another ESR priority substance. The relevant information has been summarised in the risk assessment report for that substance (ECB, 2007).

The test programme has now concluded, so this report brings together the revised exposure information and an updated review of ecotoxicity data, as an addendum to the original risk assessment report. The opportunity has been taken to include additional industry information and published data that have become available since the original risk assessment was completed[5]. The opinions of the European Commission's former Scientific Committee for Toxicity, Ecotoxicity and the Environment (CSTEE) on the original report have also been considered (CSTEE, 2002). The assessment uses the latest version of the Technical Guidance Document, which was revised after the original report's publication, so marine scenarios and a PBT assessment are included for the first time.

The format of the report is broadly in line with that of the published assessment. A brief summary of the original information is given (*the published report should be consulted for full details*), followed by all significant new data and a comment to indicate how these differ from the original report. An exception is the section on aquatic effects, which has been entirely revised (although the original data have not been re-evaluated) and reformatted (the data had previously been divided into 'toxicity test results' and 'endocrine disrupting effects').

To protect commercial confidentiality, site codes are presented in a separate confidential annex. This can be made available to regulatory authorities on request.

[3] Official Journal L 107, 28/04/2005 p. 0014 – 0016.

[4] Official Journal L 119, 09/05/2007 p. 0024 – 0026.

[5] EC (2003) was based on a review of all data published up to 2001. For this report, studies were identified independently by Industry (who provided the rapporteur with an updated reference list) and the rapporteur up to March 2007. The abstracts and, where necessary, main text of these papers were reviewed to establish their relevance. Non-relevant papers are listed in Appendix 1, with a reason for their non-inclusion. Relevant papers have been reviewed in detail and are reported in the main text.

CONTENTS

TABLES

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No: 80-05-7
EINECS No: 201-245-8
IUPAC name: 2,2-bis(4-hydroxyphenyl)propane (also known as 4,4'-isopropylidenediphenol or bisphenol-A)

Environment

Conclusion (i) There is a need for further information and/or testing.

This conclusion applies to the freshwater and marine aquatic compartments (including sediment, since the equilibrium partitioning approach has been used). Although no risks are indicated using the freshwater and marine PNEC for any scenario, there are still some uncertainties over the potential effects of bisphenol-A on snails, despite the thorough testing undertaken as part of the conclusion (i) programme. Further work being conducted by the UK Government should be taken into account when results are available in 2008.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion applies to the terrestrial and atmospheric compartments, and to secondary poisoning through the aquatic, terrestrial and marine food chains. It also applies to the risks to wastewater treatment plant micro-organisms. For these end points the conclusion applies to all life cycle steps.[6]

[6] Note that the original risk assessment also drew the same conclusion for the water and sediment compartments for five uses that had negligible emissions (i.e. because the processes are either completely dry or any aqueous effluent produced is disposed of through incineration). These have not been reconsidered in this update, and the conclusion remains valid for these applications.

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

No new information is available. 4,4'-Isopropylidenediphenol (CAS no. 80-05-7) is more commonly known as bisphenol-A, and the common name will be used throughout this report.

1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Purity

The purity of bisphenol-A is 99 – 99.8% depending upon the manufacturer (EC, 2003). Impurities typically include phenol (<0.06%), *ortho*- and *para*- isomers of bisphenol-A (<0.2%) and water (<0.2%).

Terasaki *et al.* (2004) reported the results of an analysis of commercial bisphenol-A. This had a purity of 95.3 – 96.8% from the analysis. Fifteen other components were identified, the most abundant of which was the 4,2'-isomer, at 2.95% in one sample. All of the identified components contained phenol groups. [The level of impurity is much higher than is indicated in the risk assessment. The isomer above is indicated as a possible impurity, but at <0.2%.]

1.3 PHYSICO-CHEMICAL PROPERTIES

The key physico-chemical property values are presented in Table 1.1 (EC, 2003). No values have been revised as a result of the updated literature search for this addendum. Shareef *et al.* (2006a) reported the determination of the solubility of bisphenol-A in water at a range of pHs and a range of ionic strengths. The solubility of bisphenol-A in pure water was measured as 300±5 mg/l at 25.0±0.5 °C, with no significant variation over the pH range of 4 to 10, and no change with ionic strength (up to 0.1 moles/litre KNO_3). This further supports the value used in the published assessment.

Table 1.1 Key physico-chemical properties for bisphenol-A

Parameter	Value
Physical state at normal temperature and pressure	White solid flakes or powder
Vapour pressure	5.3 x10^{-9} kPa at 25°C used in environmental models
Solubility in water	300 mg/l used in environmental models
n-Octanol-water partition coefficient (log K_{ow})	3.4 used in environmental models
Flash point	~ 207°C
Autoflammability	~ 532°C
Explosive limits (in air)	Minimum explosive concentration 0.012 g/l with oxygen > 5%
Oxidising properties	Not an oxidising agent

2 GENERAL INFORMATION ON EXPOSURE

Table 2.1 summarises the amount of bisphenol-A used within different applications according to the published risk assessment (EC, 2003). This was based upon submissions made by the bisphenol-A manufacturers and end users to CEFIC.

Table 2.1 Bisphenol-A use pattern data

Use pattern data	Tonnes/year	Percentage of EU consumption
Polycarbonate production	486,880	71.1
Epoxy resin production	171,095	25.0
Phenoplast cast resin processing	8,800	1.3
Unsaturated polyester resin production	3,000	0.4
Can coating manufacture	2,460	0.4
Use PVC production and processing	2,250	0.3
Alkyloxylated bisphenol-A manufacture	2,020	0.3
Thermal paper manufacture	1,400	0.2
Polyols/Polyurethane manufacture	950	0.1
Modified polyamide production	150	<0.1
Tyre manufacture	110	<0.1
Brake fluid	45	<0.1
Minor uses	5,990	0.9
EU Consumption	**685,000**	

Figures in the table are approximate and based upon industry submissions for the years 1996-1999.
Minor uses include sales to chemical merchants and minor sales. The uses of these minor sales are not expected to be different from those mentioned above

2.1 NEW INFORMATION

2.1.1 Tetrabromobisphenol-A

The published risk assessment included consideration of the use of bisphenol-A in the production of tetrabromobisphenol-A (TBBPA). Production of TBBPA no longer takes place in the EU and so this use of bisphenol-A is not included in this addendum. The possible formation of bisphenol-A from the breakdown of TBBPA under certain circumstances is discussed in detail in the risk assessment of that substance (ECB, 2007). The main routes by which this could lead to bisphenol-A in the environment are through degradation in anaerobic sediments and through the application of anaerobically digested sludge to soil. Information on these processes has been used in Sections 3.1.4.6.2 and 3.1.4.7 to estimate possible concentrations of bisphenol-A in sediments and soil.

2.1.2 PVC

Further more specific information has been provided on the use of bisphenol-A in PVC-related areas; this is presented in Sections 3.1.2.1, 3.1.2.3 and 3.1.2.4.

2.1.3 Thermal paper

Industry has collected additional information on thermal paper recycling and performed monitoring studies at relevant sites. This information is included in Section 3.1.2.5.

2.1.4 Revised EU consumption figures

Industry has provided new production and consumption figures for bisphenol-A for 2005/2006. These are included in Table 2.2. These new values have been taken into account in the estimation of emissions in Section 3.1.2.

Table 2.2 Revised production and use tonnages for Western Europe (2005/2006)

Application	Tonnes/year	% change from published report	Information source
BPA production	1,150,000	+64	PlasticsEurope (1)
BPA uses			
Polycarbonate	865,000	+78	PlasticsEurope (1)
Epoxy resins	191,520	+12	PlasticsEurope (2)
– can coatings	2,755	+12	PlasticsEurope (2)
– ethoxylated BPA	2,260	+12	PlasticsEurope (2)
Phenoplast cast resin processing	8,800		
Unsaturated polyesters	3,600		Cefic (1)
Thermal paper	1,890	+35	ETPA
PVC – polymerisation	0		ECVM
- stabiliser packages	450	-10	ECVM, Cefic (2), (3), EuPC
- phthalate plasticisers	900	-10	ECVM, Cefic (2), (3), EuPC
- direct stabilisation	450	-10	ECVM, Cefic (2), (3), EuPC
Others	7,245		
Net exports	65,000		PlasticsEurope (1)
Total consumption	**1,149,870**	**+68**	

Figures for BPA production and polycarbonate use are estimated volumes
Figures for other use categories are calculated from estimated percentage increase/decrease since 2003 figures as provided by relevant industry group.
Information sources:

PlasticsEurope (1)	Polycarbonate / Bisphenol A Group
PlasticsEurope (2)	Epoxy Resins Committee
Cefic (1)	Unsaturated Polyester Resin Committee
Cefic (2)	ESPA European Stabiliser Producers Association
Cefic (3)	European Council for Plasticisers and Intermediates
ETPA	European Thermal Paper Association
ECVM	European Council of Vinyl Manufacturers
EuPC	European Plastics Converters

2.1.5 Other information

Sidhu *et al.* (2005) measured a wide range of substances in diesel particle extracts and in a sample collected from an uncontrolled domestic waste burn in a steel drum. Bisphenol-A was not reported as detected in the diesel particulate extracts, but was found in the sample from waste burning. The estimated emission rate was 9.66 mg bisphenol-A per kg waste burned. The authors used estimates of waste burned in this way in the United States to estimate an emission of 79 tonnes per year from this source in the US. This is of the same order as the industry emissions reported to the Toxic Release Inventory. There is no equivalent information to allow a similar calculation to be made for the EU, but releases as reported by industry make up only a small fraction of the total estimated emissions and so this source seems unlikely to have a significant impact (if any) on the estimated concentrations. This possible source is not considered further in this assessment.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 Environmental releases – published information

The emission estimates included in the published risk assessment report were based as far as possible on information specific to the production and use of bisphenol-A. Where this was not possible, default emission factors were used, in combination with information on the likely amounts to be used. The regional and continental emissions estimated in the published risk assessment report are summarised in Table 3.1.

Table 3.1 Summary of regional and continental releases from published risk assessment

Process	Air (kg/year)		Emission to wastewater treatment plants (kg/year)		Emission to receiving waters (kg/year)	
	Regional	Continental	Regional	Continental	Regional	Continental
Bisphenol-A production [a]	575	410			277	215
Polycarbonate bottle washing [b]			0.10	1.0	0.05	0.4
Epoxy resin production [a]					216	187
Phenoplast cast resin processing [b]			4.2	38	1.8	16
Thermal paper production [a]					36	70
Thermal paper recycling [c]			35,000	315,000		
PVC – Inhibitor during production process [b]			5,810	52,290	2,490	22,410
PVC – Anti-oxidant during processing [b]			75	674	32	289
PVC – Preparation of additive packages [b]			74	668	32	286
PVC –Use of additive package [b]			75	674	32	289
PVC – Anti-oxidant in plasticiser production			81	31		
PVC – Plasticiser use [b]			10	88	4	38
Losses from PVC articles in use [a]	1,560	14,040			2,250	20,450
Total	2,135	14,450	41,129	369,464	5,371	44,250
Total in kg/day (averaged over 365 days)	5.8	39.6	112.7	1,012.2	14.7	121.2

a) Releases to receiving waters calculated in the text (taking into account any WWTP)
b) Releases to wastewater calculated in the text; these are split 70:30 between WWTP and receiving waters in the table
c) ESD indicates all emissions go to WWTP

In addition to the releases in the table, there are also releases to soil of 2,250 kg/year in the regional environment, and 20,450 kg/year in the continental environment.

3.1.2 Revised emission estimates

The information presented in this section in some cases goes beyond the estimation of emissions and considers exposure situations. The revised PEC values are however presented later in Section 3.1.4.

3.1.2.1 Production of bisphenol-A

Updated information on emissions from production sites has been provided by industry for 2006 (personal communication from PlasticsEurope, 2007). This information is included in Table 3.2.

Table 3.2 Summary of environmental releases from bisphenol-A production sites

Site	Air		Effluent (After wastewater treatment)		Receiving water type and flow rate
	Measured levels	Release	Measured levels	Release	
BPA1	<0.2 mg/Nm^3 (outlet) <0.5 μg/Nm^3 (50 m from site)	<0.012 kg/day <4.4 kg/year	5.6 μg/l	0.06 kg/day 21 kg/year	Estuary 8.64 x 10^6 m^3/day
BPA2	2.9 mg/Nm^3 (outlet discontinuous) 0.1 μg/Nm^3 (outlet)	0.00017 kg/day 0.0605 kg/year	3.13 μg/l	0.07 kg/day 27 kg/year	River 2.068 x 10^8 m^3/day
BPA3	<1 mg/Nm^3 (dust)	<1 kg/day (dust) <365 kg/year (dust)	~0.005 mg/l	0.31 kg/day 113 kg/year	Estuary 8.08 x 10^7 m^3/day
BPA4		0.03 kg/day 9 kg/year		0.096 kg/day 35 kg/year	Estuary 2.49 x 10^7 m^3/day
BPA5		1.58 kg/day (dust) 575 kg/year (dust)	Up to 45 μg/l (average 3.5 μg/l)	0.019 kg/day *6.8* kg/year	Estuary 6.1 x 10^8 m^3/day
BPA 6	10 mg/Nm^3 (dust)	0.08 kg/day (dust) 31.2 kg/year (dust)	Average 10 μg/l	0.072 kg/day 25.8 kg/year	Sea (dilution factor 100)

Values changed from the published risk assessment are underlined. The unit of Nm^3 refers to air at standard temperature and pressure (the measurements may have been made originally with hotter air and so are corrected).

3.1.2.2 Use as an inhibitor in PVC production

The published risk assessment includes the use of bisphenol-A as an inhibitor in PVC production (i.e. the polymerisation of vinyl chloride). This use ceased voluntarily in the EU in 2003 (Defra, 2003), so there are no longer any emissions from this application and it is not considered further.

3.1.2.3 PVC additive formulation

3.1.2.3.1 New information

The PVC additive industry (represented by the European Stabiliser Producers Association, ESPA) has carried out two sampling exercises at sites producing PVC additive packages containing bisphenol-A. In addition, information on other sites has been collected relating to cleaning operations, water handling and treatment, water flows, tonnage used, etc.

A total of 13 sites are involved in the production of these packages in the EU. Measurements have been conducted at seven of these, accounting for 82% of the tonnage used in this area. (The tonnage in this area is now estimated at ~1,400 tonnes, which is an increase from the value used in the original risk assessment.) For the remaining sites, some information on the site is available for all but one, and the tonnage used in 2000 is available for all sites.

3.1.2.3.2 Calculation of emission factors

The sampling exercises were timed to coincide with periods of activity at the sites, in particular in relation to cleaning activities where these were relevant. As a result they can be considered to be representative of conditions when the sites are operating normally. The results of the measurements have been used to estimate the amounts of bisphenol-A released, and hence to derive emission factors. The arrangements on the sites have led to three factors being derived:

- emissions from all sources to an off-site treatment plant;
- emissions from all sources after on-site treatment (so release to surface water); and
- release in rainwater run-off.

The factors and the types of release from which they were derived are in Table 3.3.

Table 3.3 Emission factors derived from the data

Site	Emission factor for water type (kg/tonne)				Notes on combined emissions	External MWWTP
	wash	Rain	process	combined		
1				$\underline{5.93 \times 10^{-3}}$	Flow + process + some rain, after internal treatment	n
2					No data	y
3				7.2×10^{-3}	Flow + process after internal treatment	y
4		4.4×10^{-5}		0.037	Process + wash after internal treatment	y
5				4.6×10^{-3}	Cooling + rain + surface after internal treatment	n
6					No data	
7					No data	y
8				$\underline{0.19}$	Process + wash + rain after internal treatment	y
9					No data	n

Table 3.3 continued overleaf

Table 3.3 continued Emission factors derived from the data

Site	Emission factor for water type (kg/tonne)				Notes on combined emissions	External MWWTP
	wash	Rain	process	combined		
10				$2.8x10^{-5}$	Process + rain + sewer after internal treatment	n
11		$\underline{7x10^{-5}}$				n
12					No data	
13					No data	y

Underlined values are the selected emission factors for further calculations.

MWWTP – municipal wastewater treatment plant

3.1.2.4 Anti-oxidant in plasticizer production

Site-specific data for one site were included in the published risk assessment. These were used to estimate the total emissions from this use in the EU. The calculation of the PEC then used the default size for the wastewater treatment plant and the default dilution, resulting in a C_{local} of 1.9 µg/l. It has been pointed out by Industry that a site-specific PEC could be estimated as the size of wastewater treatment plant and river flow for the site were also provided (and in fact included in the original assessment). This calculation has therefore been revised. For the site providing information the actual flows and dilution have been used to estimate the PEC. This site is located on an estuary, and so the concentration relates to marine waters. Further information on the nature of the site, the way in which bisphenol-A is handled and where emissions can arise has also been provided for this site. This indicates that the emission factor used to estimate releases from this site - $1x10^{-4}$ kg/kg - should be applicable to other sites.

Information on the other four sites that use bisphenol-A in this way has also been provided, in the form of the annual quantities used. This has been used to create a generic site to represent the remaining tonnage. The emission factor above has been used to estimate releases from this site, and the TGD default treatment plant and river flows used to estimate the PEC for this generic site. This is used for the freshwater assessment. None of these four sites discharges to marine waters.

3.1.2.5 Thermal paper recycling

The published assessment included a number of assumptions related to recycling, based largely on information provided by the European Thermal Paper Association (ETPA). In reaction to risk management activity, ETPA commissioned a number of additional studies to test these assumptions. In particular, measurements were carried out at three sites that use recovered thermal paper. These include two sites receiving waste from the thermal paper production process (known as 'broke'), and a site receiving a general mixed recovered waste paper stream. These sites include examples with and without a de-inking step in the treatment of the recovered paper.

At the same time, better information has been provided on the specific applications for which thermal paper is now used, and also on the likelihood that paper used in these areas will be found in recovered paper streams.

This new information has been used to revise the calculations of PECs for this life cycle step, and hence the risk characterisation.

3.1.2.5.1 Use pattern

The uses of thermal paper have been changing in recent years. Use for industrial fax paper was once a major application, but this is declining. The European Thermal Paper Association (ETPA) has provided more detailed information on the current use pattern of thermal paper (ETPA, personal communication). The major use area is now for point-of-sale (POS) receipts (e.g. supermarket till receipts), followed by self-adhesive labels. Two smaller uses are in lottery tickets and fax paper. The degree to which each of these types of paper is recycled has also been estimated by ETPA (in consultation with the Institute for Paper Science and Technology, Technical University of Darmstadt) and the results are presented in Table 3.4.

Table 3.4 Use and recycling pattern for thermal paper (ETPA, personal communication)

Use area	Use percentage	Fraction recycled	Percentage of total recycled
Point-of-sale receipts	50	0.3	15
Self-adhesive labels	30	0.1	3
Lottery	10	0.2	2
Fax	10	1	10

The overall estimate is that around 30% of used thermal paper will enter recycling streams.

The different types of paper are also likely to find their way into different waste paper streams. Based on discussions with ETPA and the Institute for Paper Science and Technology, used fax papers and lottery papers are considered likely to be used in making graphic papers, for which a de-inking step is necessary. In contrast, labels and POS receipts are more likely to enter the mixed waste paper stream, which is used for production of packaging, etc., and where a de-inking step will not be used. Hence both of these types of recycling process need to be considered in the assessment of bisphenol-A.

3.1.2.5.2 Amounts of bisphenol-A used in thermal paper

The amount of bisphenol-A used in thermal paper in the EU is 1,890 tonnes (figure for 2005/6). This is used to make 2.4×10^9 m^2 of thermal paper, an area which is estimated to be equivalent to ~168,000 tonnes of paper. Up to 10% of the paper from the production process is waste (due to trimmings, etc.). This waste material is called 'broke'. It is sent directly to a small number of recycling plants and so never enters actual commercial use. In other words, 190 tonnes of bisphenol-A will be sent for recycling by the thermal paper production sites each year.

The amount of bisphenol-A actually used in thermal paper in the EU is therefore 1,700 tonnes. Around 30% of this paper is estimated to enter recycling streams (see Section 3.1.2.5.1), which is equivalent to 510 tonnes of bisphenol-A. So, in total, around 700 tonnes of bisphenol-A will find its way to paper recycling sites each year.

3.1.2.5.3 Size of paper recycling sites

As already noted in the original risk assessment, a survey indicated that there were 69 paper recycling sites in Germany in 2000. The original risk assessment divided the amount of thermal paper recycled in the EU over ten sites, which would over-estimate the amount recycled on one site. It is now known that there are around 1,000 paper production sites in the EU, of which around half use recovered paper as a source material (RPA, 2003). The total amount of paper recovered in the EU (all types) is around 42 million tonnes/year (CEPI, 2001). On this basis, an average site would use around 84,000 tonnes of recovered paper[7]. This figure will be used in the generic calculations. The use of an average size of site in this instance is appropriate, as the input of bisphenol-A to a site will in general be proportional to the size of the site (in these calculations it will be based on a representative concentration in the recovered paper used). In addition, the water use at a paper recycling site is (in general) related to the amount of paper used or produced, and so it is appropriate to use an average level of water use (and hence waste water treatment plant or WWTP) with an average size of site. ETPA considered that a WWTP size of 4,000 m^3/day was appropriate for this industry. From a study of the UK paper industry (Environment Agency, 2002), typical water consumption rates are in the range 8-16 m^3/tonne of paper produced. The average value, 12 m^3/tonne, has been used in these calculations; this corresponds to a WWTP with a capacity of 2,880 m^3/day for the average site.

There is one area where the available information does not allow a straightforward selection of a representative value to fit the site. This concerns sludge production from the processing of the recovered paper (this does not relate to sludge production in the WWTP). This can vary significantly between plants, and appears to depend much more on the type of paper produced than on the amount of water used. Examples from paper mills in the UK have sludge production rates ranging from 21 kg/tonne to 6.1 tonnes/tonne of paper, although a number are in the range 200-400 kg/tonne (Environment Agency, 2002).

Bisphenol-A measurements have been made at a specific site that takes general waste paper (see Section 3.1.2.5.4). This site has an average sludge production rate of 22.9 kg/tonne paper produced. This is a low figure, related to the lack of a de-inking step in the process, and is consistent with other estimates of losses of 2% of the waste paper used at this stage. This value will be used for the calculations without de-inking. The same value and a value of 200 kg/tonne will be used for calculations with de-inking.

3.1.2.5.4 Information from specific sites

Monitoring studies have been performed at three specific sites (TNO, 2003 & 2004). The information provided consists of:

- flow charts describing the operations carried out,
- quantities handled,
- water flows within the plant, and
- measurements on the levels of bisphenol-A in water within the plant and in the effluent from the final biological treatment.

[7] For comparison, the largest paper recycling site in the UK uses ~400,000 tonnes and the larger of the two thermal paper broke recycling site that provided data uses ~115,000 tonnes.

Details are not included in this report, but the main points relevant for the assessment are presented. An important point common to all of the investigations is that they were carried out at times when the plants were operating under normal conditions.

Sites with de-inking

Two sites were selected as they each receive a high input of bisphenol-A in the form of thermal paper broke from different thermal paper manufacturers. One site is in Germany, the other in Austria. The handling of the thermal paper broke takes place on a batch basis, and the measurements were conducted during the handling of batches of this waste. They can therefore be considered to represent a worst case for each site. The study is reported in TNO (2003).

De-inking rate

De-inking takes place as part of the process to reduce the recovered paper down to fibres. Measurements were carried out on the concentration of bisphenol-A in the waste paper fed to the process and on the fibre produced. These showed a removal efficiency for the process of 95%. (The default value used in the original assessment was 100%.)

Primary treatment

The water from the de-inking process is treated before it is passed to the biological treatment plant. Here the concentrations of bisphenol-A in the water from the de-inking process were compared to the concentration following the primary treatment. This showed a removal of 95.9% from water. (The default value used in the original assessment was 50%.) The bisphenol-A removed is included in the sludge produced from this process (no specific measurements were made on this sludge at these sites).

Biological treatment

The removal rate in the WWTP was estimated by measuring the concentration of bisphenol-A in the influent and in the effluent. The measurements were carried out over a period of time so that any variation in the levels would be observed. The timing of the sampling was arranged so that it covered the expected residence time in the treatment plant where batch paper processes were used. Sampling at regular intervals was employed where the production was continuous. Some variation in the results was seen; as a result the removal rates were calculated from the lowest influent concentrations and the highest effluent[8] concentrations for each site (corrected for recovery). The results indicate a removal level of 99.99% in the WWTP for the two sites.

The results of measurements on mixed samples of effluent (taken over four-hour periods) at both sites showed similar average concentrations of around 20 ng/l. Higher levels were found in a small number of individual spot samples, with maximum concentrations of 170 and 159 ng/l. These higher levels were thought to be due to a release of bisphenol-A when pulping of a batch was completed. These reported levels were not corrected for recovery; this was >95% for the influent samples, but only 33-35% for the effluent samples. Correcting the values for the lower recovery, the maximum effluent concentrations are in fact 500 ng/l and 467 mg/l.

[8] Earlier data reported for these sites in May 2003 were based on a lack of detection in the effluent at a limit of 2 µg/l; further measurements were made with an improved detection limit of 10 ng/l which allowed the actual concentrations in the effluent to be determined.

The process sludges from both sites are incinerated. This is described as standard practice for the countries where the sites are located.

Sites with no de-inking

A site in Germany that uses a representative mixed waste paper stream was chosen for further measurements. It was identified as a representative site by the Institute for Paper Science and Technology, Technical University of Darmstadt. The site produces corrugated packaging materials and does not operate a de-inking process. It operates continuously, and the mixed waste paper stream is checked for consistency. The site also has suitable access to monitoring points, and various internal parameters are routinely monitored. The study is reported in TNO (2004).

Input

The average concentration of bisphenol-A in the waste paper input to the plant was 14.7 mg/kg over the period of the study. This is the equivalent of a level of thermal paper in the waste of 0.1%, and gives a daily input of 15 kg of bisphenol-A. The concentration of bisphenol-A in the waste paper fits the expected level based on the estimate of the amount recycled in Section 3.1.2.5.2 (432 tonnes bisphenol-A in 42,000,000 tonnes of paper is ~10 mg/kg.)

Pulping

This is the equivalent step to de-inking above, where the recovered paper is reduced to fibres. The concentration of bisphenol-A was measured in the input materials (as indicated above) and in the final paper; the difference indicates that only 10% of the bisphenol-A in the waste paper feed was removed at this stage. This shows that without a de-inking step most of the bisphenol-A is retained in the recovered paper products.

Primary treatment

A comparison of the concentrations in water before and after the flotation treatment used as a primary treatment indicates a removal rate from water of 50%. Measurements were also carried out on the sludge produced by this treatment. The concentrations measured in sludge, together with the quantity of sludge produced, indicated that 18% of the input amount was present in the sludge. This leaves 32% of the input amount not accounted for. It is not clear what happens to this. The measurements in water are considered to be reliable, and so a removal rate from water of 50% is assumed for this process. The rest of the substance is assumed to be removed with the solid material for the purposes of this assessment. This leads to a higher concentration in sludge than was actually measured. For comparison, the measured level in the sludge will also be considered in the calculations.

The amount of sludge produced at this stage is 22.68 tonnes per day, which from the pulp production rate is a rate of 22.9 kg/tonne. This is towards the low end of values found for UK mills (Environment Agency, 2002), and reflects the lower sludge production rate for sites with no de-inking.

Biological treatment

The concentrations of bisphenol-A in the influent and effluent of the WWTP were measured. The average values were 193 μg/l and 42.7 ng/l respectively, indicating a removal of 99.98%. Measurements on the sludge produced in the WWTP indicate that this contained 0.98% of the

bisphenol-A entering the WWTP. The fate of bisphenol-A in the WWTP is therefore 0.02% to water, 0.98% to sludge with 99% degraded.

All sludges and rejects from the processes are incinerated at the site. This is described as standard practice in Germany.

Summary of the use of data from the three specific sites

Both monitoring studies appear to be well conducted, and the sampling strategy takes account of site operating conditions. The sites are considered to represent the range of situations in which thermal paper may undergo recycling. At one end of the scale there are two sites that receive high loadings of thermal paper (through the inclusion of thermal paper broke in the recovered paper feedstock). At the other end is a site accepting mixed general 'near household' waste paper containing a low level of thermal paper. Therefore the information from these sites will be used to calculate possible PECs for generic paper recycling sites. These calculations will consider sites taking general waste paper, with or without a de-inking step. The number of sites taking thermal paper broke is limited, and the data for the two sites are considered to be representative for this specific scenario (the results of the surveys are consistent despite the different characteristics of the two sites). However, a calculation based on the high level of bisphenol-A input at these sites is also included for information.

Bisphenol-A input to sites

The concentration of bisphenol-A measured in the recovered paper input to the site producing corrugated packaging (14.7 mg/kg – see above) is assumed to be typical for general paper waste streams. A concentration of 15 mg/kg is therefore used in the following calculations (this represents a bisphenol-A level of around 0.001%). Considering that broke will contain higher levels, a higher figure will also be used for those sites that use broke as part of their recovered paper feed. The maximum input calculated for the two sites receiving thermal paper broke was 0.074% of bisphenol-A in the paper feed.[9]

Removal of bisphenol-A from recovered paper

A removal rate of 95% is assumed for a site with de-inking; a rate of 10% is used for a site with no de-inking.

Fate during primary treatment

For a site with no de-inking, removal of 50% from water will be assumed (Section 5.2). A sludge production rate of 22.9 kg/tonne is assumed for this stage (based on data from the site without deinking described above).

For a de-inking plant, removal of 95.9% from water at this step is assumed. As there are no specific data on paper sludge production at de-inking sites, a figure of 22.9 kg/tonne will also be assumed for calculation purposes. However, a higher value of 200 kg/tonne will also be used for comparison based on UK information from paper mills in general (Environment Agency, 2002).

[9] Around 1,600 tonnes of bisphenol-A was used to make ~140,000 tonnes of thermal paper in 2001, giving a bisphenol-A content of 1.1% by weight. The average bisphenol-A content measured in thermal paper at the two 'broke' recycling sites is 0.7% and 0.88%, which are a similar order of magnitude.

Fate during biological treatment

The values obtained from the WWTP at the corrugated packaging production site (0.02% to water, 0.98% to sludge, from above) will be used for both processing types. This is assumed to represent a worst case, since the data were obtained with a low rate of substance input, and so WWTP microbial populations are less likely to have become adapted. For comparison, SimpleTreat calculations in the original risk assessment predict an overall emission to water of 12% from this step.

Use of sludges

In all three example sites, the sludges from the paper processing steps are incinerated (as are the biological sludges at some sites). This is described as standard practice for the relevant countries (Germany and Austria). It is, however, known that these sludges are applied to land (where they function as a soil improver) in other parts of the EU. Therefore, this route has been considered in the calculations for generic sites. Calculations have been carried out for the application of the paper and biological sludges individually and as a mixture in the proportions in which they are produced.

3.1.2.6 Effect of updated tonnages on local emission estimates

The changes to the tonnages for the various use areas are not considered likely to have a significant impact on the estimates of local emissions, as explained below. The effects on regional and continental emissions are considered later in the addendum.

Bisphenol-A production and polycarbonate production take place together, i.e. there are no polycarbonate production sites at which bisphenol-A is not produced. The emission estimates in the published risk assessment are based on site-specific data. Information from industry is that the emission control measures in place at the sites have been improved such that the emissions are now lower than those reported for the published risk assessment. Some new information is presented in Section 3.1.4.1.

The local emission estimates for epoxy resin use were based on specific information; the relatively small increase in the level of use is considered unlikely to mean significant changes in these values. For can coating and ethoxylated resins, the published risk assessment concludes no emissions to water and so there are no changes.

There is no change to the tonnage used in phenoplast resins. Use in unsaturated polyesters is a dry process according to the published risk assessment and so there are no emissions to change.

The increased tonnage used in thermal paper could mean an increase in the amounts released from paper production sites. There is no specific information on this at the moment. The sites included in the published risk assessment cover a range of sizes (using from 3 to 343 tonnes per year), and these still appear to be realistic within the increased tonnage. Therefore it seems reasonable to continue to use these as representative of this use pattern. For thermal paper recycling, the local estimates are based on a representative paper site using recovered material. The input to the site was based on measured levels in feed. There is no need to change these.

The local estimates for PVC were based on a typical size of site, and this will not change as a result of the reduction in the overall tonnage used in this area, hence the same estimates are retained.

3.1.2.7 Revised regional and continental emissions

3.1.2.7.1 Changes due to revised emission estimates and tonnage

Bisphenol-A production

The new information on releases from production sites, which include polycarbonate production as well, has been used to revise the emissions for this part of the life cycle.

Thermal paper recycling

The regional emissions from thermal paper recycling have been recalculated according to the new information presented above. Recovered thermal paper from fax and lottery use is assumed to be de-inked, whereas recovered POS receipts and labels are assumed not to pass through a de-inking step. Thermal paper broke is assumed to be de-inked. The emission factors described above in Section 3.1.2.5.4 have been used to estimate the annual amounts of bisphenol-A released to surface water, to biological sludges and to paper sludges, as in Table 3.5.[10]

Table 3.5 Calculated emissions of bisphenol-A from thermal paper recycling

	De-inking route	Non-de-inking route	Thermal paper broke
Amount	204 tonnes	306 tonnes	160 tonnes
To paper sludge	187 tonnes	15.3 tonnes	-
To biological sludge	78 kg	150 kg	-
To surface water	1.9 kg	3.5 kg	1.6 kg

Sludges from sites processing thermal paper broke assumed to be incinerated

The total amount to sludge (combined) is therefore 202.5 tonnes per year, and the emissions to surface water are 7 kg/year. The emission scenario document for paper (Environment Agency, 2002) suggests that 80% of sludge from paper recycling may be applied to land, hence the emission of bisphenol-A to land from this route is 162 tonnes. Both the surface water and soil emissions are assumed to be distributed as 10% to the region and 90% to the continental scale.

PVC additive formulation

The total releases from this use have been recalculated from those in the published assessment, using the revised information included in Section 3.1.2.3.[11] The total EU emissions to wastewater treatment are estimated as 81 kg/year, and the releases to surface water as 3.45 kg/year. The regional emissions are taken as those of the largest individual source from the published risk assessment; these are 2.76 kg/year for surface water emissions and 37.1 kg/year for emissions to wastewater treatment. The remainder of the totals is allocated to the continental emissions.

[10] Note that the recalculation presented in the risk assessment update of 2005 has been further modified here to take account of the increased quantity used in thermal paper.

[11] The figures presented in the risk assessment update of 2003 have been modified for this addendum to take account of the reduced use of bisphenol-A in PVC.

3.1.2.7.2 Changes due to revised tonnages only

For the other use areas not included in Section 3.1.2.7.1 the basis for the emission estimates has not changed since the published risk assessment. The regional and continental emissions from that assessment have been adjusted to take account of the changes in quantities produced and used, as follows. Only those areas that gave rise to emissions are considered here.

For polycarbonate bottle washing, it has been assumed that the amount of bottles has increased in line with the increased amount of polycarbonate used, and so the emissions have been increased by 78% from those in the published assessment.

The amount of bisphenol-A used in epoxy resins has increased by 12%, so the regional and continental emissions have been increased by the same factor.

There has been no change to the phenoplast resin use so the emissions remain as in the published assessment.

All PVC-related uses are assumed to have had the same reduction of 10% in quantity, and so the emissions from the published assessment have been reduced by 10% (with the exception of additive packages as described in Section 3.1.2.7.1). No adjustment has been made to the releases from PVC in use during its service lifetime. A lifetime of 30 years was assumed in the published assessment, and so it is assumed that any reduction has not had time to have a significant impact on the emissions from this life cycle step.

3.1.2.7.3 Summary of revised emission estimates

The revised estimates of emissions to the regional and continental scales are presented in Table 3.6. Note that the published risk assessment used a split of 70:30 between releases to waste water treatment and to surface water, as specified in the TGD at the time the assessment was being developed. This has been changed in the current addendum to 80:20 in line with the revised TGD. Also note that for some uses the emissions are estimated after any wastewater treatment and so are presented as emissions to surface water.

Table 3.6 Revised regional and continental emissions

Process	Air (kg/year)		Emission to wastewater treatment plants (kg/year)		Emission to receiving waters (kg/year)	
	Regional	Continental	Regional	Continental	Regional	Continental
Bisphenol-A production	575	409			113	115.6
Polycarbonate bottle washing			0.23	2.05	0.05	0.52
Epoxy resin production					242	209
Phenoplast cast resin processing			4.8	43	1.2	11
Thermal paper production					49	95
Thermal paper recycling					0.68	6.25
PVC – Anti-oxidant during processing			77	693	19	174
PVC – Preparation of additive packages			37	44	2.76	0.79
PVC –Use of additive package			77	693	19	174
PVC – Anti-oxidant in plasticiser production[a]			73	28		
PVC – Plasticiser use			10	91	2.7	23
Losses from PVC articles in use	1,560	14,040			2,250	20,450
Total	2,135	14,449	279	1,594	2,699	21,260
Total in kg/day (Averaged over 365 days)	5.8	39.6	0.76	4.4	7.4	58.2

a - all emissions via WWTP.

In addition there are emissions to agricultural soil from the application of paper sludge: 16.2 tonnes/year (44 kg/day) to the region and 145.8 tonnes per year (400 kg/day) to the continent.

3.1.3 Environmental fate

3.1.3.1 Abiotic degradation

No new information is available. A short atmospheric half-life of 0.2 days is calculated for the reaction of bisphenol-A with hydroxyl radicals (EC, 2003). The physical and chemical properties of bisphenol-A suggest that hydrolysis and photolysis are likely to be negligible.

3.1.3.2 Biodegradation

Results from a number of biodegradation studies were summarised in EC (2003):

- In the OECD 301F manometric respirometry test bisphenol-A meets the criteria for ready biodegradability.
- However in the OECD 301D closed bottle test and OECD 301B modified Sturm test no biodegradation was observed.
- In a modified SCAS procedure bisphenol-A met the criteria for inherently biodegradable substances, although this test can not give any indication of the potential for bisphenol-A to undergo ready biodegradation.

Measured levels of bisphenol-A before and after wastewater treatment at chemical plant and major users of bisphenol-A suggest a high level of removal. It is not possible to say if this is via adsorption to sludge or biodegradation, although based upon its chemical properties biodegradation is likely to be the major removal mechanism.

From the biodegradation studies reported bisphenol-A would appear to be readily biodegradable, possibly with a short period of adaptation. The default rate constant for biodegradation in wastewater treatment plant is $k=1\ h^{-1}$ for a readily biodegradable substance meeting the 10-day window. This value will be used in the assessment. The resulting fate in a wastewater treatment plant as estimated by EUSES is 12% to water and 6.2% to sludge, with 81.9% degraded and a negligible fraction to air.

A number of studies on the degradation of bisphenol-A in natural waters were also summarised (EC, 2003). Removal appears to be rapid once the waters have become acclimatised to bisphenol-A. The reported lag-phases before degradation are between 3-8 days. After the lag phase removal was rapid with 50% removal in 1-2 days and 100% removal in 2 to 17 days. These data would appear to indicate that in natural waters bisphenol-A may be classed as readily biodegradable meeting the 10-day test window. The default rate constant for biodegradation of $4.7 \cdot 10^{-2}\ d^{-1}$ probably under-estimates the removal rate, as it corresponds to a half life of 15 days with 97% removal taking 75 days. However this value has been used in the risk assessment as a conservative approach.

No information was available on the degradation rate of bisphenol-A in soil. Therefore, the degradation rate was estimated from the degradation rate of bisphenol-A in surface water and the soil-water partition coefficient. The half-life for biodegradation of bisphenol-A in soil and the first order rate constant for degradation in soil were calculated by EUSES as 30 days and $0.0231\ d^{-1}$, respectively, based upon bisphenol-A being readily biodegradable in surface waters.

3.1.3.2.1 New information

Aquatic

A further ready biodegradability study is available. CERI (2004) performed a manometric respirometry test (OECD 301F) on bisphenol-A. The average percent removal by BOD was 89%, and no parent compound could be detected by HPLC after 28 days. The 10-day window was met in this test.

A CAS (continuous activated sludge) simulation test has been carried out according to the OECD 303A guideline (TNO, 2001). The guideline was adapted to use a completely closed flow

through system, and radiolabelled substance was used in order to test environmentally relevant concentrations and determine a mass balance. The activated sludge system was acclimated to unlabelled bisphenol-A for four weeks, followed by a three week period when ^{14}C-labelled substance was fed into the system. Bisphenol-A was determined in the influent, effluent, waste sludge and in CO_2 traps. Recovery of the dosed radioactivity was 94-99%. Average removal of ^{14}C-bisphenol-A was 99.1%.

Nakada *et al.* (2006) measured the concentration of bisphenol-A (among a range of substances) in 24-hour composite samples of the influent and effluent from five municipal sewage treatment plants in Tokyo. All five plants used primary and secondary treatment with activated sludge. Bisphenol-A levels in the influent were between 100 and 1000 ng/l; removal of bisphenol-A was >92% on average.

Kang and Kondo (2002) investigated the effect of temperature on the biodegradation of bisphenol-A in river water. Samples of water from fifteen rivers in Japan were spiked with 0.2 mg/l bisphenol-A. At 30°C and 20°C degradation was complete after 10-15 days (half lives from two to seven days depending on the bacterial numbers in the wares samples at the start of the exposures). At 4°C, degradation was slower and had reached 20% after 20 days. Autoclaved water samples showed no removal, demonstrating that the major removal process is biological. The same authors (2002a) isolated specific bacterial strains with a high ability to degrade bisphenol-A. They also demonstrated a lack of degradation of bisphenol A under anaerobic conditions in river water.

Ike *et al.* (2000) studied the degradation of bisphenol-A in three activated sludge microcosms and forty four river water microcosms. The river water microcosms were prepared from water samples from seven rivers, at 15 sites, with conditions ranging from “clean” to “heavily polluted”. Degradation was noted in all of the sludge systems and in forty of the river water systems. Six of the river water systems were able to mineralise the substance completely, and 34 others showed TOC removal of 40-90%. Degradation tended to be greater in microcosms from more polluted waters. In the microcosms with partial removal, common metabolites accumulated, which appeared as two peaks in the HPLC traces. Bacteria isolated from the river water experiments were able to degrade bisphenol-A, and from further work with these the two main metabolites were identified as 2,3-bis(4-hydroxyphenyl)-1,2-propanediol and p-hydroxyphenacyl alcohol.

Suzuki *et al.* (2004a) investigated the biodegradation of bisphenol-A under laboratory conditions, using river water taken from a site on the Tama River in Japan which was influenced by effluent from a sewage treatment plant. After a two or three day lag period, bisphenol-A degraded rapidly, with estimated half lives of 0.4 and 1.1 days at 1 and 10 mg/l respectively. Optical density measurements on the water showed an increase in bacteria after two days of incubation. Metabolites were detected after three days, and correspond to those found in river water at the sampling site. The metabolite in the highest amount was 2,2-bis(4-hydroxyphenyl)propanoic acid (BPA-COOH), at 4.2% of the initial bisphenol-A concentration (1 mg/l). At 10 mg/l bisphenol-A, the metabolite in highest concentration was 2,2-bis(4-hydroxyphenyl)-1-propanol (BPA-OH), which reached a concentration of 679 µg/l after six days, and declined by 14 days. All of the detected metabolites appeared to decrease in concentration over longer exposures.

Kang and Kondo (2005) studied the degradation of bisphenol-A in river water and in seawater. In river water, half-lives of 4 days and 3 days were found at 25°C and 35°C respectively. In autoclaved seawater, no degradation of bisphenol-A was observed over 60 days, indicating no abiotic removal processes. In non-autoclaved seawater samples, no degradation was observed

over the first thirty days of exposure, despite an increase in the number of bacteria over the first three or four days (the numbers of bacteria then declined slowly). Bisphenol-A was degraded after thirty days, with the concentration reducing from 1 mg/l to ~200 µg/l after sixty days at 25-35°C. Some degradation was seen at 4°C, but starting only after 40 days. The concentration had reduced to ~700 µg/l by sixty days.

Ying and Kookana (2003) carried out degradation experiments on seawater and sediments from the coast around Adelaide, South Australia. Seawater samples were spiked with bisphenol-A at a concentration of 5 µg/l. The results showed little or no degradation over the first 35 days of the experiment, followed by rapid degradation over the following seven days. Bisphenol-A was almost completely degraded (>90%) after 56 days. Sediment and water samples (5 g and 5 ml respectively) were spiked with 1 µg/g bisphenol-A and kept under aerobic conditions for seventy days. The half-life of bisphenol-A under these conditions was 14.4 days. Similar sediment and water mixes kept under anaerobic conditions (monitored with resazurin as a redox indicator) showed no degradation of bisphenol-A over 70 days.

Ying *et al.* (2003) carried out biodegradation experiments on aquifer material from South Australia. Limestone sediment samples were taken from a depth of 153-154 m, and native groundwater samples were taken from the same aquifer. The aquifer materials were spiked with bisphenol-A and four other substances (nonylphenol, octylphenol, E2 and EE2), all at 1 µg/g, and incubated at 20°C for 70 days. Aerobic conditions were maintained throughout the experiment. Samples were taken weekly. Autoclaved aquifer materials were used as a control. There was no change in bisphenol-A concentration relative to the controls over the period (there was a slight reduction in concentration in both controls and the exposures).

The same authors carried out similar experiments on the same aquifer materials but under anaerobic conditions. The samples of aquifer material were placed in tubes that were placed in an anaerobic induction chamber under nitrogen for a month, until the redox indicator resazurin indicated that anaerobic conditions had been achieved. Samples were then spiked with the mixed substances. The exposures and sampling were carried out in the anaerobic induction chamber. There were no changes in the bisphenol-A concentration over the exposure period.

Hirooka *et al.* (2005) investigated the ability of green algae *Chlorella fusca* to degrade bisphenol-A. Algae were cultured with bisphenol-A at concentrations from 10 to 160 µM (2.3 to 36 mg/l) over seven days. Removal of bisphenol-A was >95% at concentrations up to 80 µM (18 mg/l), with 70% removal at 180 µM (36 mg/l). Algal growth was promoted over that in the controls at concentrations of 10-20 µM (2.3-4.6 mg/l). The amount of bisphenol-A in the algal cells was measured, and was significantly less than the amount lost from solution; after seven days it was below the limit of detection of the HPLC analysis used. Incubation in the dark resulted in only 27% removal of bisphenol-A. A metabolite, with an additional hydroxy group on one ring, was observed; this increased in concentration up to 72 hours and then decreased. A yeast two-hybrid assay used to assess estrogenic activity showed that this decreased in parallel with the reduction in bisphenol-A concentration.

A *Streptomyces* sp. strain isolated from river water in Japan was able to degrade bisphenol-A. A solution of 1 mg/l of bisphenol-A was degraded by >90% in 10 days at 30°C by a culture of the strain. A half-life of between three and four days was calculated (Kang *et al*, 2004). Zhang *et al.* (2007) isolated a strain of *Achromobacter xylosoxidans* from the compost leachate of municipal solid waste that was able to grow on bisphenol-A. Sasaki *et al.* (2007) isolated a strain (BP-7) of *Sphingomonas* from off-shore seawater samples in Japan which was able to degrade bisphenol-A completely over a period of 40 days alone, or over seven days when combined with a *Pseudomonas* strain.

Impact of new information

The new information supports the conclusion of the published risk assessment that bisphenol-A is readily biodegradable in natural fresh surface waters.

Terrestrial

Fent *et al.* (2003) studied the adsorption and degradation of bisphenol-A in soils from Germany: three soils from North-Rhine Westphalia and one from Rhineland Palatinate. The adsorption-desorption studies were carried out according to the OECD Guideline 106, the soil degradation studies according to a SETAC design.

For the degradation study, twelve test systems were set up for each soil type. Bisphenol-A (uniformly labelled with ^{14}C) was applied at 6 µg/100 g soil. Experiments were continued for 120 days. The test systems were analysed at intervals for the amount of extractable, non-extractable and volatile radioactivity (volatiles captured in soda lime trap for CO_2 and oil-wetted quartz wool for VOCs), as well as how much bisphenol-A remained in the system. Bisphenol-A rapidly formed bound residues in soil. After one hour, 19-59% of the applied radioactivity was non-extractable under normal conditions (methanol plus 5% acetic acid). After three days, 84.7 – 88.6% was not extractable. Following hot flux extraction, only a further 2.8% was removed, so that less than 7.4% was extractable using both techniques combined. At the end of the 120 days exposure, less than 2% of the applied radioactivity was extractable.

Depending on the soil, 13.1 – 19.3% of the label was recovered as CO_2 after the incubation period. No other volatile radioactive species were found. In one soil, after 1-2 hours, 49.2% of the bisphenol-A applied could be recovered, with 33% as other extractable species (up to five different metabolites). After three days the amount was less than the detection limit (1 µg/kg). No significant metabolites could be found after three days.

The authors comment that forming bound residues is common behaviour for phenols and anilines. Rapid transformation to transient metabolites suggests that most of the bound residues are in fact transformation products.

Ying and Kookana (2005) took samples of a sandy loam soil from a depth of 0-15 cm on a farm in South Australia. Bisphenol-A was added to 5 g of soil to give a concentration of 1 µg/g, and incubated at 20°C for 70 days. Degradation was rapid, with a half-life of seven days calculated from the results. Little or no degradation was seen in sterilised soil samples. When the soil was mixed with an equal amount of river water and allowed to attain anaerobic conditions before addition of the bisphenol-A, no degradation was seen.

Oshiman *et al.* (2007) isolated a bacterial strain, identified as belonging to the *Sphingomonas* genus, from soil from a vegetable-growing field in Japan. The strain was able to utilise bisphenol-A as the sole source of carbon and to use it as an energy source under aerobic conditions. The estrogenic activity of Bisphenol-A in the test medium was ultimately reduced by the strain, although the activity increased initially.

Impact of new information

The new information supports the conclusion of the published assessment that bisphenol-A is readily biodegradable in soil.

3.1.3.3 Distribution

Adsorption coefficients for environmental media were estimated using the TGD methods as implemented in EUSES (EC, 2003). The equation used to predict the K_{oc} value is that for hydrophobic chemicals in general as described in the TGD, using a log K_{ow} value of 3.40. The derived partition coefficients are as follows:

K_{oc}	715 l/kg	Organic carbon-water partition coefficient
Kp_{soil}	14.3 l/kg	Solids-water partition coefficient in soil
Kp_{sed}	35.8 l/kg	Solids-water partition coefficient in sediment
Kp_{susp}	71.5 l/kg	Solids-water partition coefficient in suspended matter
$K_{susp\text{-}water}$	18.8 m^3/m^3	Suspended matter-water partition coefficient
$K_{soil\text{-}water}$	21.7 m^3/m^3	Soil-water partition coefficient
$K_{sed\text{-}water}$	18.7 m^3/m^3	Sediment-water partition coefficient

These data suggest that bisphenol-A is likely to be moderately adsorbed to solids upon release to the environment.

Volatilisation is not considered to be a significant removal mechanism for bisphenol-A from water. Removal of bisphenol-A in rainwater is also considered to be negligible.

3.1.3.3.1 New information

Höllrigl-Rosta *et al.* (2003) measured the sorption of radiolabelled bisphenol-A to soil on standard batch equilibrium studies according to OECD Test Guideline 106. The soil was a loamy silt soil with an organic carbon content of 1%. A low water:soil ratio of 1.41:1 was used as resembling natural conditions. The substance was analysed in both phases. The K_{oc} value determined was 890±30 l/kg. Dialysis experiments using solutions of humic and fulvic acids as dissolved organic carbon were also carried out. The distribution coefficient K_{DOC} for humic acids was 860±70 l/kg, very similar to the K_{oc} value and considered to indicate that similar binding mechanisms were operating. No formation of adducts with fulvic acids was observed.

As part of a study on bisphenol-A in German soils (see Section 3.1.3.2.2), Fent *et al.* (2003) measured the adsorption of bisphenol-A in four soils using the OECD 106 Guideline. Degradation as well as binding was seen in the adsorption studies (rapid removal was seen in the degradation studies). In studies to measure the K_{oc} values a biocide was employed to reduce the degree of degradation in the experiments. The mean K_{oc} value obtained was 795.9 (mean Kd value 11.01).

Shareef *et al.* (2006b) looked at the sorption of bisphenol-A to mineral surfaces. Little sorption (<20%) was seen to goethite and kaolinite from a 3 µM (0.7 mg/l) solution, with little effect of pH. Sorption to these two minerals was rapid and completely reversible. When montmorillonite was used, sorption was greater and took longer, and only small amounts were desorbed at pH 7. It was proposed that bisphenol-A intercalated into the inter layer spaces of montmorillonite, whereas sorption to the other two minerals was to the surface.

Ying and Kookana (2005) took samples of soils from a depth of 0-15 cm from locations in South Australia. Sorption of bisphenol-A was measured using a batch equilibrium method, shaking for two hours. The organic carbon content of the soils ranged from 0.85 to 2.9%. The resulting K_{oc} values ranged from 251 to 1507, with a mean value of 962.

Loffredo and Senesi (2006) measured the sorption of bisphenol-A to samples of two acid sandy soils using a batch equilibrium method. Surface (0-30 cm) and deep (30-90 cm) horizons of both soils were used. The organic carbon content of the soils ranged from 1.1 to 9.3 g/kg. Bisphenol-A showed linear sorption to all four soils, with no indication of saturation (up to 40 mg/l with 5 g of soil). The K_{oc} values determined ranged from 335 to 703, average value 375. Sorption was almost completely reversible for three of the four soils, the exception being the surface soil with the highest organic carbon content.

The sorption of bisphenol-A to sediments from an aquifer system in South Australia has been studied (Ying *et al*, 2003). Limestone sediment samples were taken from a depth of 153-154 m. Sorption was measured in batch equilibration experiments at room temperature. The samples were shaken over a 16-hour period and then centrifuged. No loss of bisphenol-A (<3%) was found in controls without sediment. The relation between the sorption coefficient and the bisphenol-A concentration was not linear; the data were fitted to the Freundlich equation, and a coefficient of 3.89 with n=0.85 were obtained. The organic carbon sorption coefficient can only be calculated from these data for the concentration range tests, which was 2.5 – 20 µg/l. The value obtained for the coefficient was 778. The organic carbon content of the sediment was 0.5%.

Zeng *et al.* (2006) sampled sediments from five locations on the Xiangjiang River in China. Three samples were taken from each site, mixed and sieved (88 µm). Batch sorption experiments were carried out with six concentrations of bisphenol-A. Equilibrium was reached rapidly, mostly within the first hour. The organic carbon contents of the sediments were from 2.06 to 6.29% by weight. Three functions were fitted to the data – linear, Freundlich and a dual reactive domain model; all gave a reasonably good fit, with the Freundlich model giving the best fit. The linear model gave an average K_{oc} value of 115 l/kg over the five sediments (calculated from the data in the paper); the Freundlich model gave a value of 305 l/kg. Some irreversible sorption was observed in desorption experiments, but not to a great extent. The pH of the solutions had a small effect on the sorption.

Patrolecco *et al.* (2006) sampled surface water, suspended particulate matter and bed sediments in the River Tiber in Italy, in September 2002 (summer sample) and January 2003 (winter sample). The levels of bisphenol-A measured in water and bed sediments are included in Section 3.1.4.6.3. Bisphenol-A was measured in the suspended matter in two of the four samples taken in summer, and in all four taken in the winter. The levels measured in suspended matter and the corresponding water levels were used to calculate K_{oc} values; the range of results was 11,220 to 17,000.

Hu *et al.* (2006) used the partitioning of bisphenol-A between water and solid phase micro-extractant (SPME) fibres to investigate the effect of water parameters on the availability of bisphenol-A. The measured distribution coefficients are not directly relevant to the environment. Increasing salinity of the water increased the availability of bisphenol-A by around 1.2 times (i.e. the sorption to SPME fibres was reduced). An increase in pH from 5.5 to 8.5 decreased the availability by 1-2 to 1.4 times. The technique was also used to look at the effect of humic acids (commercial form) on the free fraction of bisphenol-A. The distribution coefficients on a dissolved organic carbon basis ranged from 4.03 to 5.60 (as log D_{DOC}). The values are much higher than those reported by Hollrigl-Rosta *et al.* above. Hu *et al.* note that the concentration of DOC in their study is more lower (1-50 mg/l) than that in the Hollrigl-Rosta study (190 mg/l). If the results are extrapolated to higher DOC values then the results are much closer.

Clara *et al.* (2004) looked at the sorption of bisphenol-A to sewage sludge. Activated sludge from a municipal wastewater treatment plant was used. Sludge from the same plant inactivated

by mercury (II) sulphate was also used, to distinguish between pure absorption and biosorption. In the batch sorption tests, bisphenol-A equilibrated with the sludge in around two hours, although all sorption experiments were carried out over 24 hours. There was no significant difference between the activated and inactivated sludge in terms of the sorption of bisphenol-A. The specific adsorption coefficient K_D (and hence the derived organic matter and organic carbon partition coefficients) was concentration dependent, decreasing with increasing free concentration in water, but no saturation was seen (up to 10 mg/l). From the equation given in the paper, the K_D value at 1 mg/l would be 257 l/kg, a similar value to that used in the published assessment (265 l/kg). The authors also investigated the effect of pH on sorption. Increasing the pH above 9 led to the desorption of bisphenol-A from sludge, and the desorption was complete at pH 12. Such high pHs can occur during sludge dewatering processes where limestone is used.

Impact of new information

The new studies provide slightly different results. Batch equilibrium studies in laboratories on both sediment and soil samples give results that are generally similar to the estimated value used in the published risk assessment. There are some indications from field studies where both water and sediment samples were taken that the degree of sorption may be greater (at least sorption to suspended matter). Comments by the CSTEE prior to publication of the original report (CSTEE, 2002) pointed out that measured sediment levels appeared higher than expected from the water levels from the same locations. There is no explanation at present of what might cause this effect. There are always difficulties when comparing measured levels in different compartments to be sure that the samples can be considered to be in steady state with each other. Although there are some indications of enhanced sorption, the majority of the tests, in particular the standard batch equilibrium studies, give results with similar values to those used in the published risk assessment. The values from the published assessment have therefore been retained in the calculations for this addendum.

3.1.3.4 Accumulation and metabolism

The available measured data suggested that bisphenol-A has a low potential for bioaccumulation in fish, in contrast to the moderate potential indicated by the log K_{ow} value. A slightly higher potential was indicated by the measured bioconcentration in freshwater clams (up to 144). Measured data are preferred over calculated values when the studies are valid. A BCF of 67 for fish was therefore used in the published risk assessment, and the accumulation in clams was considered in the risk characterisation (EC, 2003).

A bioconcentration factor for earthworms of 7.9 kg/kg was estimated using QSARs (as implemented in EUSES).

3.1.3.4.1 New information

Bioaccumulation

Lindholst *et al.* (2003) studied the metabolism of bisphenol-A in zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). Adult zebrafish were exposed to 100 µg/l bisphenol-A in a flow through system for 168 hours. Exposures took place in a 100 l aquarium, with a flow rate of eight replacement volumes per day, and 150 fish. The bisphenol-A concentration was measured every two days; the actual concentration found was 97.5±5.2 µg/l. Fish were sampled

at 0, 2, 6, 12, 24, 48, 72, 120 and 168 hours. After this time the remaining fish were transferred to a system to which bisphenol-A was not added and kept for the same length of time, with sampling at the same intervals. Zebrafish tissue samples were analysed for bisphenol-A, bisphenol-A glucuronic acid (BPAGA) and bisphenol-A sulphate (BPAS).

Rainbow trout were exposed under similar conditions for eight days to 100 µg/l bisphenol-A (actual concentration from 2-day samples 107.3±6.3 µg/l). After eight days, gall bladder and blood samples were taken, and the bile fluid and blood plasma analysed for the same three substances (bisphenol-A, BPAGA and BPAS).

Uptake and excretion rates for fish were calculated by fitting data to exponential uptake and decay models (much of the data for rainbow trout came from earlier publications). Uptake was fitted to a first order model, excretion to a first or second order model depending on the goodness of fit. Bisphenol-A was detected in zebrafish after two hours' exposure, and steady sate was reached by 24 hours. Steady state concentrations were 569 ng/g for bisphenol-A, 12.6 µg/g for BPAGA and 39.3 ng/g for BPAS. The whole body uptake rate for zebrafish was calculated as 0.23; tissue specific values from rainbow trout plasma, liver and muscle were 0.73, 0.11 and 0.16, so the rates were similar between the two species despite the different matrices.

Elimination from zebrafish was fitted to a second order model; the first compartment had a half life of <1.1 hours, the second compartment half life was 139 hours. The three trout tissues had elimination half-lives of 3.7, 1.8 and 5.8 hours for plasma, liver and muscle respectively, as first order elimination. The authors suggest that in zebrafish bisphenol-A is rapidly removed from tissues, metabolised by the liver and excreted primarily as BPAGA into the gall bladder (compartment 2). Elimination from the tissues in zebrafish is much more rapid than from trout tissues. Zebrafish have a lower sensitivity to bisphenol-A than does trout when considering vitellogenin synthesis. It is suggested that this may be due to the more rapid metabolism resulting in lower bisphenol-A concentrations and a reduced response. Data on specific tissue concentrations in the liver for bisphenol-A and metabolites was needed to confirm this.

Lee *et al.* (2004) measured the accumulation of bisphenol-A in spotted halibut (*Varaspar variegates*) in a seven-day semi-static exposure, with renewal of solutions every twenty four hours. Nonylphenol was also included in the same exposures at similar concentrations. The bioconcentration factor determined at an exposure level of 70 µg/l was 38±21 l/kg, averaged over the seven days.

Killifish (*Oryzias latipes*) were exposed to bisphenol-A at 17 µg/l in a flow-through system for six days (Takino *et al*, 1999). Fish were analysed at intervals, and the results at five and six days showed that steady state had been reached. The mean BCF from these two times was 73.4 l/kg.

Koponen *et al.* (2007) studied the uptake of bisphenol-A in larvae of the common frog *Rana temporaria*. Radiolabelled substance was used at a nominal concentration of 1.84 µg/l in water, the solutions were renewed every day for the three day uptake experiment. The results are based on total radioactivity. The effect of UVB exposure was also considered in the experiments, only the results without UVB are considered here. The steady state BCF value obtained was 140±38; the value derived from uptake and depuration rates was 131. Growth correction was applied, and resulted in a small decrease in the elimination rate estimate; the revised BCF values (with growth correction by two methods) were 147 and 144. As a radiolabelled substance was used the results will include any degradation or metabolism products, hence the results have to be interpreted with caution.

Takahashi *et al.* (2003) measured the concentrations of bisphenol-A in water, periphytons and benthos in the Tama River in Japan. The range of concentrations in water was 0.02-0.15 µg/l.

The concentrations in periphytons were 2.0-8.8 µg/kg and in benthos were 0.3-12 µg/kg, giving bioaccumulation factors for periphytons of 18-650 and for benthos of 8-170.

Metabolism

Kang *et al.* (2006) have reviewed the metabolism and biodegradation of bisphenol-A in organisms – bacteria, fungi, plankton, plants, invertebrates, fish, birds and mammals. There is evidence of metabolism or biodegradation in all of these. The authors conclude that although the metabolites can enhance estrogenicity or toxicity, in general metabolism leads to detoxification of bisphenol-A. This does not mean that the substance does not show effects in organisms.

Metabolism of bisphenol-A by plant tissues has been demonstrated (Nakajima *et al.* 2002). Tobacco BY-2 cells in suspension culture reduced the concentration of added bisphenol-A rapidly after addition, with no bisphenol-A detected 2.5 hours after application. Use of radiolabelled substance allowed four metabolites to be observed, the most abundant being a glucopyranoside derivative (BPAG). When labelled bisphenol-A was administered to the roots of tobacco seedlings, radioactivity was incorporated in BPAG and three other unidentified metabolites that were accumulated in the leaves.

Schmidt and Schupan (2002) demonstrated the metabolism of bisphenol-A in plant cell suspension cultures. The products found were glycosides of bisphenol-A, non-extractable residues and highly polar, presumed polymeric, products. The proportion of each product type varied with plant species. It is possible that bisphenol-A could be liberated from the glycosides, for example under acid conditions, but the other products appeared stable for the most part.

[Note: uptake into plants is not a major route for bisphenol-A. The assumptions in the assessment are that any substance taken up into the plant is available, so metabolism is likely to reduce the level in plants.]

Spivack *et al.* (1994) carried out further work on the metabolic pathways of bisphenol-A in bacterial strain MV1 (details included in EC, 2003). The major pathway was found to account for 85% of the metabolised bisphenol-A, the minor route for the other 15%. Both routes lead to mineralisation, in whole or in part. Similar metabolic pathways with similar degrees of importance were found in a bacterial isolate *Pseudomonas paucimobilis* (Jin *et al*, 1996).

Sasaki *et al.* (2005) purified components of the cytochrome P450 monooxygenase system from a *Sphingomonas* sp. strain A01 (see also soil degradation in Section 3.1.3.2.1), which was able to degrade bisphenol-A. Two degradation products were detected by HPLC analysis and were thought to be 1,2-bis(4-hydroxyphenyl)-2-propanol and 2,2-bis(4-hydroxyphenyl)-1-propanol.

Yim *et al.* (2003) screened 26 species of micro-organisms for ability to degrade bisphenol-A. The species *Aspergillus fumigatus* KCTC 6145 was found to be able to metabolize bisphenol-A. The main product from the metabolism was bisphenol-A-*O*-β-D-glucopyranoside (BPAG). It is not clear from the report whether this was the only species to metabolize bisphenol-A, or whether it was the most successful. The process to obtain sufficient metabolite to allow identification is described as preparative scale biotransformation. Kang *et al.* (2004) isolated a strain of *Streptomyces sp*. from river water in Japan that was able to degrade bisphenol-A, with a half life of three to four days.

Impact of new information

The new BCF values for fish are generally similar to that used in the published risk assessment and so no change is necessary.

3.1.4 Predicted Environmental Concentrations (PECs)

This section presents the PEC values derived from a combination of the new data and that in the published risk assessment. The next four sections present the calculation of Clocal values for uses where the local emissions have been revised based on new information. The calculation of the regional and continental concentration follows. The PEC values for all current uses are then presented for each compartment in turn, together with the results of a survey of monitoring data. PEC values for STP ($PEC_{microorganisms}$) and for air are not included, as there were no risks for either of these compartments in the published risk assessment and the emissions have either reduced or remained the same.

3.1.4.1 Bisphenol-A production

The revised information on releases from production and polycarbonate sites has been used to calculate revised PEC values for the sites. These new values are included in Table 3.9.

3.1.4.2 PVC additive formulation

The factors presented in Table 3.3 were used to estimate emissions from the other sites, using whatever site information was provided for these. Emissions were also estimated for routes of release not covered by the measurements at the seven sites (usually rainwater run-off). Rainwater run-off estimates were only made where it was clear that the rainwater from the site was channelled into a receiving water. The resulting concentrations are presented in Table 3.6.

Table 3.7 C_{local} values for PVC additive sites

Site	C_{local}	Notes
1	0.02 µg/l	Marine.
2	4.2 ng/l	
3	0.074 µg/l	
4	0.012 µg/l	
5	0.013 µg/l	
6	0.01 µg/l	Marine
7	8 ng/l	Marine
8	<0.022 µg/l	
9	0.2 µg/l	
10	<0.32 ng/l	
11	1.1 ng/l	
12	0.065 µg/l	No information on presence of MWWTP – calculations performed for scenarios with and without MWWTP, and higher concentrations included here
13	0.48 ng/l	Marine

MWWTP Municipal wastewater treatment plant

3.1.4.3 Anti-oxidant use in plasticiser formulation

For the specific site, the actual wastewater treatment plant and river flows at the site have been used to calculate a Clocal value of 2 ng/l. For the generic site, the default wastewater treatment plant and dilution have been used to give a Clocal of 0.36 µg/l.

3.1.4.4 Thermal paper recycling

3.1.4.4.1 Site with de-inking

The concentration of bisphenol-A in the waste paper feed is 15 mg/kg. An average site will use of 84,000 tonnes of waste paper per year, over 350 days (ETPA, personal communication). This is equivalent to 240 tonnes of paper/day. The bisphenol-A input to the site is therefore 3.6 kg/day.

De-inking removes 95% from the paper, hence 3.42 kg/day is emitted to water.

At primary treatment, 95.9% is removed to the paper sludge, i.e. 3.28 kg/day. The remainder stays in the water, so 0.14 kg/day is emitted to a WWTP.

In the WWTP: 0.98% to biological sludge, so 1.4 g/day.

0.02% to effluent, so 28 mg/day.

The WWTP flow is 2,880 m^3/day, hence the effluent concentration is 9.7 ng/l. Dilution by 10 (the default factor) gives a Clocal of 0.97 ng/l.

Sites receiving thermal paper broke

An estimated maximum input of 0.074% of bisphenol-A in the paper feed corresponds to an input of 178 kg/day for the generic site. Following the same calculations as above, but using a removal rate in the WWTP of 99.99% (the value obtained from the two specific sites based on corrected concentrations, reflecting the higher level of removal at sites receiving a higher rate of input of bisphenol-A), gives a Clocal of 24 ng/l.

For comparison, the maximum concentration measured in the effluents for these sites (after correction for recovery) was 500 ng/l, giving a Clocal of 50 ng/l based on the default dilution factor. The maximum Clocal estimated from actual receiving water flow rates is 55 ng/l. Since these are maximum values, only the value for the generic site given in the preceding paragraph will be taken through to the risk characterisation section.

Sludges from the thermal paper broke recycling sites are assumed to be incinerated. There are no data on levels in sludge from these sites at the moment.

3.1.4.4.2 Site without de-inking

As above the bisphenol-A input to the site is estimated to be 3.6 kg/day.

Pulping removes 10% from the paper, hence 0.36 kg/day is emitted to water.

At primary treatment, 50% is removed to paper sludge (i.e. 0.18 kg/day). The remainder is emitted to water, so 0.18 kg/day is emitted to a WWTP.

In WWTP: 0.98% to biological sludge, so 1.76 g/day.

0.02% to effluent, so 36 mg/day.

The WWTP flow rate is 2,880 m^3/day, so the effluent concentration is 12.5 ng/l. Dilution by 10 (default factor) gives a Clocal of 1.25 ng/l. The average concentration in the effluent from the actual site measurements was 43 ng/l; assuming a ten-fold dilution gives a Clocal of 4.3 ng/l, which is in good agreement with this generic estimate. Again, only the generic estimate will be taken forward to the risk characterisation section.

3.1.4.5 Regional and continental concentrations

The regional and continental concentrations have been calculated using EUSES 2.0.3. The emissions used are summarised in Table 3.1. The resulting regional PEC values are:

$PECregional_{water}$ = 32 ng/l

$PECregional_{sediment}$ = 0.52 µg/kg wwt

$PECregional_{soil}$ = 0.07 µg/kg wwt

$PECregional_{marine\ water}$ = 2.7 ng/l

$PECregional_{marine\ sed}$ = 0.034 µg/kg wwt

The estimated regional concentration in water is comparable to the concentration of bisphenol-A found in the feed water to the general paper recycling site described in Section 3.1.2.5.4, which was 32 ng/l (average of three samples).

3.1.4.6 PEC values – water (fresh and marine)

3.1.4.6.1 Calculated PEC values

The PEC values calculated using EUSES 2.0.3 are presented in Table 3.8 (for uses where Clocal has not changed from the published assessment) and Table 3.9 (for uses where new Clocal values were calculated in the preceding sections). Values for marine waters and sediments have been added for the generic scenarios, and for specific sites where these discharge to marine or estuarine waters (for these specific sites the marine values replace the freshwater values). For the generic marine scenarios it is assumed that the effluent is not treated in a wastewater treatment plant, and a default dilution of 100 for marine waters has been used. Specific dilution rates have been used for individual sites where this information is available, otherwise the default value of 100 is used with the site-specific information.

3.1.4.6.2 Bisphenol-A from tetrabromobisphenol-A (TBBPA) in sediment

The risk assessment for TBBPA (ECB, 2007) concludes that there is strong evidence that TBBPA can degrade to give bisphenol-A under certain anaerobic conditions. This has been demonstrated conclusively for marine or saline sediments, freshwater sediments and anaerobic sewage sludge, and it is possible that it could also occur in other anaerobic systems. From Section 3.1.3.2 of this addendum, bisphenol-A is expected to be stable under anaerobic

conditions. It is therefore possible that the degradation of TBBPA in sediment could lead to the production of bisphenol-A.

An initial estimation of possible levels can be obtained by assuming that all of the TBBPA present in anaerobic sediment degrades to bisphenol-A. The results of such calculations are presented in Tables 3.10 and 3.11; the concentrations of TBBPA in sediment are taken from the TBBPA risk assessment report (ECB, 2007) and the calculation assumes that 90% of the total sediment concentration is converted (i.e. 100% conversion of TBBPA in the anaerobic part of the sediment which makes up 90% of the total sediment) . Note that only those activities taking place within the EU are included in the table (the risk assessment also has example calculations for other processes but these are not relevant to the EU).

Table 3.8 PEC values for water and sediment (fresh and marine) for uses where Clocal is unchanged from the published risk assessment[a]

	Freshwater		Marine	
	PEC_{water} (µg/l)	**PEC_{sed} (µg/kg)**	**PEC_{marine_water} (µg/l)**	**PEC_{marine_sed} (µg/kg)**
Site specific				
ER 1	0.033	0.53		
ER 2, ER 3, ER 6	0.032	0.52		
ER 4	0.99	16		
ER 5	0.062	1.0		
PAPER 1	0.31	5.1		
PAPER 2	0.14	2.3		
PAPER 3	0.10	1.6		
PAPER 4	1.03	17		
PAPER 5	1.03	17		
PAPER 6	0.97	16		
PAPER 7	0.07	1.1		
Generic scenarios				
Polycarbonate bottle washing	0.032	0.53	0.003	0.046
Phenoplast cast resin processing	1.47	24	*1.2*[a]	*20*[a]
PVC – Anti-oxidant during processing	0.19	3.0	0.13	2.1
PVC – Plasticiser use	0.14	2.3	0.09	1.5

a This scenario is included for completeness, although no relevant sites discharging to marine waters have been identified (see Section 3.3.1.2)

Table 3.9 PEC values for water (fresh and marine) for thermal paper, PVC additives and anti-oxidant use in plasticiser production

	Freshwater		Marine	
	PEC_{water} (µg/l)	**PEC_{sed} (µg/kg)**	**PEC_{marine_water} (µg/l)**	**PEC_{marine_sed} (µg/kg)**
Production				
BPA 1			0.01	0.16
BPA 2	0.032	0.53		
BPA 3			0.008	0.13
BPA 4			0.006	0.11
BPA 5			0.003	0.05
BPA 6			0.10	1.7
PVC additive package				
Site A1			0.023	0.38
Site A2	0.036	0.58		
Site A3	0.11	1.8		
Site A4	0.044	0.71		
Site A5	0.045	0.73		
Site A6			0.013	0.21
Site A7			0.011	0.18
Site A8	0.054	0.88		
Site A9	0.27	4.4		
Site A10	0.032	0.52		
Site A11	0.033	0.54		
Site A12	0.097	1.6		
Site A13			0.009	0.15
Anti-oxidant use in plasticiser production				
Specific site			0.005	0.08
Generic site	0.39	6.4		
Thermal paper recycling				
With deinking	0.033	0.54	0.003	0.045
Without deinking	0.033	0.54	0.003	0.046

Table 3.10 Estimated maximum concentrations of bisphenol-A from anaerobic degradation of TBBPA in sediment

Scenario			Estimated concentration of tetrabromobisphenol-A in sediment (mg/kg wet wt.)	Estimated maximum concentration of bisphenol-A in sediment (mg/kg wet wt.)
Reactive flame retardant use	Manufacture of epoxy and/or polycarbonate resins		0.36-0.44	0.13-0.16
	Processing of epoxy resins		2.7×10^{-3}-4.9×10^{-3}	1.0×10^{-3}-1.9×10^{-3}
Additive flame retardant use	ABS	Compounding	14.6-17.8	5.5-6.7
		Conversion	0.66-0.81	0.25-0.31

The calculations given above make a number of worst case assumptions: all of the TBBPA in aerobic sediment is degraded; the only product is bisphenol-A; there is no degradation or removal of the bisphenol-A formed; degradation of TBBPA is instantaneous. In reality the formation of TBBPA will take place over time and other processes will act on the bisphenol-A formed. A more realistic analysis of the situation has been carried out by EURAS (2006). This approach considers the adsorption/desorption of bisphenol-A and its degradation in water and aerobic sediment, as well as the similar processes for TBBPA. This approach assumes that the bisphenol-A produced is able to desorb to water and be degraded or removed; this would also be necessary for bisphenol-A to be available to have effects. A degradation rate for TBBPA (for conversion to bisphenol-A) equivalent to ready biodegradability was assumed as a worst case (this is four orders of magnitude greater than the degradation rate for TBBPA used in the risk assessment). The concentrations of TBBPA and bisphenol-A estimated using this approach are in Table 3.11. These are considered to be more realistic than the initial estimates and so will be used in the risk characterisation.

Table 3.11 Estimated concentrations of bisphenol-A from anaerobic degradation of TBBPA in sediment (more realistic approach)

Scenario			Estimated concentration of tetrabromobisphenol-A in sediment (mg/kg wet wt.)	Estimated maximum concentration of bisphenol-A in sediment (mg/kg wet wt.)
Reactive flame retardant use	Manufacture of epoxy and/or polycarbonate resins		0.36-0.44	$(1.42\text{-}1.95)\times10^{-4}$
	Processing of epoxy resins		2.7×10^{-3}-4.9×10^{-3}	$(1.23\text{-}1.7)\times10^{-6}$
Additive flame retardant use	ABS	Compounding	14.6-17.8	$(5.75\text{-}7.9)\times10^{-3}$
		Conversion	0.66-0.81	$(2.6\text{-}3.6)\times10^{-4}$

The calculations were only performed for the freshwater environment. The estimated concentrations of TBBPA in marine sediments are approximately one order of magnitude below those in freshwater (with one exception). The marine sediment concentrations of bisphenol-A resulting from TBBPA breakdown are therefore expected to be around one order of magnitude lower than those in Table 3.11.

3.1.4.6.3 Measured concentrations

Industry (PlasticsEurope, 2007) have reviewed the available monitoring studies for bisphenol-A. The studies were reviewed for their quality in two ways. Firstly, the completeness of the information reported in the studied was considered (on methods, locations, quality assurance (QA) procedures, etc.). Secondly the quality of the analytical methods and QA, etc., were assessed. Only studies that were considered as reliable or very reliable in both assessments were included in the further analysis. A total of 99 papers were reviewed initially, with 79 retained for the further analysis. The majority of the samples came from 1998 – 2003.

A number of issues arose in combining the data from different studies to categorise different regions, including high numbers of not detected results and different detection limits. A non–parametric method (Kaplan-Meier) was adopted to address these issues. The results of the evaluation are in Table 3.13 (freshwater), Table 3.14 (freshwater sediments), Table 3.15 (marine waters) and Table 3.16 (marine sediments). A summary of the data is presented in Table 3.12.

Table 3.12 Summary of measured levels data for water and sediment

	Freshwater	Freshwater sediment	Marine water	Marine sediment
Observations:				
Total number of weighted observations	848	249	115	67
Number of weighted observations below detection limit	415	75	58	44
Number of imputed observations[a]	10	65	0	0
Concentrations:	*(µg/l)*	*(ng/g dw)*	*(µg/l)*	*(ng/g dw)*
Median	0.01	16	0.0016	8.5
Mean	0.13	60	0.017	75
SD	1.5	134	0.052	209
5th percentile	0.0005	0.5	0.00005	1.1
95th percentile	0.35	256	0.088	566

a Where individual data points were not available, representative points were imputed from the summary statistics where possible.

Table 3.13 Bisphenol-A concentrations in freshwaters in the EU (all units are µg/l)

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Austria	Sattelberger and Scharf, 1999	1998	34 locations in 15 rivers	34	0.01	9/34 (26%)	Min = <0.01 Median = <0.01 95th % = 0.0559 Max = 0.075
	Paumann and Vetter, 2003; Bursch *et al*., 2004; Hohenblum *et al*., 2004	2001-2002	Monthly samples collected from 24 rivers	272	0.01	72/272 (26%)	Min = <0.005 Median = <0.005 95th % = 0.16 Max = 0.6
Belgium	Loos *et al*., 2007	2003	3 rivers, south of Ghent (Schelde, Molenbeek, Gaverbeek) each sampled upstream and downstream of wastewater discharge	18	NA	16/18 (89%)	Mean of 3 samples at each location, range: 0.003 to 0.055
Czech Republic	Stachel *et al*., 2002; Wiegel *et al*., 2004	1999 & 2000	Elbe River and mouths of its tributaries	17	0.001	15/17 (88)	Min = <0.001 Median = 0.019 95th % = 0.089 Max = 0.114
	Umweltbundesamt, 1999 and 2000; secondary reference to Gandrass, 1999	1998	4 locations (Bilina, Dolni Berkovice, Synthesia, Horenice)	4	0.0003	4/4 (100%)	Min = 0.0015 Median = 0.0278 95th % = 1.10 Max = 1.29
Denmark	Umweltbundesamt, 1999 and 2000; secondary reference to Boutrup, *et al*, 1998	1998	2 locations in Aarhus County	2	0.1	0/2 (0%)	< 0.1
	Christiansen *et al*., 2002; secondary reference for Danish reports by Christiansen and Plesner, 2001 and Boutrup and Plesner, 2001	NA	2 lakes and 3 streams	NA	0.001	NA	Min = < 0.001 Max = 0.44
France	Jeannot *et al*., 2002	NA	Orleans, downstream of wastewater effluent	1	0.0005	0/1 (0%)	< 0.0005

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Germany	Umweltbundesamt, 1999 and 2000; secondary reference for Gandrass, 1999; Voigt, 1999; Koerner, 1998	1998	River Elbe	11	0.0003	11/11 (100%)	Min = 0.0047 Median = 0.0127 95th % = 0.0369 Max = 0.0406
		1999	River Rhine - 7 locations, sampled monthly for 6 months	NA	0.01	NA	Mean ranges from <0.01 to 0.08
	Fries and Puettmann, 2002	2000-2001		NA	0.020	NA	Min = < 0.020 Mean ranges from 0.051 to 0.084
	Bolz *et al*., 2001	1998-1999	Baden-Wurttemberg - streams and rivers; evaluated the influence of STP discharges on water quality	23	0.050	NA	Min = <0.050 Max = 0.272 Median = 0.0721 for water receiving STP discharge; <0.050 with no STP discharge
	Kuch and Ballschmiter, 2001	2000	Southern Germany - Danube River, Blau River, Nau River (upstream and downstream of STP effluent discharge), Iller River, Schussen Creek, Laiblach Creek, Argen Creek	31	0.00004	31/31 (100%)	Min = 0.0005 Median = 0.0038 95th % = NA Max = 0.014
	Heemken *et al*., 2000	1998	River Elbe (10 locations) and 4 of its tributaries	18	0.00005	18/18 (100)	Min = 0.0089 Median = 0.0585 95th % = 0.223 Max = 0.776
	Stachel *et al*., 2002; Wiegel *et al*., 2004	1999-2000	River Elbe and mouths of its tributaries	35	0.001	35/35 (100)	Min = 0.003 Median = 0.023 95th % = 0.093 Max = 0.100
	Wenzel *et al*., 1998; Fromme *et al*., 2002	1997	North Rhine-Westphalia, Rheinland-Pfalz, Brandenburg & Berlin: rivers, lakes and channels. Used 2 methods of analysis: GC-MS and HPLC.	116	0.0001 (GC-MS); 0.002 (HPLC)	116/116 (100%) (GC--MS); 73/116 (63%) (HPLC)	Min = 0.0005 (GC-MS); <0.002 (HPLC) Max = 0.229 (GC-MS); 0.410 (HPLC)
	Fleig, 2000	NA	River Rhine - 3 locations (Karlsruhe, Mainz, Duesseldorf)	NA	NA	NA	Mean at each site: 0.027, 0.028, 0.033 90th % at each site: 0.052, 0.044, 0.050
Italy	Vigano *et al*., 2006	2005	River Po, upstream and 2 locations downstream of confluence of River Lambro; samples collected every other day for 3 weeks	27	0.00333	27/27 (100%)	Mean of samples at each location: 0.270, 0.302, 0.494
	Lagana *et al*., 2004	2002	Rome; Tiber River - downstream of	7	0.0002	7/7 (100%)	Min = 0.015

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
			wastewater effluent discharge				Median = 0.022 95th % = NA Max = 0.029
	Loos *et al*., 2003	2002	8 locations in River Seveso and upstream and downstream of 3 STPs near Como	15	0.002	3/15 (20%)	Min = <0.002 Median = <0.002 95th % = 0.040 Max = 0.043
	Loos *et al*., 2007	2003	2 rivers (Seveso, Lambro) upstream and downstream of wastewater discharge; located in textile industry region	6	0.002	5/6 (83%)	Min = <0.002 Median = 0.053 95th % = 0.150 Max = 0.175
	Patrolecco *et al*., 2004 and 2006	2002 & 2003	Tiber River - upstream and downstream of urban sources, including wastewater from Rome	5 locations, sampled twice	0.030	9/10 (90)	Min = < 0.030 Median = 0.080 95th% = 0.122 Max = 0.140
Netherlands	Umweltbundesamt, 1999 and 2000; secondary reference for Belfroid *et al*., 1999	1997	6 locations (Rhine at Lobith; Maas at Eijsden; Nieuwe Waterweg at Maassluis and Benelux Tunnel; Haringvliet at Haringvlietsluizen; Noordzeekanaal at Ijmuiden)	12	NA	10/12 (83%)	Min = 0.0099 Median = 0.0355 95th % = 0.108 Max = 0.16
	Belfroid *et al*., 2002	1999	River Dommel, River Meuse (Eysden), Bergermeer Lake	7	0.011l	2/7 (29%)	Min = <0.011 Median = < 0.018 95th % = 0.16 Max = 0.17
	Vethaak *et al*., 2002 and 2005	1999	Nationwide monitoring program	97	NA	50/97 (52%)	Min = <0.0088 Median = 0.018 95th % = 0.322 Max = 1.0
Norway	Pettersen and Fjeld, 2005	2005	Drammen waterway	2	0.010	1/2 (50)	Min = <0.010 Max = 43.0
Portugal	Azevedo *et al*., 2001	1999	National monitoring program; samples collected monthly for three months at 8 locations - rivers and coastal locations	24	0.002	13/24 (54%)	Min = < 0.002 Median = 0.35 95th % = 2.0 Max = 4.0
	Quiros *et al*., 2005	2001-2002	10 rivers (from Ponte Nova Barcelos to Esteiro Seixal), sampled monthly from April 2001 to Dec. 2002; summary	183	0.09	132/183 (72%)	Min = <0.01 Median = 0.10 95th % = 0.88

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
			statistics calculated using the averages for each location				Max = 0.92 (individual sample max = 5.03)
Spain	Brossa *et al*., 2005	2001-2002	Catalonia: Ebre River and irrigation canal of Ebre Delta	36	0.002	2/36 (6%)	Min = < 0.002 Median = < 0.002 95th % = 0.004 Max = 0.02
	del Olmo *et al*., 1997	NA	Granada: Santa Maria farm spring water	NA	0.6	NA	< 0.6
	Gonzalez-Casado *et al*., 1998	NA	Granada: Loja - river water	3	0.0012	0/3 (0%)	<0.0012
	Brossa *et al*., 2002	NA	Catalonia: Ebro River	NA	0.01	NA	< 0.01
	Cespedes *et al*., 2006	2001	Ter River and 2 of its tributaries (9 locations) - receive discharge from STPs	9	0.05	0/9 (0%)	< 0.05
	Cespedes *et al*., 2005	2001	Llobregat River Basin - 10 locations, including Anoia and Cardener tributaries	11	0.09	7/11 (64%)	Min = < 0.09 Median = 0.09 95th % = 1.91 Max = 2.97
	Penalver *et al*., 2002	NA	Ebro River	NA	0.06	0%	< 0.06
	Brossa *et al*., 2004	NA	Catalonia: river water	1	0.002	0/1 (0%)	<0.002
	Petrovic and Barcelo, 2001	2000	Anoia and Cardener rivers - 6 sites, upstream and downstream of STPs	6	0.1	0/6 (0%)	<0.1
	Rodriguez-Mozaz, *et al*., 2004 and 2005	2002	Llobregat River - monthly samples tested for 6 months	6	0.0063	6/6 (100%)	Min = 0.065 Median = 0.138 95th % = 0.279 Max = 0.295

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Switzerland	Voutsa *et al*., 2006	2004	Glatt River (10 locations; each sampled 3 times)	30	0.007	30/30 (100%)	Min = 0.009 Median = 0.026 95th % = 0.058 Max = 0.076
	Fleig, 2000	NA	River Rhine at Basel	NA	NA	NA	Mean = 0.010 90th % = 0.024
UK	Fawell *et al*., 2001	1998	Severn Trent water: Trent, Severn, and Derwent rivers; Tame/Trent confluence	8	5.1	0/8 (0%)	<5.1
	Liu *et al*., 2004b	2003	England, river water - East and West Sussex. Upstream, downstream and near sewage outfall (3 locations)	NA	0.0053	NA	Min = < 0.0053 Max = 0.024
	Readman *et al*., 2006	2006	Swindon: River Ray upstream of Rodbourne STP	1	0.0104	0/1 (0%)	< 0.0104
			Swindon: River Ray downstream of Rodbourne STP (3 locations; max. 8 km downstream)	3	0.0104	3/3 (100%)	Min = 0.338 Median = 0.436 Max = 0.449
			River Ock (reference site)	1	0.0104	0/1 (0%)	< 0.0104
	Environment Agency, 2003	2003	12 locations (each sampled 2 to 5 times); includes Boveney Ditch above Thames; River Aire; River Don; Manchester Ship Canal; River Douglas; River Tame; Sowe River	42	0.013	33/42 (79%)	Min = < 0.01 Median = 0.092 95th % = 0.526 Max = 0.899

NOTE:
NA - Not available
STP - Sewage treatment plant

Table 3.14 Bisphenol-A concentrations in freshwater sediments in the EU (all units are ng/g dw unless otherwise stated)

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Czech Republic	Stachel *et al*., 2005	2002	37 locations along Elbe River and mouths of tributaries after a flood	37	5	NA	Min = < 5 Median = 30 Max = 1630
Denmark	Umweltbundesamt, 1999 and 2000; secondary reference to Boutrup, *et al*, 1998	1998	5 locations in Aarhus County, including residential and agricultural areas	36	2	NA	Mean of samples at each location, range: < 10 to 150
Germany	Bolz *et al*., 1999	NA	Baden-Wurttemberg (South Germany): Lake Constance and 2 small streams (Korsch and Sulzbach)	3	10	0/3 (0)	< 10
	Bolz *et al*., 2001	1996-1999	Baden-Wurttemberg – streams and rivers; evaluated the influence of STP discharges on water quality	11	0.5	9/11 (82)	Min = <0.5 Median = 5 95th % = 13 Max = 15
	Heemken *et al*., 2000	1998	Elbe River (8 locations) and 3 of its tributaries	11	0.5	11/11 (100)	Min = 66 Median = 132 95th % = 311 Max = 343
		1998-99	Elbe River – monthly sampling at one location (Schnackenburg)	12	0.5	12/12 (100)	Min = 127 Median = 211 95th% = 288 Max = 322
	Stachel *et al*., 2002; Wiegel *et al*., 2004	2000	Elbe River and mouths of its tributaries	12	NA	12/12 (100)	Min = 10 Median = 55 95th % = 300 Max = 379
		1998	Alster River	2	NA	2/2 (100)	Min = 143 Max = 192
		1996	Berlin/Brandenburg surface waters	12	0.2	NA	Min = <0.2 Max = 190
	Wenzel *et al*., 1998; Fromme *et al*., 2002	1997	Brandenburg and Berlin – 35 waterways (rivers, lakes, and channels). Used 2 methods of analysis: GC-MS and HPLC.	35	1 (GC-MS); 5 (HPLC)	30/35 (86%)	Min = 18 (GC-MS); 10 (HPLC) Max = 190 (GC-MS); 150 (HPLC)

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Italy	Vigano *et al*., 2006	2005	River Po, upstream and 2 locations downstream of confluence of River Lambro	3	3	0/3 (0%)	< 3
	Patrolecco *et al*., 2004 and 2006	2002 & 2003	Tiber River - upstream and downstream of urban sources, including wastewater from Rome	4 locations, sampled twice	30	0/8 (0%)	< 30
Netherlands	Vethaak *et al*., 2002 and 2005	1999	Nationwide monitoring program	18	NA	14/18 (78%)	Min = 0.5 Median = 2.35 95th % = 27 Max = 43
Norway	Fjeld *et al*., 2004a	2003	Landfills or industrially contaminated sites: leachate ponds	6	NA	6/6 (100)	Min = 7.06 Median = 108.68 95th % = 346.33 Max = 371.16
			Lake Mjosa and Lake Losna	4	NA	4/4 (100)	Min = 10.64 Median = 37.10 95th % = 47.88 Max = 48.04
			Drammens River (industrialized area).	7	NA	7/7 (100)	Min = 6.07 Median = 25.48 95th % = 227.65 Max = 279.8
	Fjeld *et al*., 2004b	2004	Lagen and Vorma Rivers (inlet and outlet of Lake Mjosa)	6	NA	6/6 (100)	Min = 9.27 Median = 27.79 95th % = 50.14 Max = 50.53
		2003	Drammens River (industrialized area)	1	NA	1/1 (100)	53.14
	Pettersen and Fjeld, 2005	2005	Drammen waterway	17	1 to 5	6/17 (35)	Min = <1 Median = <1 95th % = 26 Max = 62
Spain	Petrovic and Barcelo, 2001	2000	Anoia and Cardener rivers - 6 sites, upstream and downstream of STPs	6	10	2/6 (33%)	Min = < 10 Median = < 10 95th % = 34 Max = 40
Sweden	Hedlund *et al*., 2006	2003-2004	Nationwide monitoring program	35	50	3/35 (9%)	Min = <50 Median = < 50 95th % = 83 Max = 320

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
UK	Liu *et al*., 2004a	2003	England, Sussex Rivers: River Uck, River Ouse; upstream and downstream of sewage outfall (7 locations)	15	3.4	8/15 (53%)	Min = <3.4 Max. not reported; max. ave. = 9; std dev = 2.7
	Centre for Environment, Fisheries and Aquaculture Science (CEFAS)	1999-2000	England - national monitoring program (50 locations, upstream and downstream of STP outfalls)	50	1.07 ng/g wet weight	2/50 (4%)	Min = <1.07 ng/g wet wt Median = <1.07 ng/g wet wt 95th % = <1.07 ng/g wet wt Max = 56.8 ng/g wet wt

NOTE:
NA - Not available
STP - Sewage treatment plant

Table 3.15 Bisphenol-A concentrations in marine waters in the EU (all units are µg/l)

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Germany	Heemken *et al*. 2000	1998-1999	North Sea (11 locations, sampled twice)	20	0.00005	10/20 (50%)	Min = <0.00005 Median = 0.00083 95th % = 0.0514 Max = 0.249
	Beck *et al*., 2005	2003-2004	Baltic Sea (5 locations, sampled twice)	29	0.00004	28/29 (97%)	Min = <0.00004 Max = 0.0057 Mean at each location: 0.00022 to 0.0054
	Stachel *et al*., 2002; Wiegel *et al*., 2004	2000	Elbe River (mouth)	1	0.001	1/1 (100%)	0.0038
Italy	Famiglini *et al*., 2005	NA	Adriatic Coast (middle-western) - near shore and mouths of rivers and canals	20	0.0032	2/20 (10%)	Min = < 0.0032 Median = < 0.0032 95th % = 0.074 Max = 0.0845
	Pojana *et al*., 2004a	2001-2002	Venice Lagoon - 2 locations near the historical centre and 1 location near the industrial area; bimonthly samples collected for 10 months	15	0.001	14/15 (93%)	Min = <0.001 Mean at each location: 0.010, 0.0045, 0.0044 Max = 0.030
	Pojana *et al*., 2004b	2001-2002	Venice Lagoon - 25 locations sampled 4 times	NA	0.001	NA	Min = <0.001 Max = 0.066
Netherlands	Umweltbundesamt, 1999 and 2000; secondary reference for Belfroid *et al*., 1999	1997	5 locations (Zeehavenkanaal at Delfzijl; Westerschelde at Hansweert and Terneuzen; Kanaal Gent-Terneuzen; Oosterschelde Oesterput)	5	NA	5/5 (100%)	Min = 0.0035 Median = 0.0038 95th % = 0.019 Max = 0.023
	Belfroid *et al*., 2002	1999	Wadden Sea (Bocht van Wattum, Dantziggat, and Den Oever)	10	0.01	5/10 (50%)	Min = <0.01 Median = 0.016 95th % = 0.33 Max = 0.33

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Spain	del Olmo *et al*., 1997	NA	Malaga - sea water	NA	0.6	0%	< 0.6
	Brossa *et al*., 2005	2001-2002	Catalonia: industrial port of Tarragona	12	0.002	2/12 (17%)	Min = < 0.002 Median = < 0.002 95th % = 0.015 Max = 0.02
	Gonzalez-Casado *et al*., 1998	NA	Granada: Motril - sea water	3	0.0012	0/3 (0%)	<0.0012
	Brossa *et al*., 2004	NA	Catalonia: industrial port of Tarragona	1	0.002	0/1 (0%)	<0.002
	Petrovic and Barcelo, 2001	NA	Barcelona, Almeria, Tarragona - 14 sites near outfalls of industrial plants and municipal wastewater discharges	10	0.1	0/10 (0%)	<0.1

NOTE:
NA - Not available
STP - Sewage treatment plant

Table 3.16 Bisphenol-A concentrations in marine sediments in the EU (all units are ng/g dw)

Country	Author	Sample dates(s)	Sample Description	No. of Samples	Minimum Detection Limit	Frequency Detection (%)	Summary Statistics
Denmark	Umweltbundesamt, 1999 and 2000; secondary reference to Boutrup, *et al*, 1998	1998	Aarhus County; 2 locations	10	2	NA	Mean at each location: < 2 and 13 ng/g
Norway	Fjeld *et al*. 2004a	2003	Norwegian coast from near Russian border to the outer Oslofjord	12	NA	12/12 (100%)	Min = 0.01 Median = 89.13 95th % = 448.0 Max = 623.38
	Fjeld *et al*., 2004b	2003	Drammersfjord	1	NA	1/1 (100%)	51.05
Spain	Petrovic and Barcelo, 2001	NA	Barcelona, Almeria, Tarragona - 14 sites near outfalls of industrial plants and municipal wastewater discharges	14	10	0/14 (0%)	< 10
	Morales-Munoz *et al*., 2005a and 2005b	NA	Aguadulce (near Almeria) - composite sample collected near outfall of an urban STP; used 2 different extraction methods	1	0.2	1/1 (100%)	Mean = 19.6
Sweden	Hedlund *et al*., 2006	2003-2004	Nationwide monitoring program; 10 locations	14	50	0/14 (0%)	<50
UK	Kelly *et al*., 2006	NA	National monitoring program; 22 locations near urban or industrial areas (includes Tees River, Tyne River, Belfast Lough, Burbo Bight, River Dee)	22	10	6/22 (27%)	Min = < 10 Median = < 10 95th % = 1030 Max = 1140

NOTE:
NA - Not available
STP - Sewage treatment plant

3.1.4.6.4 Comparison of measured and calculated levels

In general it is not possible to assign the measured values to a corresponding calculated value, either by specific use or by scale. The measured values are considered to provide a good picture of the bisphenol-A levels in industrial/urban areas and can be considered as a mixture of local and regional concentrations. Hence only a general comparison is possible.

For freshwater, the calculated values fall within the range of the 95%ile or maximum values from the measurements. The calculated regional concentration falls toward the lower end of the range of 95%ile values. The calculated regional PEC value of 34 ng/l is higher than the median value from the whole freshwater data set, 10 ng/l, but is just below the 75%ile value from the data set, 42 ng/l.

For freshwater sediment, the measured values are reported as dry weight, so need to be reduced by a factor of 2.6 to be on the same wet weight basis as the calculated values (using the standard water content of sediment in the TGD). The highest of the 95%iles from the measured values are above the range of calculated values. The higher calculated values are similar to the middle of the range of measured levels, 10-20 µg/kg wwt (25-50 ng/g dwt).

The amount of measured data for marine waters is more limited, but covers a similar range to the calculated values, the highest calculated values being a little above the highest measured levels reported. Measured and calculated levels for marine sediment also cover largely similar ranges (after adjusting to the same basis as above). The highest measured values are somewhat above the highest calculated levels, and the lowest calculated level (the regional background) is below the range of measurements.

Overall the calculated and measured values are comparable. There are some exceptions, but these cannot be related to specific situations. Therefore the risk assessment will be based on the calculated values.

3.1.4.7 PEC values - terrestrial

PEC values for the terrestrial compartment have been calculated using EUSES 2.0.3. The only significant route to the terrestrial environment is through the application of sewage sludge. As in the published assessment, values have not been calculated for most of the site-specific scenarios, as the information obtained indicates that disposal of the sludge from many of these is either by incineration or as controlled waste to landfill[12]. Where there is no specific information then a calculation has been performed, but this does not necessarily mean that sludge from the site is actually applied to land. Sites with no on-site biological treatment that do not release to an off-site treatment plant are also excluded.

The resulting PEC values are in Table 3.17.

[12] A calculation for one epoxy resin site (ER4) with sludge application was included in the published assessment. Information provided for this update shows that sludge from this site is no longer applied to land. There is therefore no sludge application related to epoxy resin production and this scenario has been deleted from the terrestrial assessment.

Table 3.17 PEC values for the terrestrial compartment (agricultural soil at 30 days)

	PEC (µg/kg wwt)
Site specific	
PVC additive package: A2	1.43
A3	0.86
A4	1.38
A6	3.46
A8	0.77
A13	0.07
Generic scenarios	
Phenoplast cast resin processing	20
PVC – anti-oxidant during processing	2.2
PVC – plasticiser use	1.6
Anti-oxidant in plasticiser production	5.0
Thermal paper recycling with deinking	633 (p); 1.4 (b); 534 (c)
Thermal paper recycling without deinking	35 (p); 1.7 (b); 29 (c)

p Paper sludge;

b Biological sludge;

c Combined paper and biological sludges (in ratio produced)

Note that these PECs are calculated using the usual TGD method, i.e. sludge is assumed to be applied once per year for 10 years, and the PEC represents the situation 30 days after the final application. In this case, the bisphenol-A concentration is effectively zero after one year, so the PEC is equivalent to the concentration 30 days after an initial application of sludge. However, the $PNEC_{soil}$ is derived from toxicity data based on the initial concentrations of bisphenol-A applied to soil (see Section 3.2.3.1). The concentrations in soil for the risk characterisation must therefore also be expressed on the same basis. This is considered further in Section 3.3.2.

3.1.4.7.1 Thermal paper recycling

Some specific considerations in relation to thermal paper recycling are included here.

Concentrations in sludges from sites with de-inking

From paper treatment, 5.5 tonnes of sludge are produced per day. A total bisphenol-A content of 3.28 kg gives a sludge concentration of 597 mg/kg dry weight (dwt).

From biological treatment, 1,022 kg of sludge are produced per day. A total bisphenol-A content of 1.4 g gives a sludge concentration of 1.4 mg/kg dwt.

If sludges are mixed in the proportions in which they are produced, then the mixed sludge concentration is 504 mg/kg dwt.

The bisphenol-A concentrations in soil at 30 days after application of these sludges[13] (using the TGD method, with input from air neglected) is as follows:

- paper sludge: 633 μg/kg wet weight (wwt)
- biological sludge: 1.5 μg/kg wwt
- combined: 534 μg/kg wwt.

For comparison, if the paper sludge production rate at the site were 200 kg/tonne, then the combined sludge concentration would be 66 mg/kg, and the resulting soil concentration would be 70 mg/kg wwt.

Sludges from the thermal paper broke recycling sites are assumed to be incinerated. There are no data on levels in sludge from these sites at the moment.

Concentrations in sludges from non-deinking sites

From paper treatment, 5.5 tonnes of sludge are produced per day. A total bisphenol-A content of 0.18 kg gives a sludge concentration of 33 mg/kg dwt.

From biological treatment, 1,022 kg of sludge are produced per day. A total bisphenol-A content of 1.76 g gives a sludge concentration of 1.7 mg/kg dwt.

If sludges are mixed in the proportions in which they are produced, then the mixed sludge concentration is 28 mg/kg dwt.

The bisphenol-A concentrations in soil at 30 days after application of these sludges[13] (using the TGD method, with input from air neglected) is as follows:

- paper sludge: 35 μg/kg wwt
- biological sludge: 1.8 μg/kg wwt
- combined: 29 μg/kg wwt.

For comparison the measured concentrations in the paper sludge and biological sludge from the actual site producing corrugated packaging were 12 mg/kg and 5.5 mg/kg respectively. Combining the two sludges gives a calculated concentration of 8.1 mg/kg. The resulting soil concentrations would be 13 μg/kg wwt (paper sludge), 5.9 μg/kg (biological sludge) and 8.6 μg/kg (combined). These figures are broadly in line with the generic calculation, but note that in reality these sludges are incinerated at the site.

3.1.4.7.2 Bisphenol-A in sludge from the degradation of TBBPA

A further possible exposure route for the soil compartment is the degradation of TBBPA during anaerobic wastewater treatment processes, in particular anaerobic sludge digestion[14]. If this did occur, then it is possible that the bisphenol-A formed would be applied to agricultural land with

[13] As bisphenol-A is readily biodegradable there is no residual substance in soil from previous applications.

[14] Anaerobic digestion of sewage sludge is carried out at elevated temperatures (e.g. 35-37°C (mesophilic digestors) or 55°C (thermophilic digesters)) and the residence time of sludge within the system is usually in the range 10-20 days but can be longer).

the digested sludge. The available experimental evidence on this is discussed in the TBBPA risk assessment (ECB, 2007) and is conflicting. A recent study has shown that TBBPA can be degraded to bisphenol-A by anaerobic sewage sludge when incubated at 35°C, but the rate of degradation was relatively slow, for example the half-life for the initial loss of TBBPA from the system was around 19 days, and total yield of bisphenol-A was around 48% after 120 days. However much more rapid disappearance of TBBPA has been demonstrated in a test system using digested sewage sludge (the half-life for degradation of tetrabromobisphenol-A was reported to be 0.59 days) but this study has not yet been fully validated. In contrast to this, a further (unpublished) study has reported little or no degradation of TBBPA in bench-scale reactor systems based on the contact anaerobic process and the conventional aerobic activated sludge process.

A rough estimate has been made of the maximum possible concentration of bisphenol-A that would be present in soil if it was formed from TBBPA during sludge digestion. The concentrations of TBBPA in sludge are taken from the TBBPA risk assessment. The calculation assumes that all the TBBPA present in sludge is converted to bisphenol-A on a molar basis, and that when the sludge is applied to agricultural soil, bisphenol-A will be susceptible to biodegradation in the aerobic conditions present. The resulting concentrations in soil have been estimated with EUSES and are shown in Table 3.18. These figures are speculative, as it is not certain that this reaction occurs during anaerobic sludge digestion, and the calculations do not take into account the rate of the reaction.

3.1.4.7.3 Measured levels

No information on measured levels in soil was available for the published risk assessment and no new information has been located. Limited data on measured levels in sewage sludge were included in the published assessment and showed values which were around three orders of magnitude lower than those calculated; the values calculated for this addendum are similar to those calculated for the published assessment and so are also higher than the available measurements.

3.1.4.8 PEC values - Secondary poisoning

EUSES 2.0.3 has been used to calculate concentrations of bisphenol-A in freshwater fish and earthworms. For the site specific calculations, the site with the highest emission to the appropriate compartment (water or soil) has been used as the basis for the calculations. Concentrations in worms are only calculated for those uses with releases to soil via sludge, as in Section 3.1.4.7. The results are in Table 3.19.

Table 3.18 Estimated maximum concentrations of bisphenol-A in soil that could potentially result from application of sewage sludge from TBBPA processes

Scenario			Estimated concentration of TBBPA in sewage sludge (mg/kg dry weight)	Estimated maximum concentration of bisphenol-A	
				in sewage sludge (mg/kg dry wt.)	in agricultural soil (30-day average) (mg/kg wet wt.)
Reactive flame retardant use	Manufacture of epoxy and/or polycarbonate resins		28-30 (sludge from the eight major brominated epoxy resin companies in the EU is not applied to agricultural land)	12.6	0.013
	Processing of epoxy resins		0.052-0.056	0.024	2.5×10^{-5}
Additive flame retardant use	ABS	Compounding	1.13×10^{3}-1.22×10^{3}	512	0.54
		Conversion	51.6-55.5	23.3	0.025

Table 3.19 PECs for secondary poisoning

	PEC in freshwater fish for predators (µg/kg)	PEC in worms for predators (µg/kg)
Site specific		
Bisphenol-A production (BPA 2)	2.2	-
Epoxy resin (ER 4)	29	-
Thermal paper production (PAPER 6)	28	-
PVC additive package (A6)	8.9	1.6
Generic scenarios		
Polycarbonate bottle washing	2.2	-
Phenoplast cast resin processing	35	7.8
PVC – anti-oxidant during processing	5.7	1.2
PVC – plasticiser use	4.7	0.92
Anti-oxidant use in plasticiser production	11	2.2
Thermal paper recycling with deinking	2.2	237 (p); 0.84 (b); 200 (c)
Thermal paper recycling without deinking	2.2	13 (p); 0.98 (b); 12 (c)

p Paper sludge;

b Biological sludge;

c Combined paper and biological sludges (in ratio produced)

For those scenarios with possible releases to the marine environment, concentrations for exposure of predators and top predators have also been calculated, and are included in Table 3.20. For production, only the highest PEC is presented.

Table 3.20 PECs for secondary poisoning for marine predators

	PEC in food for marine predators (µg/kg)	PEC in food for marine top predators (µg/kg)
Site specific		
Bisphenol-A production (BPA 6)	3.5	0.85
PVC additive package (A1)	0.72	0.29
Generic scenarios		
Polycarbonate bottle washing	0.2	0.2
Phenoplast cast resin processing	*28*[a]	*5.8*[a]
PVC – anti-oxidant during processing	3.2	0.77
PVC – plasticiser use	2.3	0.6
Anti-oxidant use in plasticiser production	7.1	1.6
Thermal paper recycling with deinking	0.2	0.2
Thermal paper recycling without deinking	0.2	0.2

a This scenario is included for completeness, although no relevant sites discharging to marine waters have been identified (see Section 3.3.1.2)

3.1.4.8.1 Measured levels in biota

There is a limited amount of data on levels of bisphenol-A in biota. Belfroid *et al.* (2002) reported levels of bisphenol-A in fish from the Netherlands. Fish were taken from seven locations, both freshwater and marine. The fish species sampled were bream (*Abramis brama*) and flounder (*Platichthys flesus*). The range of concentrations measured was 2 to 75 ng/g dry weight in liver samples, and 1 to 11 ng/g dry weight in muscle samples. These are based on pooled fish samples.

In addition to the fish data reported by Belfroid *et al.* (2002), Vethaak *et al.* (2002) included data on levels in freshwater mussels (*Dreissena polymorpha*) and saltwater mussels (*Mytilus edulis*). These were sampled at locations where higher concentrations of bisphenol-A had been found in the water. In freshwater mussels the concentrations (at two sites) were 0.22 and 0.36 ng/g wet weight; in saltwater mussels (three sites) levels were 0.26 – 1.8 ng/g.

Fjeld *et al.* (2004a) report the results of a screening study in Norway for a range of substances, including bisphenol-A. Sampling for the study took place largely in 2003. Samples were taken from a variety of locations, including lakes and associated rivers, a fjord and its associated river and coastal marine sites (harbours, a contaminated site and open waters). Biota was sampled at a subset of these sampling sites. The bisphenol-A concentration measured in freshwater fish are summarised in Table 3.21.

Table 3.21 Concentrations of bisphenol-A in freshwater fish in Norway

Location	n	Concentration (ng/g)			
		wet weight		Lipid	
		min	max	min	max
Lake Mjøsa (whole fish)	5	1.4	13.7	58	466
Lake Vorma (muscle)	2	6.1	10.4	201	1,350
Lake Øyeren (whole fish)	2	1.0	1.2	32.9	74.5
Inner Drammensfjord (brackish water, muscle)	4	1.9	14.1	24.6	407.8

n Number of samples

In the marine samples (cod liver), bisphenol-A was not detected (at 2-4 ng/g wet weight) in four samples, and was measured at 7 and 62 ng/g wwt in two other samples.

Fjeld *et al.* (2004b) reported further measurements on concentrations in fish from the Drammensfjord, Norway. The levels of bisphenol-A in the fish samples varied between 0.61–13,73 ng/g wwt, lowest in the muscle sample from ide (*Leuciscus idus*) from 1998 and highest in the liver sample from flounder (*Platichthys flesus*) from 2004.

Levels of bisphenol-A in biota from Germany have also been reported (Fraunhofer, 1999). The majority of the samples were collected between 1990 and 1996. The values found in freshwater organisms were: bream (*Abramis brama*) muscle <1-3.3 µg/kg; and zebra mussel (*Dreissena polymorpha*) 1-5.3 µg/kg; and for marine samples: eel pout (*Zoarces viviparus*) muscle <1-3.3 µg/kg; blue mussels (*Mytilus edulis*) <1-1.3 µg/kg; and brown algae (*Fucus vesiculosus*) <1-2.8 µg/kg.

3.1.4.8.2 Comparison with calculated values

The measured values are of the same order of magnitude as the calculated concentrations in fish for the secondary poisoning scenario, and cover a similar range when considering the whole fish values (the calculated values are for whole fish). Individual values are higher, in particular for specific tissues. The data are not extensive, and it is not possible to allocate the measured data directly to either a local or a regional situation. The calculated values are therefore used in the risk characterisation, supported by the measured values.

3.2 ENVIRONMENTAL EFFECTS

3.2.1 Aquatic compartment

This section is a complete reformat and update of the aquatic effects sections presented in EC (2003). Given the rapid biodegradability of bisphenol-A in aquatic systems, studies that do not involve confirmation of exposure concentrations are of limited usefulness for PNEC derivation, especially over longer durations (since the concentration that causes any observed effect cannot be established; this is particularly the case for static tests). Nevertheless, such studies may still be considered qualitatively.

3.2.1.1 Micro-organisms

3.2.1.1.1 Toxicity data

Experiments have been performed with two *Pseudomonas* species, and these are summarised below.

1. Dow (1988) report the determination of an acute bacterial toxicity test carried out using bisphenol-A. The test was performed to good laboratory practice guidelines. Cultures of *Pseudomonas putida* from an agar-solidified medium were added to culture vessels and incubated at 25°C for 18 hours with bisphenol-A. The growth rate of the bacteria was measured by turbidimetry. The highest concentration tested was 320 mg/l, and at this concentration no inhibition of cell growth was observed. (Note that this concentration is slightly above the water solubility of 300 mg/l.)
2. Stone and Watkinson (1983) conducted a growth inhibition test on *P. fluorescens* as part of their studies on bisphenol-A biodegradation. They reported an IC_{50} of 54.5 mg/l for the inhibition of the growth of *P. fluorescens* by bisphenol-A.

Data on micro-organisms are not usually included in a species sensitivity distribution to protect the freshwater compartment. No data were found on the effects of bisphenol-A on saltwater micro-organisms.

3.2.1.1.2 PNEC derivation for WWTP microorganisms

The TGD indicates that tests with *P. fluorescens* should not be used to determine the $PNEC_{WWTP}$ because it uses glucose as a substrate. Results of a cell multiplication test with *Pseudomonas putida* may be used with care. For *P. putida* a NOEC based on cell growth of ≥320 mg/l is reported. This is not a true NOEC since it is the highest concentration used in the test and no effects were observed at this concentration. However, in the absence of any other data this value will be used as the NOEC for the derivation of a PNEC for microorganisms. For a NOEC from a specific population the $PNEC_{WWTP}$ is set equal to the NOEC value. Therefore the $PNEC_{WWTP}$ for bisphenol-A is taken as 320 mg/l.

3.2.1.2 Primary producers

3.2.1.2.1 Freshwater primary producers

Experiments have been performed with one algal and one higher plant species, and these are summarised below.

1. Alexander *et al*. (1985b, 1988) report 96-hour EC_{50} values, based upon cell count and total cell volume of 2.73 mg/l and 3.10 mg/l respectively for the green alga *Pseudokirchneriella subcapitata* (previously known as *Selenastrum capricornutum*). Both of the test results are based upon changes in biomass. In addition to the EC_{50} values reported, the percentage inhibition of cell count and cell volume is reported for the concentrations tested. From these data it is possible to derive an EC_{10} using probit analysis. The calculated 96-hour EC_{10} values

are 1.36 mg/l based upon cell count and 1.68 mg/l based upon cell volume. The test report describes the test methods and test concentrations were measured.

Stephenson (1983) reports a 96-hour EC_{50} of 2.5 mg/l, based upon cell count, for *Ps. subcapitata*. The test report describes the test method used, but does not give details of the test conditions. The test concentration is based upon nominal concentrations. This result supports the data reported by Alexander *et al*. (1985b).

For algae studies it is generally accepted that a 72-hour (or longer) NOEC value can be considered as a chronic result. The TGD indicates that if a long-term NOEC is not available then an EC_{10} obtained by extrapolation using appropriate statistics, such as probit analysis, can be considered as if it were a NOEC.

In summary, the 96-h EC_{10} of 1.36 mg/l for *Ps. subcapitata* is considered valid for use in the PNEC derivation and SSD.

1. Putt (2003) reports a 7-d frond density, biomass and growth rate NOEC of 7.8 mg/l for the duckweed *Lemna gibba*. The static-renewal study was performed to GLP according to OECD Guideline 221 and analytical measurement of bisphenol-A showed that test concentrations remained between 79-100% of nominal. The test report describes the test methods and test concentrations.

The 7-d NOEC of 7.8 mg/l for *L. gibba* is considered valid for use in the PNEC derivation and SSD.

3.2.1.2.2 Saltwater primary producers

Experiments have been performed for two algal species, and these are summarised below.

1. Springborn Bionomics Inc. (1985c) (also published in Alexander *et al*. (1988)) report 96-hour EC_{50} values, based upon cell count and chlorophyll content, of 1.0 mg/l and 1.8 mg/l, respectively for the marine alga *Skeletonema costatum*. The test report describes the test methods and test concentrations were measured. The method used to estimate the effect concentrations was non-linear interpolation. The percentage inhibition of cell count and chlorophyll content is reported for the concentrations tested. These original data have been analysed by the rapporteur using probit analysis in accordance with the OECD Guideline. The resulting EC_{50} for cell count is 1.1 mg/l, and that for chlorophyll content is 1.4 mg/l. It is also possible to derive EC_{10} values using the probit analysis. The calculated 96-hour EC_{10} values are 0.69 mg/l based on chlorophyll content and 0.40 mg/l based upon cell count.

The 96-h EC_{10} of 0.40 mg/l for *S. costatum* is considered valid for use in the derivation of a saltwater PNEC if required.

1. Ishii *et al*. (2003) report a study in which the marine microalga *Nannochloropsis oculata* (ST-3 strain) was exposed to bisphenol-A for more than 15 days. The paper is in Japanese and a translation is currently unavailable, so experimental details could not be determined (and its suitability for PNEC derivation is unknown). However, the abstract, figures and tables are in English and show that there were no effects over 12 days at concentrations up to 3 mg/l, but substantial effects occurred after 1-3 days at concentrations at 6 mg/l and above. Figures in the paper show that exponential growth occurred over a period of about seven days in this study, so the effects occurred during the exponential growth phase.

3.2.1.3 Invertebrates

Many species have been studied, and these are grouped below in taxonomic sequence.

3.2.1.3.1 Freshwater invertebrates

Sponges (Poriferans)

Hill *et al*. (2002) exposed individual gemmules of the freshwater sponges *Heteromyenia* sp. and *Eunapius* sp. to bisphenol-A for nine days in 24-well tissue culture plates with renewal of test medium every 2-3 days. The highest test concentration (160 mg/l) was chemically analysed. The growth of *Heteromyenia* was significantly reduced at 16 mg/l, with no significant effects at 1.6 mg/l. The authors report that there was a similar response in *Eunapius*, but no statistical tests were performed due to small sample sizes. The precise amount of bisphenol-A to which the sponges were exposed for three days between renewals is unknown. However, due to the general robustness of the design (five replicates per treatment, frequent renewal of test solutions), this study is classed as "valid with restriction".

The 9-d growth NOEC of 1.6 mg/l for *Heteromyenia* sp. is considered valid for use in the PNEC derivation and SSD.

Hydra

Experiments have been performed for two *Hydra* species, and these are summarised below.

1. The effects of bisphenol-A on the cnidarian *Hydra vulgaris* have been studied by Pascoe *et al*. (2002). Chemical analysis of test concentrations was performed, and polyp survival, structure, regeneration and mortality were monitored over 96 hours. The 96-h LC_{50} was 6.9 mg/l. The structure and physiology of polyps (considered to be a growth-related end point for the purposes of this assessment) was adversely affected at concentrations greater than 42 µg/l over 6 weeks, and a concentration-dependent inhibition of regeneration was seen above 460 µg/l. The effect levels for 17α-ethinylestradiol were similar to those of bisphenol-A. The authors concluded that the signalling processes responsible for control and regulation of cell movement and differentiation during normal development, regeneration and sexual reproduction were not disrupted by either of the chemicals at low concentrations.

The 6-week polyp structure NOEC of 42 µg/l for *H. vulgaris* is considered valid for use in the PNEC derivation and SSD.

1. The reproductive effects of bisphenol-A on another cnidarian, *H. oligactis*, were studied by Fukuhori *et al*. (2005). Mature polyps were exposed individually in 5 ml of medium for 35 days (males) or 50 days (females) to study effects on sexual reproduction, with renewal of test medium on the 12th, 24th and 36th days. The incidence and number of gonads (testes or eggs) in each polyp were counted at the end of the experiment. Male polyps were also exposed in a separate treatment to examine asexual reproduction, with medium renewal three times a week. The number of buds detached from each parent polyp was recorded at the end of the 35-day asexual study. Concentrations of bisphenol-A were analysed in stock solutions at the start of both experiments only. Testis formation was unaffected at 0.5 mg/l but declined significantly and dose-dependently at 1-4 mg/l in unfed males exposed at 10^{o}C. However, males that were fed during the study showed significant effects (25-30% reduction) on the number of testes at the lowest test concentrations of 0.5 and 1 mg/l, with no differences at higher test concentrations of 2 and 3 mg/l, but complete inhibition at 4 mg/l. In

starved females, a significant decline in egg production began at 2 mg/l, with a substantial albeit non-significant reduction at 1 mg/l, and no effects at 0.5 mg/l. Results for fed females were not reported in this study. In the group of male polyps used to examine asexual budding, there were no effects at 0.5 mg/l, stimulation of budding occurred at 1 mg/l and a suppression of budding occurred at 2-4 mg/l when animals were exposed at 20°C. When exposure was at 10°C, there was stimulation of budding at both 0.5 and 1 mg/l. These effects at different temperatures could be explained by greater uptake of bisphenol-A at 10°C, which analysis of radiolabelled bisphenol-A showed was 1.4 times higher than at 20°C. The lowest test concentration of 0.5 mg/l elicited a response in some endpoints. Since this response was between 20-30% of the control, a NOEC could be derived by dividing this value by a factor of 3 (i.e., 0.5/3 = 0.17 mg/l), as has been done in some ESR assessments, e.g. for zinc. This study is not used for estimating a PNEC because test solutions were not analysed as frequently, the level of replication was lower, and the final result was less sensitive than in the Pascoe *et al*. (2002) study.

Nematodes

Kohra *et al*. (2002a) exposed young nematode worms *Caenorhabditis elegans* to bisphenol-A in agar plates. On transfer to clean plates the movement of worms to a food source was monitored. Both levels of exposure (10 µM and 0.1 µM) resulted in a reduction in the number of worms reaching the food source after time periods of up to 24 hours. A similar level of effect was seen in both exposures. Kohra *et al*. (2002b) also reported that the respiration of *C. elegans* declined when exposed to 0.1 and 10 µM bisphenol-A in agar. The results cannot be used to derive a no-effect level, and the exposure conditions cannot be easily related to the environment.

Several similar studies with *C. elegans* have been reported in which nematodes were exposed to bisphenol-A incorporated into agar. Ji *et al*. (2004) exposed different strains to 70, 80 and 90 mM of bisphenol-A in agar plates to investigate resistant mutants, and Watanabe *et al*. (2005) reports on studies with a mutant *C. elegans* strain that is particularly sensitive to bisphenol-A and could be used as a sensitive screening assay. Hoshi *et al*. (2003) found a significant increase in the relative percentage of *C. elegans* germ cells when they were exposed to $\geq 10^{-9}$ M bisphenol-A in agar for six days, with no effects at 10^{-10} M. Tominaga *et al*. (2003) exposed *C. elegans* to bisphenol-A on agar plates over four generations and found sublethal effects on fourth generation abundance at 1 nM. Once again, none of these results can be used to derive a no-effect level because the exposure conditions cannot be related to environmental concentrations. However, the results do show that multigenerational effects may occur at concentrations much lower than the LC_{50} from a short-term test.

Rotifers

A 48-hour test was performed with the rotifer *Brachionus calyciflorus* to determine the effects of bisphenol-A on the intrinsic rate of population increase (Springborn Smithers, 2006a). Chemical concentrations were analysed in replicates at the start and end of the exposure period and were in close agreement (95-97%) with nominal concentrations. The NOEC was 1.8 mg/l based on measured concentrations. This study was performed to GLP and is fully reported.

The 48-h reproduction NOEC of 1.8 mg/l for *B. calyciflorus* is considered valid for use in the PNEC derivation and SSD.

Molluscs

Experiments have been performed with two species, and these are summarised below.

1 *Marisa cornuarietis*

Three groups of experiments have been performed on the ramshorn snail *Marisa cornuarietis* (a tropical species of prosobranch snail, not found in Europe).

a) Studies of Oehlmann and co-workers

First test series: Oehlmann *et al*. (2000), Schulte-Oehlmann *et al*. (2001) and Oehlmann *et al*. (2001) report the effect of bisphenol-A on *M. cornuarietis*. Adults were exposed to nominal concentrations of bisphenol-A (1, 5, 25, and 100 µg/l) under semi-static laboratory conditions (with renewal every 24 hours) for five months and in a complete life-cycle test for 12 months. Both experiments included a solvent control, and were carried out at 22ºC. No analysis of the exposure solutions was carried out in these experiments. In both experiments a complex number of alterations referred to as "superfeminisation" occurred. Effects included the enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production. These effects were statistically significant at each test concentration when compared to the control, and were concentration dependent with the exception of mortality, which was virtually the same in all four bisphenol-A exposure groups (13.3-15.7% compared to control mortality of 3.8%). The cumulative numbers of eggs and the cumulative number of egg masses increased with increasing bisphenol-A concentrations. The hatching success of eggs from the organisms in the five-month experiment (used to start the life cycle test) was not affected by exposure to bisphenol-A.

Second test series: Schulte-Oehlmann *et al*. (2001) and Oehlmann (2001) also report a further experiment using lower bisphenol-A concentrations. The same semi-static exposure system was used, and the duration of exposure was 180 days. The nominal exposure concentrations were 0.05-1.0 µg/l, and the concentrations were checked by analysis following sampling on three occasions. The initial concentrations in the exposures were close to the nominal values. Observations over the 24-hour period between the changes of solution showed that the concentration of bisphenol-A decreased with time. After two months of the experiment, the half-life of bisphenol-A in the exposure solutions was around six hours. After four months the half-life had decreased to two hours and a similar value was found after six months. The concentrations were measured at 2-hour intervals; these were used to calculate average exposure concentrations over a 24-hour period as a time-weighted average. The detection limit was 30 ng/l.

The phenomenon of superfeminisation was again observed in all of the treated groups (with the exception of the 0.05 µg/l (nominal) group). The incidence was at a lower level than in the high concentration experiment (although the level of incidence in the one concentration common to both studies, 1 µg/l, was the same). Mortality was not significantly enhanced in any of the bisphenol-A groups in comparison to the controls. Egg production was also stimulated as in the previous experiment, although the results over the whole 180-day exposure period showed a significant increase only at the two highest concentrations. The authors observe that the exposure period in this second experiment included the season of the year (October to February) when spawning activity in this population of *M. cornuarietis* increases naturally. It was therefore considered that the effect of bisphenol-A might be masked to some degree by the natural increase. (The first experiment took place completely outside this active season.) The experimental results were therefore split into three periods of 60 days, with the middle period containing the season of greatest natural spawning activity. The initial 60-day period showed an increase in the cumulative numbers of eggs and spawning masses in the exposed organisms, with a significant increase over the control for

all but the lowest exposure level. Over the middle period, the animals exposed to bisphenol-A showed a reduction in the cumulative number of spawning masses in all treated groups when compared to the control; in the final 60-day period the pattern was similar to that in the first period. Based on the cumulative egg production over the first 60 days of exposure, the following effect concentrations were obtained: LOEC 48.3 ng/l; NOEC 7.9 ng/l; EC_{10} 13.9 ng/l (all based on the average exposure levels calculated from the measured concentrations).

Third test series: Follow-up studies with ***M. cornuarietis*** are reported by Oehlmann *et al*. (2006) with the stated objective of resolving identified shortcomings in their earlier studies (see discussion below). Exposures were semi-static, with medium renewal every one or two days. The first of the two experiments reported in this paper is identical to that reported in Oehlmann *et al*. (2000) and Schulte-Oehlmann *et al*. (2001) (i.e., these data are not new).

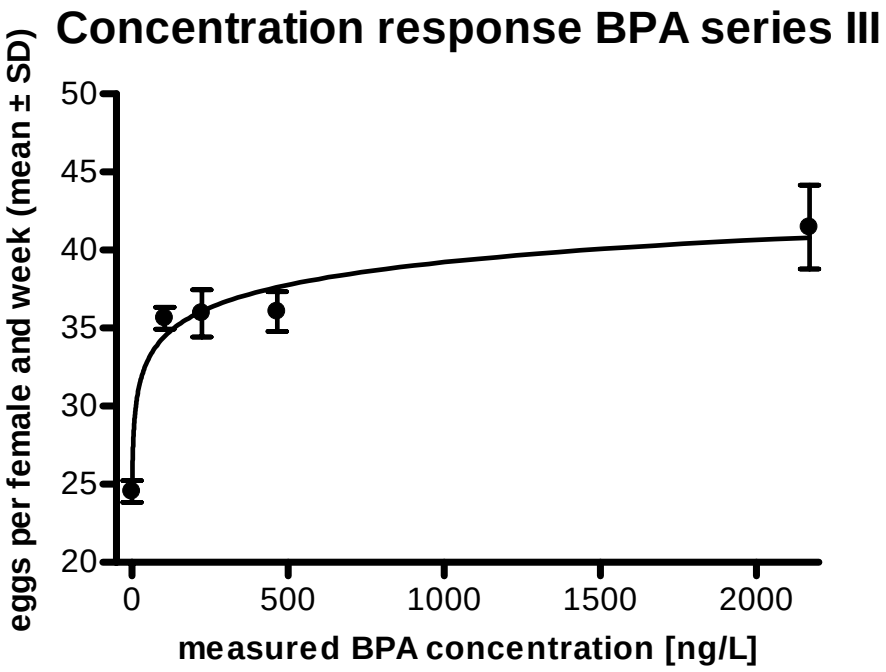
The results of the second experiment have not been published previously. In that study, two replicates, each of 30 sexually mature snails, were exposed to five concentrations of bisphenol-A (0, 0.25, 0.5, 1, and 5 µg/l). Two further treatments of 5 µg/l bisphenol-A with either 3 µg/l of the anti-estrogen ICI 182 780 or 10 µg/l of the anti-estrogen tamoxifen were also used. Exposure was for five months (February-July, which is outside the main spawning period for this population of snails) at two different temperatures (20°C or 27°C) with analysis of all surviving animals at the end of the study. Survival, numbers of eggs and clutches, and numbers of eggs per clutch were recorded daily. Analytical determinations of exposure concentrations over a 24-h period were undertaken in month 1. Measured initial concentrations were very close to nominals, but concentrations declined between renewals. Results were therefore expressed in terms of median (rather than time-weighted) measured concentrations, which were between 39.0% and 48.3% of nominal levels. Bisphenol-A half-lives were slightly lower at 27°C when compared with those at 20°C.

Snails exposed to bisphenol-A at 20°C produced significantly more clutches and eggs compared to controls. A NOEC could not be calculated because there were significant effects (compared to the control) at the lowest test concentration of 106 ng/l. EC_{10} values were estimated to be 14.8 ng/l (95% confidence interval 6.07 – 36.2 ng/l) and 18.0 ng/l (95% confidence interval 6.2-52.5 ng/l) for egg and clutch production, respectively. The dose-response curve derived by Oehlmann *et al*. (2006) is provided in Figure 3.1 (this is a replot of Figure 2C in the original paper provided by the lead author, but the y-axis is the estimate of eggs laid per female per week, rather than the overall egg numbers as in the paper). The curve shown is a fitted Weibull distribution as used by the authors.

At 27°C, none of the treatment groups produced significantly more clutches, or eggs on a per female basis, than the control. A significant increase in egg production could only be detected if measured in terms of cumulative egg number, for the nominal 1 and 5 µg/l exposure groups. Based on measured concentrations, the NOEC for egg production was 205 ng/l (EC_{10} = 998 ng/l; 95% confidence interval 161-6,200 ng/l) and the NOEC for clutch production rose to $\geq$1,990 ng/l (EC_{10} = 2,090 ng/l; 95% confidence interval 796-5,460 ng/l).

There were no significant differences in egg numbers per female for any of the exposed groups when comparing the output at both temperatures (around 700-800 over the study period), i.e. no dose-response relationship was evident. The temperature-related differences in NOECs are a direct consequence of the lower egg production in controls observed at 20°C (~500 eggs/female over the 5-month period).

Figure 3.1 Dose-response curve for snails exposed to bisphenol-A at 20°C (from Oehlmann *et al*., 2006)



Females with oviduct malformations were only found at 20°C, with an incidence of 4.8%, 8.0%, 14.8% and 11.5% in the groups receiving 0.25, 0.5, 1, and 5 µg/l bisphenol-A respectively. Increased mortality was observed in those groups experiencing oviduct malformation (numbers are not cited, but from Figure 2E in the paper, around 10 deaths were observed in each treatment group at 20°C, compared to 3 in the control – like egg production, there was no clear dose-response).

Some anti-androgenic effects (e.g., a significant concentration-dependent decrease in penis length of males at 20°C) were also observed (neither the magnitude of this change nor a NOEC/EC_{10} for the effect are indicated in the paper).

When snails were simultaneously exposed to bisphenol-A and an anti-estrogen, the stimulatory effect of bisphenol-A on egg production was completely antagonised. Competitive receptor displacement experiments with cytostolic preparations showed the existence of androgen- and estrogen-specific binding sites. Bisphenol-A appeared to have a higher binding affinity for the *M. cornuarietis* estrogen receptor than for fish estrogen receptors.

The first and second test series above were included in the published risk assessment (ECB, 2003). At that time it was concluded that these were not suitable for use in deriving a PNEC for the assessment, and that further work on this species was necessary. A method development and testing programme has been carried out, and the results from this are presented below.

b) Conclusion (i) programme

The conclusion (i) programme was made up of three phases. In the first phase, *Marisa cornuarietis* were obtained from a pristine habitat in the wild[15], and colonies established in three laboratories. The effects of temperature, photoperiod, population density, food, etc., on reproduction, fecundity and juvenile growth were investigated to establish husbandry conditions. It was found in this work that external characteristics of the snails could be used to reliably sex the animals, which allowed a test design based on breeding pairs (Oehlmann *et al*. have reported that reliable sexing based on external characteristics is not possible with the *Marisa* in their laboratory). Partial life cycle tests without chemical exposure were conducted at each of the three laboratories to investigate inter and intra-laboratory variability in endpoints. The main finding was that the primary source of variability was at the level of the breeding pairs. Following the fecundity of adult snails over a 12-month period showed a decrease over the first few months and a plateau thereafter (but no evidence of seasonal variation). This phase of the work is reported in Aufderheide *et al.* (2006) and Selck *et al.* (2006).

The second phase of the work was a preliminary toxicity test to define the range of bisphenol-A concentrations to be used in the definitive test. This was carried out at one laboratory in flow-through apparatus using breeding pairs of snails, with the adult pairs exposed over three months at 25°C. An analytical method to determine bisphenol-A concentrations was developed. This phase investigated the degree of replication and the statistical power needed to identify substance-induced effects. The exposure concentrations used were 0.1, 1, 16, 160 and 640 µg/l. No effects were seen on any of the endpoints studied – reproduction (eggs/female/month), egg hatchability (percent hatch, time to first hatch, time to 50% hatch) and growth rate. There was no increase in fecundity on exposure to bisphenol-A in this phase. This phase of the work is reported in Forbes *et al.* (2007a and 2007b).

The third phase of the work was the definitive toxicity study based on the methodology and results of the earlier phases (Warbritton *et al.*, 2007a). For the adult fecundity trial, replicate exposure aquaria were divided into ten equal size chambers using perforated glass partitioning. Each chamber randomly received a breeding pair of snails. Six replicate exposure aquaria were used for each bisphenol-A exposure concentration, with twelve replicate aquaria for the control. The test temperature was 25°C. Four bisphenol-A concentrations were used: 0.1, 1, 25 and 640 µg/l (nominal), with an intermittent flow-through dosing system, and exposure was for six months. The number of egg masses and the number of eggs per egg mass were counted.

An egg hatchability trial was also conducted. Five females in each of three of the replicate exposure aquaria (and in six of the control aquaria) were randomly selected, and five consecutive egg masses were collected starting at two months after the beginning of the fecundity trial. The egg masses were placed individually in the appropriate test solutions in glass-nylon mesh baskets. The percent hatch, the time to first hatch and the time to 50% hatch were recorded.

A juvenile growth trial was also conducted. One egg clutch was selected randomly from each of five females in each replicate vessel (three replicates per bisphenol-A treatment and six from the controls). The eggs were exposed as in the hatchability trial. At 32 days post hatch five juvenile snails were selected from each female's offspring, giving 25 young per

[15] The snails were taken from a reservoir in Puerto Rico, to which they had been introduced in 1960 (Aufderheide *et al*, 2006).

replicate, and were placed in aquaria (one aquarium for each of the three replicates at each concentration). Snails were individually marked, and were weighed weekly over the three-month exposures. Animal gender was determined for each individual based upon internal examination of the gonads at the termination of the exposure.

An additional fecundity trial was carried out at a lower temperature of 22°C over a twelve-week period, at a single bisphenol-A concentration of 25 µg/l (Warbritton *et al*., 2007b). Four replicates were used for the exposure, along with four controls. The other conditions were the same as those for the main fecundity trial.

The concentrations of bisphenol-A in the exposures were measured on a weekly basis throughout the adult fecundity trials and the juvenile growth trial, and more frequently during the hatchability trials. The mean measured concentrations were 74-135% of the nominal concentrations. No bisphenol-A was detected in the control exposures at a detection limit of 0.06 µg/l. The results are expressed in terms of the nominal concentrations.

There was no significant effect of bisphenol-A exposure on adult egg production at any of the tested concentrations. Statistical tests were carried out on the number of eggs per female per month. Comparisons with the controls were carried out by calculating the mean value for each replicate vessel and then testing the means of the replicates using a two-sided Dunnett's test (looking for either increases or decreases compared to the control). Differences in bisphenol-A concentration explained only 1.6% of the total variation in egg production.

There was no significant effect of bisphenol-A on the percentage of eggs hatching, and no significant difference between the controls and any of the bisphenol-A treatments (based on the mean percentage hatch per treatment). ANOVA suggested a significant effect of bisphenol-A on the time to first hatching, but the two-sided Dunnett's test showed no significant difference between any of the treatments and the control. First hatch occurred over a narrow period of time, generally not much more than 24 hours. The timing of the observations in the lab could have meant just including or just excluding snails from a time period, and so could have affected the result quite strongly. No significant effects on the time to 50% hatch were found.

In the juvenile growth trial, bisphenol-A had a significant effect on female growth rate, female wet weight and male growth rate. Growth rates were calculated by fitting a third degree polynomial curve to the data, and taking the slope of the function at 60 days post hatch as the growth rate. The point on the curve at 60 days was noted as the wet weight at this time. Comparison with the control values (using the mean growth rate or wet weight for each replicate) found a significant reduction in female growth at the highest exposure level. There was also a marginal effect (reduction) on female weight at the same level. A significant increase in male growth rate, and a marginal increase in wet weight, was found for the 1 µg/l treatment group. However, a much greater proportion of the variability in the data was explained by variation between pairs, and between siblings from the same pair, than by bisphenol-A treatment (for example, for male growth rate 42% of the variation was between siblings from the same breeding pair, 24% between breeding pairs, 21% related to bisphenol-A). The effect was not seen in the Phase 2 data when these were re-examined with separated data for males and females. The growth endpoint had a high level of variability, showing the highest difference for an endpoint between the three laboratories in the Phase 1 study. The test facility also noted variation between the growth rates in Phases 2 and 3. The difference in growth rates between Phases 2 and 3 at the test facility was greater than the difference between the growth rates at the BPA exposure levels in Phase 3. Different ages of

the adult snails used to produce the egg masses may account for some of this (between laboratories and/or phases).

There were no significant differences between the fecundity (as eggs/female/month) of the controls and snails exposed to 25 µg/l at 22°C and 25°C. The increase in temperature increased the number of eggs/female/month by 5.1% in the controls and by 11.6% in the exposed snails. Exposure to bisphenol-A increased egg production by 6.6% at 22°C and by 13.2% at 25°C. These differences were not statistically significant.

The overall NOEC from the study was concluded to be 25 µg/l, related to the growth of juvenile female snails. It should be noted that this is a conservative value since the LOEC is significantly higher (i.e. the true NOEC lies somewhere between 25 and 640 µg/l).

c) Studies of Schirling *et al*.

Schirling *et al*. (2006a) reported the development of a test method using eggs of *Marisa cornuarietis* to assess the effects of potential developmental and endocrine disruptors. Eggs were exposed to bisphenol-A in Petri dishes, with 15-20 eggs per dish. Two exposure concentrations were used, 50 and 100 µg/l, with nine replicates for each concentration and the control. Exposure solutions were renewed at least every third day, as was the stock solution. Eggs were observed under a microscope from the day of laying until the hatching of the snail. Endpoints monitored were mortality, formation of eyes, formation of tentacles, hatching (all as percentages of the exposed organisms) and heart rate. The weight of the snails on hatching was also recorded (hatching took place from 9-14 days after laying in the controls.

Bisphenol-A had no effect on the development of eyes or tentacles compared to the controls. There was a significant reduction in the heart rate in the 100 µg/l exposure at nine days. Slightly more animals hatched in the exposed than the control groups at 11 days, but this was reversed at 12 and 13 days, and none of the differences from the controls were statistically significant. The weight of newly hatched snails was significantly higher at 100 µg/l than in the controls. Similar results to those at 100 µg/l bisphenol-A were seen with 17α-ethinylestradiol at 10 µg/l. The study was intended as a development of the method and not for the determination of dose-responses, but a NOEC of 50 µg/l could be tentatively drawn.

2) *Potamopyrgus antipodarum*

As part of studies to investigate the relative sensitivities of fish and molluscs, Jobling *et al*. (2003; corrected version published 2004) exposed *Potamopyrgus antipodarum* (a temperate species of prosobranch snail, common in Europe) to bisphenol-A in water. The exposures lasted up to 90 days, in a semi-static system with 50% of the dosed water being replaced every four days. The exposure levels were nominally 1, 5, 25 and 100 µg/l. No analysis of the exposure solutions was performed. The endpoints monitored were growth and embryo production. Growth was measured by the length of the shell and the width of the shell opening. For embryo production, the number of embryos in the brood pouch was counted, distinguishing between shelled and unshelled embryos. The former are at a much later stage of development, and so the latter gave a measure of new embryo production.

No effects were seen on survival at any concentration of bisphenol-A (or for octylphenol at the same concentrations, or for 17α-ethinylestradiol (EE_2) at concentrations three orders of magnitude lower). Shell height and operculum width were also little affected for the most part, but at nine weeks the shell height in the 5 µg/l exposure were significantly increased over the six week value. There were no significant effects at higher doses. Embryo

production was significantly increased over that in the controls after three weeks at 5 µg/l. At 63 days, the 5 and 25 µg/l exposures had significantly more embryos than the controls, the 1 µg/l exposure had higher numbers but not significantly so, and the numbers at 100 µg/l were lower than in the controls. This indicates an inverted U-shaped response, similar to that seen with EE_2 (at ng/l levels). From the studies reported here and from other studies, the authors concluded that fish appear to be more sensitive to disruption in reproductive output caused by EE_2, but that snails may be more responsive to low concentrations of some xenoestrogens than fish.

This study cannot be used directly for the PNEC derivation or SSD because of the lack of confirmation of exposure concentrations. However, it does suggest that the NOEC for this species might be below that for fish (see Section 3.2.1.4.1), providing additional evidence that snails could be more sensitive than other groups of freshwater organisms. It may be noted that this study was performed in the same German laboratory that has performed a number of tests with *M. cornuarietis* reported above (S Jobling, personal communication to the Environment Agency).

The toxicity of bisphenol-A in sediments to *Po. antipodarum* has also been reported by Duft *et al*. (2003), and this study is discussed in section 3.2.2.1 of this report. In summary, stimulation of embryo production occurred at all test concentrations (i.e., the NOEC was below 1 µg/kg nominal dry weight) after eight weeks' exposure.

Discussion of the mollusc studies

Studies on three species of prosobranch snails (*Po. antipodarum*, *M. cornuarietis* and *Nucella lapillus* - see Section 3.2.1.3.2) – mostly performed in a single laboratory – indicate effects of bisphenol-A on reproductive parameters. The main underlying effect seems to be a stimulation of production of embryos, eggs and/or spawning masses[16]. In the case of *M. cornuarietis,* which appears to be particularly sensitive based on the work of Oehlmann and co-workers, this can lead to a rupture of internal organs and death in a proportion of the snails (this appears to depend on the morphology of the pallial oviduct, and the observation is so far restricted to this one species). Changes to other organs in the animals were also observed. These types of effects were not observed in the extensive conclusion (i) study, which had much greater statistical power (the most sensitive endpoint identified was growth of juvenile females; tissues were not examined for histopathological changes).

There are a number of drawbacks in the experimental methodology of some of the tests performed by Oehlmann and co-workers that makes it difficult to interpret the results with confidence. For example:

- The source of the snails is not well documented. The breeding population was originally established using snails obtained from Dusseldorf Zoo, with occasional inputs of snails from an unnamed source in Florida (J Oehlmann, personal communication).

- In the initial experiments with *M. cornuarietis* (Oehlmann *et al*., 2000; Schulte-Oehlmann *et al*., 2001; Oehlmann *et al*., 2001) two concentration ranges were used, i.e. high (1-100 µg/l) and low (0.05-1 µg/l). Concentrations were not measured in the first (high concentration) experiment. Measurements in the second (low concentration) experiment showed that the

[16] It may be noted that bisphenol-A has also been found to increase egg production in the copepod *Acartia tonsa* – see main text under Section 3.2.1.3.2 (saltwater *Crustacea*).

concentrations of bisphenol-A decreased rapidly over the 24-hour period between renewals of solution. The rate of disappearance was more rapid at later times in the test, despite the renewal of the solutions each day. Bisphenol-A is not susceptible to rapid abiotic degradation in solution, so it would appear that biodegradation or metabolism occurred in the solutions, with some indication of adaptation over the course of the experiment. In view of the rapid disappearance of the substance, the nature of the chemical species present in the exposures is unclear, particularly in the later parts of the experiment. For example, for a half-life of six hours only 6% of the substance would remain after 24 hours; for a half-life of two hours there would be effectively none of the substance left after twelve hours.

- The report of the second (low concentration) experiment also indicates that the control exposure solutions initially contained 30-40 ng/l of bisphenol-A at the first time of sampling for analysis. This was found to be due to leaching of the test compound from the plastic tubes used in the filter systems of the exposure tanks. Following replacement of these tubes with glassware, bisphenol-A could not be detected in the control group in the subsequent sampling.

- As noted in the description of the studies above, the period of the second (low concentration) experiment included the natural spawning season of the snails. This makes it difficult to compare the two studies. Both experiments included a nominal exposure concentration of 1 μg/l. The cumulative egg production over the first 60 days of the second experiment (taken as a period less affected by the natural spawning) was much higher than that seen over the 180 days of the high concentration experiment at the same exposure level, but the incidence of females with malformed oviducts was identical in both 1 μg/l exposure groups. The cumulative egg production in the control of the low concentration experiment was similarly higher than that for the high concentration experiment. These observations presumably relate to the difference in the natural spawning rate, and make it difficult to distinguish the effects due to the substance. The conclusion (i) study found no indications of seasonality in spawning in the *Marisa* used in that study.

- Snail density and volume per snail can both affect the growth, natality rates and calcium uptake rates of aquatic snails. This is due for example to competition for nutrients and food, ammonia formation, and the removal or dilution of beneficial growth factors secreted by the snails (JD Thomas, personal communication). In this case, snail densities were as high as 3.5 snails/litre, and this changed as snails were removed for analysis. Densities above about 1 snail/litre have been shown to have a negative effect on reproductive output of this species even under flow-through conditions (Aufderheide *et al*., 2006). Density-dependent effects may therefore have confounded the results.

- The level of replication should be as high as possible because aquatic snail reproductive traits are generally highly variable (JD Thomas, personal communication). However, only one replicate test chamber was used per exposure concentration. The absence of replication means that inferential statistics cannot be applied, i.e., trends can be observed but they cannot be extrapolated to the 'population' of interest (Leidi, 2005). Further detailed criticisms of the statistical methods are presented in van der Hoeven (2005). In summary, statistical experts consider it invalid to calculate a NOEC from the data or to test the effect of concentration on snail mortality. The main point is that there could be many other factors causing mortality in a given test vessel that have nothing to do with exposure concentration, which is why replicates are needed.

In view of the apparent instability of the substance under the exposure conditions used, statistical uncertainties, issues over snail density and other test conditions, and the possible overlap with natural changes, the effect concentrations from the initial *Marisa* studies are not considered suitable for use in the PNEC derivation.

This led the research group to conduct further studies, which have been reported recently (Oehlmann *et al*., 2006). Despite the criticisms of the original studies, the new study appears to indicate very similar effects, with a 5-month egg production EC_{10} of 14.8 ng/l at 20°C. (This value was inferred since significant effects occurred at the lowest median measured test concentration of 106 ng/l, equivalent to an increase in egg production of ~40% compared to control females.) At 27°C, depending on how the data are interpreted, there are either no effects on egg production (in terms of eggs per female), or a NOEC of 205 ng/l can be derived based on cumulative egg numbers (with an estimated EC_{10} of 998 ng/l or 0.998 µg/l). This paper and the underlying dataset were reviewed by members of an expert group that was set up to guide the conclusion (i) testing programme for snails (as reported above). This review identified a number of issues, as follows:

- Snails were exposed in duplicate tanks at each treatment level. This level of replication permits better statistical analysis than the earlier experiments. However, interpretation of the results from work with groups of snails (rather than paired individuals) remains difficult because it is known from the conclusion (i) test programme that there can be considerable animal-to-animal variability in egg production. By presenting the cumulative egg production per tank and not per female, intra- and inter-female variability is ignored, i.e. apparent differences in egg production among treatments and controls may in fact be related to natural intra- and inter-female variability. The test design still does not allow separation of breeding pairs and analysis of the intra- and inter-female variability in reproductive output. The conclusion (i) study required a very high degree of replication to overcome this problem.

- There are still significant criticisms about the application of the statistical techniques that were used in the paper (van der Hoeven, 2005 and Leidi, 2005 & 2006). For example:
  - The analysis assumed that identical sex ratios were employed in the replicates and exposures, and that mortality did not affect these ratios, nor the fecundity. This influences calculations of egg production per female, and adds uncertainty to the reported results that is not addressed.
  - Van der Hoeven (2005) and Leidi (2005 & 2006) were unable to replicate the derivation of the EC_{10} value from the raw data provided by the authors for this purpose. Whilst the Weibull distribution appears to fit the data very well ($r^2 = 0.936$), the small number of data points means that this could simply be the result of overfitting (van der Hoeven, 2005). Unfortunately there are no details in the paper about how this curve was calculated to permit independent validation.
  - Several alternative statistical models could have been used. For example, simple linear correlation has been found to be significant ($p=0.02$), although the fit is not as good ($r^2 = 0.718$) (van der Hoeven, 2005). This is not necessarily the correct distribution for the data, but it is the simplest. A quadratic relationship was also used, but did not provide a better fit compared with the linear model.

- The authors used the control group repeatedly in their tests of significance, making the results of the individual comparisons dependent on each other. The p-values should have been adjusted accordingly.

- The variability in the data set is surprisingly small. The mean coefficient of variation (CV) for reproductive output is ~3% for all treatment groups. In comparison, van der Hoeven (1998) found that the median CV in a ring test of a highly standardised method with clonal *Daphnia magna* was 14.4%; the median CV in less-standardised tests with *Folsomia candida* and *Eisenia fetida* was about 50%. Leidi (2006) has also pointed out that the observed variability was much lower than expected. This suggests that the dataset is unusual in that the tanks can not be considered to be truly independent replicates: no variability was observed between a few tanks at the same dose. One explanation might be that each individual snail is negatively correlated with the rest (Collett, 2002).

- Some of the experimental issues identified for the original studies still remain, for example:
  - the varying snail density (although this did not vary as much as in the earlier experiments, with an initial density of 1.11 snails/l, dropping to 0.72-1.00 snails/l at the end, depending on the mortality in the different treatment groups); and
  - the very rapid loss of bisphenol-A in the test system, which is a major additional complication. The only analytical measurement of test solution stability occurred after one month, over one test media renewal cycle. The test substance half-life is reported as 3.0-3.9 hours, and in all but the highest dose group, no bisphenol-A was detectable in the media at the end of the renewal period. It is therefore not clear what substance concentration actually caused the apparent effects in each treatment. The authors used the median measured concentrations to derive an EC_{10} value. For example, the lowest nominal concentration of 0.25 µg/l had a median measured concentration of 0.106 µg/l, but the standard deviation for this value is 0.113 µg/l, demonstrating the wide variation observed. Given the lack of information to derive proper time-weighted average concentrations over the whole course of the experiment, the rapporteur considers that this approach is unreliable.

- The paper suggests a possible mode of action in which bisphenol-A binds more strongly to *M. cornuarietis* receptors than to the vertebrate estrogen receptor, which helps to explain why this species is so sensitive. However, some experts have identified experimental drawbacks about this part of the study, which raises some doubt about its reliability (e.g. Dietrich *et al*, 2006). Despite this, the elimination of effects when snails were exposed to a mixture of bisphenol-A and an anti-estrogen lend some support to the hypothesis. In any case, this mechanistic information does not directly affect the interpretation of the bisphenol-A toxicity data themselves.

The Oehlmann *et al*. (2006) study clearly suggests that there are temperature-driven differences in the observed effects[17], and this is linked to an apparently natural increase in egg production at higher temperatures. It should be noted that reproductive traits are more variable in this species at lower temperatures, which could have some influence on the findings due to the test design and method of statistical analysis. Oviduct malformations and associated mortality are not seen

[17] It has been suggested that the lower temperature might not be relevant for this species. However, although mainly tropical in distribution, *M. cornuarietis* is tolerant of a wide range of temperatures, and can exist in Florida (at the edge of its range) at an average temperature of 20-22°C. The snail population used for this experiment was a mixture of laboratory-derived stock and snails obtained from Florida. Control mortality at 20°C was very low during the experiment (5% over a period of 5 months) and even slightly lower than in controls at 27°C of the same experiment (J Oehlmann, personal communication). The lower temperature therefore seems to be appropriate.

at the higher temperature of 27°C (the study authors state that the oviduct becomes widened during natural periods of higher egg production).

Comparison of the studies performed by Oehlmann and co-workers with the conclusion (i) study is not straightforward because the latter was not intended to be an exact replica of the original work. Rather, it was developed to specifically take account of the influence of key husbandry parameters such as water quality, food type, snail density and temperature on snail responses. The findings of the initial phases were used to develop a statistically robust test based on breeding pairs of snails which allowed the intra- and inter-female variability to be investigated, with sufficient replication to detect an increase in fecundity of ~20% under the conditions of the test.

An obvious difference between the two groups of studies is temperature. A temperature of 25°C was chosen for the main test of the conclusion (i) study because:

- of practical considerations such as timing and the laboratory space available to house the required number of aquaria (the snails showed a higher degree of variability (i.e. more control tanks are needed) and grow more slowly at lower temperatures); and
- this is closer to the temperatures that this population of *Marisa* normally encounters in the wild (the original stock of snails was collected from a tropical lake).

There was no increase in egg production at any of the tested concentrations at this temperature. The most sensitive endpoint was growth of female juveniles, which had a NOEC of 25 µg/l.

A trial at 22°C at the same concentration also showed no significant difference in fecundity compared to controls (and only slightly lower egg production than at 25°C). This test concentration and temperature is the same as one in the first test series by Oehlmann and co-workers, when effects on both egg production and mortality were seen.

Another obvious difference is the exposure regime. Bisphenol-A is readily biodegradable, and a flow-through test system is usually preferred for such substances. This was the regime chosen for the conclusion (i) study. The studies of Oehlmann and co-workers used semi-static exposures, and there was rapid disappearance of the parent compound. It is therefore possible that metabolites could have been present at higher concentrations in the semi-static studies. The same metabolites may have been present in the conclusion (i) study, but the actual levels of any metabolites, and their role in the observed effect, is unknown.

Clearly there is a significant difference in the responses of the *Marisa* used in the two major groups of studies. This does not appear to be simply due to temperature differences, because the lower temperature trial in the conclusion (i) study did not result in any effect. Whilst differences in experimental design will obviously have influenced the results, it may be noted that the snails in the studies by Oehlmann and co-workers showed seasonal spawning behaviour, which was not present in the animals used for the conclusion (i) study (no stimulation of egg production was seen in the Oehlmann's snails during their active spawning phase). The fact that these two groups of snails come from different geographical sources has been suggested as a possible contributory factor to this difference, and this demonstrates the difficulties in working with species that have not been properly ring-tested.

In summary, the studies performed by Oehlmann and co-workers have major limitations, which make the numerical results derived from them of questionable reliability. However, whilst preference would normally be given to the fully valid conclusion (i) study, the findings of mortality and morphological changes cannot easily be discounted. This is also supported by the

observation of similar types of effects (in relation to stimulation of aspects of reproduction) in other species such as *Potamopyrgus* and *Nucella*, although none of these other studies provides a value that is suitable for use in the risk assessment (and the tests were performed in the same laboratory and so could be subject to similar drawbacks). In addition, the snails used by Oehlmann and co-workers have a seasonal breeding cycle. It is therefore possible that an effect was missed by using a strain of snails that did not have a seasonal breeding cycle in the conclusion (i) study.

The calculated EC_{10} reported by Oehlmann *et al*. (2006) may be an artefact of the statistical approach and the choice of values used to represent the exposure concentrations. For example, van der Hoeven (2005) calculated an alternative EC_{10} of 2.1 µg/l at 20°C (with a 95% confidence interval from 1.0 to 11 µg/l), based on a simple linear two-parameter model and nominal test concentrations[18]. If the mean measured concentrations were used, the values would be approximately halved, but the rapporteur believes that the lack of proper chemical analysis makes these too unreliable.

One approach to overcome the problem of which value to use for *M. cornuarietis* would be to derive a geometric mean of the available NOECs to take some account of both studies (which would be consistent with the approach used for the *Xenopus* studies reported below). This is not preferred since if seasonality were a key factor in snail sensitivity, the importance of the 'low dose' findings would be diluted. Both studies will therefore be taken into account in the PNEC derivation.

In summary, the 5-month egg production EC_{10} of 0.0148 µg/l at 20°C from Oehlmann *et al.* (2006), the recalculated EC_{10} value of 2.1 µg/l for the same endpoint by van der Hoeven (2005), and the NOEC of 25 µg/l at 25°C for juvenile female growth from the conclusion (i) programme for *M. cornuarietis* are considered in the PNEC derivation and SSD.

Crustacea

Experiments have been performed with three species, and these are summarised below.

1) Four 48-hour EC_{50} values based upon immobilisation of *Daphnia magna* are reported. Stephenson (1983) reports a value of 3.9 mg/l, Chen *et al*. (2002) report a value of 10 mg/l, and Hirano *et al*. (2004) report a value of 12.8 mg/l, all based upon nominal concentrations. Alexander *et al*. (1985c) report a 48-h EC_{50} of 10.2 mg/l based upon measured concentrations. The methods used are documented in all of these studies, but the latter test result based upon measured concentrations is considered to be the most reliable, and is supported by two of the other studies.

   A 21-day $NOEC_{reproduction}$ of ≥3.146 mg/l for *Daphnia magna* is reported by Bayer AG (1996). The method used is fully documented in the test report and the test concentration is measured. At the highest concentration tested (nominal concentration of 3.2 mg/l) no effect on reproduction was observed. The $NOEC_{reproduction}$ is therefore given as ≥3.146 mg/l (measured concentration).

   Caspers (1998) studied the moulting behaviour of parthenogenetic *D. magna* females in what is likely to be the same study as Bayer AG (1996). The author did not observe any change in

[18] Other relationships could be used to fit the data. For example, the EC_{10} estimate is 0.38 µg/l using a quadratic relationship. This does not provide a significantly better fit than the linear one (the *p*-value was 5.6%).

moulting behaviour at exposure concentrations of 0.316 and 3.16 mg/l. Moulting behaviour has been claimed to be a toxicological endpoint that is able to reflect effects of endocrine disruption.

Mu *et al*. (2005) report both acute and chronic effects of bisphenol-A on *D. magna* in semi-static test systems with daily renewal. The EC_{50} for juveniles less than 24 hours old was 16 mg/l (95% confidence interval of 15.9-16.4 mg/l). The authors report a chronic toxicity threshold of 1.3 mg/l for effects on female fecundity after a 21-day exposure period. However, this threshold is based upon a second order regression model fitted to the data. Figure 6 in the paper shows that a plausible model could be fitted that would lead to a higher threshold (i.e., a NOEC) of approximately 5 mg/l. At concentrations >5 mg/l there was evidence of an increase in the intermoult period for neonates, and at 10 mg/l there was an increase in neonatal abnormalities. The authors propose that bisphenol-A effects on daphnid reproduction are due to anti-ecdysteroidal activity. There was no chemical analysis of exposure concentrations in this study, so these results are not themselves suitable for inclusion in an SSD but can be used to support the Bayer AG (1996) study.

Brennan *et al.* (2006) carried out acute and chronic tests on *Daphnia magna*. The acute test were carried out over 48 hours, measuring immobilisation at 24 and 48 hours, and the number of discarded carapaces as a measure of the moulting frequency, also at 24 and 48 hours. Three replicates were used at each concentration, with five *Daphnia* in each. The results were EC_{50} (24 hours) 8.57 mg/l (95% confidence limits 8.28 – 8.86 mg/l), and EC_{50} (24 hours) 7.75 (7.65 – 7.85) mg/l. Results are based on nominal concentrations. There were no effects on the moulting frequency from bisphenol-A exposure, up to the highest tested concentration of 10.5 mg/l. This contrasts with the results reported by Mu *et al*. (2005), where a longer time between birth and first moult was found at concentrations above 5 mg/l. The organisms in the Mu *et al.* study were <1 hour old at the start of the exposures; those in the current study were <24 hours old in line with the ISO guideline.

In the chronic studies, single *Daphnia* were exposed to bisphenol-A over 21-day exposures, with ten replicates for each concentration. The test solutions were renewed three times per week. The endpoints monitored were survival, moulting frequency and the number of offspring produced. Again results are based on nominal concentrations. There were no effects on the cumulative number of moults or the cumulative number of offspring per female at concentrations up to 1.0 mg/l, the highest used, in either the first or second generations. The LC_{50} for mortality in the first generation was 0.806 mg/l over the 21 days. (The second generation value is not given, but the exposure at 0.6 mg/l had 50% mortality in the second generation. Probit analysis of the second generation data carried out for this assessment gives an EC_{10} value of 0.2 mg/l.)

Wang *et al.* (2005) developed a short-term assay with Daphnia to screen for endocrine effects. Specifically, the screen was for the ability to stimulate the production of male offspring under conditions when the offspring would normally be all female. This is promoted by juvenoid hormones in crustaceans. Bisphenol-A had no agonistic effect in Daphnia, and no males were produced. When a low level of methyl farnesoate, a potent agonist, was used together with bisphenol-A, the effect was potentiated compared to that of methyl farnesoate alone. The bisphenol-A level used was 10 mg/l. A possible mechanism was suggested in which bisphenol-A inhibits the enzyme that degrades methyl farnesoate. As only a single concentration was used and the endpoints are indicators, the study is not suitable for use in the risk assessment.

In summary, the 21-d reproduction NOEC of 3.146 mg/l (as a limit value) for *D. magna* is considered valid for use in the PNEC derivation and SSD.

2) Watts *et al*. (2001a) exposed the freshwater amphipod *Gammarus pulex* to bisphenol-A and examined toxicity over a 10-day period and precopulatory behaviour over a 24-hour period. Chemical analysis of test concentrations showed good agreement with nominal concentrations. The LC_{50} was 12.8 mg/l after 24 hours, 5.6 mg/l after 48 hours, and plateaued at around 1.5 mg/L after 120 hours. Precopulatory guarding behaviour was only affected at concentrations close to these lethal levels (0.83 mg/l).

   Johnson *et al*. (2005) exposed *G. pulex* to 0, 1, 10, 100 and 1,000 µg/l bisphenol-A for 14 days in a semi-static exposure system. Ten pairs of animals were exposed to each concentration in the form of precopula pairs (i.e., males clasping the females during the pre-mating period) and observed daily to determine whether they had moulted or died, and to count any juveniles produced. There was reduced survival at the highest test concentration (1,000 µg/l), but there were no significant effects on female moulting rate or juvenile production at any concentration. However, the results were variable, so there would have been little statistical power to detect differences. In addition, although the authors state that samples were taken for analysis at the start of the study, no analytical results are reported, so true exposure concentrations are uncertain. Short-term data reported by Johnson *et al*. (2005) also suggest effects on *G. pulex* survival only at higher concentrations, with mortality over 96 hours of 6.7% in controls, 10% in solvent controls and at 1,000 µg/l, 30% at 3,200 µg/l and complete lethality at concentrations above 10,000 µg/l.

   Both of these studies provide information on short-term or lethal effects of bisphenol-A on *G. pulex*, so are unsuitable for inclusion in an SSD.

3) Springborn Smithers (2006b) report the results of a 42-day reproduction test with the amphipod *Hyalella azteca* (known as the scud) during aqueous exposures. Mean measured concentrations during the test were 0.12, 0.22, 0.49, 1 and 2.2 mg/l, which were 63%, 58%, 65%, 67%, and 73% respectively of nominal concentrations. The NOEC for cumulative number of offspring per female was 0.49 mg/l. There were no effects on body length or dry weight up to the highest concentration tested. This study was performed to GLP using US EPA guidelines and a full study report is available.

   The 42-d reproduction NOEC of 0.49 mg/l for *H. azteca* is considered valid for use in the PNEC derivation and SSD.

Insects

Experiments have been performed with two *Chironomus* species, and these are summarised below.

1) Sayers (2005) reports a 96-h LC_{50} of 2.7 mg/l (95% confidence interval of 2.1 - 3.2 mg/l) and a 96-h NOEC for survival of 1.4 mg/l for the midge *Chironomus tentans* exposed to bisphenol-A under flow-through conditions. The test was performed according to US EPA guidelines and was GLP compliant, and mean measured concentrations ranged between 84% and 110% of nominal. The test report describes the test methods and test concentrations, but this is only a short-term study.

2) Hahn *et al*. (2002) examined the effect of bisphenol-A on the yolk protein content of *Ch. riparius* and found a significant reduction in vitellogenin in males at all test concentrations of 1, 100 and 3,000 µg/l, and in females at 3,000 µg/l. Test concentrations were not chemically

analysed. These results, which the authors found surprising, may have been influenced by cross-reactivity in the immunoassay that was used, although the authors regarded this as unlikely. This study is not suitable for PNEC derivation because the method is in development and it also lacked confirmation of exposure concentrations.

Watts *et al.* (2003) report the effects of bisphenol-A on larval moulting and mouthpart structure in *Ch. riparius*. Midges were exposed throughout their entire life-cycle, first in repli-dishes when exposed as eggs, and then in glass vials containing 10 ml of test solution when the eggs hatched into larvae. Chironomids require a substrate for normal development and this was provided in the form of a minimal amount of filter paper, previously soaked for 24 hours in the relevant test solution. Test solutions were renewed daily and chemical analysis of the 1 mg/l concentration confirmed that it was within 20% (830 µg/l) of the nominal concentration. Time to first moult and mean wet weights of first instar larvae were only affected at the highest test concentration of 1 mg/l, with no effects at the next lowest concentration of 100 µg/l. There is a substantial difference between the two concentrations, and clearly the actual NOEC could be significantly higher than 100 µg/l. The incidence of mouthpart mentum deformities was significantly higher than controls at lower and intermediate exposure concentrations (10 ng/l and 1 µg/l). There were no significant differences at higher exposure concentrations (or at the intermediate concentration of 100 ng/l), and there were no significant effects on other mouthparts.

Exposure of chironomids in this study could have been via both overlying water and pre-soaked filter paper, but equilibrium partitioning theory suggests that the fugacity of bisphenol-A should be similar from both sources, and the amount of filter paper used in the test was small. In addition to this, analytical confirmation of one exposure concentration plus daily renewal is not an ideal experimental design, but would have been adequate to characterise exposure concentrations sufficiently for use in risk assessment. The ecological consequences of the deformities observed in the study remain unclear, and the lack of a clear linear or U-shaped dose-response makes these data unsuitable for PNEC derivation. However, the data on time to first moult and first instar larval weight are valid.

In summary, the growth NOEC of 100 µg/l (as a limit) for *Ch. riparius* is considered valid for use in the PNEC derivation and SSD.

3.2.1.3.2 Saltwater invertebrates

Annelids

Biggers and Laufer (2004) used a rapid settlement and metamorphosis assay with the polychaete *Capitella* sp. to assess the juvenile hormone activity of bisphenol-A and other phenolic compounds. Two-day old metatrochophore larvae were exposed in 10 ml of artificial seawater (salinity 30 ppt) and the number of larvae that settled and metamorphosed was assessed after 1 hour. The authors reported an EC_{50} of 0.05 µM, equivalent to 11.5 µg/l. There was no chemical analysis of exposure concentrations, and coupled with the short study duration, this means that the study is unsuitable for PNEC derivation.

Molluscs

Experiments have been performed with three species, and these are summarised below.

1) Adult Dogwhelks *Nucella lapillus* (a prosobranch gastropod) from the field were exposed for three months in the laboratory to concentrations of 1, 25 and 100 µg/l, with renewal every 24

hours (Oehlmann *et al*., 2000). Superfeminisation with enlarged pallial sex glands and an enhancement of oocyte production was observed. No oviduct malformations were found (it was noted that there are differences in gross anatomical structure of the pallial oviduct between this species and *M. cornuarietis*). A lower percentage of exposed specimens had ripe sperm stored in their vesicula seminalis and males exhibited a reduced length of penis and prostate gland when compared to the control. Statistically significant effects were observed at all the test concentrations. The authors concluded that the results show that prosobranchs are sensitive to endocrine disruption at the lowest concentrations of bisphenol-A tested (1 µg/l nominal).

The lack of confirmation of exposure concentrations means that these results cannot be used directly for PNEC derivation. However, the study does provide additional evidence of toxicity to prosobranch molluscs in support of the findings for freshwater species.

2) Canesi *et al*. (2005) injected 0.1 and 0.5 µM of bisphenol-A into the mussel *Mytilus galloprovincialis* and examined effects on lysosomal stability and kinase-mediated cell signalling in haemocytes after 6, 12 and 24 hours. Canesi *et al*. (2004) also exposed haematocyte monolayers from the same species to 25 µM of bisphenol-A. These data cannot be used for this assessment because the method of exposure cannot be related to environmental concentrations, and the end points cannot be related to demographically important factors.

3) Blue mussels *Mytilus edulis* were exposed to bisphenol-A, diallylphthalate (DAP) and tetrabromodiphenyl ether (congener 47) for three weeks in filtered seawater at 10-12°C (Ortiz-Zarrogoitia and Cajaraville, 2006). The exposure level for bisphenol-A was 50 ppb. At the end of the exposures 20 animals were sampled. Bisphenol-A exposure did not significantly induce Acyl-CoA oxidase activity in comparison with the controls, and there were no significant changes in the peroxisomal volume density. These two endpoints were used as a measure of exposure to general pollution. Resorption of gametes was observed in 35% of the female and male animals in the bisphenol-A exposure group. Alkali-labile phosphate levels (considered as a measure of vitellogenin-like proteins) were not affected by exposure to bisphenol-A. There were no changes in oocyte athresia after exposure to bisphenol-A. The authors note that the mussels used in this experiment were at the mature gonad stage, and that they may be more sensitive at earlier stages of gonad development. The study appears to have exposed all of the animals in one vessel, hence there were no true replicate exposures. Only one concentration was used, and so no NOEC value can be determined for the one examined endpoint that showed effects. The study is not suitable for use in the risk assessment.

   Aarab *et al.* (2006) exposed blue mussels *Mytilus edulis* to one concentration of bisphenol-A (50 µg/l) for three weeks in a flow through system. Mussels were obtained from a pristine site in Norway. After exposure, mussels were dissected and the gonadal tissue in the mantle was sampled. Histological examination revealed that the control female animals were in a late pre-spawning stage. Mussels exposed to bisphenol-A exhibited two different patterns; half were considered to be in a post-spawning stage, while the other half had atreitic ovocytes, interpreted as relating to spawning delay. Male control mussels showed no evidence of spawning, while mussels exposed to bisphenol-A showed evidence of spawning having taken place. An alkali-labile phosphate assay to determine total phosphate protein was used as an indicator of vitellogenin-like protein levels. Bisphenol-A exposed female mussels had slightly increased levels over the controls, males had similar levels to the controls. The authors comment that this method may not be suitable for assaying VTG levels in mussels. With only one concentration tested the result cannot be used in the risk assessment.

Crustacea

Experiments have been performed with three species, and these are summarised below.

1) Andersen *et al*. (1999) report a 72-hour immobilisation EC_{50} of 0.96 mg/l for the saltwater copepod *Acartia tonsa*. Kusk and Wollenberger (1999) report 24 and 48-hour EC_{50} values of 5.1-6.3 and 3.4-5.0 mg/l, respectively, for the same species.

   Andersen *et al*. (1999, 2001) studied the effects of a range of substances on the development of nauplii of *A. tonsa*. In Andersen *et al*. (1999) semi-static exposures of copepod eggs were performed with nominal bisphenol-A concentrations of 0.2, 2 and 20 µg/l and medium renewal on days 2, 4 and 6. On day 8 hatched juveniles were divided into groups and placed in vessels with new test medium, and egg production was monitored every day for the next three days. Exposure to 20 µg/l bisphenol-A caused a significant increase in egg production on day 10 of the study, but not on days 9 or 11. In Andersen *et al*. (2001) semi-static exposures were also used, with solution renewal after three days; there was no monitoring of test concentration. The exposures were carried out for five days or until at least 50% of the organisms had undergone metamorphosis from the nauplius to copepodite stage, whichever was the longer. The larval development rate was expressed as the ratio of copepodites to the sum of nauplii and copepodites. The EC_{50} value established for this effect was 0.55 mg/l, and the EC_{10} value was 0.10 mg/l. Although this test is of relatively short duration, it assesses what is considered to be a sensitive endpoint.

   None of these studies is considered suitable for the PNEC derivation or SSD, because of the lack of confirmation of exposure concentrations.

2) The effects of bisphenol-A on the harpacticoid copepod *Tigriopus japonicus* have been studied (Marcial *et al*., 2003). This is an intertidal organism, which thrives at a wide range of temperatures and salinities. It has six naupliar and six copepodid stages, of which the last is the adult. Tests were conducted at 2.5% salinity. Stock solutions of bisphenol-A were changed every week. Acute toxicity was determined in 48-hour exposures, the result being an LC_{50} of 4.32 mg/l (95% confidence limits 4.25 – 4.39 mg/l).

   Longer-term exposures were carried out at four concentrations: 0.01, 0.1, 1.0 and 10 µg/l, with three replicates at each concentration. Nominal concentrations are reported, and the test solutions were renewed daily by replacing around 50% of the working volume each time. Twenty nauplii less than 24 hours old were used at each exposure level. The survival and developmental stage of the organisms was assessed at the renewal of the solutions, at which time they were also fed. For the first eight days the exposures were in 24-well plates; after this time the surviving copepodids were transferred to the chambers of 6-well plates, with food and fresh solution, to initiate copulation. After two to three days, six mature females (bearing ovisacs) were randomly selected from the population and transferred individually to new plates. The number of nauplii produced up to the third brood was monitored for each organism. After 21 days, the sex ratio of the copepodids and the percentage survival were determined. The first brood of nauplii were cultured in the same conditions and the same parameters were monitored for 21 days.

   Survival rates were not affected by bisphenol-A exposure in either the parent or the F1 generation. A significant delay in completion of the naupliar stages (compared to the controls) was seen at concentrations of 0.1 µg/l and above in the parent generation, and at all concentrations for the F1 generation. The time to sexual maturity was increased at 1 µg/l in the parent generation, and at all concentrations for the F1 generation. The sex ratios of copepodids were not significantly different from the controls at any concentration, for either

of the generations. There were also no effects on fecundity (as measured by the average number of nauplii per female) at any concentration. The authors concluded that bisphenol-A (and the other chemicals tested, alkylphenols and 17β-estradiol) had no extensive effect on reproductive parameters, and would have little impact on the demographic profile of the copepod. However, the effects on development could be a potential indicator of exposure to estrogens for crustacean species.

This study is not considered to be suitable for the PNEC derivation or SSD because of the lack of confirmation of exposure concentrations.

3) The most sensitive acute result reported for the mysid shrimp *Americamysis bahia* is a 96-hour LC_{50} of 1.1 mg/l (NOEC = 0.51 mg/l) (Springborn Bionomics, 1985b; Alexander *et al*., 1988). The test conditions and methods are fully described in the test report, concentrations were measured and the test is considered to be valid. This value is supported by a 96-hour LC_{50} of 1.03 mg/l reported by Hirano *et al*. (2004) in a study without analytical confirmation of test concentrations. Both of these studies report short-term lethality results, which are not suitable for inclusion in an SSD (but the 96-h LC_{50} of 1.1 mg/l could be used in the derivation of a saltwater PNEC).

Echinoderms

1) Roepke *et al*. (2005) exposed sea urchin *Strongylocentrotus purpuratus* embryos to bisphenol-A for 96 hours post-fertilisation and measured the percentage achieving the pluteus stage when compared to controls. The EC_{50} was 0.227 mg/l (95% confidence interval 0.122 – 0.324 mg/l). The concentration of bisphenol-A in the 20 ml glass vials used to run these tests was not measured. Addition of tamoxifen, an estrogen receptor agonist, substantially reduced these teratogenic effects, while addition of ICI 182 780, a complete estrogen receptor antagonist in mammals, increased developmental abnormalities by 10-20%.

2) Kiyomoto *et al.* (2006) investigated the effect of ethynylestradiol and bisphenol-A on the development of sea urchin embryos and juveniles. Two species of sea urchin were used, *Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus*; both were collected from the wild in Japan. Eggs and sperm were obtained from the collected animals in the laboratory. Exposures were started either with newly fertilised eggs, or with embryos following hatching at 12 hours post fertilisation. In both cases 500 organisms (eggs or embryos) were used. Exposures took place over different durations, up to 48 hours post fertilisation. The exposure levels ranged from 1.25 to 10 μM (0.29 to 2.3 mg/l). The development of the embryos was observed and categorised in five stages.

   The control eggs hatched at 12 hours after fertilisation, and reached stage 5 (pluteus larvae) after 48 hours. In the bisphenol-A exposed animals, this normal development was only affected at the higher concentrations 1.1 and 2.3 mg/l, and only when the exposure began with the eggs. Embryos exposed from 12 hours after fertilisation showed no effects. Ethynylestradiol produced effects at much lower concentrations and in exposures beginning with embryos as well as with eggs. Although the concentrations were not measured the exposures were relatively short (in particular those over 12 hours from fertilisation), and this part of the study indicates a NOEC of 0.71 mg/l for effects on eggs.

   The authors also exposed juvenile sea urchins (*H. pulcherrimus*) following metamorphosis (~45 days after fertilisation) to bisphenol-A at 0.5 μM (0.11 mg/l) for 80 days. The exposure solutions were renewed every week. After 80 days the test diameters of the juveniles were measured. Animals exposed to bisphenol-A had an average test diameter only half that in the

controls. Ethynylestradiol exposure produced animals with a larger diameter than the controls. As only one concentration was tested no NOEC can be derived and so the result cannot be used in the assessment. This part of the study showed effects at lower concentrations than the egg and embryo exposures.

These studies are not considered to be suitable for the PNEC derivation or SSD because of the lack of confirmation of exposure concentration.

3.2.1.4 Vertebrates

3.2.1.4.1 Fish

Given the widespread interest in the effects of endocrine disrupting substances on organisms, it is not surprising that many studies have been performed using fish, including both whole organisms and isolated tissues. For example, Smeets *et al*. (1999) determined the *in vitro* estrogenic potential of bisphenol-A using cultured hepatocytes from the male carp (*Cyprinus carpio*). Estrogenicity was measured as induction of vitellogenin. Bisphenol-A was found to induce vitellogenin production with a relative potency of 1.10^{-4} to 17β-estradiol and a LOEC of 50 μM (11 mg/l). Bisphenol-A was also found to exhibit cytotoxic effects at 100 μM (22 mg/l) the highest concentration of bisphenol-A tested. Pawlowski *et al*. (2000) studied the estrogenic response of bisphenol-A in cells from rainbow trout (*Oncorhynchus mykiss*) and the variation of the response with temperature. Estrogenic response was measured *in vitro* using cultured hepatocytes from male rainbow trout using a non-radioactive dot blot/RNAse protection assay and by RT-PCR. They found that bisphenol-A was estrogenic with a relative potency of 10^{-4} to 10^{-5} of that of 17β-estradiol. They also found that a higher response rate was measured at 18°C than 14°C with a LOEC of 10 μM (2.3 mg/l) after 48 hours exposure at 14°C and a LOEC of 1 μM (0.23 mg/l) after 48 hours exposure at 18°C. The lowest LOEC measured for vitellogenin induction was 0.1 μM (23 μg/l) after 96 hours exposure at 18°C. Jurgella *et al*. (2006) incubated fragments of liver and kidney tissue from immature lake trout (*Salvelinus namaycush*) with bisphenol-A, and found that 100 μM (23 mg/l) inhibited the production of water soluble (conjugated) metabolites of 17β-estradoil. Several other recent papers have also investigated the effects of bisphenol-A on fish hepatocytes or other cells (e.g., Gushiken, 2002; Hassanin *et al*., 2003; Letcher *et al*., 2005; Rouhani Rankouhi *et al*., 2004; Suzuki and Hattori, 2003), or have investigated bisphenol-A binding affinities and interactions with ligands in fish (e.g., Alo' *et al*., 2005; Ohkimoto *et al*., 2003; Tollefsen *et al*., 2004).

Whilst such *in vitro* studies are useful for elucidating mechanisms of action or for developing environmental screening tools, they are not useful for deriving a PNEC for protecting populations of organisms. However, a similar type of study that may be of greater environmental relevance is reported by Thomas and Doughty (2004). They collected sperm from Atlantic croaker (*Micropogonius undulatus*) and treated it with a progestin in the presence or absence of several potential endocrine disrupting chemicals. The lowest concentration of bisphenol-A that significantly reduced upregulation of sperm motility by the progestin was 0.1 μM, which is equivalent to 23 μg/l. This result may be significant for the functioning of fish reproduction, but the authors acknowledge that the environmental relevance of their findings remain unclear. In this case, the lack of confirmation of exposure concentrations also makes the study unsuitable for the PNEC derivation.

The following sections discuss the available *in vivo* studies, grouping the data for individual species together (freshwater first, followed by saltwater).

Freshwater species

1) Carp *Cyprinus carpio*

Bowmer and Gimeno (2001) have studied the effects of bisphenol-A on the development of the male carp reproductive tract when exposed during sexual differentiation (only an extended abstract of this study was available at the time of writing and no published paper has been found subsequently). Males were exposed to nominal concentrations of 10, 32, 100, 320 and 1,000 µg/l bisphenol-A under flow through conditions, during the period of sexual differentiation (from 45 to 55 days post hatch onwards). Two experiments were performed, the first conforming to the OECD principles of GLP. In both experiments nominal concentrations were confirmed by analysis. In the first experiment 28- and 49-day NOECs for growth (wet weight) were >600 and 100 µg/l bisphenol-A; in the second experiment 28- and 56-day NOECs were both 226 µg/l. In the first experiment 28- and 49-day NOECs for oviduct formation were 100 and 16 µg/l bisphenol-A while in the second experiment they were 60 and 17 µg/l. These results should be considered as "valid with restriction" because a full report is not available. The effects on oviduct formation cannot be related directly to demographic parameters such as survival, growth or reproduction, and so are not used in the PNEC derivation directly.

The 49-d growth NOEC of 100 µg/l for *Cy. carpio* is considered suitable for use in the PNEC derivation and SSD.

2) Goldfish *Carassius auratus*

Suzuki *et al*. (2003) exposed immature fish to a single concentration of 10^{-6} M (~230 µg/l) bisphenol-A for 8 days and measured effects on plasma vitellogenin, calcium and calcitonin levels. Vitellogenin was detected in the exposed fish. Calcium concentrations were significantly higher than controls on day 4 and significantly lower on day 8. Plasma calcitonin concentrations were significantly lower than controls on day 8. This shows that bisphenol-A can affect calcium metabolism in teleost fish, but the single dose and lack of confirmation of exposure concentration means that these results are not suitable for use in deriving a PNEC.

3) Zebrafish *Danio rerio*

Bayer AG (1999a) report a 14-day NOEC of 3.2 mg/l and a LOEC of 10.15 mg/l from semi-static tests performed according to OECD guideline 204. The endpoints studied were mortality and visual effects on appearance and behaviour; the specific effect on which the NOEC was defined is not given.

Schäfers *et al*. (2001) studied the estrogenic impact of bisphenol-A in a full life cycle study (only an extended abstract on the work has been seen, but see further references later in this sub-section). They found that bisphenol-A exposure affected juvenile growth, time until first spawning, egg production and fertilisation rate. The EC_{50} and NOEC for fertilisation rate were 1.45 mg/l and 0.76 mg/l, respectively. Bisphenol-A showed a lower estrogenic potency than ethinylestradiol. Similar results were found by Fenske *et al*. (2001) who looked at alterations in vitellogenesis and reproduction in zebra fish exposed to ethinylestradiol and bisphenol-A.

Segner *et al*. (2003a)[19] report a full life cycle study in which zebrafish were exposed to 94-1,500 µg/l bisphenol-A, with analytical confirmation of exposure concentrations. The LOEC for vitellogenin induction and changes in gonad histology was 375 µg/l (the NOEC was 188 µg/l). The LOEC for juvenile growth, time to spawning, mating behaviour, eggs per female and fertilisation success was 1,500 µg/l (the NOEC was 750 µg/l). There was no effect on the hatching rate of offspring. The absence of a considerable number of pertinent experimental design details, lack of discussion on analytical details and sparse presentation of results mean that this study should be classed as "valid with restriction". Nevertheless, it is considered suitable for the PNEC derivation and SSD, even though the most sensitive end points cannot be related directly to demographic parameters such as survival, growth or reproduction.

Lindholst *et al*. (2003) examined the toxicokinetics of bisphenol-A in zebrafish and rainbow trout. It was suggested that zebrafish may be less sensitive than rainbow trout because of more rapid metabolism of bisphenol-A in the zebrafish liver. However Van den Belt *et al*. (2003) compared vitellogenin induction in zebrafish and rainbow trout after a three week semi-static exposure to 40, 200 and 1000 µg/l bisphenol-A and found no evidence for a difference in sensitivity, with significant induction in both species occurring at only the highest concentration.

Drastichová *et al.* (2005) exposed zebrafish (*Danio rerio*) to bisphenol-A in their food. The exposures were begun with 20-day old fry, a stage which is prior to differentiation between males and females. Bisphenol-A in ethanol solution was mixed with decapsulated brine shrimp (*Artemia salina*) eggs at 500, 1000 and 2000 mg/kg. The fish were fed three times a day over the 45 day exposure period, and the water in the exposure vessels was changed three times per week. The sex of each fish was established from the morphology of the gonads at the end of the exposures. The sex ratio in the controls was 1:1. The ratios in the exposures were (female:male) 1.4:1 at 500 mg/kg, 3.8:1 at 1000 mg/kg and 11.5:1 at 2000 mg/kg. The ratios were significantly different from the controls at 1000 and 2000 mg/kg. Fish exposed to 20 mg/kg 17β-estradiol as a positive control all developed as females. The exposure route means that the result is not suitable for use in the risk assessment.

In summary, the NOEC of 750 µg/l for multiple end points from a *D. rerio* full life cycle study is considered suitable for use in the PNEC derivation and SSD. It is noted that vitellogenin induction and changes in gonad histology were observed at this concentration.

4) Rainbow trout *Oncorhynchus mykiss*

Lysak and Marcinek (1972) report a 24-hour LC_{100} of 7 mg/l bisphenol-A and a 48-h NOEC of 5 mg/l for rainbow trout (*Oncorhynchus mykiss*), while Reiff (1979) reports a 96-hour LC_{50} of 3-5 mg/l. Test methods were not stated and concentrations of bisphenol-A were not measured.

Bayer AG (1999b) report the results of a 28-day juvenile growth test on rainbow trout using bisphenol-A. The test followed the proposed OECD guideline 215 for "Fish, Juvenile growth test". The NOEC and LOEC for growth rate were 3.64 and 11.0 mg/l respectively (both

[19] Segner *et al*. (2003b) also report the results of a life cycle test with zebrafish, which is very likely the same test reported in Segner *et al*. (2003a). For example, the exposure concentrations appear to be identical (94, 188, 375, 750 and 1,500 µg/l). This study might also be the same as that reported by Schäfers *et al*. (2001). For example, the reproduction EC_{50} is stated to be 6,140 nM (equivalent to approximately 1.4 mg/l), which is similar to the value reported by Schäfers *et al*. (2001). Schäfers is one of the co-authors of the Segner *et al*. papers.

arithmetic means of analytical values). This chronic study is fully valid, was conducted under full GLP and followed an OECD Guideline.

Lindholst *et al*. (2000) studied the estrogenic response to bisphenol-A in rainbow trout, which were exposed to bisphenol-A via a continuous flow-through system. Vitellogenin concentrations were measured during the exposure period (12 days). A significant induction of vitellogenin synthesis was observed in the 500 µg/l bisphenol-A exposure group over the study period. In lower exposure groups (40 and 70 µg/l) steadily increasing levels of vitellogenin were observed between 6 and 12 days only. Based upon the data the NOEC for vitellogenin production is taken as 40 µg/l. In a further study, Lindholst *et al*. (2001) again exposed rainbow trout to bisphenol-A in a continuous flow-through system (at 100 µg/l), and also through intraperitoneal injection (at a tissue concentration of 35 mg/kg). Both male and female fish showed increased levels of vitellogenin in the injection exposures, with a lag period of 3-5 days for females and 5-7 days for males. Measured levels of bisphenol-A in the livers of the fish had decreased almost to the detection limit before the increase in vitellogenin was noted. Fish in the continuous exposures did not show significantly higher levels of vitellogenin up to the end of the experiment after seven days. Neither of these two studies can be used for the PNEC derivation or SSD since the exposure concentrations were not measured and the end point cannot be related to population effects.

As mentioned above for zebrafish, Lindholst *et al*. (2003) considered that rainbow trout may be more sensitive than zebrafish due to differences in liver metabolism, yet Van den Belt *et al*. (2003) found no evidence for a difference in sensitivity regarding vitellogenin induction (with significant induction only occurring at 1,000 µg/l after a three week semi-static exposure).

In summary, the 28-d NOEC for juvenile growth rate of 3.64 mg/l for *O. mykiss* is considered suitable for use in the PNEC derivation and SSD.

5) Atlantic salmon *Salmo salar* (m. *sebago*)

Honkanen *et al*. (2004) exposed 8-day old yolk-sac fry to nominal concentrations of 10, 100 or 1,000 µg/l bisphenol-A for 42 days. Test media were renewed every 48 hours, but there was no chemical confirmation of exposure concentrations. After 6 days, yolk-sac oedemas and haemorrhages around the gill arches and in the front part of the yolk sac were observed in fry exposed at the highest concentration. After 8 days fry at the highest concentration were lethargic and remained inactive, and by 17 days they were darker in colour than fry at other concentrations. At the end of the experiment fry at the highest concentration remained lethargic, were darker, and some still had yolk-sac oedemas, although others appeared to have recovered. There was also some evidence that fry in the highest concentration weighed significantly more than other fry, although they did not weigh significantly more than those in the solvent control. Histological analysis of fry livers revealed strongly stained fragments in the nuclei of hepatocytes in the 100 and 1,000 µg/l groups, plus a reduction in liver cell storage substances and a difference in the shape of cytoplasmic vacuoles. This study is not considered to be of direct use for the PNEC derivation because of the lack of confirmation of exposure concentration.

6) Brown trout *Salmo trutta*

Lindholst *et al.* (poster presentation; 2002) exposed eggs to 50 µg/l of bisphenol-A from fertilisation through to 64 days post fertilisation, corresponding to the time of first feeding. The concentrations of bisphenol-A and bisphenol-A glucuronic acid (BPAGA) were

measured in the eggs and developing fry. A rapid increase in the concentration of BPAGA was observed at 35 days post fertilisation, on hatching, possibly due to increased uptake of bisphenol-A through the gills at this time. The bioconcentration factor measured in developing eggs was 10, and in the fry it was 14. After hatching, fish were kept for up to 400 days in clean water, and observations were made on the gonads and sex of the fish. No changes were seen in the sex ratio compared to the controls. Generally there were a higher number of males in both exposures and controls, but there was no increase in the percentage of mature males at 400 days. There were no significant differences in the gonadosomatic index of male fish in this study. This study is not considered to be suitable for PNEC derivation because only a single exposure concentration was used, and the actual exposure level was apparently unconfirmed.

7) Japanese medaka *Oryzias latipes*

Several short-term results are available. MITI (1977) report a 48-hour LC_{50} of 15 mg/l, Tabata et al. (2001) report 72-hour LC_{50} values of 5.1 mg/l for embryos and 7.5 mg/l for adults exposed in semi-static systems.

Several longer-term studies have been reported as follows:

a) Shioda and Wakabayashi (2000) exposed male *Or. latipes* to a natural estrogen (17β-estradiol) and three estrogenic substances including bisphenol-A. After 14 days' exposure, one male was kept with two females for spawning. The results indicated that bisphenol-A caused a decrease in the number of hatchlings at a concentration of 2.3 mg/l. No effects were observed at the lower concentrations that were tested (68, 230 and 680 µg/l). This study was designed to look at the effects of endocrine disrupters on reproduction due to *in vivo* exposure. Due to the different study protocols used it is not possible to compare the estimated potency of bisphenol-A with that of 17β-estradiol.

b) Tabata *et al*. (2001) studied the effect of bisphenol-A on mature male *Or. latipes*. No concentration monitoring was undertaken to confirm the exposure levels. After two weeks' exposure to 100 µg/l bisphenol-A, female specific proteins could be detected in the fish, but no effects were observed at 0.1 or 10 µg/l exposure. After five weeks' exposure female specific proteins were found in the 10 µg/l exposure group but not in the 0.1 µg/l exposure group. Abnormalities in the gonad tissue were observed in the 100 µg/l exposure group in one animal of the sixteen exposed. There was no observation of any sex bias towards females in any of the bisphenol-A exposure groups. Tabata *et al*. (2003a&b, 2004) also report results for *Or. latipes* exposed in flow-through systems to bisphenol-A for five weeks in what appear to be different studies to Tabata *et al*. (2001) which produced less sensitive results. Tabata *et al*. (2003a) and Tabata *et al*. (2004) report the same study in which the NOEC for vitellogenin induction was 200 µg/l and the LOEC was 500 µg/l, with significant induction at 500 µg/l beginning on day 3 of the study. Weekly chemical analysis confirmed that test concentrations remained within 77.2 – 102.6% of nominal. Tabata *et al*. (2003b) report a study in which the effect of chlorination on bisphenol-A estrogenicity was assessed when fish were exposed to 100 or 1,000 µg/l for five weeks. Chlorination appeared to remove bisphenol estrogenicity.

c) Na *et al.* (2002) studied the effects of bisphenol-A on sex differentiation and gonadal development in *Or. latipes*. Fish were obtained from a stream in the wild, and breeding fish were cultured for three months. Fertilised eggs were removed and incubated until hatching. Newly hatched larvae were exposed to bisphenol-A in a static renewal system at nominal concentrations of 50, 100 and 200 µg/l (there is no indication that actual

concentrations in the exposures were measured). Solutions were renewed every 72 hours for the first month and then every 48 hours thereafter. The total length of the exposures was 70 days. Fish were sampled at 10, 20, 30 and 70 days. Saggital sections of the gonads of the fish (male and female) were examined and the proportion of the section occupied by each type of germ cell was determined. Length and weight (at 70 days only) were also determined.

No differences of gonadal development in the process of sexual differentiation were observed between any groups until 30 days after exposure began. At 70 days, bisphenol-A exposed female fish had greater proportions of the later stages of oocytes, including mature eggs, which were not present in the controls. In males, the proportions of the later stages of spermatogenesis were reduced; at the highest exposure level there were very few spermatocytes or spermatids.

A chi-square analysis of numbers of females and males showed a 1:1 ratio in the controls and the 200 μg/l exposure, but a 2:1 (female:male) ratio in the other two exposures. However, this section of the paper is unclear. The ratios are based on the sum of the numbers at each time interval and a much larger number was sampled at 30 days, so the ratio is dominated by the numbers then. At 20 days, the control ratio was 3:1 f:m.

The lengths of fish exposed to 50 or 100 μg/l were not significantly different from the controls, but fish exposed to 200 μg/l were longer than those in the other groups after 30 and 70 days exposure. Fish in the high exposure group were also heavier. The NOEC for a growth effect is therefore 100 μg/l (for promoting growth).

The authors conclude that bisphenol-A appears to contribute to the accumulation of vitellogenin in oocytes in females. In males, it inhibits spermatogenesis, and exposed fish had less developed testicular structure. Lack of chemical analysis means that these results are not suitable for direct use in deriving a PNEC.

d) Yokota *et al*. (2000) report a fully valid and thoroughly described extended early life stage study that examined a number of ecologically critical endpoints. *Or. latipes* were exposed to bisphenol-A from fertilised eggs through to 60 days post-hatch. Five concentrations were used: 2.28, 13.0, 71.2, 355 and 1,820 μg/l (as mean measured concentrations, detection limit 2.5 μg/l.) Semi-static exposures were used for the embryos, with flow-through exposures for larvae. The low exposure concentrations were more stable in the semi-static exposures (which is unexpected). The parameters monitored were egg hatchability, time to hatching, cumulative mortality and growth (total length and body weight).

An initial pre-test established a 96-hour LC_{50} of 13.0 mg/l. In the main study, no significant effects were seen on hatchability, time to hatch, or mortality at any exposure. (Hatching of some embryos was significantly delayed in the 13 μg/l exposure, but there was no dose response and higher exposures showed no differences from controls.) Growth was reduced at 60 days - this was a dose-related effect, with only the highest exposure level producing a significant reduction ($p=0.005$). Sex ratios were determined from external secondary sexual characteristics. Ratios were 1:1 at concentrations of 71 μg/l and below; at 355 μg/l there were more females than males (5:13); and there were no males at the highest concentration. Sex ratios were also determined through gonadal histological investigation (this allowed some of the fish that did not show clear secondary sexual characteristics to be sexed). These results showed a similar pattern, with the ratio at the highest concentration significantly different from that in the controls (which was

2:1 male to female). The sexual characteristic results were not treated statistically as they are not considered reliable indicators of sex. The examination also looked for cases of testis-ova; cases were only found at the highest exposure concentration. The authors concluded that the lowest effect concentration was between 355 and 1,820 µg/l, although they noted that the study did not investigate whether early life exposure would impair reproduction as adults.

e) Kang *et al*. (2002) examined the effects of bisphenol-A on the reproductive capacity (fecundity and fertility) and estrogenic response of adult *Or. latipes* and studied the transgenerational effects (F1 generation growth and sex) of this substance on the F1 offspring. The test methods used are now being recommended by the OECD for elucidation of effects on survival, growth, and reproduction of potential endocrine disrupting compounds (paired breeding assay and extended early life stage test). Sexually mature *Or. latipes* at four months after hatching (300 mg body weight, 33 mm length) were acclimated to flow through conditions for three weeks in 56 breeding pairs in individual 1 litre chambers. The fecundity of each pair was checked daily, and over the last week of the acclimation period eggs were collected daily, a few hours after deposition, counted and assessed for fertility. From these fish, 32 pairs were selected which spawned every day, with ≥15 eggs per day and mean fertility >90%. These fish were exposed to bisphenol-A for three weeks, at nominal concentrations of 0, 1,000, 2,000 and 4,000 µg/l. The concentrations in the exposure chambers were measured twice each week during the exposures. The levels varied, falling as low as 60% of the nominal concentration on one day, but the average concentrations were 78-86% of nominal. The average measured levels were 837, 1,720 and 3,120 µg/l.

   Eggs were collected daily and assessed for fecundity and fertility under a light microscope. All fish were sacrificed at day 21, and histological evaluation and determination of vitellogenin were carried out.

   Eggs collected on days 18-20 from the exposure and control groups were used to assess trans-generational effects. Eggs were incubated in dechlorinated tap water and hatched larvae were transferred randomly to four test chambers for each treatment. One week after the mean time for hatching across all treatments, 15 larvae from each chamber were selected randomly and kept until day 60 to assess abnormal development and mortality.

   There was no decrease in fecundity or fertility in any of the treatments compared to the controls. One male died in the 1,720 µg/l exposure on day 11, and one female fish died in the 837 µg/l exposure on day 17. Neither of these dead fish showed any pathological changes. The gonadosomatic and hepatosomatic indices of both sexes of fish were unaffected by bisphenol-A exposure. Intersex gonads (testis-ova) were observed in males at all three of the exposure levels – one case at 837 µg/l, six at 1,720 µg/l and four at 3,120 µg/l. No instances of testis-ova were observed in the controls. Cells indicative of normal spermatogenesis were also found in all of the gonads examined. No histological abnormalities were noted in the ovaries of any of the female fish exposed, or the controls.

   Vitellogenin levels were significantly elevated above the controls in male fish at the highest exposure concentration used. The levels at this concentration were similar to those found in female fish in the controls and at all exposure levels. Levels of vitellogenin in four of the seven male fish examined from the 1,720 µg/l exposure exceeded the detection limit but were not significantly elevated above the controls.

Bisphenol-A had no observable effect on the survival and growth of offspring at 60 days after hatching. Low mortality was seen at all three exposure levels and was not significant. Length and weight were not significantly affected. The sex ratios did not differ significantly between the controls and the exposed fish, although the controls contained more males than females (1.52:1), the 837 µg/l exposure had more females than males (0.71:1) and the two higher exposure levels had approximately equal ratios (1:1). The authors concluded that although bisphenol-A induced hepatic vitellogenin and gonadal intersex in male fish, these effects are not associated directly with effects on reproduction.

f) Metcalfe *et al*. (2001) examined growth and sexual differentiation endpoints over the course of an extended early life stage test. These are ecologically relevant endpoints and the study is well reported. *Or. latipes* fry were exposed to bisphenol-A in a static renewal system from one day after they had hatched until they reached a length of 1.5 cm (approximately 90 days post-hatch). The fry were then examined histologically to determine phenotypic sex and incidence of testis-ova. Exposure concentrations of bisphenol-A were 0, 10, 50, 100 and 200 µg/l, and test medium was replaced every 48 hours; chemical analysis showed that average concentrations over 48 hours were 59.6% of nominal. Fish exposed to the two highest test concentrations had a significantly higher condition factor (weight divided by total length) than fish in the other groups, but there were no significant differences between treatments in either total length or wet weight alone. Sex ratios did not differ after exposure to any of the bisphenol-A concentrations. Testis-ova were observed in only two males from the lowest test concentration, but not in any fish from the higher concentrations. Male fish at the higher concentrations (50, 100 and 200 µg/l) showed several morphological changes in testes, and female fish at the highest concentration had ovaries in advanced stages of oogenesis in comparison to control females.

g) Kashiwada *et al*. (2002) exposed *Or. latipes* eggs, embryos or adults to nominal concentrations of bisphenol-A for three days, with daily medium renewal. 72-hour LC_{50} values were 9 mg/l for eggs (95% confidence interval 7.1-11 mg/l), 5.1 mg/l for embryos (95% confidence interval 4.2-6.7 mg/l), 6.8 mg/l for adult males (95% confidence interval 5.9-7.7 mg/l) and 8.3 mg/l for adult females (95% confidence interval 7.4-9.4 mg/l). Adult males were also exposed for five weeks under flow-through conditions to nominal concentrations of 0.1, 10 or 100 µg/l bisphenol-A, with chemical analysis confirming that measured concentrations remained within 10% of nominals. Fish were sampled at the end of weeks 1, 2, 3 and 5 for analysis of female specific proteins. These proteins were detected in males exposed to 10 µg/l after 4-5 weeks and in males exposed to 100 µg/l after two weeks.

h) Embryos of medaka (*Or. latipes*) were exposed to bisphenol-A in scintillation vials, with five embryos per vial (Pastva *et al*, 2001). Two concentration of bisphenol-A were used, 20 and 200 µg/l, with five replicates per concentration. Solutions were renewed every 24 hours for nine days, and a new standard solution of bisphenol-A was prepared every day. Individual embryos were observed daily and compared to a published atlas of normal medaka development. Abnormalities for each embryo were recorded and a score (severity index) for each test vessel. Larval stages were also exposed to 200 µg/l for 96 hours with a similar solution renewal pattern.

The data for the severity index were analysed using a repeated measures function, to take account of the non-independence of the observations – if a fish had a deformity on day 6 it was likely to have the same one on day 7. No deformities were noted until after day 3.

The severity index in the 200 µg/l exposures was greater than that in the controls for days 5 to 8, but by day 9 it was not significantly different, due to reduced severity of lesions in some individual embryos. Hence the effects were largely transient. Most embryos did not show deformities. There were no mortalities in the exposed larvae.

i) Ishibashi *et al.* (2005) exposed eggs of medaka (*Or. latipes*) from a few hours after fertilisation to bisphenol A at nominal concentrations of 1563, 3125, 6250, 12500, 25000 and 50000 µg/l for 14 days. There were two replicates at each concentration, with 30 eggs per replicate. A semi-static exposure regime was used, with solutions changed every 24 hours. The 14-day LC_{50} value determined in these exposures was 14.8 mg/l. The hatchability of eggs was decreased compared to the controls at concentrations above 12,500 µg/l. The time to hatching was not affected.

The same authors also exposed eggs under similar conditions to MBP (4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, a metabolite). Concentrations used were 313, 625, 1250, 2500 and 5000 µg/l. This study gave a 14-day LC_{50} of 1,730 µg/l; hatchability was affected at concentrations above 1,250 µg/l and the time to hatching increased at all exposure concentrations.

Adult male medaka were exposed from three months old to nominal concentrations of 250, 500, 1000 and 2000 µg/l bisphenol-A for 21 days. Seven males were used per concentration, and the solutions were changed every 24 hours. At the end of the exposures the body weight, and total length were measured. Livers and gonads were sampled, and the HIS and GSI were calculated as the ratios of the organ to body weight. Hepatic vitellogenin levels were measured. Bisphenol-A did not affect growth (weight or length) and there were no effects on survival at 2,000 µg/l. Similarly there were no effects on HIS or GSI over 21 days. Vitellogenin levels in the liver were significantly higher in the 1,000 and 2,000 µg/l exposures.

The metabolite MBP was also tested under similar conditions to the above. The 21-day LC_{50} was estimated as 63.4 µg/l. There were no effects on the GSI up to 1,000 µg/l, but the HIS was significantly higher than in the controls at 37 µg/l. Vitellogenin levels in the liver were significantly increased over the controls at 4.1 µg/l and above.

j) Three other studies on Japanese medaka have recently been completed in Japan. Full details are unavailable, but the text for the results tables is available (Japanese Ministry of the Environment, 2006):

- In one study fish were exposed for 21 days to 0, 58.5, 141, 334, 772 and 1,740 µg/l mean measured concentrations of bisphenol-A. All fish survived at concentrations up to 334 µg/l; there was 3.3% and 13.3% mortality at 772 and 1,740 µg/l, respectively. At 21 days the hepatosomatic index increased significantly at 334 µg/l and vitellogenin concentrations were significantly higher at the same level.
- In a second study (a partial life-cycle test) groups of 20 fish were exposed to 0, 220, 470, 890, 2120 and 4,410 µg/l mean measured bisphenol-A. Hatching rate was unaffected at all concentrations, but time to hatching was significantly greater at the highest concentration. Body length was significantly greater than controls at 470 and 890 µg/l, but not at the two highest concentrations. Body weight was significantly greater than controls at 220 and 470 µg/l, and significantly lower at 4,410 µg/l, with no significant difference at 890 and 2,120 µg/l. The gonadosomatic index was unaffected in this study, but there were significant effects on incidence of testis-ova at 890 µg/l, male hepatosomatic index at 4,410 µg/l, female hepatosomatic index at

2,120 µg/l, male liver vitellogenin at 470 µg/l and female live vitellogenin at 2,120 µg/l.

- In a third study (a full life-cycle test), groups of 20 medaka were exposed to 0, 2, 9.3, 49.7, 247 and 1,179 µg/l mean measured bisphenol-A. There were no effects on F0 hatching rate, days to hatching, body length, body weight, gonadosomatic index, number of eggs, incidence of testis-ova, liver vitellogenin and female hepatosomatic index, but there was a significant increase in mortality at the highest test concentration and in the male hepatosomatic index at 49.7 µg/l. In the F1 generation there were no significant effects on survival, days to hatching, gonadosomatic index or female liver vitellogenin. There were effects in the F1 generation on testis-ova and male liver vitellogenin at the highest concentration. Differences in body length and weight were small and although some were significant there were no linear or U-shaped dose-response relationships. There was a significant reduction in hatching rate at 2 µg/l of about 10%, but no significant reductions at any of the other test concentrations. Taken together, these results suggest that the most sensitive reliable NOEC for *Or. latipes* is 247 µg/l for F0 survival (28% mortality compared with 11-12% in the controls) with a LOEC of 1,179 µg/l. Measured effects below this NOEC are either difficult to interpret or do not appear to have influenced survival, growth or reproduction.

k) Several other authors have also measured vitellogenin induction or gene expression in *Or. latipes* exposed to bisphenol-A. Yamaguchi *et al*. (2005) exposed adults to nominal concentrations of 800 or 8,000 µg/l bisphenol-A for 8 hours and then analysed liver vitellogenin. There was significant induction of vitellogenin II and ERα at 8,000 µg/l, but not at 800 µg/l, and no induction of vitellogenin I at either concentration. Nagae *et al*. (2005) exposed fish to nominal vitellogenin concentrations of 100, 500, 1,000, 5,000 and 10,000 µg/l bisphenol-A for three days, with renewal of test medium every day. Induction of vitellogenin I and II was only apparent at concentrations above 5,000 µg/l. Chikae *et al*. (2003) exposed adult males to 0, 0.02, 0.2, 2, 20 and 40 mg/g bisphenol-A in their diet for 14 days and estimated an EC_{50} for plasma vitellogenin induction of 1.6 mg/g. Lee *et al*. (2002) exposed adult males to nominal concentrations of 5, 50, 100, 200 or 500 µg/l bisphenol-A for 144 hours and found that choriogenin mRNA, which is involved in egg formation in females, began to be expressed in males exposed to ≥50 µg/l.

Many of the studies reported above provide results for secondary supplemental end points (such as vitellogenin, other proteins, histology and other biomarkers) which provide useful information on possible mechanisms of action. However, only long-term end points related to mortality, growth and reproduction (i.e., endpoints of demographic importance) should be used to derive the PNEC. In this regard several of the studies are highly relevant and of high quality, and include measurement of exposure concentrations (particularly Yokota *et al*. (2000), Kang *et al*. (2002), Metcalfe *et al*. (2001) and Japanese Ministry of the Environment (2006)). Inclusion of more than one study for a single species in an SSD would introduce bias, and calculation of a geometric mean for *Or. latipes* is not possible because the study durations differ. After consideration of all of the studies, the new results from the Japanese Ministry of the Environment (2006) are considered to be the most useful for inclusion in an SSD. Although full details are lacking, results are presented for end points of clear demographic importance from a multi-generation study with measured test concentrations.

The NOEC of 247 µg/l for multiple end points in a full life cycle study with *Or. latipes* is considered the most relevant for use in the PNEC derivation and SSD. Effects observed below this NOEC are either difficult to interpret or do not

appear to have influenced survival, growth or reproduction.

8) Fathead minnow *Pimephales promelas*

Two short-term results are available. Alexander et al. (1985a and 1988) report 96-hour LC_{50} values of 4.7 (static) and 4.6 mg/l (flow-through) (nominal concentrations). The test conditions and methods are fully described in the test report, and the studies are considered valid.

Sumpter et al. (2001) (partly published as Sohoni et al., 2001) report a multigenerational study on P promelas that examined effects of bisphenol-A on the F0, F1 and F2 generations. Exposure was to nominal concentrations of bisphenol-A (1 µg/l, 16 µg/l, 160 µg/l, 640 µg/l and 1,280 µg/l) in a flow-through system. Nominal test concentrations were confirmed by measurements of bisphenol-A in the test media. Fish were also exposed to a dilution water control throughout the experiment. The study began with adult fish at 120 days post hatch, with 60 fish per treatment level. At day 42 of the study, eight breeding pairs per treatment were randomly selected and used to assess the fecundity of the F0 generation. Spawnings of 50 embryos from single females were used in hatchability trials. Two cohorts of eggs from these breeding pairs were taken and used in two separate early life stage studies (commencing on days 56 and 155 of the study). Fish larvae from the hatchability trials were discarded at the end of the trials, but those from the early life stage studies were transferred to the progeny tanks to form the F1 generation. The F0 breeding pairs were sacrificed on day 164 of the study. Other adult fish in the F0 generation were sacrificed after 43 and 71 days of the exposure.

Fish of the F1 generation were continuously exposed through to sexual maturity. On day 275 of the study (when the F1 fish were an average of 150 days old) eight breeding pairs were randomly selected and a similar series of tests to those above conducted: fecundity measurements on the F1 generation; hatchability trials on the F2 generation; and an early life stage test on the F2 generation. Adults from the F1 generation not selected for breeding were sacrificed on day 295 of the study. The study was terminated at 431 days from the start with the sacrifice of the F1 breeding pairs.

During the experiment information was recorded on fish survival, fecundity and hatchability of eggs. Upon sacrifice, intact fish, dissected gonads and blood plasma of the F0 and F1 fish were analysed for vitellogenin, gonad growth and histology of the gonads. For male fish, the gonad histology included a scoring of the various testicular cell types in order to assess the progression of spermatogenesis. The study concluded that bisphenol-A acts as a weak estrogen in vivo to fathead minnow exposed to bisphenol-A via water. The overall NOEC for conventional endpoints of survival, growth and reproduction based on the hatchability of the F2 generation is 16 µg/l. For vitellogenin production a NOEC of 16 µg/l is determined. Some growth endpoints, including gonad size, show NOEC values of <16 µg/l at individual monitoring points, but not consistently over the course of the experiment.

The observations on the testes of the male fish showed that exposure to bisphenol-A had a significant effect on the development of sex cell types compared to the controls. Measurements were made on the relative proportions of each cell type in the tissue, not the absolute numbers of cells. The cells develop from spermatogonia through spermatocytes and spermatids to spermatozoa. For the F0 generation, regression analysis showed that there were dose-related effects of bisphenol-A on the proportion of different cell types. The lowest effective concentration for these responses was 640 µg/l (spermatogonia) and 16 µg/l (spermatozoa). The highest exposure concentration (1,280 µg/l) caused a five-fold decrease

in the relative occurrence of mature spermatozoa while spermatocytes, spermatids and other cell types varied by up to 10%. The relative proportion of spermatogonia increased from ~12% in the controls to 83% at the highest concentration. The NOEC for a reduced proportion of spermatozoa is 1 µg/l. For the F1 generation, there was a positive dose-related effect of bisphenol-A on the proportion of spermatogonia, and an inhibitory effect on the proportion of the testes occupied by spermatozoa. The lowest effect concentration for these responses was 1 µg/l for both spermatogonia and spermatozoa. (This aspect of the study has since been questioned, see comments below following the study conclusions.)

From the data it is not possible to say that inter-generational sensitivity increased or decreased because the F0 generation fish were introduced to the test system as sub-adults, whereas the F1 generation was exposed to bisphenol-A throughout their lives.

From the data the report derived the following conclusions:

- LOEC (survival, 60 days) 640 µg/l (for F1, LOEC>640 µg/l for F0 and F2)
- NOEC (growth, 164 days) 160 µg/l.
- The size of the gonads of female F0 fish, were significantly greater than that of the controls at 1 µg/l on day 43. However, no significant effects were seen at 16 µg/l and subsequently the NOEC rose to 1,280 µg/l (day 71) and 160 µg/l (day 164). In males the NOEC for effects on gonad size was <1 µg/l on day 43 but subsequently rose to 1,280 µg/l (day 71) and 160 µg/l (day 164). Therefore, the NOEC for consistent or dose-related effects is taken as 160 µg/l.
- NOEC (egg production) 160 µg/l for the F1 generation and 640 µg/l for the F0 generation. This is based upon the number of eggs produced per female per day.
- NOEC (hatchability of eggs) 160 µg/l for the F1 generation and 16 µg/l for the F2 generation.
- NOEC (vitellogenin production) 16 µg/l, for F0 males and F1 generation males and females.
- Effects on the different stages of male spermatozoa development were seen at lower concentrations, with a NOEC value for the proportion of spermatogonia and spermatozoa of 1 µg/l for the F0 generation and a LOEC of 1 µg/l for the F1 generation. The hatchability of eggs was affected only at 160 µg/l or greater.

Overall, effects based upon the survival, and reproductive fitness of fathead minnows exposed to bisphenol-A from F0 breeding adults to F2 offspring occurred at concentrations of 640 µg/l bisphenol-A and higher, with hatchability of F2 eggs slightly but significantly reduced at 160 µg/l.

Two independent experts in fish histopathology subsequently reviewed the parts of this study relating to spermatogenesis (D Dietrich, personal communication). It was noted that the study was designed to look for effects on reproduction, hatching and growth. The sampling and examination of gonad tissues for sperm cell types was added after the study design had been implemented, and so the experimental design was not optimised to look at these effects. Some short-comings of this part of the study were identified in relation to the number of fish sampled from each exposure level, the taking of tissue samples from the testes and their preparation for counting, and the number of cells counted in each sample. In addition, the statistical methods used to compare the proportions of cell types in the controls and exposed fish were not appropriate, as the relative proportions of each cell type are not independent of

each other. While these short-comings and general test design are not considered to make the study invalid for population effects in terms of reproduction, hatchability and growth, the experts concluded that the weaknesses in the spermatogenesis data make them unsuitable as the basis for deriving a PNEC. This view was supported by one of the main authors of the study.

The published risk assessment for bisphenol-A (EC, 2003) concluded that further work was needed on this endpoint. As a result a series of studies has been carried out on fathead minnows to investigate further the possible effects on sperm cells and on ovarian cells (Rhodes *et al*, 2007). The initial phase of the work was to develop the methods necessary to visualise and quantify the individual gonadal cell types (Wolf *et al*., 2004). In this phase of the work the fish were exposed to 17β-estradiol. Techniques were developed for the optimal preparation, preservation and processing of gonadal tissues. These tissue samples were used to develop manual tagging procedures for the identification and quantification of gonadal cell types. The developed method allows for a permanent record of all cells identified and counted and therefore facilitates peer review of gonadal cell type assessment.

The second phase of the studies (Caunter *et al*., 2006) was a 42-day range finding study exposing fathead minnows to bisphenol-A in order to demonstrate the above methods. This part of the work also looked at the natural variability of the distribution of cell types, and the consequences for the number of replicates needed in the final study to allow determination of statistically significant effects on gonadal cell type distribution as a result of treatment.

The third phase of the studies was a partial life cycle test over 164 days, with a similar duration and similar bisphenol-A concentrations to those in the P (F0) and F1 parts of the Sumpter *et al.* (2001) study (Rhodes *et al*., 2007). In addition to the cell types, endpoints covered included survival, growth, reproduction, gonadosomatic index and vitellogenin levels. The study involved flow-through exposures to nominal concentrations of 1.0, 16, 64, 160 and 640 µg/l, together with controls. Concentrations were measured regularly during the exposures, and the mean measured levels were 1.19, 13.4, 52.8, 130 and 567 µg/l. These were 81-89% of nominal with the exception of the lowest concentration which was higher than nominal; all exposure levels were reasonably consistent throughout the test. Concentrations in this summary refer to the nominal. The concentration of bisphenol-A in the controls was less than 0.293 µg/l (the analytical limit of quantitation). Other environmental properties were also monitored routinely – dissolved oxygen, temperature, pH, conductivity, alkalinity and hardness – with no notable deviations from the required values.

Testes were fixed, embedded, sectioned and stained according to the methods developed in the earlier phases of the work. Four digital images of each testis (left and right) were obtained at 40X magnification (hence eight images per fish). A grid with 400 intersection points was superimposed on the images, and the intersection points were manually identified as a cell type. The cell types included were spermatozoa, spermatid, spermatocyte, spermatogonium, vacuolated cell (VC), apoptic body cell (ABC), interstitial (Leydig) cell, Sertoli cell, interstitial tissue or unknown cell (ITUC), or empty space. A total of 3,200 points were counted per male fish. The relative frequency of each sperm cell type was expressed as the number of cells of a given type as a proportion of the number of cells that were equally or less mature – the sequence runs from more mature to less mature above, from spermatozoa to spermatogonium. The median frequencies of the other cell types (VC, ABC, Leydig, Sertoli, ITUC) were also determined.

Ovaries were fixed, embedded, sectioned and stained according to the methods developed in the earlier phases of the work. Two digital images were obtained from each of the left and

right ovary sections at 4X magnification (hence four images per fish). All ovarian follicles in the images were identified and tagged as one of six types from least to most mature – perinuclear, cortical alveolar, early vitellogenic, late vitellogenic, mature/spawning and atretic. An average of 408.5 follicles from both left and right ovaries combined were counted from the four images per fish. The relative frequency of each oocyte type was expressed as the number of a given type as a proportion of those equally or less mature.

The gonadal tissue slides were examined microscopically for morphological abnormalities and potential exposure-related changes. These were graded on a severity scale from 1 (minimal) to 5 (severe/high). Gross observations were also made during necropsy and sample preparations, and were related to microscopic observations where possible.

Statistical assessments were carried out on 37 variables for males and 27 for females, using a variety of statistical techniques (for most variables several techniques were employed). Variable-wise comparisons with the control values were carried out for each variable at a significance level of 5%. At this level of significance, on average one in twenty comparisons will show a difference by chance, i.e. a false positive will be detected. To address this, simultaneous significance tests were also carried out, where the level of significance was distributed across the number of endpoints addressed. This was done by initially grouping the variables into five types – size variables (weight, length etc), histopathological tissue lesions, gonad cell type frequencies, reproduction variables (e.g. fecundity, egg production), and others (survival, vitellogenin etc). The significance level was split equally between the five groups, and then further divided between the variables within each group. Splitting the significance level between groups of similar endpoints, such as size or histopathology endpoints, maintains an equal importance or power to detect an effect for each group so that a group with more variables (e.g. histopathology) does not dilute the significance of anther group of endpoints. An effect was considered to be statistically significant if it was significant in both the variable-wise and the simultaneous tests. If an effect was only significant in a variable-wise comparison this was considered an indication of an effect, but not sufficient to conclude an effect at the 5% significance level[20].

For studies with similar methodology and replication, one possible consequence of splitting the significance across the variables in this way is a reduction in the power of the tests to identify a real effect, i.e. an increased possibility of a false negative. A comparison of the statistical power available in the Phase 3 study with the power available in the Sumpter *et al.* study clearly demonstrates a much higher power (lower minimum significant difference which can be detected) in the Phase 3 study. This is due to the improved methodology used for gonadal cell determinations as well as a result of the high replication employed. Thus in the Sumpter *et al*. study, up to eight fish were available for assessment per treatment, compared to up to 32 fish of the same sex in the Phase 3 study. The minimum significant difference of the one-sided Dunnett test in logit units ranged from 1 to 1.2 for the male gonadal cell types in the Sumpter *et al*. study, compared with 0.5 to 0.8 for the same endpoints in the Phase 3 study (calculated on the assumption that the fish were independent of each other). Consequently, even with the splitting of the significance level the Phase 3 study had a much higher likelihood of finding a treatment related effect than the original Sumpter *et al.* study.

[20] Variable-wise significant effects which were not simultaneously significant in any statistical test were observed for male wet-weight at 640 µg/l, for the effects on the number of spawns and the time of first spawning at 640 µg/l, the VTG level in males at 16 µg/l and in females at 16 and 1 µg/l, for the lesion testicular cysts in testis at 640 µg/l, the lesion cellular infiltrates, mononuclear cells in ovaries at 640, 160 and 64 µg/l, and the frequency of VC in the group of VC and ABC in testis at 640 µg/l.

The survival of male fish was significantly reduced at 640 µg/l; no effect was seen on survival of females at any concentration. No statistically significant effects on growth were seen in either males or females, or on gonad weight or gonadosomatic index. Among the breeding pairs of fish there were no significant differences from controls at any concentration in the mean numbers of eggs, the mean number of spawns and the mean number of eggs per spawn. There were no statistically significant effects on hatching, with no significant trend with concentration and no significant differences between treatments.

The levels of vitellogenin were significantly elevated above those in the control fish in both males and females at concentrations of 64 µg/l and above.

The incidence and severity of the histopathological lesions observed increased in male fish at 160 and 640 µg/l, and in female fish at 640 µg/l. The main observation was proteinaceous fluid in the testes and ovaries. These observations may be related to the induction of vitellogenin. In male fish the bisphenol-A concentration accounts for 65% of the variance in the results. In the majority of male fish exposed to BPA at 640 µg/l, and to a lesser extent at 160 µg/l, macroscopic enlargement of the kidneys was associated with a variety of degenerative changes that are considered to be consistent with chronic protein (vitellogenin) overload. Other histopathological observations were not considered to be related to bisphenol-A exposure (no dose responses, occurrence in the controls, historical incidence).

For the sperm cells, the relative proportions of cell types to those of an equal or lesser stage of development were calculated as indicated above. A comparison of these relative frequencies between the controls and treatments is not the best way to express the size of a treatment effect. Small changes in the relative frequency are more important when the relative frequency is very small or very large – so in the margins - compared to when the relative frequency is intermediate. To address this, the relative frequency values for each gonad were converted to logit values, the logit values for the left and right gonads were averaged, and the comparisons between controls and treatments carried out on these values. From these comparisons, there was a statistically significant shift to less mature cell types at 160 and 640 µg/l in male fish (for spermatocytes as a proportion of equally and less developed cells at both concentrations, for spermatids as a proportion of equally and less developed cells at 640 µg/l). There was also a decrease in the proportion of Leydig cells relative to the ITUC at the same concentrations. In female fish, a shift to less mature cell types in the ovaries was observed at 640 µg/l.

From this study, the lowest effect concentration for a "conventional" endpoint was 640 µg/l for survival in male fish, giving a NOEC of 160 µg/l. The gonadal cell distribution was significantly affected at concentrations of 160 and 640 µg/l in male fish, hence a NOEC of 64 µg/l, and vitellogenin induction was significant at 64 µg/l, giving a NOEC of 16 µg/l.

The results from the two long-term studies on fathead minnow are compared in Table 3.22.

The Phase 3 study is comparable to that on the F0 generation in the Sumpter *et al.* study in terms of the concentration range used and the duration of exposure. The concentrations used in the comparison are nominal values as in most cases the same nominal levels were used. The overall effect levels for survival are the same for both studies, though for different generations. Growth was not significantly affected in the Phase 3 study, whereas effects were seen at 160 µg/l in the earlier study. Both studies reported a NOEC of 16 µg/l (nominal) for the production of vitellogenin[21]. The results for egg production are similar for the F0

[21] The levels of vitellogenin in the control fish in the Phase 2 and 3 studies were notably higher than those in the Sumpter *et al.* study. Work by the OECD and others has shown that there can be considerable variation between

generation. There is a difference in the results for hatchability. No effects were seen in the new study at 640 µg/l, whereas effects were seen at 640 µg/l in the F1 eggs and at 160 µg/l in the F2 eggs in the earlier study. The F2 eggs in the Sumpter *et al.* study come from fish which had been exposed to bisphenol-A throughout their lifetime up to egg laying, and so had a longer exposure than those in either of the two F0 cases. Although the replication was not as great in the Sumpter *et al.* study, and hence the possibility of a false positive finding is greater, the original study is considered to be suitable for use in the assessment for this endpoint, and so the NOEC of 16 µg/l for hatchability will be taken.

VTG levels in control fish in different studies, even when similar assays are used. As a result, comparisons within studies are considered relevant but comparisons between studies are not.

Table 3.22 Comparison of results from Phase 3 study with original Sumpter *et al.* study

	NOEC values (µg/l)			LOEC values (µg/l)		
Endpoint	**Phase 3 (F0)**	**Sumpter F0**	**Sumpter F1**	**Phase 3 (F0)**	**Sumpter F0**	**Sumpter F1**
Survival	160 (m)	NA	160, 640[d]	640 (m)	NA	640, >640[d]
Growth (length, weight)	>640	160 (m)	160 (m)	>640	640 (m)	640 (m)
Gonad weight	>640	160	NA	>640	640	NA
GSI	>640	160 (m)	NA	>640	640 (m)	NA
Egg production	>640 (f)	640 (f)	160 (f)	>640 (f)	1280 (f)	640 (f)
Hatchability	>640[a]	160[a]	16[b]	>640[a]	640[a]	160[b]
Vitellogenin	16	16 (m)	16	64	160 (m)	160
Histopathological lesions	64 (m)	NA	NA	160 (m)	NA	NA
Gonad cell distribution	64 (m)	c	c	160 (m)	c	c

Notes: m, f – male, female, if neither included then value applies to both
>: no effect at highest concentration tested
<: effect at lowest concentration tested
NA: not assessed
a: relates to F1 eggs
b: relates to F2 eggs
c: figures for this endpoint not considered reliable
d: results from two separate early life stage studies at 60 days. No results for F0 survival reported. F2 ELS studies had LOEC >640 µg/l.

Brian *et al*. (2005) exposed *Pi. promelas* to bisphenol-A in a study designed to assess the effects of mixtures of endocrine disrupters on plasma vitellogenin induction. The individual dose-response curves for vitellogenin induction were fully characterised for bisphenol-A, estradiol, ethinylestradiol, nonylphenol and octylphenol by exposing adult fish under flow-through conditions for two weeks, with chemical analysis of test concentrations. There were problems with the analysis of test concentrations during this phase of the study, resolved later for the mixture stage, so nominal concentrations were used to report the single substance studies. The EC_{50} for vitellogenin induction by bisphenol-A was 158 µg/l (95% confidence interval 119-205 µg/l).

Prediction of the overall effect on vitellogenin induction of a mixture of the five chemicals, based on concentration addition, was supported by the empirical results. This shows that the co-occurrence of several estrogenic chemicals in the environment may lead to greater biological effects than individual substance risk assessments might suggest.

The NOEC of 16 µg/l for F2 generation egg hatchability in a full life cycle study with *Pi. promelas* is considered suitable for use in the PNEC derivation and SSD.

9) Guppy *Poecilia reticulata*

Haubruge et al. (2000) exposed adult males to bisphenol-A at 274 and 549 µg/l. The exposure solutions were renewed every 48 hours. After 21 days a significant decline on total sperm count was noted, by 40-75%. It was considered that the short-term decline in sperm count was unlikely to be due to endocrine mediated alteration of the germ line, and no change was found in testis size or sperm length. The authors speculate that the effect may be due to interference with the function of Sertoli cells, which facilitate the transport of maturing sperm. These cells are directly sensitive to the action of xenobiotics.

Kinnberg and Toft (2003) exposed groups of 30 sexually mature males for 30 days to nominal concentrations of 5, 50, 500 and 5,000 µg/l bisphenol-A in a flow-through system. Concentrations were analysed and were all higher than nominals, with a maximum difference of 21%, except for one sample which was 129% higher. During the first 21 days of exposure 77% of the fish in the highest concentration died and this treatment was terminated. No fish died in any of the other treatments. The gonad histology of fish in the highest treatment showed pronounced effects, with testes filled with spermatozeugmata, some of which had ruptured resulting in free spermatozoa, and virtually no spermatogenic cysts. No effects were reported at the other treatment levels.

The 30-d survival NOEC of 500 µg/l for *Po. reticulata* is considered suitable for use in the PNEC derivation and SSD.

10) Swordtail *Xiphophorus helleri*

Kwak et al. (2001) conducted short-term tests (72 hours) to determine the effect of bisphenol-A on vitellogenesis and damage to testes, and long-term tests (60 days) to examine the effect on sword (tail) length (a secondary sexual characteristic in males). Semi-static exposure conditions were used, but no concentration monitoring was undertaken. Vitellogenin expression was noted in a dose dependant manner with no induction at 0.4 mg/l bisphenol-A, but induction was observed at 2 and 10 mg/l. Binding studies to detect cell damage (apoptosis or necrosis) showed a reduction in the proportion of healthy cells at all three exposure concentrations. However, histological examination of testis tissue taken from

fish exposed to 0.4 or 2 mg/l bisphenol-A failed to show any apoptotic cells. Apoptotic cell masses and other injured cells were observed at 10 mg/l bisphenol-A though no lesions were observed. In tests on swordtail length, a significant reduction in length was observed at 0.002 and 0.02 mg/l bisphenol-A but not at 0.0002 mg/l bisphenol-A. The authors also determined a 96-hour LC_{50} of 17.93 mg/l, based upon OECD guideline 204 and semi-static exposure.

The significance of the changes in sword length is not understood, but it is thought that the length of the sword has an influence on mating success, with female fish preferring males with longer swords. It is not clear what degree of change should be considered to be significant. The separation between exposure levels was an order of magnitude, and there was no measurement of concentration during the exposures. The study is therefore not considered suitable for use in PNEC derivation, but it is noted that the LOEC from this study is higher than the NOEC from the full life cycle test with Pi. promelas.

Summary of freshwater fish studies

Toxicity data are now available for ten species of freshwater fish. Whilst some of these are warmwater or even tropical species, most are 'standard' species for ecotoxicological assessment. Bisphenol-A is estrogenic to fish as shown by the concentration-related increase in the plasma vitellogenin concentration observed in the fathead minnow (Sumpter *et al*., 2001), rainbow trout (Lindholst *et al*., 2000), male carp (Smeets *et al*., 1999), and other studies reported above. Vitellogenin synthesis in fish is widely considered to be a reliable and sensitive indicator of exposure to estrogenic chemicals (Sumpter and Jobling, 1995). Sumpter *et al*. (2001) report a LOEC of 160 µg/l and a NOEC of 16 µg/l for vitellogenin production. The conclusion (i) test programme also found a NOEC of 16 µg/l for the same endpoint. These values fit with the LOECs of 23 µg/l and 40 µg/l for vitellogenin production reported by Pawlowski *et al*. (2000) and Lindholst *et al*. (2000). While both of these studies did not test concentrations low enough to derive NOEC values they did measure the relative potency of bisphenol-A to 17β-estradiol. All of the studies showed a similar relative potency of bisphenol-A to that of 17β-estradiol of around 10^{-4}. This and the similar concentration for LOEC values between the studies suggests that the NOEC values from these other studies should be in a similar concentration range to the NOEC of 16 µg/l. Based upon the available data a NOEC of 16 µg/l can be assumed for vitellogenin production in fish.

Bisphenol-A can also bind to the estrogen receptor of fish (Kloas *et al*., 2000) though with a lower affinity than estradiol has for the receptor.

Although vitellogenin is a biomarker for exposure to estrogenic substances, the ecological significance of its presence is not yet known - the relationship between biomarkers for endocrine disruption and ecological effects is currently being investigated by a number of workers. The most sensitive end point for population-relevant effects from the existing fish studies is the NOEC for egg hatchability for the F2 generation of 16 µg/l reported by Sumpter *et al*. (2001). This is the same as the NOEC for vitellogenin production from the same study.

Other parameters can be sensitive to both estrogens and xenoestrogens. These include inhibition of testis growth by natural and synthetic estrogens and xenoestrogens (Panter *et al*., 1998; Jobling *et al*., 1996). Estrogens are also known to inhibit spermatogenesis in male fish (Billard *et al*., 1981) and exposure of fish to estradiol and nonylphenol has been shown to affect testicular structure (Miles-Richardson *et al*., 1999; Flammarion *et al*., 2000; Jobling *et al*., 1996). High concentrations of both estrogens and xenoestrogens can induce the development of ovotestes in male fish or cause complete feminisation (Hartley *et al*., 1998; Gary and Metcalfe, 1997).

Bowmer and Gimeno (2001) observed a NOEC for oviduct formation in male carp of 16 µg/l. It is noted that this is the same as the NOEC for egg hatchability observed by Sumpter *et al*. (2001).

The study on swordtail fish (Kwak *et al*., 2001) largely showed responses at similar concentrations to other studies and support these data. As noted above, this study is not considered suitable for use in defining the PNEC, but it is noted that the LOEC from this study is higher than the NOEC from the full life cycle test with fathead minnow *Pi. promelas*.

To summarise, the results from many tests indicate that bisphenol-A acts as a weak estrogen in fish, though it is a lot less active than either estradiol or ethinylestradiol. The NOEC for egg hatchability in fathead minnows (16 µg/l) will be taken forward for further discussion in the PNEC derivation as the most sensitive measure of fish toxicity. Long-term results for a further five fish species may also be considered for the derivation of an SSD:

- *Cyprinus carpio* 49-d growth NOEC of 100 µg/l,
- *Danio rerio* NOEC of 750 µg/l for multiple end points (full life cycle study),
- *Oncorhynchus mykiss* 28-d juvenile growth rate NOEC of 3,640 µg/l,
- *Oryzias latipes* NOEC of 247 µg/l for multiple end points (full life cycle study), and
- *Poecilia reticulata* 30-d survival NOEC of 500 µg/l.

Saltwater species

1) Sheepshead minnow *Cyprinodon variegatus*

   Emmitte (1978) reports a 96-hour LC_{50} of 7.5 mg/l (measured concentration) in a flow-through exposure. The test method used appears to be acceptable, although no information is given on temperature, pH or dissolved oxygen during the test. Whilst apparently reliable, this test only considers short-term lethality.

2) Atlantic silverside *Menidia menidia*

   Springborn Bionomics (1985a) and Alexander *et al*. (1988) report a 96-hour LC_{50} of 9.4 mg/l measured bisphenol-A in a flow-through test. Whilst valid, this study only considers short-term lethality.

3) Japanese common goby *Acanthogobius flavimanus*

   Mochida *et al*. (2004) exposed *A. flavimanus* to 0.2, 1, 5 or 25 µg/l bisphenol-A for three weeks in a flow-through system. Chemical analysis at least once per week showed that mean concentrations were 0.28, 0.79, 3.02 and 19.1 µg/l. Exposure had no effect on histology, serum vitellogenin or the expression of ubiquitin C-terminal hydrolase mRNA in either the testis or brain. This study is not considered to be suitable for PNEC derivation because the measured parameters cannot be directly related to demographically important end points.

4) Killifish *Fundulus heteroclitus*

   Pait and Nelson (2003) investigated the production of vitellogenin in males following injection with bisphenol-A. The injection levels used were 0, 10, 50, 100 and 150 mg/kg body weight fish. They compared the results obtained for fish from "clean" stocks with fish taken from contaminated areas (general contamination, not specifically with bisphenol-A). In

the clean fish there were significant increases in vitellogenin levels at 50, 100 and 150 mg/kg doses. In fish from contaminated areas the production of vitellogenin was reduced compared to that in the clean fish, although only significantly so at the middle two doses. The administration route makes this study unsuitable for use in the risk assessment, and the measured parameters cannot be related directly to demographically important end points either.

1) Turbot *Psetta maxima*

Labadie and Budzinski (2006) exposed juvenile turbot (Psetta maxima) to bisphenol-A for three weeks under flow through conditions in natural seawater. The one exposure concentration was confirmed by GC-MS as 59±11 µg/l. The levels of sex steroids in the fish were measured at the end of the exposure. Bisphenol-A had no effect on androgen levels. It increased levels of E1 (estrone), which the authors suggested was due to up-regulation of aromatase activity.

2) Korean rockfish *Sebastes schlegeli*

Lee et al. (2003) exposed fry of the Korean rockfish (Sebastes schlegeli) to bisphenol-A in their diet. Food was prepared by adding a solution of bisphenol-A in ethanol to powdered fish diet at the appropriate level, drying at room temperature and storing in a refrigerator until used. Exposure levels were 0.05, 0.5, 5, 50 and 100 µg/g. Fish were fed 2.52 g of food per day. Three replicates for each exposure were used, with 70 fry in each, with exposures over 29 days. The fry were exposed from 51 days old, a time at which they have undifferentiated gonads. The study found no difference in the male to female ratio between the controls and any of the exposure levels. No effects on fry length were noted. The exposure route means that the result is not suitable for use in the risk assessment.

In summary, the limited data available for saltwater species does not suggest any significant difference in sensitivity compared to freshwater species during short-term exposures.

3.2.1.4.2 Amphibians

Experiments have been conducted with five species.

1) European common frog *Rana temporaria*

Koponen and Kukkonen (2002) investigated the effect of bisphenol-A alone and together with artificial UVB radiation. Eggs and larvae were exposed for 20 days in Pyrex dishes, 30 embryos to a dish. The exposures were ended after 20 days because by this time almost all of the UVB-exposed larvae had died. Control larvae were at Gosner stage 25-27 at this time. In the exposures without UVB, bisphenol-A had no effects on survival at concentrations up to 100 µg/l, with significant reduction in survival at 1,000 µg/l. The UVB exposures showed mortality with or without bisphenol-A. The authors concluded that the combined effect at the highest bisphenol-A concentration was greater than that from UVB alone. This study is not considered to be suitable for PNEC derivation because there was no chemical analysis of exposure concentrations.

Rouhani Rankouhi *et al*. (2005) found that bisphenol-A did not induce estrogen-receptor mediated vitellogenesis in *R. temporaria* primary hepatocytes at concentrations up to 100 µM.

2) African clawed frog *Xenopus laevis*

Several studies have been performed with this species, and these are summarised below.

a) Kloas *et al*. (1999) reported the development of a model for the investigation of endocrine-disrupting chemicals using this species. As part of this work tadpoles at 2-3 days post-hatch were exposed to nominal concentrations of bisphenol-A. Solutions were renewed three times per week, and exposure continued until metamorphosis occurred in approximately 90% of all animals - this took around 12 weeks. The two exposure concentrations used were 10^{-7} M (23 µg/l) and 10^{-8} M (2.3 µg/l). After exposure the animals were examined for differentiation into males and females. The higher exposure concentration produced a statistically significant increase in the number of female phenotypes in relation to the controls. The ratio of the sexes in the control exposures was 60:40 male:female and in the 23 µg/l exposure group was 36:64 male:female. A decreased male:female ratio was also observed in the 2.3 µg/l test group though the result was not significant comparable to the controls.

   It should be noted that this was only a method development study, which was not optimally designed to establish a NOEC (e.g., only two exposure concentrations were used, separated by an order of magnitude). The lack of information on test conditions (e.g., temperature, water quality), limited test vessel replication and lack of analytical confirmation of test concentrations means that this study cannot be used directly in the PNEC derivation or SSD.

b) Pickford *et al*. (2000; published in Pickford *et al*., 2003) reported the results of a study investigating the effects of bisphenol-A on larval growth, development and sexual differentiation on *X. laevis*. This study was conducted in an attempt to repeat the original findings by Kloas *et al*. (1999) and establish a dose-response relationship. The test was initiated with 4-day-old larvae. Hatching of larvae occurred principally on day 2 post-fertilisation; exposure to the test substance therefore commenced approximately 2 days post-hatching. A dynamic flow-through test system was used with four replicate test vessels for each test concentration, dilution water and positive control. In the experiments 17β-estradiol was used as a positive control. Larvae were exposed to 1, 2.3, 10, 23, 100 and 500 µg/l nominal concentrations of bisphenol-A. The larvae were observed daily for mortality, behaviour and appearance. Test conditions were monitored throughout the study. Growth and development assessments were performed on all larvae from one replicate per treatment group on exposure days 32 and 62. Larvae were sacrificed upon reaching the froglet stage of development for analysis. The test was terminated at day 90, which corresponds to 94 days after fertilisation.

   The NOEC for larval survival was calculated as 500 µg/l based upon pooled data results. At 32 days and 62 days post fertilisation there were no significant differences in growth or development between the test concentrations, the positive control or the dilution water control. The sex ratios were assessed pre- and post- fixation to allow comparison with the method used by Kloas *et al*. (1999), with statistical analysis being undertaken on the post-fixation results only. No significant difference from the expected 50:50 sex ratio were observed in any of the test concentrations or the dilution water control, while a significant feminisation was observed in the positive control group. The exposure of larvae to bisphenol-A did not result in an increase in gross gonadal abnormalities in stage 66 froglets. There was no significant difference in time to metamorphosis in any of the test concentrations of bisphenol-A compared to the dilution water control. There was no significant difference in total lengths in any of the test concentrations compared to the dilution water control. There were no significant differences in weight between any of the test concentrations of bisphenol-A, and the dilution water control.

This 90-d study was conducted under full GLP and was thoroughly reported, so is considered fully valid.

c) Kloas and co-workers (Levy *et al*. 2004) carried out a further study on *X. laevis* as a follow-up to their original 1999 study. The bisphenol-A used in the experiments was 99% pure. Tadpoles were raised in tanks to development stage 42/43, in deionised distilled water with 2.5 g/l added sea salt. They were then randomly assigned to groups of forty tadpoles. Two experiments were then conducted with different exposure regimes.

Experiment 1: This consisted of exposures to 10^{-7} and 10^{-8} M bisphenol-A (23 µg/l and 2.3 µg/l respectively) in duplicate. The test medium was changed three times per week, on Monday, Wednesday and Friday, and food and test substance were added at the same time to give a semi-static exposure regime. Exposures at the same molar concentrations were also conducted with 17β-estradiol (E2). After metamorphosis was complete, the froglets were sacrificed, and their gonads fixed *in situ*. Sex determination was based on gross morphology. The same procedure was applied to tadpoles that had not completed metamorphosis by 120 days after the first chemical application.

There were no signs of general toxicity at either of the applied concentrations. An average of 75% of the surviving animals reached metamorphosis in the experiment, with mortality at 20-30%. There were no significant differences between the replicates in terms of mortality, time to metamorphosis, and other end points so the groups were pooled. The control organisms had 56% male and 44% female froglets. The organisms from the 23 µg/l nominal exposure were 69% female (significantly different from controls at $P<0.005$), and at 2.3 µg/l nominal 65% were female (not statistically significant). For comparison, E2 produced 81% and 84% females respectively.

Experiment 2: Three exposure levels were used (10^{-6}, 10^{-7} and 10^{-8} M, or 230, 23 and 2.3 µg/l), in duplicate, with 10^{-7} M E2 as a positive control. A different food was used in this experiment. After metamorphosis, the froglets were weighed, and sex determined by gross morphology. The gonads and kidneys were removed completely and fixed. Tadpoles not completing metamorphosis after 120 days were treated in the same way, but without weighing. Testes from this experiment were examined to confirm the identification as males, and to look for possible cellular irregularities. Only a random sub sample of ovaries was examined, as bisphenol-A is not expected to produce effects on ovaries.

The content of bisphenol-A in the water from all vessels from experiment 2 was analysed. Samples were taken immediately after the application of the nominal concentration and at six-hour intervals over the 48 hours between solution changes. Samples were taken from the experimental solutions, solutions without tadpoles, and from test medium with only bisphenol-A. Water samples were sterilised with mercuric chloride after collection and stored at -20°C before analysis, which was by solid phase extraction and HPLC determination. The concentration of bisphenol-A in the controls and the pure test medium was below the limit of detection, which was 0.2 µg/l (9×10^{-9} M). The measured concentrations in the exposures at time zero (immediately after the solution change) were 90-105% of nominal levels. Samples from the actual exposures (containing medium, food and tadpoles) decreased to 15-30% of nominal over the 48-hour period between solution changes. Samples of the medium with food, and the medium alone, remained at 70% or higher of nominal (with one exception, the 10^{-8} M solution with food dropping to 50% nominal).

An average of 80% of the surviving animals reached metamorphosis, and the mortality in the exposures was 10-20%. As for experiment 1, the data for the duplicate exposures were pooled. The mean body weight of the organisms increased at all three bisphenol-A exposure levels, with a possible trend of higher weights at lower concentrations, but the changes were not statistically significant. Animals exposed to E2 had a similar mean weight to the controls. The middle bisphenol-A nominal concentration of 23 µg/l resulted in a statistically increased proportion of females (70%) compared to the controls (48% in this experiment). The other two bisphenol-A exposures resulted in ratios not significantly different from the controls (51% female at 2.3 µg/l; 53% female at 230 µg/l). No morphological irregularities were noted in the gonads from any of the bisphenol-A exposure groups (nor from the E2 exposure) following examination of gross morphology. None of the developed testes were affected by bisphenol-A or E2 exposure. The incidence of testis-ova was only 1.1%.

The paper gives no information on metabolism, but studies on mRNA also reported in this paper suggest that bisphenol-A may bind effectively to the endocrine receptor *in vivo*. The authors conclude that bisphenol-A is responsible for sex reversal in this species.

It is not entirely straightforward to derive a NOEC from this study. In the first experiment, the 10^{-8} M exposure affected sex ratio (although the effect was not statistically significant), while the same concentration had no effect in the second experiment. The 10^{-7} M exposure showed effects in both experiments, so could be considered to be the LOEC. If the lower concentration were taken as showing no effect, then the NOEC would be 10^{-8} M, or 2.3 µg/l. There is a large gap between the concentrations (one order of magnitude), so the geometric mean will be taken to indicate a NOEC of 7.3 µg/l (and it is noted that it could be higher still). The validity of this study is discussed at the end of this section.

d) Iwamuro *et al*. (2003) carried out studies on embryos and larvae of *X. laevis*. Adult frogs were mated and eggs were removed the next morning and kept for sixteen hours in dechlorinated water. Fertilised embryos were transferred to containers with the experimental solutions, or kept in dechlorinated water for experiments at more advanced stages of development. Embryos from different parents were well mixed when larger numbers of individuals were needed for the experiments.

Embryos (60-100, development stage 7) were exposed for 72 hours to concentrations of bisphenol-A of 10^{-5}, 2 x 10^{-5}, 2.5 x 10^{-5}, 3 x 10^{-5}, 5 x 10^{-5} and 1 x 10^{-4} M (i.e., 2.3 – 23 mg/l). They were transferred to dechlorinated water, and the number of surviving embryos counted at 48, 96 and 120 hours. The survival rates at 96 and 120 hours were expressed as a percentage of those alive after 48 hours. The number of tadpoles showing morphological abnormalities was recorded at 5-7 days after fertilisation.

Tadpoles developed to stage 52 were immersed in 2.5 x 10^{-5} M bisphenol-A for 22 days, with the exposure solution being changed every two days. Development was monitored every three days under a microscope. *In vitro* studies were also carried out on tails removed from stage 52-54 tadpoles.

The survival rate of embryos exposed to bisphenol-A was reduced at 2.5 x 10^{-5} M, with no significant effect at 2 x 10^{-5} M. The median lethal dose was calculated as 2.1 x 10^{-5} M (4.8 mg/l). No apparent abnormalities were seen in embryos immersed in 10^{-5} M bisphenol-A for seven days. At 2.5 x 10^{-5} M, most of the abnormalities were seen in dead

tadpoles. The abnormalities seen were winding vertebrae (scoliosis) or malformation of the head region (reduced distance between the eyes).

Stage 52 embryos kept in bisphenol-A solution (10^{-5} M or above) showed a retardation of metamorphosis by 1-2 stages compared to the controls, and the effect was dose-dependent. The hormone T_4 (L-thyroxine) promoted metamorphosis by four stages from the controls; in combination with bisphenol-A this was retarded by 2-4 stages, again dependent on concentration. The hormone T_3 promoted the reduction of the length of tail sections taken from stage 52-54 larvae; 10^{-5} and 10^{-4} M bisphenol-A blocked this shortening in a concentration dependent manner.

The parameters measured in this study cannot be readily related to demographic effects, so are unsuitable for use in PNEC derivation. In any case, the test concentrations were significantly higher than the NOECs derived in the other studies reported above.

e) Oka *et al*. (2003) exposed *X. laevis* embryos to 10-100 µM bisphenol-A until the early tadpole stage, when they had reached stage 6 or late stage 10. Developmental abnormalities were absent at 10 µM (2.3 mg/l) but began to occur at 20 µM (4.6 mg/l), with crooked vertebrae and development defects of the head and abdomen. At 40-100 µM embryos died rapidly during the gastrula stage. When embryos were exposed to similar molar concentrations of ethinylestradiol different abnormalities were induced, suggesting to the authors that the effects of bisphenol-A were due to non-estrogenic effects on developmental processes. The paper does not report whether the test medium was renewed and there does not appear to have been any chemical analysis of test concentrations, so it cannot be used for PNEC derivation.

f) Trudeau *et al*. (2005) exposed *X. laevis* tadpoles for 48-hours to nominal concentrations of 50 nM bisphenol-A (11.5 µg/l). They then injected an estrogen response element-thymidine-kinase-luciferase (ERE-TK-LUC) construct into the tadpoles' brains before returning them to fresh test medium for a further 48 hours before sacrifice and determination of total brain luciferase activity. Bisphenol-A increased luciferase activity 1.5-fold over controls. This study showed that exposure to bisphenol-A can modulate tadpole brain activity. However, the individual or demographic consequences remain unknown, so the study cannot be used for PNEC derivation.

g) Several papers evaluate the ability of receptors of *X. laevis* and other amphibians to bind bisphenol-A *in vitro* or *in vivo*. Kudo and Yamauchi (2005) suggest that bisphenol-A could interfere with the *X. laevis* thyroid system at environmentally realistic concentrations. However, this conclusion was based solely on results from *in vitro* transthyretin and thyroid hormone receptor β binding assays with bisphenol-A, and the relevance of this *in vivo* remains uncertain. Suzuki *et al*. (2004b) developed an *in vitro* estrogen binding assay and found a relative binding affinity of bisphenol-A of 0.957% when compared with diethylstilbestrol. This is rather a low value, but greater than the binding affinity of bisphenol-A to quail ERα. Lutz *et al.* (2005) studied the time course of free estrogen receptor (ER) in cultures of primary cultured hepatocytes of *X. laevis*. Bisphenol-A, nonylphenol and E2 all led to immediate drops in the free ER levels, followed by significant increases. Bisphenol-A produced a significant increase at 10^{-7} M (~23 µg/l).These studies provide useful information on possible mechanisms of action but are not relevant for PNEC derivation.

3) Wrinkled frog *Rana rugosa*

Goto *et al.* (2006) carried out a number of studies on the effects of bisphenol-A on the T_3-induced tadpole tail regression in *Rana rugosa* (the wrinkled frog). Tadpoles were exposed to bisphenol-A (2.3, 23 and 230 µg/l) for five days with a chlorine-free water control. After five days, T_3 ($5x10^{-8}$ M) was added to half of the vessels in the treatment and control groups for one day. This addition resulted in induced metamorphosis (measured by tail shortening) in the treated tadpoles not exposed to bisphenol-A. Tadpoles in the untreated water controls and the untreated bisphenol-A exposures did not exhibit tail shortening. Tadpoles in the treated bisphenol-A exposures showed a lesser degree of tail shortening, significantly less than the T_3-treated tadpoles at 23 and 230 µg/l.

DNA fragmentation in the tails of the tadpoles was also investigated. T_3-treated tadpoles showed marked fragmentation and the development of a ladder-like profile. This was not seen in untreated controls (tadpoles kept in water), those treated with bisphenol-A alone (230 µg/l) and those treated with both T_3 and bisphenol-A (230 µg/l).

These results were interpreted as showing possible competition between bisphenol-A and T_3 for the thyroid hormone receptor. The study is not suitable for use in the risk assessment as the metamorphosis was artificially induced.

4) Tropical clawed frog *Silurana tropicalis*

Goto *et al.* (2006) investigated the effects of bisphenol-A exposure on spontaneous metamorphosis, tail shortening and hindlimb elongation in tropical clawed frog *Silurana tropicalis* tadpoles. Stage 57 tadpoles were exposed to either 230 µg/l bisphenol-A or a 1 mM solution of methimazole (thyroid hormone synthesis inhibitor) for up to ten days. Tadpoles raised in chlorine-free tap water acted as the control population. At various times during the test, the stage of metamorphosis, tail length and hind limb length was determined. Exposure to bisphenol-A at 230 µg/l elicited a similar response as methimazole, and resulted in suppressed spontaneous metamorphosis, tadpole tail length shortening and hindlimb elongation compared with the control population. As only one concentration was used this result cannot be used in the risk assessment but will be considered in the discussion.

5) Black spotted pond frog *Rana nigromaculata*

Yang *et al.* (2005) exposed tadpoles of the black spotted pond frog *Rana nigromaculata* to bisphenol A from five days after hatching. Embryos were collected from the field. Concentrations of 2, 20 and 200 µg/l were used, and half of the exposure solution in each vessel (one vessel per concentration) was replaced every three days. Five tadpoles were sampled from each vessel on days 15, 30 45 and 60 of the exposures. These were weighted individually, then pooled for analysis. The samples were analysed for testosterone, total thryoxin (TT4) and plasma vitellogenin (as alkaline-labile phosphate). (Note: the paper indicates that the five tadpoles were pooled for analysis, but the results are presented as the average for five tadpoles, so the basis of the results is not clear.)

Malformations of tail flexure were noted in the highest exposure concentration at a level of 10%. These animals grew into young frogs. Inhibition of TT4 compared to the controls at 60 days was noted, but not significant. Testosterone levels were not different from those in the controls. Alkaline-labile phosphate levels were increased at all concentrations, but again not significantly. The lack of clarity in what the results represent and the lack of measurement of concentrations means this study is not suitable for the risk assessment.

Discussion of amphibian toxicity data

It is not clear why the three experiments by Kloas *et al*. (1999), Pickford *et al*. (2000 & 2003) and Levy *et al*. (2004) on the same species of amphibian produced such different results. The original study by Kloas *et al*. (1999) was aimed at developing a method to investigate endocrine effects rather than to determine a no-effect level, and can be disregarded given the other two studies now available.

Differences in experimental design might be an important factor. The range of concentrations in the Pickford *et al*. study covers the range of nominal concentrations used by Levy *et al*. (2004), as well as the actual range based on measurements (with the exception of the lowest concentration towards the end of the period between changes). The only clear difference between Levy *et al*. (2004) and Pickford *et al*. (2000 & 2003) is the exposure regime, with semi-static renewal used in the former instead of a flow-through design. As the bisphenol-A concentration declined significantly during the period between solution changes, it is likely that degradation was occurring. It is noted that this did not appear to occur in solutions unless tadpoles were present. However, this does not necessarily suggest that tadpole metabolism was the cause: the tadpoles have a microbial flora on their skin, and in any case, uptake would have occurred in both studies, so metabolism should also have been the same.

Pickford (2003) identified a number of other issues from a pre-publication manuscript of the Levy *et al*. (2004) study, as follows:

- There were two replicate vessels per test concentration, but pooling the data effectively reduced the number of replicates to one. The experimental error associated with the sex ratio end point therefore cannot be estimated.
- The most appropriate statistical method for analysis of sex ratio data is a test based on a binomial distribution of frequency data (e.g., Chi-squared test). In contrast, Levy *et al*. reduced the sex ratio of the duplicate tanks to one average numeric value for per cent males, and compared this by a non-parametric method to the solvent control value (as in Kloas *et al*., 1999). Since the solvent control sex ratio is slightly skewed in favour of males, this could have introduced a statistical bias.
- No concentration-response relationship is apparent. The experiments were not conducted with sufficient replication and statistical rigour to infer that a non-monotonic (i.e., inverted-U) response was involved.
- The absence of significant incidences of gonadal abnormalities at the histological level does not seem to be consistent with the presumed effect of bisphenol-A on gonadal development. Evidence of intersex condition in some larvae would be expected.
- Given the generally lower estrogen receptor binding affinity of bisphenol-A compared to estradiol, the similarity in level of mRNA induction at the same concentration is rather surprising. As there are no complementary protein expression data (e.g., immunohistochemistry), it is not clear whether this level of upregulation has any biological significance. It is not even clear whether the upregulation was in the presumptive (gonad) target tissue. Consequently it is not possible to relate the apparent mRNA upregulation mechanistically to the apparent effect on the sex ratio.

The Pickford *et al*. (2000 & 2003) study is of high quality and was specifically designed to establish a no-effect level for a range of effects. The Levy *et al*. (2004) study suggests a lower NOEC, but there were some drawbacks to the methodology used, and the apparent effect could

be an artefact. The true NOEC value is also uncertain given the wide separation of test concentrations. However, given that an effect on sex ratio is a potentially important finding, and the results cannot be rejected as invalid, the study is still considered in the PNEC derivation, though classed as 'valid with restriction'.

This means that two NOEC values are considered for this species. Since there is a large gap between them (two orders of magnitude), the geometric mean NOEC of 60.4 µg/l is used as the preferred value for PNEC derivation. It should be noted that the highest NOEC of 500 µg/l was in fact the highest concentration tested in the study, so the true NOEC could be higher. The geometric mean is therefore considered to be a conservative value. Finally, there are also still questions in relation to the use of a parameter such as the sex ratio in risk assessment: for example, what other factors influence the ratio, and what is the normal range of values for the ratio in healthy populations? The geometric mean value is below the concentration which retarded metamorphosis in *Silurana tropicalis*.

In summary, the geometric mean chronic sex ratio NOEC of 60.4 µg/l for *X. laevis* is considered in the PNEC derivation and SSD. This is derived from two significantly different NOECs, i.e., 7.3 and 500 µg/l.

3.2.1.4.3 Reptiles

Stoker *et al*. (2003) studied the effect of topical administration of bisphenol-A to eggs of the broad snouted caiman *Caiman latirostris* (a member of the *Crocodylidae* family). Sex determination in this species is temperature dependent. Bisphenol-A was applied to the eggs at doses of 1.4 and 140 ppm, and exposures were at two temperatures, i.e., 30°C (female producing temperature) and 33°C (male producing temperature).

No effects were seen on animals exposed to bisphenol-A at 30°C. At 33°C, all animals hatched as females at a bisphenol-A dose of 140 ppm. The same result was observed with 17β-estradiol at a dose of 1.4 ppm at this temperature. At the lower bisphenol-A dose of 1.4 ppm, all of the animals hatched as males, but significant disruption of the seminiferous tubule histoarchitecture was observed.

The topical method of application of bisphenol-A in this study means that these results are not suitable for derivation of a PNEC, although it does provide additional evidence of an endocrine effect.

3.2.1.4.4 Mesocosm studies

One stream mesocosm study is available in which the effect on aufwuchs biomass was examined (Licht *et al*., 2004) ("aufwuchs" is a term used to describe the organisms and detritus that coat rock and plant surfaces in aquatic systems). In this study the aufwuchs were collected from a German river and allowed to colonise unglazed ceramic tiles. These tiles were then placed in unreplicated artificial streams (3.7 m long x 0.5 m wide) located in a greenhouse. The bisphenol-A was added at nominal concentrations of 5, 50 and 500 µg/l as weekly pulses and analysis of concentrations showed that nominal concentrations were achieved initially, with rapid degradation (DT50 ~1 day) leading to almost complete disappearance of the substance between doses. The authors converted nominal concentrations into what they termed 'effective concentrations' by using the geometric mean of the initial concentration and the concentration after 7 days (or the limit of detection). Three of the aufwuchs-colonised tiles were removed from

each stream at 14-day intervals and the ash-free dry weight of the aufwuchs was determined. The overall exposure period was 103 days.

There was some evidence that exposure of aufwuchs to 500 µg/l bisphenol-A was associated with significantly lower ash-free dry weights, but there was little evidence of effects at 50 or 5 µg/l. However, the authors report an EC_{10} of 11 µg/l and EC_{50} of 46 µg/l for the area under the aufwuchs biomass/time curve, or an EC_{10} of 20 µg/l and EC_{50} of 73 µg/l when these values were normalised to the percentage of initial biomass in each treatment. These low values result from the calculation of exposure concentrations as the geometric means of initial and 7-day concentrations. If nominal concentrations are used then the EC_{10} and EC_{50} for the area under the aufwuchs biomass/time curve are 38 and 450 µg/l respectively, or 239 and 806 µg/l, respectively, if normalised to the percentage of initial biomass.

This study is not suitable for derivation of a PNEC because treatments were not replicated, and the relationship between exposure and effects on aufwuchs is difficult to interpret because of the pulsed dosing design.

The same artificial streams were used for exposures of the crustacean *Gammarus fossarum* to bisphenol-A at the same nominal concentrations (Schirling *et al*, 2006b). The effects noted in the study were an accelerated maturation of oocytes in females and a reduction in the size and number of early vitellogenic oocytes. The pulsed dosing system again makes the actual exposures difficult to estimate, and this and the lack of replication mean that the results are not suitable for the risk assessment.

3.2.1.4.5 Field studies

Vethaak *et al*. (2005) report a field monitoring study in which concentrations of several xenoestrogens, including bisphenol-A were measured at several freshwater and saltwater sites across the Netherlands. The *in vitro* reporter assay ER-CALUX was used to assess the estrogenic activity of the water samples, and samples of flounder (*Platichthys flesus*) and bream (*Abramis brama*) were also collected from selected sites for assessment of blood vitellogenin and ovotestis in males, and muscle concentrations of xenoestrogens. Two case studies were also performed as part of this study in which caged rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) were exposed on-site to sewage effluent. Multivariate statistical techniques were then used to relate measured concentrations of xenoestrogens to estrogenic activity and effects on fish. Steroid hormones (particularly ethinylestradiol), alkylphenols and alkylphenol ethoxylate concentrations were associated with estrogenic effects, but the authors state that 'Estrogenic effects of [bisphenol-A] can be more or less ruled out.'

This study is not suitable for either deriving a PNEC or for assessing the environmental realism of the PNEC based on laboratory-derived data, since a mixture of substances was present, and only fish were analysed.

3.2.1.5 PNEC derivation for fresh surface water

3.2.1.5.1 Assessment factor approach

In deriving the $PNEC_{water}$ consideration needs to be given to short-term and chronic toxicity studies for fish, amphibians, aquatic invertebrates and algae. The guidelines given in the TGD

are based upon population effects (e.g. effects on ability to reproduce and species mortality) and do not directly cover endocrine disruption as an endpoint. Ideally, the data used to derive the $PNEC_{water}$ should be obtained from studies conducted to standard guideline methods that have been adequately ring tested, and performed to appropriate quality assurance standards. In this case, data are available for many species but most do not involve standardised methods. Nevertheless, these studies can still be relevant provided that the methods and results are sufficiently described.

A number of acute toxicity studies are available for fresh and saltwater fish, invertebrates and algae. No group appears to be significantly more sensitive than the others, and $L(E)C_{50}$ values are typically in the range 1-10 mg/l. There are no new acute studies with $L(E)C_{50}$ values below 1 mg/l, so the environmental classification proposal is unaffected.

For bisphenol-A the most sensitive effect that has a clear ecological relevance is egg hatchability in the fathead minnow, with a NOEC of 16 µg/l. This is also the NOEC for vitellogenin production in males of the same species (seen as an indicator of endocrine effects) and oviduct formation in male carp, and the study is of high quality and is considered reliable.

As there are long-term NOEC values available for fish, invertebrates and algae a factor of 10 can be used on the NOEC in accordance with the usual TGD method to give a **$PNEC_{water}$ of 1.6 µg/l**.[22]

This leaves out the consideration of effects on snails, in particular *Marisa cornuarietis*. The (conservative) NOEC from the most reliable study is 25 µg/l, and so is covered by the derivation above. The other, less reliable, values identified in Section 3.2.1.3.1 relate to the stimulation of egg production during a period of non-spawning. Such an effect is clearly important for seasonally breeding species, when chemical stimulation of breeding could effectively be forcing the organism to use energy reserves at a time of sexual repose, with a consequent possible reduction in fecundity during the following normal breeding season. There may also be an impact on offspring survival since eggs may hatch during periods of low natural food availability. Finally, the female-specific mortality caused by oviduct malformations might have an impact on sex ratio. Therefore this is a potentially important adverse effect, although ideally a full life cycle study would help clarify the actual relevance of each of these considerations. If the lowest EC_{10} value of 0.0148 µg/l[23] from Oehlmann *et al.* (2006) were used with an assessment factor of 10, the $PNEC_{water}$ would be 1.48 ng/l, which is extremely low. The derivation of this value is not clear in the paper and could not be duplicated (e.g. van der Hoeven, 2005). An alternative value of 2.1 µg/l was derived from the same raw data; this would give a $PNEC_{water}$ of 0.21 µg/l with an assessment factor of 10. It should be noted that this study is not considered to be as reliable as the conclusion (i) study, but this is discussed further in the risk characterisation section.

[22] For the majority of industrial chemicals, the $PNEC_{water}$ can be derived from just five tests perhaps involving only three species – one algal test (providing both acute and chronic data), an acute and a chronic *Daphnia* test, and an acute and a chronic fish test. For bisphenol-A, chronic NOECs for fish, *Daphnia* and algae are 3,640 (from the juvenile fish growth test), >3,146 and 1,360 µg/l respectively. If these were the only data available, the $PNEC_{water}$ would be 136 µg/l based on an assessment factor of 10 (with algae being the most sensitive trophic level). This shows the importance of measuring reproductive effects for suspected endocrine disrupters.

[23] The German competent authority believes that this value is reliable enough for the PNEC derivation.

3.2.1.5.2 Statistical approach

The TGD allows the use of statistical extrapolation to derive a $PNEC_{water}$ if there are sufficient data. The suggested minimum data requirements are that there should be at least 10 NOECs from at least 8 taxonomic groups, including:

- Fish
- A second family in the phylum Chordata (fish, amphibian, etc.);
- A crustacean (e.g., cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.);
- An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.);
- A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca);
- A family in any order of insect or any phylum not already represented;
- Algae; and
- Higher plants.

Suitable data (including some from studies that are classed as valid with restriction) are listed in Table 3.20, and these show that the above requirements are met. With the exception of molluscs, the data are the most reliable long-term values for each of the species, and the reasons for their selection are discussed in Sections 3.2.1.2 to 3.2.1.4. Fish provide around one third of the chronic data points. It could be argued that only data from full life cycle tests should be used, since these should cover all relevant life stages. It should also be noted that this table only considers data for freshwater organisms. At least one marine species has a chronic value that could be suitable for inclusion (*Skeletonema costatum*).

The data in Table 3.20 were used to construct species sensitivity distributions (SSDs) using the software program ETX 2.0, available from RIVM in the Netherlands. The value selected for *Marisa* snails is open to challenge since it is not from the most reliable study, and represents a recalculated value. Therefore, calculations have also been performed with the 5-month egg production EC_{10} as derived by the original study authors (for illustrative purposes only, given the low reliability of the actual value), as well as the result of the fully valid conclusion (i) study, to illustrate how the SSD would be affected. Plots of the resulting SSDs are shown in Figure 3.2 to Figure 3.4, and the resulting HC5 values are presented in Table 3.24. The table also contains the results of the estimation of the goodness of fit to a normal distribution using three different measures.

Table 3.23 Freshwater toxicity data used to construct a species sensitivity distribution for bisphenol-A

Taxonomic group	Species	Common name	Endpoint	Result (µg/l)	Reference
Fish	*Cyprinus carpio*	Common carp	49-d growth NOEC	100 [a]	Bowmer & Gimeno (2001)
	Danio rerio	Zebrafish	Full life-cycle multiple end point NOEC	750	Segner *et al.* (2003a)
	Oncorhynchus mykiss	Rainbow trout	28-d juvenile growth NOEC	3,640	Bayer AG (1999)

Taxonomic group	Species	Common name	Endpoint	Result (µg/l)	Reference
	Pimephales promelas	Fathead minnow	Multi-generation F2 egg hatchability NOEC	16	Sumpter *et al*. (2001)
	Oryzias latipes	Japanese medaka	Multi-generation multiple end point NOEC	247 [a]	Japanese Ministry of the Environment (2006)
	Poecilia reticulata	Guppy	30-d survival NOEC	500	Kinnberg & Toft (2003)
Amphibia	*Xenopus laevis*	African clawed frog	12-week sex ratio NOEC	60.4	Geometric mean of 500 (Pickford *et al*., 2000 & 2003) and 7.3 (Levy *et al*., 2004)
Crustacea	*Daphnia magna*	Water flea	21-d reproduction NOEC	3,146	Bayer AG (1996)
	Hyalella azteca	Scud	42-d reproduction NOEC	490	Springborn Smithers (2006b)
Insects	*Chironomus riparius*	Midge	Life-cycle time to moult & growth NOEC	100	Watts *et al*. (2003)
Rotifers	*Brachionus calyciflorus*	Rotifer	48-h intrinsic rate of increase NOEC	1,800	Springborn Smithers (2006a)
Molluscs	*Marisa cornuarietis*	Ramshorn (apple) snail	5-month egg production EC_{10}	2.1	Van der Hoeven (2005), recalculated from Oehlmann *et al*. (2006)
Cnidarians	*Hydra vulgaris*	Hydra	6-week polyp structure NOEC	42	Pascoe *et al*. (2002)
Poriferans	*Heteromyenia sp.*	Sponge	9-d growth NOEC	1,600	Hill *et al*. (2002)
Algae	*Pseudo-kirchneriella subcapitata*	Alga	96-h cell count EC_{10}	1,360	Alexander *et al*. (1985b & 1988)
Macrophytes	*Lemna gibba*	Duckweed	7-d growth NOEC	7,800	Putt (2003)

a) A full study report is not currently available, but the data are considered adequate based on abstract information

Figure 3.2 Species sensitivity distribution using whole data set with mollusc value of 0.0148 µg/l

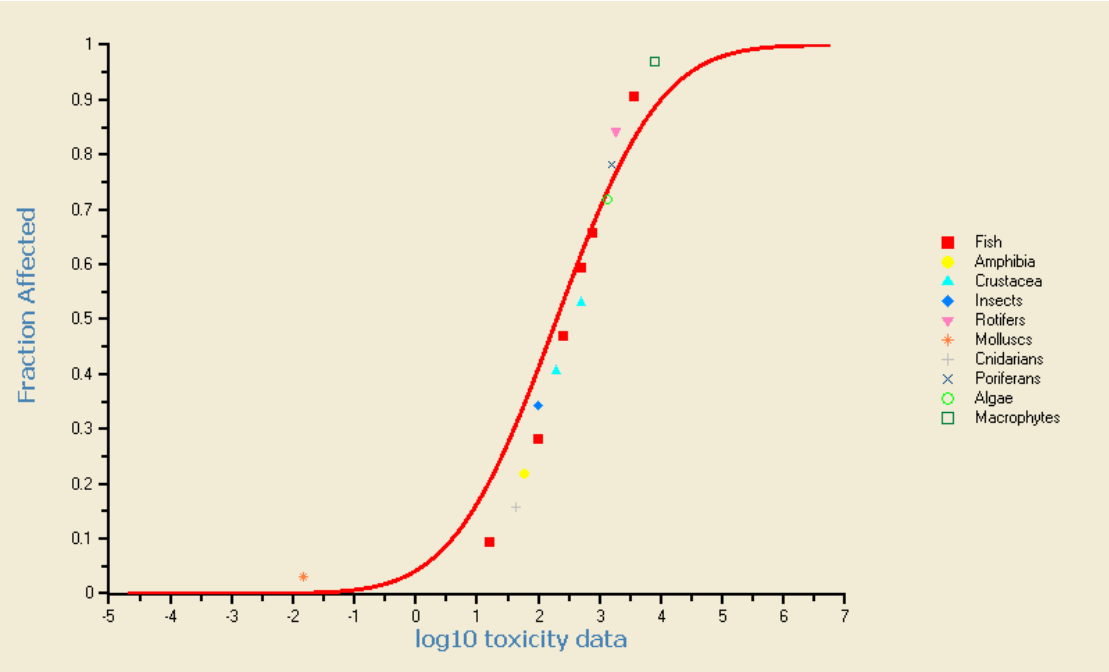


Figure 3.3 Species sensitivity distribution using whole data set with mollusc value of 2.1 µg/l

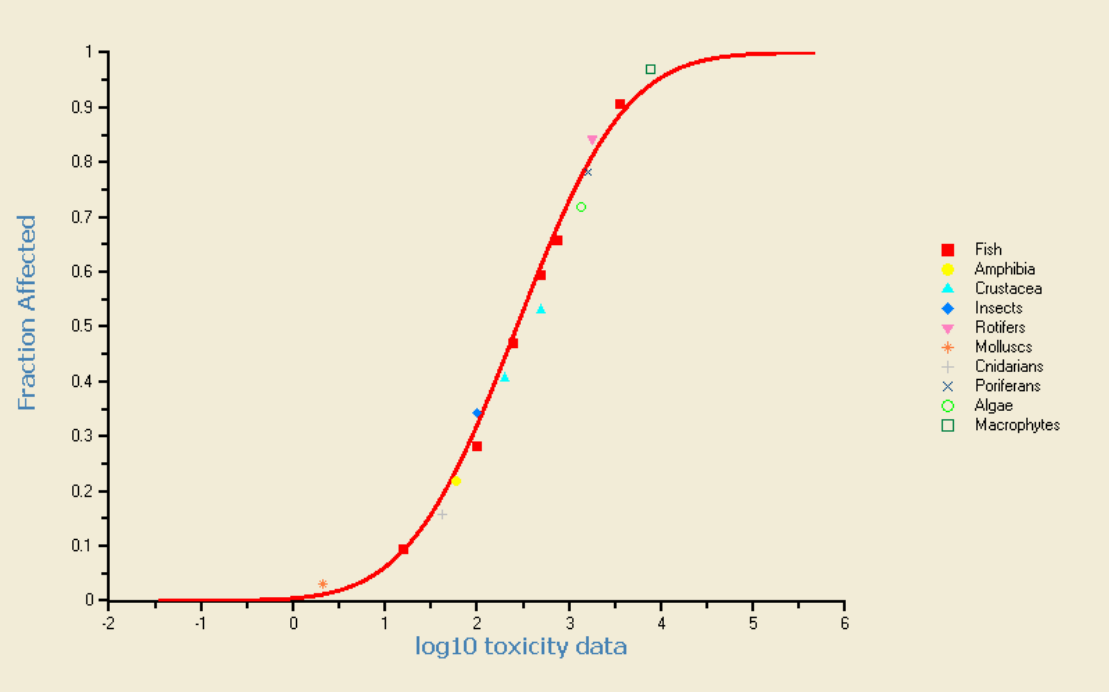
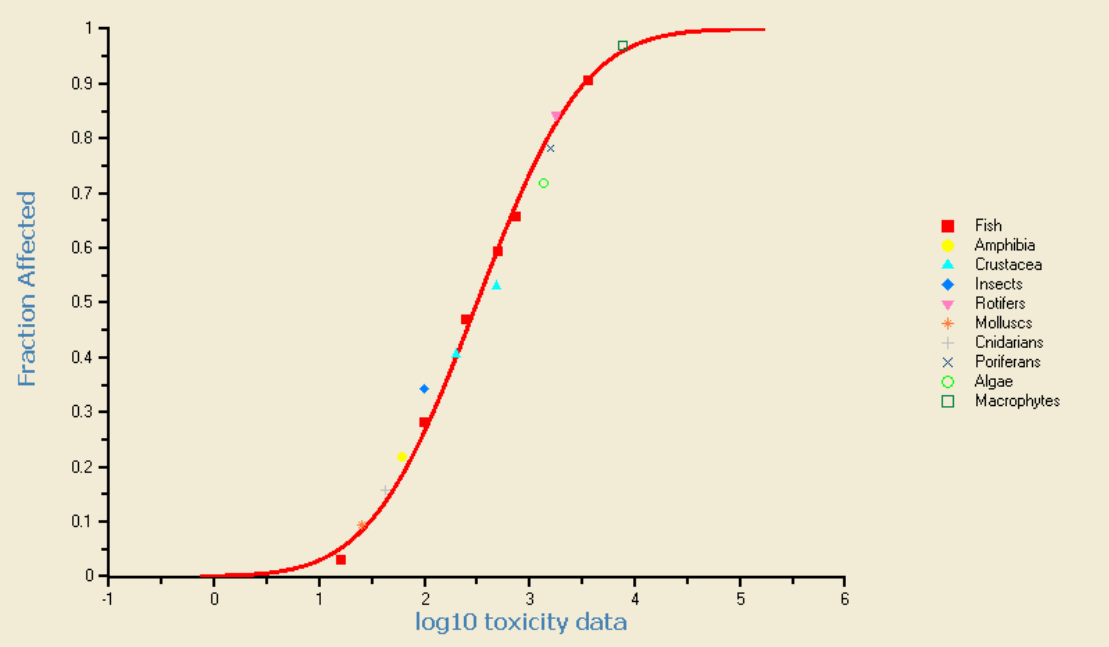


Figure 3.4 Species sensitivity distribution using whole data set with mollusc value of 25 µg/l



There are indications (not necessarily in relation to bisphenol-A) that many invertebrates have physiological systems that could potentially be affected by endocrine-disrupting substances. Fish are known to be affected by such compounds, so it is considered valid to combine the data for the invertebrates and fish in the SSD. It might be expected that algae and plants could respond differently to bisphenol-A. The results of leaving the data for these out of the data set are therefore also shown in Table 3.21 (plots are not shown).

Table 3.24 HC5 values

Data set	HC5	90% interval	Goodness of fit
Mollusc 2.1 µg/l	7.5	1.23 – 24.3	All accepted
Mollusc 2.1 µg/l Algae and macrophytes removed	6.36	0.97 – 20.7	All accepted
Mollusc 25 µg/l	14.7	3.12 40.2	All accepted
Mollusc 25 µg/l Algae and macrophytes removed	13.6	2.87 – 36.2	All accepted
The following results are included for comparative purposes			
Mollusc 0.0148 µg/l	1.19	0.09 – 6.4	A-D rejected at 0.025 K-S all accepted CvM rejected at 0.1
Mollusc 0.0148 µg/l Algae and macrophytes removed	0.79	0.046 – 4.7	A-D rejected at 0.025 K-S rejected at 0.1 CvM rejected at 0.05

A-D Anderson-Darling;
K-S Kolmogorov-Smirnov;
CvM Cramer von Mies

The choice of value for *Marisa* has a large influence on the HC5 resulting from the calculation, the difference between the highest and the lowest being over an order of magnitude. In contrast, removing the algal and macrophytes values produced a change of only 35% at most. The complete data set has therefore been used.

If the *Marisa* EC_{10} of 2.1 µg/l is used, the data pass all three tests for normality at the p=0.1 level; this value is also the lowest in the data set. Using the conclusion (i) programme NOEC of 25 µg/l, the data also pass all three tests at the p=0.01 level, but fish are more sensitive.

The lowest EC_{10} value of 0.0148 µg/l for *Marisa* is considered to be the least reliable value for a variety of reasons, as outlined in Section 3.2.1.3.1. When it is included in the data set for the SSD, the data pass the Kolmogorov-Smirnov test for normality at the p=0.1 significance level, but only pass the Anderson-Darling test at the p=0.01 level and the Cramer von Mies test at the p=0.05 level. These latter two tests focus on the fit in the tails of the distribution, and this *Marisa* result is inconsistent with a normal distribution of data. Regardless of the validity of the numerical value of this EC_{10}, the HC5 from this SSD would have to be treated with caution because the assumptions of normality are likely to be violated and the value therefore has a high level of uncertainty. It is therefore not considered further in this assessment.

The TGD proposes the use of an assessment factor of between one and five on the HC5 value to arrive at the $PNEC_{water}$. The choice of assessment factor depends on the quality and extent of the data. The data set meets the minimum requirements of 10 NOEC values for eight taxonomic groups, there are in fact 16 values for 10 groups. The main group is fish, with six values, and hence fish could be considered to have too large an influence on the result; however, the individual values for fish are spread out through the data set fairly evenly. There are no valid mesocosm or field data to use to support or confirm the result of the SSD. The data set overall is smaller than that used for some metals where a low factor of one or two has been proposed. There is also the remaining uncertainty over the appropriate value to use for molluscs. It is therefore proposed to use an assessment factor of five.

Applying a factor of five to the HC5 from the data set including the conclusion (i) programme result would give a $PNEC_{water}$ of 3.0 µg/l. Using the recalculated result of 2.1 µg/l in the data set and an assessment factor of five gives a $PNEC_{water}$ of 1.5 µg/l. This lower value is preferred for the risk characterisation given the possibility that the conclusion (i) study might have missed an effect because the snails did not exhibit a seasonal breeding pattern. It is very similar to the $PNEC_{water}$ derived using the assessment factor approach without consideration of the snail data, and is also close to the lower limit of the 90^{th} percentile confidence interval on the HC5 value.

3.2.1.6 PNEC for marine waters

Information is available on a number of tests with marine organisms. None of these tests provide results that are suitable for direct use in the risk assessment. The results for fish appear to show effects at similar levels to those in freshwater fish and no indication of increased sensitivity in the marine species. The most sensitive organisms appear to be molluscs, with effects at similar levels to those with *Marisa* in freshwater (with the same reservations about the limitations of the studies). There is no specific guidance on adapting an SSD-based PNEC from freshwater organisms to the marine environment. For the assessment factor approach, an additional factor of 10 would be used on a PNEC from freshwater data only, to take account of the wider range of species in the marine environment. The same approach will be adopted here, giving a $PNEC_{marine\ water}$ of 0.15 µg/l.

3.2.2 Sediment compartment (fresh and saltwater)

3.2.2.1 Invertebrate toxicity data

3.2.2.1.1 Molluscs

The freshwater snail *Potamopyrgus antipodarum* was exposed to bisphenol-A in artificial sediments for up to eight weeks (Duft *et al*., 2003). The sediment was composed of 95% quartz sand and 5% ground beech leaves. Bisphenol-A was added to the sediment dissolved in ethanol and the sediment left for one day for the solvent to evaporate. Water was added and the sediment allowed to equilibrate for five days with aeration. Exposure concentrations were 1, 10, 30, 100 and 300 µg/kg dry weight. Experiments were carried out at 15±1°C.

Eighty snails were added to the flasks containing sediment at the start of the exposures, and twenty were removed after 0, 2, 4 and 8 weeks. Embryos were removed from the brood pouch and the number of 'grown up' embryos (with shells) and 'new' embryos (without shells) were counted. The occurrence of egg cells in the oviduct and the maturity of the ovary were noted, as was any mortality in the treatments.

Two weeks' exposure to bisphenol-A concentrations of 30 µg/kg and above resulted in the increased production of unshelled embryos. A similar result was found for the total number of embryos, but not for the number of shelled embryos. At eight weeks, the stimulation of embryo production was significant at all concentrations tested in comparison to the controls. The total number of embryos and the number of shelled embryos varied very little in the controls; the number of unshelled embryos showed a slight but not significant decrease over the eight weeks. (Non-linear regression was used to fit the results and to derive concentrations giving 10% and 50% stimulation. For embryo production at two weeks the values were 0.22 and 24.5 µg/kg, at eight weeks they were 0.001 and 0.004 µg/kg. Note that these latter values are extrapolated by three orders of magnitude below the lowest concentration tested, and so have a very high degree of uncertainty.)

No analysis of the sediment was undertaken because of the reported short half-life of bisphenol-A in sediment. In addition, all of the exposure concentrations were below the reported detection limit for bisphenol-A in sediment of 5 mg/kg. Attempts were made to analyse the soft tissues of the snails to obtain a measure of the levels in the organisms, but insufficient tissue was recovered to allow the analysis to be performed.

The lack of confirmation of exposure concentrations means that these data cannot be used directly in the $PNEC_{sediment}$ derivation. However, given the apparent sensitivity of snail species to aqueous exposures, it is notable that effects were observed, and it should also be recalled that a NOEC of 1 µg/l (nominal) was obtained for stimulation of embryo production with this species via water phase exposure (Jobling *et al*., 2004; discussed in Section 3.2.1.3.1).

3.2.2.1.2 Crustacea

Whale *et al*. (1999) studied the acute toxicity of bisphenol-A to the saltwater benthic amphipod *Corophium volutator*. Artificial sediment was prepared following guidelines in the OECD (1984) earthworm acute toxicity test. Bisphenol-A with 98% purity was added to the sediment by a spiking procedure with and without the presence of acetone as a carrier solvent. Animals were

added to the test system and exposed to bisphenol-A for 10 days. The condition of the organisms was assessed daily as active, immobilised or dead. The resultant LC_{50} (based on mortality) and EC_{50} (based on total adverse effects) values were calculated using probit analysis. The concentration of bisphenol-A in sediment was measured using solvent extraction and liquid chromatography. The pore-water concentration of bisphenol-A was estimated from the sediment concentration using the equilibrium partitioning model approach. The 10-day LC_{50} values calculated for acetone and direct spiked tests based on bulk sediment concentrations were 46 and 60 mg/kg dry weight, respectively. The corresponding 10-day EC_{50} values were 31 and 36 mg/kg dry weight for acetone and direct spiked tests, respectively. The endpoints of the toxicity tests based upon interstitial water concentrations were also determined; the 10-day LC_{50} values were 1.4 and 1.6 mg/l for acetone and direct spiked tests, respectively and the 10-day EC_{50} values were 1.1 and 1.3 mg/l for acetone and direct spiked tests, respectively.

3.2.2.1.3 Insects

Watts *et al*. (2001b) studied the effect of bisphenol-A on development and reproduction in the freshwater midge *Chironomus riparius*. Larvae were exposed to a range of sediment concentrations and raised until the adults emerged. The time to emergence, sex ratio, number of adults, egg production and egg viability were all measured. The sediments in the experiment were spiked with stock solutions of bisphenol-A, and the concentrations of bisphenol-A in the stock solution were confirmed by analysis. The sediment was artificial, containing 15% organic matter; the resultant bisphenol-A concentrations were not measured in the sediment or the exposure water. The authors found that emergence of male and female adults were significantly delayed in the second generation of adults at bisphenol-A concentrations of 78 ng/l to 0.75 mg/l (these are stock solution concentrations and not the actual exposure concentrations in sediment). There was no observable effect on the first generation adults, and no effect on sex ratio or total number of adults produced in either generation. The authors noted that although time of emergence of adults was affected, the results in general do not suggest that the criteria examined, although validated as indicators of general sediment toxicity, could be used to detect oestrogenic effects. In this experiment it is not possible to estimate the actual level of exposure in the test system which may be substantially different from the stock solution concentrations due to adsorption and degradation of bisphenol-A. This study is not considered valid for further use in the risk assessment.

3.2.2.2 PNEC derivation for sediment

For bisphenol-A there are limited data on the toxic effects of bisphenol-A to benthic organisms. Based upon a 10-day EC_{50} for *Corophium volutator* of 36 mg/kg dry weight (lowest value for direct spiked tests) and using an assessment factor of 1,000 a $PNEC_{sediment}$ of 36 µg/kg dry weight is calculated. It should be noted that this result is from a test on a saltwater organism carried out in a saltwater sediment medium. As the data set is very limited a $PNEC_{sediment}$ derived from the PNECwater using the equilibrium partitioning method has also been calculated for comparison. The calculated $PNEC_{sediment}$ value is 24 µg/kg wet weight (63 µg/kg dry weight) using the $PNEC_{water}$ of 1.5 µg/l.

The PNECs derived by the two methods are reasonably similar. The equilibrium partitioning approach should be suitable for a substance such as bisphenol-A. The database for aquatic organisms is much more extensive than that for sediment organisms, and so more confidence can be placed in the result. In addition, the sediment study also derived acute toxicity values based

on the measured interstitial water concentrations in the test. The resulting $L(E)C_{50}$ values (1.1-1.4 mg/l) are the same as the lower end of the values for aquatic invertebrates. There are indications from one study that snails may also be more sensitive when exposed to bisphenol-A in sediment. This also supports the importance of developing a result or results for molluscs that can be used with confidence. Whether the snail data are confirmed or not, taking all the evidence together the assessment for aquatic organisms can be considered to be protective for the sediment compartment.

For the marine compartment, there are no additional specific data to that used above, so the equilibrium partition method is used on the marine aquatic PNEC of 0.15 µg/l, giving a $PNEC_{marine\ sediment}$ of 2.4 µg/kg wet weight (6.3 µg/kg dry weight).

3.2.3 Terrestrial compartment

3.2.3.1 Terrestrial toxicity data

There were no terrestrial toxicity results available at the time the original risk assessment was developed, and so the PNEC for the terrestrial compartment was derived using the equilibrium partition method. A testing programme for the terrestrial compartment was developed following the publication of the original risk assessment. The degradability of bisphenol-A leads to difficulties in maintaining a constant concentration in all media, but especially in soils where renewal of the soil is both difficult and unrealistic. It was agreed by the Technical Committee for New and Existing Substances that these tests should be performed with a single addition of bisphenol-A to the soil at the start of the exposures. This approach would mimic the only likely route of exposure for soil, which is through the application of sewage sludge containing the substance. Deposition from air is estimated to be negligible in comparison. Effect concentrations would be expressed in terms of the initially added concentration.

3.2.3.1.1 Springtails

The toxicity of bisphenol-A to the collembolan *Folsomia candida* in a test according to ISO 11267 has been reported (ECT, 2007a)[24]. The test was conducted using an artificial soil, composition based on the OECD Guideline 207 (10% sphagnum peat, 20% kaolin clay, 68-69% quartz sand, 1% calcium carbonate). A mixture of radiolabelled (^{14}C ring-labelled) and non-labelled bisphenol-A was used. Solutions of bisphenol-A in acetone were applied to portions of the quartz sand and the solvent allowed to evaporate; the treated sand was then incorporated into the rest of the artificial soil. The soil was wetted to 40-60% of its maximum water holding capacity at the start; the weight was checked once per week, and water added if the weight loss was greater than 2% of the water content. Samples of the treated soil were taken for analysis before the organisms were added. The recovery of the radiolabel from the initially treated soils (expressed as dpm in soil/dpm in dosage solution, where dpm is disintegrations per minute) ranged from 101-109%, so the actual concentrations at the start corresponded to the nominal concentrations.

[24] The rapporteur originally proposed a test on *Folsomia fimetaria* as this is a sexually reproducing species of collembolan. Information from the literature on the toxicity of nonylphenol to *F. fimetaria* and *F. candida* shows them to have similar sensitivities in terms of effects following exposure to a weakly estrogenic substance (nonylphenol). It is therefore considered that the test on *F. candida* is appropriate for the purpose of the risk assessment.

Five exposure concentrations were used, with solvent and deionised water controls, with five replicate vessels for each treatment and control. Collembolans were introduced to the treated soil after the soil was added to the test vessels; ten animals were added to each vessel. The animals were 10-12 days old (juveniles), from a synchronised culture. They were fed with ~30 mg of granulated dry yeast at the start of the exposures, and again after 14 days. The exposures lasted for 28 days.

At the end of the exposures, the soil from each vessel was mixed with water and the collembolans floated to the surface. The appearance and behaviour of the adults was observed, and the number of adults counted directly. Digital photos of the test vessels were taken, and the number of juveniles on these images was counted.

Mortality among the adults in the solvent and water controls was 12%, that in the exposures was 2-12%, so there was no effect on survival at any exposure level. (The test guidelines performance criterion for survival is less that 20% mortality in the controls.) The number of juveniles was reduced at the highest exposure level of 1,000 mg/kg dw, down to 61% of the number in the solvent control. The NOEC for this endpoint is therefore 500 mg/kg dw.

3.2.3.1.2 Earthworms

a) Eisenia andrei

As part of a project to investigate endocrine disruption in key invertebrate taxonomic groups, Johnson *et al*. (2005) carried out tests on the earthworm *Eisenia andrei* exposed to a number of substances (individually), including bisphenol-A. Both short-term (14-day) screening tests and longer-term (56-day) reproduction studies were conducted. For both types of test a stock solution of bisphenol-A was prepared in acetone, and the appropriate amount added to OECD earthworm soil. After spiking with the substance, 0.5 kg amounts of soil were added to replicate test vessels, which were left to stand overnight to allow the acetone to evaporate. Ten animals were added to each replicate and allowed to burrow; dried ground rabbit droppings (5 g) were placed on the surface for the worms to feed on. The weight of the test vessels was recorded at this stage; twice a week distilled water was added to restore the weight to the starting value to compensate for water lost by evaporation. The control and solvent controls contained twenty animals each. All worms used in the study were sexually mature with clitella. The environmental conditions were 25±2°C and a photoperiod of 16 hours light and eight hours dark. The test procedures are not described in detail in the report, but for the most part there appear to be only minor variations from the OECD guideline, and these would not be expected to affect the test. A possible exception is the feeding of the worms; the test report indicates this was done once at the start of the exposures, whereas the OECD guideline indicates weekly additions of food.

The nominal concentrations used in the short-term test were 1.0, 3.2, 10, 32, 100, 320, 1,000, 3,200 and 10,000 mg/kg, with two replicates. After 14 days, the live and dead worms were removed from the test vessels by sieving the soil. The numbers of live and dead worms were recorded and the live worms were snap frozen in liquid nitrogen and stored at -80°C for analysis[25]. Concentration-dependent mortality was observed in the study; the responses were 10% at 10 mg/kg, 20% at 32 mg/kg, 90% at 100 mg/kg, 40% at 320 mg/kg, and 100% at 1,000 mg/kg and above. Given the 10% mortality observed in the solvent control (which is within the validity criteria for the OECD guideline), a NOEC of 32 mg/kg and a LOEC of 100 mg/kg are

[25] The report comments that analysis was carried out on samples where significant effects on toxicity indices were recorded, but no analytical results are included in the report

presented in the report. A probit analysis of the data for this paper gives an LC_{50} of 61 mg/kg. No comment is given on the high mortality at the 100 mg/kg dose level, but this may just reflect the natural variation in such tests; it does mean the result will be more uncertain.

The long-term test used nominal concentrations of 1.0, 3.2, 10, 32 and 100 mg/kg, with four replicates at each concentration. Exposures were started in the same way as for the short-term studies; after 28 days the soil was sieved and the adult worms were removed, snap frozen in liquid nitrogen and stored at -80°C. The sieved soil containing cocoons was returned to the test vessels and left for a further 28 days to allow the cocoons to hatch. The number of juvenile worms in each vessel was determined at 56 days after wet sieving the soil. The report notes that soil samples were taken for analysis at the start of the test and at 28 days (when the soil was sieved) and stored at -20°C. Analyses were to be carried out on those samples from vessels where significant effects on toxicity indices or biomarkers were seen. No statistically significant effects were seen on the numbers of hatched cocoons, unhatched cocoons or live worms at any of the exposure levels. In the absence of any significant effects, no soil analyses were conducted. The number of worms in the controls was below the value of 30 set as a validity criterion in the OECD test guideline. Similar low numbers of worms were recorded for three of the other substances. For the other two substances tested the control responses met the validity criteria. The report notes that the production of cocoons varied in a seasonal manner, and that two of the experiments had to be repeated due to the low numbers of cocoons in the original tests. These repeats were the two tests that met the OECD criterion. It is not clear whether the limited addition of food in this study would have an influence on cocoon production. These observations are not considered to affect the overall conclusion of the study.

The study also investigated possible biomarkers for endocrine effects. A robust assay was developed for the gene expression of annetocin, a protein involved in the release of cocoons. No significant effects on the expression of this protein were observed in either the short- or long-term exposures with bisphenol-A. Long-term experiments under the same conditions with oestradiol, ethinylestradiol, testosterone, nonylphenol and propoxur also showed no effect on the gene expression. All but propoxur had no effect on the numbers of cocoons or live young worms; the effects caused by propoxur were not considered to be endocrine related.

The report concludes that earthworms may not be the most appropriate terrestrial species with which to assess the effects of endocrine disrupting chemicals. Earthworms are hermaphroditic, and they appear to have inherent homeostatic mechanisms to compensate for internal fluctuations in the levels of oestrogen and androgen that may result from exposure to chemicals.

Discussion

Although earthworms may not be suitable to demonstrate effects arising from endocrine disruption, they are still susceptible to “classical” toxicity, and effects on reproduction were seen with one of the test substances. No effects were seen with bisphenol-A at the highest concentration tested of 100 mg/kg in the long-term study. However, in the short-term study lethality was observed, beginning at a lower concentration than this, with significant lethality being seen at 100 mg/kg.

This apparent discrepancy may be due to degradation of bisphenol-A in the soil during the course of the exposures. Bisphenol-A is considered to be readily biodegradable in aquatic tests (the corresponding half-life in soil from the Technical Guidance Document is 30 days, although it should be noted that this is only a very approximate guide for modelling purposes). It is therefore likely that some degradation occurred over the 14-day study. However, as the soils were dosed in the same way for both the short- and longer-term studies, the extent of degradation

would be expected to be similar at the same times, and hence similar effects would be expected at the same time points. Since no concentrations were measured there is no information to indicate whether the test substance degraded at different rates in the two studies.

It should also be noted that the results obtained with nonylphenol in the same study follow a similar pattern to those with bisphenol-A, in that effects were seen at 14 days in the 100 mg/kg exposure, but not in the long-term study at the same level. The short-term effects for nonylphenol agree fairly well with other results available for that substance (Enviroment Agency, 2005). However, the long-term result for nonylphenol is not consistent with other data, which suggests that there may have been a problem with the longer test.

b) Enchytraeus crypticus

As a consequence of the unclear results with *Eisenia andrei*, a study on the effects of bisphenol-A on the enchytraeid species *Enchytraeus crypticus* has been conducted following OECD Guideline 220 (ECT, 2007b). The same artificial soil was used as in the collembolan study above, and the test soil samples were prepared in the same way. Samples were again taken for analysis at the start of the exposures. The recovery (as dpm in soil/dpm in dosage solution) ranged from 110 to 148% of the nominal levels. These were considered to be in reasonable agreement with the nominal levels, and the results are presented in terms of the nominal values.

Six concentrations of bisphenol-A were used, together with a solvent and a deionised water control. Four replicates were used for each treatment level and for the water control, with eight replicates for the solvent control. Ten adult enchytraeids were added to each test vessel. The animals were fed ~50 mg of ground oats at the start of the exposures, and once a week for the 28-day duration.

At the end of the exposures each soil sample was transferred to a shallow plastic tray and ethanol added to fix the juveniles. Water and Bengal red stain were added, the components mixed and left for 12 hours, after which the worms were stained red and lying on the surface where they were counted.

The mortality in the water control was 5% and in the solvent control 7.5% (the test guideline specifies a maximum mortality for validity of 20%). Mortality in the bisphenol-A treatments was 5-12.5%, hence there was no effect on survival. There were no significant differences between the numbers of juveniles in the controls and any of the bisphenol-A exposures (the controls met the validity criterion of >50 per vessel). Hence the NOEC for the study is ≥100 mg/kg dw (the top dose).

3.2.3.1.3 Plants

The effect of bisphenol-A on the emergence and growth of six species of plant has been determined using a protocol meeting the requirements of OECD Test Guideline 208 (Springborn Smithers, 2007). The plant species tested were three monocotyledons: corn (Zea mays); oats (Avena sativa); and wheat (Triticum aestivum); and three dicotyledons: cabbage (Brassica oleracea); soybean (Glycine max); and tomato (Lycopersicum esculentum).

The sandy loam used for the test came from Fairhaven, Massachusetts, and contained 85% sand, 12% silt and 3% clay, with an organic carbon content of 1.1% (1.9% organic matter). The soil was heat sterilised before use.

A master stock solution of bisphenol-A was prepared in acetone, and a series of dilutions was prepared from this, also in acetone. The appropriate volume of each dilution was applied to silica sand and the acetone allowed to evaporate. Once dried, the sand was mixed with soil (0.5 kg sand to 11.5 kg soil for all except wheat, where 6 kg of soil were used). Different exposure levels were used for each plant species based on range finding tests. Solvent controls were prepared in the same way without addition of bisphenol-A to the acetone; controls without the addition of acetone were also used.

Approximately 1.2 kg of treated soil was placed in each pot. Seeds were planted in each pot at the start of the test. The number of seeds per pot was based on the seed and expected plant size (see table headings). Pots were placed on saucers, and well water or nutrient solution was added to the saucer at regular intervals. The tests were conducted in a greenhouse, with a 16-hour light period (sunlight augmented by sodium vapour lights as necessary). The temperature was monitored and ranged from 24 to 42°C; the relative humidity range was 18-100%. Although these exceed the recommended ranges in OECD 208, the same conditions have been used by the laboratory in the past with no negative impact on plants.

The control pots were observed daily until 50% emergence or greater was seen. At 7, 14 and 21 days after this, the number of emerged plants, mortalities and morphological abnormalities (e.g. chlorosis of leaves) were recorded. After 21 days from 50% control emergence, all exposures were terminated, and the above ground portions of living plants were removed and dried to determine dry shoot weights.

Each dosing solution was analysed prior to application to sand. An earlier range finding study using radiolabelled bisphenol-A added to soil in the same way as above demonstrated that the concentration after application ranged from 78-152% of nominal. This range was considered to be within the expected range for mixing a solid substance (sand) into soil, and therefore the nominal concentrations are taken as representative of the initial concentration in soil.

The results for each species are presented in Table 3.25 to Table 3.30. The derived effect levels are summarised in Table 3.32 and Table 3.31.

Table 3.25 Cabbage (10 replicates, 4 seeds per replicate)

Concentration (mg/kg dw)	21 day shoot dry weight (g)	SD	Percent emergence at 21 days
Control	0.2964	0.0862	59
20	0.2165[a]	0.0915	70
50	0.3058	0.0457	88
130	0.1622[b]	0.0570	50
320	0.0251[c]	NA[c]	2.5[b]
800	NA[c]	NA[c]	0[b]

a) Significantly reduced compared to control, but not considered treatment related as next concentration is not reduced

b) Significantly reduced compared to control

c) Statistics not calculated due to lack of emerged plants.

Table 3.26 Corn (10 replicates, 2 seeds per replicate)

Concentration (mg/kg dw)	21 day shoot dry weight (g)	SD	Percent emergence at 21 days
Control	2.2619	1.0197	80
3.8	1.6899	0.4695	90
10	1.7635	0.1831	95
20	1.9959	0.7395	80
50	1.8348	0.4213	90
130	1.1461	1.0883	65
320	0.7472[a]	0.1563	80

a) Significantly reduced compared to control

Table 3.27 Oat (10 replicates, 8 seeds per replicate)

Concentration (mg/kg dw)	21 day shoot dry weight (g)	SD	Percent emergence at 21 days
Control	0.2889	0.0513	76
9.4	0.2802	0.0247	86
19	0.3133	0.0269	98
47	0.2736	0.0532	68
120	0.0894[a]	0.0775	18[b]
300	0.0530[a]	0.0230	84
800	0.0100[a]	0.0026	64

a) Significantly reduced compared to control

b) Significantly reduced compared to control, but not considered treatment related as next two concentrations are not reduced.

Table 3.28 Soybean (10 replicates, 2 seeds per replicate)

Concentration (mg/kg dw)	21 day shoot dry weight (g)	SD	Percent emergence at 21 days
Control	2.1009	0.6438	100
20	1.4529	0.7335	100
50	2.0287	0.4238	100
130	1.7787	0.7694	90
320	1.4084	0.3400	90
800	0.1869[a]	0.1250	75

a) Significantly reduced compared to control

Table 3.29 Tomato (10 replicates, 2 seeds per replicate)

Concentration (mg/kg dw)	21 day shoot dry weight (g)	SD	Percent emergence at 21 days
Control	1.1308	0.2914	85
4.0	1.0774	0.3001	85
10	0.9528	0.0994	85
20	0.8137	0.1208	95
50	0.6790[a]	0.1390	90
130	0.1568[a]	0.0753	90
320	0.0206[a]	0.0288	25[a]

a) Significantly reduced compared to control

Table 3.30 Wheat (5 replicates, 8 seeds per replicate)

Concentration (mg/kg dw)	21 day shoot dry weight (g)	SD	Percent emergence at 21 days
Control	0.1609	0.0178	99
3.8	0.1685	0.0206	98
9.4	0.1541	0.0046	100
20	0.1858	0.0085	98
47	0.1678	0.0161	100
120	0.1230[a]	0.0176	98
300	0.0297[a]	0.0101	98

a) Significantly reduced compared to control

Table 3.31 Results based on dry shoot weight (mg/kg nominal)

Species	EC25[a]	EC50[a]	LOEC	NOEC
Cabbage	82 (52-120)	>130 NA[b]	130	50
Corn	83 (14-180)	160 (80-280)	320	130
Oat	69 (57-81)	100 (87-130)	120	47
Soybean	220 (72-360)	460 (370-520)	800	320
Tomato	19 (9.8-32)	67 (52-79)	50	20
Wheat	120 (98-140)	200 (180-210)	120	47

a) 95% confidence limits in parentheses

b) NA = not applicable; EC25 and EC50 values were estimated empirically, so 95% confidence limits could not be calculated.

Table 3.32 Results based on percent emergence (mg/kg nominal)

Species	EC25[a]	EC50[a]	LOEC	NOEC
Cabbage	130 (83-180)	190 (120-230)	320	130
Corn	>320 NA[b]	>320 NA[b]	>320	320
Oat	>800 NA[b]	>800 NA[b]	>800	800
Soybean	650 (370-800)	>800 NA[b]	>800	800
Tomato	190 (160-210)	260 (230-300)	320	130
Wheat	>300 NA[b]	>300 NA[b]	>300	300

a) 95% confidence limits in parentheses

b) NA = not applicable; EC25 and EC50 values were estimated empirically, so 95% confidence limits could not be calculated.

The lowest EC_{25} value from the study is 19 mg/kg dw, for dry shoot weight in tomato plants. This endpoint also has a NOEC value of 20 mg/kg dw. The value of 20 mg/kg will be used in the derivation of the PNEC.

Ferrera *et al.* (2006) exposed seeds and seedlings of four plant species (broad beans, *Vicia faba* L., var. maior; tomato, *Lycopersicum esculentum* Mill.; lettuce, *Lactuca sativa* L.; and durum wheat, *Triticum durum* Desf.) to bisphenol-A in solution (not in soils). Seeds were exposed on filter paper in Petri dishes, and germination and early growth were evaluated. Seedlings from these exposures were inserted into holes in aluminium lids on glass jars filled with nutrient medium containing bisphenol-A or medium alone for 21 days. The length and weight (wet and dry) of the roots and shoots of the seedlings were measured. The exposure concentrations were 10 and 50 mg/l.

Germination of seeds and root and shoot lengths of seedlings up to six days (Petri dish exposures) were not affected by bisphenol-A, with the exception of the root length of tomato seedlings which was reduced by over 50% at 50 mg/l. The root and shoot lengths and weights (wet and dry) were reduced at both bisphenol-A levels in the 21-day growth tests for tomato, durum wheat and lettuce, but not for broad bean. The bisphenol-A concentration reduced in the nutrient medium over the 21-day exposure by ~90% at the lower concentration and by between 80% and 96% at the higher concentration. Solutions stored under the same conditions with no seedlings showed only limited decreases. Analysis of broad been and tomato seedlings for bisphenol-A was carried out. The tomato seedlings contained measurable levels in both shoots and roots; broad bean seedlings had lower levels that the tomatoes in the roots, and bisphenol-A was not detected in the shoots.

These exposures are through water only, and no NOEC can be determined, so they are not suitable for use in the assessment.

3.2.3.2 Terrestrial PNEC

As discussed in Section 3.2.1.3.1, aquatic snail species might be sensitive to bisphenol-A, and so terrestrial molluscs could be an important group to protect. However, no relevant data are available, and a standardised reproductive toxicity test method is not available either. Unlike the aquatic environment, terrestrial exposures are intermittent, and since bisphenol-A is readily biodegradable, there is no potential for continuous exposure. It is therefore recommended that the standard soil assessment scheme is followed, which is consistent with the approach taken for other endocrine active chemicals, e.g. nonylphenol (EC, 2002).

The results of long-term tests with earthworms, springtails and plants are available, with NOEC values of >100, 500 and 20 mg/kg dw respectively. The first two values were obtained in soils with an organic matter content of 10%. Normalising these values to the standard TGD soil organic matter content of 3.4% gives NOEC values of >34 and 170 mg/kg dw. The value for plants was obtained from a soil with 1.82% organic matter[26]. Normalising the value to the standard TGD soil organic matter content gives a NOEC value of 37 mg/kg dw.

As three NOEC values are available covering a suitable range of organisms[27], an assessment factor of 10 is appropriate, giving a $PNEC_{soil}$ of 3.7 mg/kg dw. For comparison with the calculated concentrations in soil, which are presented on a wet weight basis, this corresponds to a value of 3.2 mg/kg wet weight using the standard TGD. A NOEC of 32 mg/kg has also been reported for mortality in *Eisenia* in a short-term test. As no effects were seen with the same species in a longer-term test with the same test conditions, this short-term result is not considered to have a high reliability. As the $PNEC_{soil}$ derived here is well below the reported value, it is considered to be suitably protective.

3.2.4 Secondary poisoning

The $PNEC_{oral}$ was based on the mammalian data reviewed in the human health risk assessment. A NOAEL of 50 mg/kg bw/day was used (related to reduction in litter size in a three-generation feeding study with rats).

3.2.4.1 New information

The effects of bisphenol-A on fertility and reproductive performance in CD-1 mice have been investigated in a two-generation study, conducted in response to the published risk assessment conclusions (Tyl *et al*, 2007). This study has been reviewed for the human health assessment. Overall, the study NOAEL for both general and reproductive toxicity is 50 mg/kg bw/day.

No avian toxicity data were previously available. Male White Leghorn *Gallus domesticus* chicks were administered bisphenol-A orally from two weeks of age to 25 weeks (Furuya *et al*, 2006). The doses used were 2 µg/kg bw to 200 mg/kg bw with administration every two days. The weights of the comb, wattle and testes were examined; cell counts on testes sections were used to monitor spermatogenesis.

[26] The OM content of the test soil as collected was 1.9%. The test medium as used was made up by mixing 11.5 kg of soil with 0.5 kg of sand, hence the final OM content was 1.82%.

[27] Although no data are available for soil micro-organisms, this is considered to be acceptable since bisphenol-A is readily biodegradable and possible endocrine effects are of more importance.

There were no differences in the body weights of birds between the control animals and those exposed to bisphenol-A. No pathological lesions were observed in the liver or kidneys of exposed birds. Decreases in the weight of the comb and wattle were seen in birds exposed to 2 µg/kg bw at ten weeks, but not at other times. At 20 µg/kg bw, similar effects were seen at ten weeks, and at 15 weeks reduced weights in the combs and testes were observed. These had recovered to control levels at 20 and 25 weeks. Higher concentrations tended to show greater effects, although only the 200 mg/kg bw exposure did not recover back to levels not statistically significantly different from the controls after 15 weeks.

The counts of spermatogonia per lumen were reduced in birds exposed to 20 µg/kg bw and above at ten weeks and longer exposures. Spermatocytes per lumen were reduced after 15 weeks and longer at 20 µg/kg and above, and the counts of spermatids per lumen were reduced from ten weeks on at 20 µg/kg bw and above.

The variation in effects over time makes the interpretation of the results difficult. There appears to be a delaying effect on the development of the comb, wattle and testes at most of the exposure levels, but this is temporary in all but the highest exposure. Spermatogenesis was affected at all but the lowest exposure level, but the effect of this on ability to reproduce was not investigated in the study and is not clear. If the highest dose was considered as a LOAEL then the NOAEL for the effects on the comb, wattle and testes weights would be 20 mg/kg bw. As this appears to have been given every two days, the daily dose should be taken as 10 mg/kg bw.

3.2.4.2 PNEC for secondary poisoning

The $PNEC_{oral}$ derived in the published risk assessment was 33 mg/kg food, based on a NOAEL of 50 mg/kg bw/day from a three-generation rat study with a conversion factor of 20 and an assessment factor of 30.

The new two-generation study on mice also has a NOAEL of 50 mg/kg bw/day. However, the conversion factor for mice is 8.3, giving a NOEC of 415 mg/kg; applying the same assessment factor of 30 gives a $PNEC_{oral}$ of 13.8 mg/kg food.

The NOAEL from the study on chickens is 10 mg/kg bw/day. The conversion factor is 8, giving a NOEC of 80 mg/kg; applying an assessment factor of 30 gives a $PNEC_{oral}$ of 2.67 mg/kg food.

The lowest $PNEC_{oral}$ value of 2.67 mg/kg food will be used in the risk characterisation.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

3.3.1.1 Surface water

The PNEC for surface water derived in Section 3.2.1.5 is 1.5 µg/l. The PEC/PNEC ratios obtained using this value are presented in Table 3.33 and a) This scenario is included for completeness, but see the main text for further discussion

Table 3.34. Values above 1 are highlighted in bold.

Table 3.33 Risk characterisation ratios for freshwater and marine water – part 1

	Freshwater		Marine	
	PEC_{water} (µg/l)	**PEC/PNEC**	**$PEC_{marine\ water}$ (µg/l)**	**PEC/PNEC**
Site specific				
BPA 1			0.01	0.07
BPA 2	0.032	0.02		
BPA 3			0.008	0.05
BPA 4			0.007	0.05
BPA 5			0.003	0.02
BPA 6			0.10	0.67
ER 1	0.033	0.02		
ER 2, ER 3, ER 6	0.032	0.02		
ER 4	0.99	0.66		
ER 5	0.062	0.04		
PAPER 1	0.31	0.2		
PAPER 2	0.14	0.09		
PAPER 3	0.10	0.07		
PAPER 4	1.03	0.67		
PAPER 5	1.03	0.67		
PAPER 6	0.97	0.65		
PAPER 7	0.07	0.05		
Generic scenarios				
Polycarbonate bottle washing	0.032	0.02	0.003	0.02
Phenoplast cast resin processing	1.47	0.98	*1.2*[a]	***7.74***[a]
PVC – Anti-oxidant during processing	0.19	0.12	0.13	0.87
PVC – Plasticiser use	0.14	0.09	0.09	0.61

a) This scenario is included for completeness, but see the main text for further discussion

Table 3.34 Risk characterisation ratios for freshwater and marine water – part 2

	Freshwater		Marine	
	PEC_{water} (µg/l)	**PEC/PNEC**	**$PEC_{marine\ water}$ (µg/l)**	**PEC/PNEC**
PVC additive package				
Site A1			0.023	0.15
Site A2	0.036	0.02		
Site A3	0.11	0.07		
Site A4	0.044	0.03		
Site A5	0.045	0.03		
Site A6			0.013	0.08
Site A7			0.011	0.07
Site A8	0.054	0.04		
Site A9	0.27	0.18		
Site A10	0.032	0.02		
Site A11	0.033	0.02		
Site A12	0.097	0.06		
Site A13			0.009	0.06
Anti-oxidant use in plasticiser production				
Specific site			0.005	0.03
Generic site	0.39	0.26		
Thermal paper recycling				
With deinking	0.033	0.02	0.003	0.02
Without deinking	0.033	0.02	0.003	0.02

No PEC/PNEC ratio is above one for the freshwater compartment for any life cycle stage. The $PNEC_{water}$ of 1.5 µg/l is also above the 95%ile value of 0.35 µg/l from the whole freshwater monitoring data set as presented in Section 3.1.4.6.3.

Nevertheless, there remains a possibility that the $PNEC_{water}$ does not take full account of the potential effects of bisphenol-A on snails. The UK Government funded some additional research with native European[28] gastropod mollusc species in 2006 (the pulmonate snail *Planorbarius corneus* and the prosobranch snails *Bithynia tentaculata* and *Viviparus viviparus*). Much of this work has involved method development, since the selected species are not typically cultured in laboratories (and *V. viviparus* could not be cultured successfully). Initial results (unpublished) are as follows:

[28] The original work on snails involved a tropical species – it was decided early in the development of the conclusion (i) programme that the same species should be tested since:
- there was little experience in testing European freshwater snails at the time, and
- if a test with a different species failed to show any effect, there would still have been an open question about the original findings.

- Adult *P. corneus* were exposed to estradiol over an 8-week period in a semi-static system. At exposure concentrations of 1, 10 and 100 ng/l there was evidence of an increase in egg production relative to the controls at 15°C, whilst at 20°C there appeared to be a small decrease.
- A preliminary experiment exposed adult *P. corneus* to bisphenol-A at concentrations of 0.2, 2 and 20 µg/l at 15°C. Compared to the estradiol study, a much lower egg production rate was obtained for both exposed and control snails, which might have been caused by operational problems. The results were inconclusive as to whether the bisphenol-A exposure resulted in a change in egg production rate, although the mean egg productivity of the control groups was below all the bisphenol-A exposed groups.

There is therefore a residual concern that the $PNEC_{water}$ might be too high, despite the thorough testing conducted under the conclusion (i) programme. The following conclusion is therefore drawn for the freshwater compartment:

Conclusion (i) There is a need for further information and/or testing.

The rapporteur has commissioned an additional study that will expose adult *P. corneus* over a 6-month period using a more robust test design than that used for the original trial. If an effect is indeed apparent, further work will need to be considered (such as a life-cycle test). The implications for other priority substances will need to be considered at the same time.

3.3.1.2 Marine water

The $PNEC_{marine\ water}$ derived in Section 3.2.1.5 is 0.15 µg/l, although this should also be considered provisional for the same reasons as for freshwater (it is noted that effects of bisphenol-A on marine molluscs have been recorded).

The resulting risk characterisation ratios are presented in Table 3.33 and a)This scenario is included for completeness, but see the main text for further discussion

Table 3.34. The generic scenario for phenoplast resins is the only scenario to give a potential risk for marine discharges; however, consultation with the European Phenolic Resins Association has confirmed that the sites previously identified as having marine discharges no longer use bisphenol-A for this purpose. Hence this scenario is not relevant for the marine environment.

The marine PNEC is above the 95%ile value from the measured levels in marine waters reviewed in Section 3.1.4.6.3.

The considerations with regard to toxicity to snails described in the freshwater assessment are also relevant for the marine assessment. Therefore the conclusion is:

Conclusion (i) There is a need for further information and/or testing.

No scenarios pose a potential risk using the current $PNEC_{marine\ water}$, but this should be reconsidered once the results of further toxicity testing with freshwater snails are available.

3.3.1.3 Sediment

The sediment PNEC and the sediment PEC values are obtained through the equilibrium partition method, and so the risk characterisation ratios are the same as for the freshwater and marine compartments and the same conclusions apply.

There are also additional scenarios related to the possible degradation of TBBPA in anaerobic sediments to give bisphenol-A. The PEC values estimated for the relevant freshwater TBBPA scenarios and the risk characterisation ratios (using a PNEC of 24 µg/kg wwt) are provided in Table 3.35 (marine sediment concentrations are expected to be around one order of magnitude lower). All ratios are below 1.

Table 3.35 Risk characterisation ratios for sediment (scenarios for TBBPA degradation)

Scenario			PEC (mg/kg wwt)	PEC/PNEC
Reactive flame retardant use	Manufacture of epoxy and/or polycarbonate resins		$(1.42\text{-}1.95)\times10^{-4}$	<0.01
	Processing of epoxy resins		$(1.23\text{-}1.7)\times10^{-6}$	<0.01
Additive flame retardant use	ABS	Compounding	$(5.75\text{-}7.9)\times10^{-3}$	0.24-0.32
		Conversion	$(2.6\text{-}3.6)\times10^{-4}$	0.01-0.02

3.3.2 Terrestrial compartment

The PNEC for the terrestrial compartment is 3.2 mg/kg wwt. This is derived from the initial concentrations of bisphenol-A applied to the soils as described in Section 3.2.3.1. The concentrations in soil for the comparison therefore also need to be on the same basis. The values presented in Section 3.1.4.7 are on the standard TGD basis at a time 30 days after application. From the calculation method in the TGD, the ratio between the initial concentration and the 30-day value is 1.39:1, and so the calculated concentrations have been increased by a factor of 1.39. These revised concentrations and the resulting risk characterisation ratios are in Table 3.36 for bisphenol-A uses and Table 3.37 for possible formation of bisphenol-A through the degradation of TBBPA during sludge digestion. All of the ratios are below one.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

There are no risks for the terrestrial compartment from the current production and use of bisphenol-A, nor from the possible degradation of TBBPA to bisphenol-A during sludge digestion.

Table 3.36 Risk characterisation ratios for soil from bisphenol-A uses

	PEC (µg/kg wwt)	Revised PEC	PEC/PNEC
Site specific			
Epoxy resin ER4	13.3	18.5	<0.01
PVC additive package: A2	1.43	2.0	<0.01
A3	0.86	1.2	<0.01
A4	1.38	1.9	<0.01
A6	3.46	4.8	<0.01
A8	0.77	1.1	<0.01
A13	0.07	0.1	<0.01
Generic scenarios			
Phenoplast cast resin processing	20	28	0.01
PVC – anti-oxidant during processing	2.2	3.1	<0.01
PVC – plasticiser use	1.6	2.2	<0.01
Anti-oxidant in plasticiser production	5.0	7.0	<0.01
Thermal paper recycling with deinking	633 (p); 1.5 (b); 534 (c)	880 (p); 2.1 (b); 742 (c)	0.28 (p); <0.01 (b); 0.23 (c)
Thermal paper recycling without deinking	35 (p); 1.8 (b); 29 (c)	49 (p); 2.5 (b); 40 (c)	0.02 (p); <0.01 (b); 0.01 (c)

p Paper sludge;

b Biological sludge;

c Combined paper and biological sludges (in ratio produced)

Table 3.37 Risk characterisation ratios for soil from TBBPA uses

Scenario			PEC	PEC/PNEC
Reactive flame retardant use	Manufacture of epoxy and/or polycarbonate resins		0.013	<0.01
	Processing of epoxy resins		2.5×10^{-5}	<0.01
Additive flame retardant use	ABS	Compounding	0.54	<0.01
		Conversion	0.025	<0.01

3.3.3 Secondary poisoning

The PNEC for secondary poisoning is 2.67 mg/kg food based on a non-standard test with birds (Section 3.2.4.2). The resulting risk characterisation ratios for freshwater and terrestrial predators are in Table 3.38 and for marine predators are in Table 3.39. All of the ratios are below one, the majority below 0.01. This would be the case even if the slightly higher BCF for clams were used for the aquatic food chain.

Table 3.38 Risk characterisation ratios for secondary poisoning – freshwater and soil

	PEC fish (µg/kg)	PEC/PNEC	PEC worms (µg/kg)	PEC/PNEC
Site specific				
Bisphenol-A production (BPA 2)	2.2	<0.01	-	
Epoxy resin (ER 4)	29	<0.01	5.3	<0.01
Thermal paper production (PAPER 6)	28	<0.01	-	
PVC additive package (A6)	21	<0.01	1.6	<0.01
Generic scenarios				
Polycarbonate bottle washing	2.3	<0.01	-	
Phenoplast cast resin processing	36	<0.01	7.8	<0.01
PVC – anti-oxidant during processing	5.8	<0.01	1.2	<0.01
PVC – plasticiser use	4.8	<0.01	0.92	<0.01
Anti-oxidant use in plasticiser production	11	<0.01	2.2	<0.01
Thermal paper recycling with deinking	2.3	<0.01	237 (p); 0.84 (b); 200 (c)	0.09 (p); <0.01 (b); 0.07 (c)
Thermal paper recycling without deinking	2.3	<0.01	13 (p); 0.98 (b); 12 (c)	<0.01 (p); <0.01 (b); <0.01 (c)

p Paper sludge;

b Biological sludge;

c Combined paper and biological sludges (in ratio produced)

Information on avian reproductive toxicity is important for endocrine disrupting chemicals, since mammalian toxicity data are of limited predictive value (birds are fundamentally different in certain aspects of their physiology, e.g. the control of sexual differentiation, egg laying, etc.). In this case a standard test guideline study is not available. However, it is not considered appropriate to request a further multi-generational study with birds because:

- bisphenol-A is readily biodegradable and has a low bioaccumulation potential,
- the existing study addressed several relevant end points, and
- the PEC/PNEC ratios are all significantly below 1.

Table 3.39 Risk characterisation ratios for secondary poisoning - marine

	PECpredators (µg/kg)	PEC/PNEC	PEC top predators (µg/kg)	PEC/PNEC
Site specific				
Bisphenol-A production (BPA 6)	3.5	<0.01	0.85	<0.01
PVC additive package (A1)	0.73	<0.01	0.3	<0.01
Generic scenarios				
Polycarbonate bottle washing	0.2	<0.01	0.2	<0.01
Phenoplast cast resin processing	28	<0.01	5.8	<0.01
PVC – anti-oxidant during processing	3.2	<0.01	0.78	<0.01
PVC – plasticiser use	2.3	<0.01	0.61	<0.01
Anti-oxidant use in plasticiser production	7.1	<0.01	1.6	<0.01
Thermal paper recycling with deinking	0.2	<0.01	0.2	<0.01
Thermal paper recycling without deinking	0.2	<0.01	0.2	<0.01

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

There are no risks for secondary poisoning from the current production and uses of bisphenol-A

3.3.4 PBT assessment

Persistence: Bisphenol-A is readily biodegradable, and so does not meet the P criterion.

Bioaccumulation: The measured BCF values in fish for bisphenol-A are in the range 30-75, with slightly higher values for other aquatic organisms (tadpoles, clams). These values are well below the threshold, and so bisphenol-A does not meet the B criterion.

Toxicity: There are no reliable chronic NOEC values below 0.01 mg/l, although there are some less reliable values and indications of possible effects at this level. Bisphenol-A has been shown to have effects on the endocrine systems of a number of organisms. It is therefore considered to meet the T criterion.

Conclusion: Bisphenol-A is not a PBT or vPvB substance; it meets the T criterion but not the P or B criteria.

3.3.5 Uncertainties

The exposure assessment is based on data provided by Industry, much of which is based on site-specific considerations. It is possible that some suppliers or users exist outside of the main trade associations, particularly in those Member States that joined the EU in recent years. For example, the rapporteur has been informed that there is a producer in Poland that is not part of the Industry consortium (no data have been supplied from this site). Competent Authorities may therefore need to check that the scenarios presented in this report are appropriate for their national situation.

As discussed in Section 3.2.1.5, the $PNEC_{water}$ can be derived in several ways, depending on how the *Marisa cornuarietis* data are viewed. The conclusion (i) study is reliable, and has not confirmed the original findings of Oehlmann and co-workers. Nevertheless, there are certainly strain differences in the snail stocks used in the different laboratories, and it is possible that the role of seasonality was not sufficiently investigated. Differences in exposure regimes might also have an influence if metabolites are more potent than the parent substance (though there is no evidence for this).

It is also apparent that reproductive effects have been observed at apparently low concentrations in more than one aquatic snail species (*Nucella lapillus* and *Potamopyrgus antipodarum*), although the available data are not sufficiently robust for direct use in the PNEC derivation. Whilst some of these effects might be an artefact of the experimental design, histopathological changes are difficult to dismiss in this way (although these are not necessarily directly related to effects that could influence population growth).

There therefore remains a possibility that the $PNEC_{water}$ does not take full account of the potential effects of bisphenol-A on snails. Further work being conducted by the UK Government should be taken into account when results are available in 2008. The implications for other endocrine active compounds will also need to be considered at the same time.

The PNEC for the marine compartment should also be considered as provisional for the same reasons as for freshwater; it is noted that effects of bisphenol-A on marine molluscs have been recorded.

If the aquatic PNEC is revised, then the sediment ratios would also change.

4 REFERENCES

Aarab N, Lemaire-Gony S, Unruh E, Hansen PD, Larsen BK, Andersen OK and Narbonne JF (2006). Preliminary study of responses in mussel (*Mytilus edulis*) exposed to bisphenol A, diallyl phthalate and tetrabromodiphenyl ether. Aquat. Toxicol., **78S**, S86-S92.

Alexander HC, Bartlett EA, Boggs GU (1985a). Bisphenol-A: Flow-through acute toxicity to the fathead minnow. Dow Company Report: ES-764.

Alexander HC, Dill DC, Milazzo DP, Boggs GU (1985b). Bisphenol-A: Algal Toxicity Test. Dow Company Report: ES-762. 1985.

Alexander HC, Milazzo DP and Boggs GU (1985c). Bisphenol-A: daphnid static acute toxicity test. Dow Company Report. ES-763.

Alexander HC, Dill DC, Smith LW, Guiney PD and Dorn P (1988). Bisphenol-A: Acute aquatic toxicity. Environmental Toxicology and Chemistry **7**, 19-26.

Alo' R, Facciolo RM, Madeo M, Giusi G, Carelli A and Canonaco M (2005). Effects of the xenoestrogen bisphenol A in diencephalic regions of the teleost fish *Coris julis* occur preferentially via distinct somatostatin receptor subtypes. Brain Res. Bull., **65**, 267-273.

Andersen HR, Halling-Sørensen B and Kusk KO (1999). A parameter for detecting estrogenic exposure in the copepod *Acartia tonsa*. Ecotoxicol. Environ. Safe., **44**, 56-61.

Andersen HR, Wollenberger L, Halling-Sørensen B and Kusk KO (2001). Development of copepod nauplii to copepodites – A parameter for chronic toxicity including endocrine disruption. Environmental Toxicology and Chemistry, **20**(12), 2821-2829.

Aufderheide, J., Warbritton, R., Pounds, N., File-Emperador, S., Staples, C., Caspers, N., Forbes, V. (2006). The effects of husbandry parameters on the life-history traits of *Marisa cornuarietis*: effects of temperature, photoperiod and population density. Invert. Biol., **125**, 9-20.

Azevedo DA, Lacorte S, Viana P and Barcelo D (2001). Occurrence of nonylphenol and bisphenol A in surface waters from Portugal. J Brazilian Chemical Society, **12**(4), 532-537.

Bayer AG (1996). Studies on the ecological behaviour of bisphenol-A. Study Number 600 A/96.

Bayer AG (1999a). Fish, prolonged toxicity test (*Brachydanio rerio*) 14 day study of Bisphenol-A. Study Number 707 A/98 Fl.

Bayer AG (1999b). Fish, juvenile growth test (*Oncorhynchus mykiss*) of Bisphenol-A. Study Number: 707 A/98FF.

Beck IC, Bruhn R, Gandrass J and Ruck W (2005). Liquid chromatography-tandem mass spectrometry analysis of estrogenic compounds in coastal surface water of the Baltic Sea. J Chromatography A., **1090**(1-2), 98-106.

Belfroid A, Velzen MV, Horst BV and Vethaak D (2002). Occurrence of bisphenol A in surface water and uptake in fish: evaluation of field measurements. Chemosphere, **49**, 97-103.

Biggers WJ and Laufer H (2004). Identification of juvenile hormone-active alkylphenols in the lobster *Homarus americanus* and in marine sediments. Biol. Bull., **206**, 13-24.

Billard R, Breton B, Richard M (1981). On the inhibitory effect of some steroids on spermatogenesis in adult rainbow trout (*Salmo gairdneri*) Can. J. Zool., **59**, 1479-1487.

Bolz U, Hagenmaier H and Koerner W (2001). Phenolic xenoestrogens in surface water, sediments, and sewage sludge from Baden-Wurttemberg, south-west Germany. Environmental Pollution., **115**(2), 291-301.

Bolz U, Koerner W and Hagenmaier H (1999). Determination of phenolic xenoestrogens in sediments and sewage sludges by HRGC/LRMS. Organohalogen Compounds, **40**, 65-68.

Bowmer T and Gimeno S (2001). The effects of Bisphenol-A on the development of the male carp reproductive tract when exposed during sexual differentiation – implications for test design and risk assessment. Unpublished TNO Report.

Brennan SJ, Brougham CA, Roche JJ and Fogarty AM (2006). Multi-generational effects of four selected environmental oestrogens on *Daphnia magna*. Chemosphere, **64**, 49-55.

Brian JV, Harris CA, Scholze M, Backhaus T, Booy P, Lamoree M, Pojana G, Jonkers N, Runnalls T, Bonfà A, Marcomini A and Sumpter JP (2005). Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. Environ. Health Perspect., **113**, 721-728.

Brossa L, Marce RM, Borrull F and Pocurull E (2005). Occurrence of twenty-six endocrine-disrupting compounds in environmental water samples from Catalonia, Spain. Env Tox and Chem., **24**(2), 261-267.

Brossa L, Pocurull E, Borrull F and Marce RM (2002). A rapid method for determining phenolic endocrine disrupters in water samples. Chromatographia, **56**(9/10), 573-576.

Brossa L, Pocurull E, Borrull F and Marce RM (2004). Solid-phase extraction-high-performance liquid chromatography-electrospray mass spectrometry to determine endocrine disruptors in water samples. Chromatographia, **59**(7-8), 419-423.

Bursch W, Fuerhacker M, Gemeiner M, Grillitsch B, Jungbauer A, Kreuzinger N, Moestl E, Scharf S, Schmid E, Skutan S and Walter I (2004). Endocrine disrupters in the aquatic environment: the Austrian approach - ARCEM. Water Sci and Technology, **50**(5), 293-300.

Canesi L, Betti M, Lorusso LC, Ciacci C and Gallo G (2005). 'In vivo' effects of bisphenol A in *Mytilus* hemocytes: modulation of kinase-mediated signalling pathways. Aquatic Toxicol., **71**, 73-84.

Canesi L, Lorusso LC, Ciacci C, Betti M, Zampini M, Gallo G (2004). Environmental estrogens can affect the function of mussel hemocytes through rapid modulation of kinase pathways. Gen. Comp. Endocrinol., **138**, 58-69.

Caspers N (1998). No estrogenic effects of bisphenol-A in *Daphnia magna* Straus. Bull. Environ. Contam. Toxicol., **61**, 143-148.

Caunter JE, Panter GH, Bamforth JE, Hutchinson TH and Brown AR (2006) Bisphenol A: 42 day exposure to mature adult fathead minnows (*Pimephales promelas*) – Phase 2 of the study "Effects of BPA on fathead minnow gonadal histology". Brixham Environmental Laboratory study number 02-0413A, Report No BL 7556/B, April 2006.

Centre for Environment, Fisheries and Aquaculture Science (CEFAS). Extract of CEFAS contract report C1337 to Defra.

CEPI (2001). Special Recycling - 2000 Statistics. Confederation of European Paper Industries. Published on-line (August 2001) at http://www.cepi.org

CERI (2004). Biodegradation study of BPA by microorganisms. Kerume Laboratory, Chemicals Evaluation and Research Institute, Japan. Study number 14294.

Cespedes R, Lacorte S, Ginebreda A and Barcelo D (2006). Chemical monitoring and occurrence of alkylphenols, alkylphenol ethoxylates, alcohol ethoxylates, phthalates and benzothiazoles in sewage treatment plants and receiving waters along the Ter River basin (Catalonia, N. E. Spain). Analytical and Bioanalytical Chemistry, **385**(6), 992-1000.

Cespedes R, Lacorte S, Raldua D, Ginebreda A, Barcelo D and Pina B (2005). Distribution of endocrine disruptors in the Llobregat River basin (Catalonia, NE Spain). Chemosphere, **61**(11), 1710-1719.

Chen M-Y, Ike M and Fujita M. (2002). Acute toxicity, mutagenicity and estrogenicity of bisphenol-A and other bisphenols. Environmental Toxicology, **17**(1), 80-86.

Chikae M, Ikeda R, Hasan Q, Morita Y, Tamiya E (2003). Effect of alkylphenols on adult male medaka: plasma vitellogenin goes up to the level of estrous female. Environ. Toxicol. Pharmacol., **15**, 33-36.

Christiansen LB, Winther-Nielsen M and Helweg C (2002). Feminisation of fish: The effect of estrogenic compounds and their fate in sewage treatment plants and nature. Danish Environmental Protection Agency, No. 729.

Clara M, Strenn B, Saracevic E and Kreuzinger N (2004). Adsorption of bisphenol-A, 17β-estradiole and 17α-ethinylestradiole to sewage sludge. Chemosphere, **56**, 843-851.

Collett D (2002). Modelling binary data. 2nd edition. Chapman & Hall.

CSTEE, 2002. Opinion on the results of the Risk Assessment of: BISPHENOL A ENVIRONMENTAL PART. Opinion expressed at the 32nd CSTEE plenary meeting. Brussels, 22 June 2002. Scientific Committee on Toxicity, Ecotoxicity and the Environment.

Defra, 2003. Interim Risk Reduction Strategy and Analysis of Advantages and Drawbacks for Bisphenol-A, Stage 4 Report. July 2003. Prepared by Risk & Policy Analysts Ltd for the Department for Environment, Food and Rural Affairs, UK.

del Olmo M, Gonzalez-Casado A, Navas NA and Vilchez JL (1997). Determination of bisphenol A (BPA) in water by gas chromatography-mass spectrometry. Analytica Chimica Acta, **346**, 87-92.

Dietrich D R, O'Brien E, Hoffmann S, Balaguer P, Nicolas J-C, Seinen W, Depledge M (2006). Effects of BPA in Snails. Letter to the editor. Environmental Health Perspectives, **114**. http://www.ehponline.org/docs/2006/8942/letter.html.

Dow (1988). Bisphenol-A: Determination of acute bacterial toxicity. Internal Company Report.

Drastichová J, Svobodová Z, Groenland M, Dobšiková R, Žlábek V, Weissová D and Szotkowská M (2005). Effect of exposure to bisphenol A and 17β-estradiol in zebrafish (*Danio rerio*). Acta. Vet. Brno, **74**, 287-291.

Duft M, Schulte-Oehlmann U, Weltje L, Tillman M and Oehlmann J (2003). Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. Aquatic Toxicology, **64**, 437-449.

EC (2003) European Union Risk Assessment Report: 4,4'-Isopropylenediphenol (bisphenol-A). 3rd Priority List, Volume 37. European Commission Joint Research Centre, EUR 20843 EN.

ECB (2007). Draft risk assessment report for 2,2', 6,6'-Tetrabromo-4,4'-isopropylidenediphenol (Tetrabromobisphenol-A). Report R402_0706_env.

ECT (2007a). Bisphenol A: reproduction toxicity to the collembolan species *Folsomia candida* in artificial soil according to the international standard ISO 11267 (1999) "Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants". ECT Oekotoxikologie GmbH, Study number AS1CR, April 2007.

ECT (2007b). Bispehnol A: reproduction toxicity to the enchytraeid species Enchytraeus crypticus in artificial soil according to the OECD Guideline No 220 for the testing of chemicals "Enchytraeid reproduction test" adopted April 13 2004. ECT Oekotoxikologie GmbH, Study number AS1EN, April 2007.

Emmitte JA (1978). The acute toxicity of Parabis A and Bisphenol-A to the sheepshead minnow, *Cyprinodon variegatus*. Dow Company Report, TWC 102.

Environment Agency (2002). "National R&D Project P2-203: Development of Emission Scenario Documents for Use in Environmental Risk Assessment - Pulp, Paper and Board Industry." Environment Agency of England and Wales.

Environment Agency (2003). Surface water monitoring data. Supplemental information provided by Mark Wilkinson, National Laboratory Service, Environment Agency of England and Wales.

Environment Agency (2005). Nonylphenol - additional terrestrial toxicity studies for future update. Unpublished report.

EURAS (2006). Local exposure assessment of TBBPA and BPA. Short report to BSEF, October 2006.

Famiglini G, Palma P, Siviero A, Rezai MA and Cappiello A (2005). Determination of endocrine disrupting compounds in marine water by nanoliquid chromatography/direct-electron ionization mass spectrometry. Analytical Chemistry, **77**(23), 7654-7661.

Fawell JK, Sheahan D, James HA, Hurst M and Scott S (2001). Oestrogens and oestrogenic activity in raw and treated water in Severn Trent water. Water Research, **35**(5), 1240-1244.

Fenske M, Maack G, Schäfers C and Segner H (2001). Assessment of estrogenic effects on reproduction and sexual differentiation in zebrafish (*Danio rerio*): Alterations in vitellogenesis and reproductive physiology. Poster presentation.

Fent G, Hein WJ, Moendel MJ and Kubiak R. (2003). Fate of ^{14}C-bisphenol-A in soils. Chemosphere, **51**, 735-746.

Ferrara G, Loffredo E and Senesi N (2006). Phytotoxic, clastogenic and bioaccumulation effects of the environmental endocrine disruptor bisphenol A in various crops grown hydroponically. Planta, **223**, 910-916.

Fjeld E, Schlabach M, Berge JA, Eggen T, Snilsberg P, Kallberg G, Rognerud S, Enge EK, Borgen A and Gundersen H (2004a). Screening of selected new organic contaminants - brominated flame retardants, chlorinated paraffins, bisphenol-A and triclosan. Norsk institutt for vannforskning (NIVA).

Fjeld E, Schlabach M, Rognerud S and Kallberg G (2004b). Environmental pollutants in sediments and fish from Lake Mjosa and the Drammens River and Drammensfjord, follow-up studies in 2004. Norsk institutt for vannforskning (NIVA).

Flammarion P, Brion F, Babut M, Garric J, Migeon B, Noury P, Thyband E, Tyler CR, Palazzi X (2000). Induction of fish vitellogenin and alterations in testicular structure: preliminary results of estrogenic effects in chub (*Leuciscus cephalus*). Ecotoxicology, **9**, 127-135.

Fleig M, Brauch H-J and Kuehn W (2000). Ergebnisse der AWBR-Untersuchungen im Jahr 2000. Resultats des analyses effectuees en 2000; Results of the Investigations by the AWBR in 2000.-Jahresbericht 2000 / Arbeitsgemeinschaft Wasserwerke Bodensee-Rhein (AWBR).2000. S.S. 39-58.

Forbes, V.E., Selck, H., Palmqvist, A., Aufderheide, J., Warbritton, R., Pounds, N., Thompson, R., van der Hoeven, N., Caspers, N. 2007a. Does bisphenol A induce superfeminization in *Marisa cornuarietis*? Part I: Intra- and inter-laboratory variability in test endpoints. Ecotoxicol. Environ. Saf., **66**, 309-318.

Forbes, V.E., Aufderheide, J., Warbritton, R.,van der Hoeven, N., Caspers, N. 2007b. Does bisphenol A induce superfeminization in *Marisa cornuarietis*? Part II: Toxicity test results and requirements for statistical power analysis. Ecotoxicol. Environ. Saf., **66**, 319-325.

Fraunhofer (1999). Verfolgung von Umweltbeslastungen durch Alkylphenole, Bisphenol A und organische Zinnverbindungen in repräsentativen Umweltproben. Teilbericht C1: Analytik von Bisphenol A. Prüf-Code: K-UBA-001/7-61. Fraunhofer-Institut für Umweltcheme und Ökotoxicologie, Schmallenberg. November 1999.

Fries E and Puettmann W (2002). Eintrag von Nonylphenol und Bisphenol A aus der Oder in den anaeroben Grundwasserleiter des Oderbruch. Input of Nonylphenol and Bisphenol A from the Odra into the anaerobic aquifer of the Oderbruch.-Endokrin wirksame Substanzen in Abwasser und Klaerschlamm - neueste Ergebnisse aus Wissenschaft und Technik : Tagungsband zum Workshop vom 22./23. April 2002. S. S. 186-197.

Fromme H, Kuechler T, Otto T, Pilz K, Mueller J and Wenzel A (2002). Occurrence of phthalates and bisphenol A and F in the environment. Water Research, **36**(6), 1429-1438.

Fukuhori N, Kitano M, Kimura H (2005). Toxic effects of bisphenol A on sexual and asexual reproduction in *Hydra oligactis*. Arch. Environ. Contam. Toxicol., **48**, 495-500.

Furuya M, Adachi K, Kuwahara S, Ogawa K and Tsukamoto Y (2006). Inhibition of male chick phenotypes and spermatogenesis by Bisphenol-A. Life Sciences, **78**, 1767-1776.

Gary MA and Metcalfe CD. (1997) Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to p-nonylphenol. Environ. Tox. Chem., **16**, 1082-1086.

Gonzalez-Casado Navas AN, del Olmo M and Vilchez JL (1998). Determination of bisphenol A in water by micro liquid-liquid extraction followed by silylation and gas chromatography-mass spectrometry analysis. J Chromatographic Science, **36**(11), 565-569.

Goto Y, Kitamura S, Kashiwagi K, Oofusa K, Tooi O, Yoshizato K, Sato J, Ohta S and Kashiwagi A (2006). Suppresion of amphibian metamorphosis by bisphenol A and related chemical substances. J Health Sci., **52**(2), 160-168.

Gushiken Y, Watanuki H and Sakai M (2002). In vitro effect of carp phagocytic cells by bisphenol A and nonylphenol. Fish. Sci., **68**, 178-183.

Hahn T, Schenk K and Schulz R (2002). Environmental chemicals with known endocrine potential affect yolk protein content in the aquatic insect *Chironomus riparius*. Environ. Poll., **120**, 525-528.

Hartley WR, Thiyagarajah A, Anderson MB, Broxson MW, Major SE, Zell SI (1998). Gonadal development in Japanese Medaka (*Oryzias latipes*) exposed to 17β-oestradiol. Marine Environment Research, **46**, 145-148.

Hassanin A, Kuwahara S, Nurhidayat, Tsukamoto Y, Ogawa K, Hiramatsu K and Sasaki F (2003). Functional changes of the LH-immunoreactive cells in adenohypophysis of the common carp (*Cyprinus carpio*) from rivers contaminated with estrogenic chemicals. J. Vet. Med. Sci., **65**, 485-490.

Haubruge E, Petit F and Gage MJG (2000). Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyl tin and bisphenol A. Proc. R. Soc. Lond. B, **267**, 2333-2337.

Hedlund B, Rodhe J, Arner M, Forsberg J, Taaler M, Forsgren A and Eriksson M (2006). Screeninguppdrag inom nationell miljoovervakning. Screening av: Bisfenol A. NV Diarienr. 721-1173-03Mm, WSP 10033045, 35 pp. English translations provided by Lars Andersson and Maria Linderoth of Kemi.

Heemken OP, Reincke H, Stachel B and Theobald N (2000). The occurrence of xenostrogens in the Elbe River and the North Sea. Chemosphere, **45**, 245-259.

Hill M, Stabile C, Steffen LK and Hill A (2002). Toxic effects of endocrine disrupters on freshwater sponges: common developmental abnormalities. Environ. Poll., **117**, 295-300.

Hirano M, Ishibashi H, Matsumura N, Nagao Y, Watanabe N, Watanabe A, Onikura N, Kishi K and Arizono K (2004). Acute toxicity responses of two crustaceans, *Americamysis bahia* and *Daphnia magna*, to endocrine disrupters. Journal of Health Science, **50**(1), 97-100.

Hirooka T, Nagase H, Uchhida K, Hiroshige Y, Ehara Y, Nishikawa J-I, Nishihara T, Miyamoto K and Hirata Z (2005). Biodegradation of bisphenol-A and disappearance of its estrogenic activity by the green alga *Chlorella fusca* var. *vacuolata*. Environ. Toxicol. Chem., **24**(8), 1896-1901.

Hohenblum P, Gans O, Moche W, Scharf S and Lorbeer G (2004). Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria. Science of the Total Env., **333**(1-3), 185-193.

Höllrigl-Rosta A, Vinken R, Lenz M and Schäffer A (2003). Sorption and dialysis experiments to assess the binding of phenolic xenobiotics to dissolved organic matter in soil. Environ. Toxicol. Chem., **22**(4), 746-752.

Honkanen JO, Holopainen IJ and Kukkonen JVK (2004). Bisphenol A induces yolk-sac oedema and other adverse effects in landlocked salmon (*Salmo salar* m. *sebago*) yolk-sac fry. Chemosphere, **55**, 187-196.

Hoshi H, Kamata Y and Uemura T (2003). Effects of 17β-estradiol, bisphenol A and tributyltin chloride on germ cells of *Caenorhabditis elegans*. J. Vet. Med. Sci., **65**, 881-885.

Hu X, Peng J-F, Liu J-F, Jinag G-B and Jönsson JA (2006). Evaluating the impacts of some environmentally relevant factors on the availability of bisphenol A with negligible-depletion SPME. Chemosphere, **65**, 1935-1941.

Ike M, Jin CS and Fujita M (2000). Biodegradation of bisphenol A in the aquatic environment. Water Science and Technology, **42**(7), 31-38.

Ishibashi H, Watanabe N, Matsumura N, Hirano M, Nagao Y, Shiratsuchi H, Kohra S, Yoshihara S-I and Arizono K (2005). Toxicity to early life stages and an estrogenic effect of a bisphenol A metabolite, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene on the medaka (*Oryzias latipes*). Life Sciences, **77**, 2643-2655.

Ishii H, Saito H, Akiyama N and Kobayashi Y (2003). Effects of endocrine disrupter chemicals bisphenol A and p-nonylphenol on marine macroalga *Nannochloropsis oculata* ST-3. J. School Mar. Sci. Technol., **1**, 93-98.

Iwamuro S, Sakakibara M, Terao M, Ozawa A, Kurobe C, Shigeura T, Kato M and Kikuyama S (2003). Teratogenic and anti-metamorphic effects of bisphenol A on embryonic and larval *Xenopus laevis*. General and Comparative Endocrinology, **133**, 189-198.

Japanese Ministry of the Environment (2006). Results of Assay and Tests in Evaluation of the Endocrine Disrupting Activities in Fish (Medaka), 26 January 2006. http://www.env.go.jp/en/chemi/ed/rt_medaka.pdf

Jeannot R, Sabik H, Sauvard E, Dagnac T and Dohrendorf K (2002). Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry. J Chromatography A, **974**(1-2), 143-159.

Ji YJ, Song C-S and Ahnn J (2004). Isolation and characterization of bisphenol-A resistant mutants in *Caenorhabditis elegans*. Korean J. Genetics, **26**, 107-114.

Jin C, Ike M and Fujita M (1996) Metabolic pathway of bisphenol A by *Pseudomonas paucimobilis* strain FJ-4. Japan Water Treatment Biological Association Journal, **32**, 199-210.

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkyphenolic chemicals. Environmental Toxicology and Chemistry. **15**, 194-202.

Jobling S, Casey D, Rodgers-Gray T, Oehlmann J, Schulte-Oehlmann U, Pawlowski S, Baunbeck T, Turner AP and Tyler CR (2003). Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquat. Toxicol., **65**, 205-220.

Jobling S, Casey D, Rodgers-Gray T, Oehlmann J, Schulte-Oehlmann U, Pawlowski S, Baunbeck T, Turner AP and Tyler CR (2004). Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. Aquat. Toxicol., **66**, 207-222. [Note: this is a corrected and reprinted version of Jobling *et al*. (2003)]

Johnson I, Weeks JM, and Kille P (2005). Endocrine Disruption in Aquatic and Terrestrial Invertebrates – Final Report. WRc-NSF Ltd, Report No UC 4906/6 for the Department of the Environment, Food and Rural Affairs.

Jurgella GF, Marwah A, Malison JA, Peterson R and Barry TP (2006). Effects of xenobiotics and steroids on renal and hepatic estrogen metabolism in lake trout. General and Comparative Endocrinology, **148**, 273-281.

Kang IJ, Yokota H, Oshima Y, Tsuruda Y, Oe T, Imada N, Tadokoro H, Honjo T (2002). Effects of bisphenol A on the reproduction of Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem., **21**(11), 2394-2400.

Kang J-H and Kondo F (2002). Effects of bacterial counts and temperature on the biodegradation of bisphenol-A in river water. Chemosphere, **49**, 493-498.

Kang J-H and Kondo F (2002a). Bisphenol A degradation by bacteria isolated from river water. Arch. Environ. Contam. Toxicol., **43**, 265-269.

Kang J-H and Kondo F (2005). Bisphenol A degradation in seawater is different from that in river water. Chemosphere, **60**, 1288-1292.

Kang J-H, Ri N and Kondo F (2004) *Streptomyces sp*. strain isolated from river water has high bisphenol A degradability. Letters in Applied Microbiology, **39**, 178-180.

Kang J-H, Katayama Y and Kondo F (2006). Biodegradation or metabolism of bisphenol A: from microorganisms to mammals. Toxicology, **217**, 81-90.

Kashiwada S, Ishikawa H, Miyamoto N, Ohnishi Y and Magara Y (2002). Fish test for endocrine-disruption and estimation of water quality of Japanese rivers. Wat. Res., **36**, 2161-2166.

Kelly C, Langford K, Mellor P and Rowland S (2006). Preliminary investigations of bisphenol-A in marine sediments. Extract of Report to DEFRA on OSPAR Priority Substances.

Kinnberg K and Toft G (2003). Effects of estrogenic and antiandrogenic compounds on the testis structure of the adult guppy (*Poecilia reticulata*). Ecotoxicol Environ. Safe., **54**, 16-24.

Kiyomoto M, Kikuchi A, Unuma T and Yokota Y (2006). Effects of ethynylestradiol and bisphenol A on the development of sea urchin embryos and juveniles. Marine Biology, **149**, 57-63.

Kloas W, Lutz I, Einspanier R (1999). Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. Sci. Total Environ., **225**, 59-68.

Kloas W, Schrag B, Ehnes C, Segner H (2000). Binding of xenobiotics to hepatic estrogen receptor and plasma sex steroid binding protein in the teleost fish, the common carp (*Cyprinus carpio*). Gen. Comp. Endocrinol., **119**, 287-299.

Kohra S, Kuwahara K, Takao Y, Ishibashi Y, Lee HC, Arizono K and Tominaga N (2002a). Effects of bisphenol A on the feeding behaviour of *Caernorhabditis elegans*. J. Health Sci., **48**, 93-95.

Kohra S, Tominaga N, Takao Y, Nagae M, Ishibashi Y, Ueda K and Arizono K (2002b). A rapid respiratory toxicity test using *Caenorhabditis elegans* with an oxygen electrode system. J. Health Sci., **48**, 269-272.

Koponen PS and Kukkonen JVK (2002). Effects of bisphenol A and artificial UVB radiation on the early development of *Rana temporaria*. J. Toxicol. Environ. Health A, **65**, 947-959.

Koponen PS, Tuikka A and Kukkonen JVK (2007). Effects of ultraviolet-B radiation and larval growth on toxicokinetics of waterborne bisphenol A in common frog (*Rana temporaria*) larvae. Chemosphere, **66**, 1323-1328.

Kuch HM and Ballschmiter K (2001). Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per litre range. Environ. Sci and Tech., **35**(15), 3201-3206.

Kudo Y and Yamauchi K (2005) In vitro and in vivo analysis of the thyroid disrupting activities of phenolic and phenol compounds in *Xenopus laevis*. Toxicol. Sci, **84**, 29-37.

Kusk KO, Wollenberger L (1999). Fully defined saltwater medium for cultivation of and toxicity testing with marine copepod *Acartia tonsa*. Environmental Toxicology and Chemistry, **18**(7), 1564-1567.

Kwak HI, Bae MO, Lee MH, Lee YS, Lee BJ, Kang KS, Chae CH, Sung HJ, Shin JS, Kim JH, Mar WC, Sheen YY, Cho MH (2001) Effects of nonylphenol, bisphenol-A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). Environmental Toxicology and Chemistry, **20**(4), 787-795.

Labadie P and Budzinski H (2006). Alteration of steroid hormone balance in juvenile turbot (Psetta maxima) exposed to nonylphenol, bisphenol A, tetrabromodiphenyl ether 47, diallylphthalate, oil and oil spiked with alkylphenols. Arch. Environ. Contam. Toxicol., **50**, 552-561.

Lagana A, Bacaloni A, De Leva I, Faberi A, Fago G and Marino A (2004). Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. Analytica Chimica Acta, **501**(1), 79-88.

Lee C, Na JG, Lee K-C and Park K (2002). Choriogenin mRNA induction in male medaka, *Oryzias latipes* as a biomarker of endocrine disruption. Aquat. Toxicol., **61**, 233-241.

Lee W-K, Lee K-W, Kwak E-J, Yang S-W, Yang K-S, Park J-C, Joo H-S, Lee W-J and Lee W-B (2003). Effects of environmental endocrine disruptors on the sex differentiation in Korean rockfish, *Sebastes schlegeli*. Water Sci. Technol., **47**(9), 65-70.

Lee HC, Soyano K, Ishimatsu A, Nagae M, Kohra S, Ishibashi Y, Arizono K and Takao Y (2004) Bisphenol A and nonylphenol bioconcentration in spotted halibut *Varaspar variegates*. Fisheries Science, **70**, 192-194.

Leidi S (2005). A review of the statistical aspects of the article by Oehlman J. *et al*. (in press) "Bisphenol A induces superfeminization in the ramshorn snail (*Marisa cornuarietis*) at environmentally relevant concentrations". Report for the Environment Agency. Statistical Services Centre, University of Reading, UK, November 2005.

Leidi S (2006). A review of the reply by Prof. Oehlmann in the letter dated 31 December 2005 on the re-analyses of both unreplicated and replicated experiments. Report for the Environment Agency. Statistical Services Centre, University of Reading, UK, March 2006.

Letcher RJ, Sanderson JT, Bokkers A, Giesy JP and van den Berg M (2005). Effects of bisphenol A-related diphenylalkanes on vitellogenin production in male carp (*Cyprinus carpio*) hepatocytes and aromatase (CYP19) activity in human H295R adrenocortical carcinoma cells. Toxicol Appl Pharmacol., **209**(2), 95-104.

Levy G, Lutz I, Krüger A and Kloas W (2004). Bisphenol A induces feminization in *Xenopus laevis* tadpoles. Environ. Res., **94**, 102-111.

Licht O, Jungmann D, Ladewig V, Ludwichowski K-U and Nagel R (2004). Bisphenol-A in artificial indoor streams: I. Fate and effects on aufwuchs. Ecotoxicol., **13**, 657-666.

Lindholst C, Pedersen KL, Pedersen SN (2000). Estrogenic response of bisphenol-A in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology, **48**, 87-94.

Lindholst C, Pedersen SN and Bjerregaard P (2001). Uptake, metabolism and excretion of bisphenol-A in the rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology **55**, 75-84.

Lindholst C, Korsgaard B, Bjerregard P and Christiansen LB (2002). Hormonal and histological changes in brown trout (*Salmo trutta*) exposed to 17β-estradiol and bisphenol-A. Poster presentation.

Lindholst C, Wynne PM, Marriott P, Pedersen SN and Bjerregaard P (2003). Metabolism of bisphenol A in zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*) in relation to estrogenic response. Comparative Biochemistry and Physiology Part C, **135**, 169-177.

Liu R, Zhou JL and Wilding A (2004a). Microwave-assisted extraction followed by gas chromatography-mass spectrometry for the determination of endocrine disrupting chemicals in river sediments. J Chromatography A, **1038**(1-2), 19-26.

Liu R, Zhou JL and Wilding A (2004b). Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry. J Chromatography A, **1022**, 179-180.

Loffredo E and Senesi N (2006). Fate of anthropogenic organic pollutants in soils with emphasis on adsorption/desorption processes of endocrine disruptor compounds. Pure Appl. Chem., **78**(5), 947-961.

Loos R, Hanke G and Eisenreich SJ (2003). Multi-component analysis of polar water pollutants using sequential solid-phase extraction followed by LC-ESI-MS. J Environmental Monitoring, **5**(3), 384-394.

Loos R, Hanke G, Umlauf G and Eisenreich SJ (2007). LC-MS-MS analysis and occurrence of octyl- and nonylphenol, their ethoxylates and their carboxylates in Belgian and Italian textile industry, waste water treatment plant effluents and surface waters. Chemosphere, **66**(4), 690-699.

Lutz I, Blodt S and Kloas W (2005). Regulation of estrogen receptors in primary cultured hepatocytes of the amphibian *Xenopus laevis* as estrogenic biomarker and its application in environmental monitoring. Comp. Biochem. Physiol. C Toxicol. Pharmcol., **141**(4), 384-392.

Lysak A and Marcinek J (1972). Multiple toxic effects of simultaneous action of some chemical substances on fish. Roczniki nauk rolniczych, **94**(3), 53-63.

Marcial HS, Hagiwara A and Snell TW (2003). Estrogenic compounds affect development of harpacticoid copepod *Tigriopus japonicus*. Environ. Toxicol. Chem., **22**(12), 3025-3030.

Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE and Potter T (2001). Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem., **20**, 297-308.

Miles-Richardson SR, Kramer VJ, Fitzgerald SD, Render JA, Yamini B, Barbee SJ, Giesy JP (1999). Effects of waterborne exposure to 17β-estradiol on secondary sex characteristics and gonads of fathead minnow (*Pimephales promelas*). Aquatic Toxicology, **47**, 129-145.

MITI (1977). Results of bisphenol-A bioaccumulation test.

Mochida K, Ohkubo N, Matsubara T, Ito K, Kakuno A and Fujii K (2004). Effects of endocrine-disrupting chemicals on expression of ubiquitin C-terminal hydrolase mRNA in testis and brain of the Japanese common goby. Aquat. Toxicol., **70(2)**, 123-136.

Morales-Munoz S, Luque-Garcia JL, Ramos MJ, Fernandez-Alba A and Luque de Castro MD (2005a). Sequential superheated liquid extraction of pesticides, pharmaceutical and personal care products with different polarity from marine sediments followed by gas chromatography mass spectrometry detection. Analytica Chimica Acta, **552**(1-2), 50-59.

Morales-Munoz S, Luque-Garcia JL, Ramos MJ, Martinez-Bueno MJ, and Luque de Castro MD (2005b). Sequential automated focused microwave-assisted soxhlet extraction of compounds with different polarity from marine sediments prior to gas chromatography mass spectrometry detection. Chromatographia, **62**(1-2), 69-74.

Mu X, Rider CV, Hwang GS, Hoy H and Leblanc GA (2005). Covert signal disruption: anti-ecdysteroidal activity of bisphenol A involves cross talk between signaling pathways. Environ. Toxicol. Chem. **24**, 146-152.

Na OS, Lee YD, Baek HJ and Kim HB (2002). Effects of bisphenol-A on sex differentiation and gonadal development of medaka, *Oryzias latipes*. Korean J Biol. Sci., **6**, 75-80.

Nagae M, Shiroyama K, Inoue M, Hara A, Takao Y, Kohra S, Ishibashi Y, Tominaga N, Yoshihara S and Arizono K (2005). Estrogenic potency of a bisphenol A metabolite on vitellogenin synthesis in medaka, *Oryzias latipes*. J. Health Sci., **51**, 93-95.

Nakada N, Tanishima T, Shinohara H, Kiri K and Takada H (2006). Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res., **40**, 3297-3303.

Nakajima N, Oshima Y, Serizawa S, Kouda T, Edmonds JS, Shirashi F, Aono M, Kubo A, Tamaoki M, Saji H and Morita M (2002). Processing of bisphenol A by plant tissue: glucosylation by cultured BY-2 cells and glucosylation/translocation by plants of *Nicotiana tabacum*. Plant and Cell Physiology, **43**(9), 1036-1042.

Oehlmann J, Schulte-Oehlmann U, Tillmann M and Markert B (2000). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol-A and Octylphenol as xenoestrogens. Ecotoxicology, **9**, 383-397.

Oehlmann J, Schulte-Oehlmann U, Duft M and Tillmann M (2001). Effect of Environmental Hormones in Prosobranch Molluscs. Presentation at 2nd Status Seminar on Endocrine Disrupters held in Berlin, April 2001.

Oehlmann J, Schulte-Oehlmann U, Bachmann J, Oetken M, Lutz I, Kloas W and Ternes TA (2006). Bisphenol A induces superfeminization in the ramshorn snail *Marisa cornuarietis* (Gastropoda: Prosobranchia) at environmentally relevant concentrations. Environmental Health Perspectives, **114**, 127-133

Ohkimoto K, Sugahara T, Sakakibara Y, Suiko M, Liu M-Y, Carter G, Liu M-C (2003). Sulfonation of environmental estrogens by zebrafish cytosolic sulfotransferases. Biochem. Biophys. Res. Comm., **309**, 7-11.

Oka T, Adati N, Shinkai T, Sakuma K, Nishimura T and Kurose K (2003). Bisphenol A induces apoptosis in central neural cells during early development of *Xenopus laevis*. Biochem. Biophys. Res. Comm, **312**, 877-882.

Ortiz-Zarragoitia M and Cajaraville MP (2006). Biomarkers of exposure and reproduction-related effects in mussels exposed to endocrine disruptors. Arch. Environ. Contam. Toxicol., **50**, 361-369.

Oshiman K, Tsutsumi Y, Nishida T and Matsumura Y (2007). Isolation and characterization of a novel bacterium, *Sphingomonas bisphenolicum* strain A01, that degrades bisphenol A. Biodegradation, **18**(2), 247-255.

Pait AS and Nelson JO (2003). Vitellogenesis in male *Fundulus heteroclitus* (killifish) induced by selected estrogenic compounds. Aquat. Toxicol, **64**, 331-342.

Panter GH, Thompson RS, Sumpter JP (1998). Adverse reproductive effects in male fathead minnows (*Pimephales promelas*) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. Aquatic Toxicology **42**, 243-253.

Pascoe D, Carroll K, Karntanut W and Watts MM (2002). Toxicity of 17α-ethinylestradiol and bisphenol A to the freshwater Cnidarian *Hydra vulgaris*. Archives of Environmental Contamination and Toxicology, **43**, 56-63.

Pastva SD, Villalobos SA, Kannan K and Giesy JP (2001). Morphological effects of bisphenol-A on the early life-cycle stages of medaka (*Oryzias latipes*). Chemosphere, **45**, 535-541.

Patrolecco L, Capri S, De Angelis S, Polesello S and Valsecchi S (2004). Determination of endocrine disrupting chemicals in environmental solid matrices by extraction with a non-ionic surfactant (Tween 80). J Chromatography A,. **1022**(1-2), 1-7.

Patrolecco L, Capri S, De Angelis S, Pagnotta R, Polesello S and Valsecchi S (2006). Partition of nonylphenol and related compounds among different aquatic compartments in Tiber River (central Italy). Water, Air and Soil Pollution, **172**, 151-166.

Paumann R and Vetter S (2003). Endocrine disrupters in Austria's waters - a risk?: Results from three years of research --SYNOPSIS (Introduction-Results-Conclusion). Austrian Research Cooperation on Endocrine Modulators (ARCEM). Federal Ministry of Agriculture, Forestry, Environment and Water Management.

Pawlowski S, Islinger M, Völkl A and Braunbeck T (2000). Temperature-dependent vitellogenin-mRNA expression in primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes at 14°C and 18°C. Toxicology *in Vitro*, **14**, 531-540.

Penalver A, Pocurull E, Borrull F and Marce RM (2002). Method based on solid-phase microextraction-high-performance liquid chromatography with UV and electrochemical detection to determine estrogenic compounds in water samples. J Chromatography A, **964**(1-2), 153-160.

Petrovic M and Barcelo D (2001). Determination of phenolic xenoestrogens in environmental samples by liquid chromatography with mass spectrometric detection. J AOAC International, **84**(4), 1074-1085.

Pettersen A and Fjeld E (2005). Miljogifter i Drammensvassdraget 2005. Contaminants in the Drammen waterway 2005. Norges Geotekniske Institutt (NGI) and Norsk institutt for vannforskning (NIVA).

Pickford DB (2003). Critique of the manuscript "BPA induces feminization in *X. laevis* tadpoles" by Levy *et al.* Brixham Environmental Laboratory, AstraZeneca. (17th March 2003).

Pickford DB, Caunter JE, Hetheridge MJ, Hutchinson TH (2000). Bisphenol-A: Determination of effects on larval growth, development and sexual differentiation of the African clawed frog (*Xenopus laevis*). Brixham Environmental Laboratory.

Pickford DB, Hetheridge MJ, Caunter JE, Hall AT, Hutchinson TH (2003). Assessing chronic toxicity of bisphenol A to larvae of the African clawed frog (*Xenopus laevis*) in a flow-through exposure system. Chemosphere, **53**, 223-235.

PlasticsEurope (2007). Evaluation of environmental monitoring data for bisphenol A: European data. April 2007. Provided by David.Thomas@plasticseurope.org.

Pojana G, Bonfa A, Busetti F, Collarin A and Marcomini A (2004a). Determination of natural and synthetic estrogenic compounds in coastal lagoon waters by HPLC-electrospray-mass spectrometry. International Journal of Environmental and Analytical Chemistry, **84**(10), 717-727.

Pojana G, Bonfa A, Busetti F, Collarin A and Marcomini A (2004b). Estrogenic potential of the Venice, Italy, lagoon waters. Env Tox and Chem., **23**(8), 1874-1880.

Putt AE (2003). Bisphenol A – 7 day toxicity to duckweed, *Lemna gibba*, under static renewal conditions. Springborn Smithers Laboratories Study No. 13796.6101 submitted to the American Plastics Council.

Quiros L, Cespedes R, Lacorte S, Viana P, Raldua D, Barcelo D and Pina B (2005). Detection and evaluation of endocrine-disruption activity in water samples from Portuguese rivers. Env Tox and Chem., **24**(2), 389-395.

Readman JW, Guitart C and Frickers T (2006). Inputs and fates of pharmaceuticals, synthetic and natural endocrine disruptors and faecal steroids downstream of a sewage treatment plant. Plymouth Marine Laboratory, Plymouth, UK. Draft Paper, 24 pp.

Reiff B (1979). The acute toxicity of disphenylolpropane to rainbow trout (*Salmo gairdneri*). Shell Company report. GRR-TLGR.79.146.

Rhodes JE, Wolf JC and van der Hoeven N (2007). Bisphenol A: partial life-cycle toxicity test with the fathead minnow, *Pimephales promelas*. ABC Laboratories, Inc. and Experimental Pathology Laboratories, Inc., ABC Study number 48972 (in preparation, draft used).

Rodriguez-Mozaz S, Lopez del Alda MJ and Barcelo D (2004). Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry. J Chromatography A, **1045**(1-2), 85-92.

Rodriguez-Mozaz S, Lopez de Alda M and Barcelo D (2005). Analysis of bisphenol A in natural waters by means of an optical immunosensor. Water Research, **39**(20), 5071-5079.

Roepke TA, Snyder MJ, Cherr GN (2005). Estradiol and endocrine disrupting compounds adversely affect development of sea urchin embryos at environmentally relevant concentrations. Aquat. Toxicol., **71**, 155-173.

Rouhani Rankouhi T, Sanderson JT, van Holsteijn I, van Leeuwen C, Vethaak AD and van den Berg M (2004). Effects of natural and synthetic estrogens and various environmental contaminants on vitellogenesis in fish primary hepatocytes: comparison of bream (*Abramis brama*) and carp (*Cyprinus carpio*). Toxicol. Sci., **81**, 90-102.

Rouhani Rankouhi T, Sanderson JT, van Holsteijn I, van Kooten P, Bosveld ATC, van den Berg M (2005). Effects of environmental and natural estrogens on vitellogenin production in hepatocytes of the brown frog (*Rana temporaria*). Aquat. Toxicol., **71**, 97-101.

RPA (2003). Interim Risk Reduction Strategy and Analysis of Advantages and Drawbacks for Bisphenol-A: Stage 4 Report, unpublished report for Department of Environment, Food and Rural Affairs.

Sasaki M, Akahira A, Oshiman K, Tsuchido T and Matsumura Y (2005). Purification of cytochrome P450 and ferredoxin, involved in bisphenol A degradation, from *Sphingomonas* sp. strain A01. Appl. Environ. Microbiol., **71**(12), 8024-8030.

Sasaki K, Yamanaka H, Moriyoshi K, Ohmoto T and Ohe T (2007). Biodegradation of bisphenol A and related compounds by Sphingomonas sp. Strain BP-7 isolated from seawater. Biosci. Biotechnol. Biochem., **71**(1), 51-57.

Sattelberger R and Scharf S (1999). Hormonell wirksame substanzen in fliessgewassern. Umweltbundesamt. BE-150.

Sayers LE (2005). Bisphenol A (BPA) – Acute toxicity to midges (*Chironomus tentans*) under flow-through conditions. Springborn Smithers Laboratories Study No. 13796.6107 submitted to the American Plastics Council.

Schäfers C, Wenzel A, Schmitz A, Böhmer W (2001). Effects of Xenoestrogens on the life cycle of fish. Poster Presentation.

Schirling M, Bohlen A, Triebskorn R and Köhler H-R (2006a). An invertebrate embryo test with the apple snail *Marisa cornuarietis* to assess effects of potential developmental and endocrine disruptors. Chemosphere, **64**, 1730-1738.

Schirling M, Jungmann D, Ladewig V, Ludwichowski K-U, Nagel R, Köhler H-R and Triebskorn R (2006b). Bisphenol A in artificial indoor streams: II. Stress response and gonad histology in *Gammarus fossarum* (Amphipoda). Ecotoxiicology, **15**, 143-156.

Schmidt, B and Schuphan, I. (2002). Metabolism of the environmental estrogen bisphenol A by plant cell suspension cultures. Chemosphere, **49**, 51-59.

Schulte-Oehlmann U, Tillmann M, Casey D, Duft M, Markert B Oehlmann J (2001). Östrogenartige Wirkungen von Bisphenol A auf Vorderkiemenschnecken (Mollusca: Gastropoda: Prosobranchia). - UWSF - Z Umweltchem Ökotox., **13**, 319-333

Segner H, Caroll K, Fenske M, Janssen CR, Maack G, Pascoe D, Schäfers C, Vandenburgh GF, Watts M and Wenzel A (2003a). Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. Ecotoxicol. Environ. Safe., **54**, 302-314.

Segner H, Navas JM, Schäfers C and Wenzel A (2003b). Potencies of estrogenic compounds in in vitro screening assays and in life cycle tests with zebrafish in vivo. Ecotoxicol. Environ. Safe., **54**, 315-322.

Selck H, Aufderheide J, Pounds N, Staples C, Caspers N, Forbes VE. (2006). Effects of food type, feeding frequency, and temperature on juvenile survival and growth of *Marisa cornuarietis* (Mollusca: Gastropoda). *Invert Biol*., **125**, 106-116.

Shareef A, Angove MJ, Wells JD and Johnson BB (2006a). Aqueous solubilities of estrone, 17β-estradiol, 17α-ethynylestradiol, and bisphenol-A. J Chem Eng data, **51**(3), 879-881.

Shareef A, Angove MJ, Wells JD and Johnson BB (2006b). Sorption of bisphenol-A, 17alpha-ethynylestradiol and estrone to mineral surfaces. J. Colloid Interface Sci., **297** (1), 62-69.

Shioda T and Wakabayashi M (2000). Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). Chemosphere, **40**, 239-243.

Sidhu S, Gullett B, Striebich R, Klosterman J, Contreras J and De Vito M (2005). Endocrine disrupting chemical emissions from combustion sources: diesel particulate emissions and domestic waste open burn emissions. Atmospheric Environment, **39**, 801-811.

Smeets JMW, van Holsteijn I, Giesy JP, Seinen W, and van der Berg M (1999). Estrogenic potencies of several environmental pollutants, as determined by vitellogenin induction in carp hepatocyte assay. Toxicological Sciences, **50**, 206-213.

Sohoni P, Tyler CR, Hurd KS, Caunter JE, Hetheridge MJ, Williams TD, Woods C, Evans M, Toy R, Gargas M, Sumpter JP (2001). Reproductive effects of long-term exposure to Bisphenol A in the fathead minnow (*Pimephales promelas*). Environ. Sci. Technol., **35**, 2917-2925.

Spivack J, Leib TK and Lobos JH (1994) Novel pathway for bacterial metabolism of bisphenol A. J Biol Chem., **289**, 7323-7329.

Springborn Bionomics Inc. (1985a). 96 hour flow-through acute toxicity test with the Atlantic silverside. Report Number BW-85-6-1802. Commissioned by the Society of the Plastics Industry.

Springborn Bionomics Inc. (1985b). Acute toxicity of Bisphenol-A to the mysid (*Mysidopsis bahia*) under flow-through conditions. Report Number BW-85-8-1825. Commissioned by the Society of the Plastics Industry.

Springborn Bionomics Inc. (1985c). Acute toxicity of Bisphenol-A to the marine alga *Skeletonema costatum*. Report Number BW-85-8-1829. Commissioned by the Society of the Plastics Industry.

Springborn Smithers (2006a). Bisphenol A (BPA) - Chronic Toxicity to Rotifers (Brachionus calyciflorus) Under Static Conditions, Unpublished report, No. 13796.6108.

Springborn Smithers (2006b). Bisphenol A (BPA) - Chronic Toxicity to Amphipods (Hyalella azteca) Under Flow-Through Conditions. Unpublished report, No. 13796.6106

Springborn Smithers (2007). Bisphenol A – Determination of effects on seedling emergence and seedling growth (definitive test). Study number 13761.6124. Commissioned by American Chemistry Council. August 2007.

Stachel B, Ehrhorn U, Heemken OP, Lepom P, Reincke H, Sawal G and Theobald N (2002). Xenoestrogens in the river Elbe and its tributaries. Environmental Pollution, **124**(3), 497-507.

Stachel B, Jantzen E, Knoth W, Krueger F, Lepom P, Oetken M, Reincke H, Sawal G, Schwartz R and Uhlig, S (2005). The Elbe flood in August 2002 - Organic contaminants in sediments samples taken after the flood event. J Env Sci and Health - Part A, **40**(2), 265-287.

Stephenson RR (1983). Diphenylol propane: Acute toxicity to *Daphnia magna* and *Selenastrum capricornutum*. Shell Group Research Report SBGR.83.216.

Stoker C, Rey F, Rodriguez H, Ramos JG, Sirosky P, Larriera A, Luque EH and Muñoz-de-Toro M (2003). Sex reversal effects on *Caiman latirostris* exposed to environmentally relevant doses of the xenoestrogen bisphenol A. General and Comparative Endocrinology, **133**, 287-296.

Stone CM and Watkinson RJ (1983). Diphenylol propane: an assessment of ready biodegradability. Shell Group Research Report SBGR.83.425.

Sumpter JP and Jobling S (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Perspect., **103** (suppl. 7), 173-178.

Sumpter JP, Tyler CR, Sherazi A (2001). Bisphenol-A: Multigeneration study with the fathead minnow (*Pimephales promelas*). Brunel University. Part of study published as Sohoni *et al*, 2001.

Suzuki N and Hattori A (2003). Bisphenol A suppresses osteoclastic and osteoblastic activities in the cultured scales of goldfish. Life Sci., **73**, 2237-2247.

Suzuki N, Kambegawa A and Hattori A (2003). Bisphenol A influences the plasma calcium level and inhibits calcitonin secretion in goldfish. Zool. Sci., **20**, 745-748.

Suzuki T, Nakagawa Y, Takano I, Yaguchi K and Yasuda K (2004a). Environmental fate of bisphenol-A and its biological metabolites in river water and their xeno-estrogenic activity. Environ. Sci. Technol., **38**(8), 2389-2396.

Suzuki E, Kunimoto M, Nishizuka M, and Imagawa M (2004b). Evaluation of ability of chemicals to bind frog (*Xenopus laevis*) estrogen receptor by in vitro binding assay. J. Health Sci., **50**, 685-688.

Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M and Magara Y (2001). Estrogenic influences of estradiol-17β, p-nonylphenol and bisphenol-A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. Water Science and Technology, **43**(2), 109-116.

Tabata A, Kamei T, Magara Y, Watanabe N, Miyamoto N, Ohnishi Y and Itoh M (2003a). Effects of nonylphenol, bisphenol A, 17β-estradiol, and their mixture on induction of serum vitellogenin in Japanese medaka. J. Japan Soc. Wat. Environ., **26**, 671-676.

Tabata A, Miyamoto N, Ohnishi Y, Itoh M, Yamada T, Kamei T and Magara Y (2003b). The effect of chlorination of estrogenic chemicals on the level of serum vitellogenin of Japanese medaka (*Oryzias latipes*). Wat. Sci. Technol., **47**, 51-57.

Tabata A, Watanabe N, Yamamoto I, Ohnishi Y, Itoh M, Kamei T, Magara Y and Terao Y (2004). The effect of bisphenol A and chlorinated derivatives of bisphenol A on the level of serum vitellogenin in Japanese medaka (*Oryzias latipes*). Wat. Sci. Technol., **50**, 125-132.

Takahashi A, Higashitani T, Yakou Y, Saitou M, Tamamoto H and Tanaka H (2003). Evaluating bioaccumulation of suspected endocrine disruptors into periphytons and benthos in the Tama River. Water Science and Technology, **47**(9), 71-76.

Takino A, Tsuda T, Kojima M, Harada H, Muraki K and Wada M (1999) Bioconcentration factor of bisphenol A for killifish (*Oryzias latipes*) Rep Shiga Prefecture. Public Health & Environ Sci, **34**, 65-67.

Terasaki M, Nomachi M, Edmonds JS and Morita M. (2004). Impurities in industrial grade 4,4'-isopropylidene diphenol (bisphenol-A): possible implications for estrogenic activity. Chemosphere, **55**, 927-931.

Thomas P and Doughty K (2004). Disruption of rapid, nongenomic steroid actions by environmental chemicals: interference with progestin stimulation of sperm motility in Atlantic croaker. Environ. Sci. Technol., **38**, 6328-6332.

TNO (2001). Bisphenol A: Degradation kinetics and removal in a continuous activated sludge (CAS) simulation test (OECD 303A). TNO Report Executive Summary V99. 1179.

TNO (2003). Monitoring of emissions of bisphenol A from recycling of thermal paper. For the European Thermal Paper Association (EPTA). TNO Report V4074/01.

TNO (2004). Monitoring of the fate of bisphenol A in a generic paper recycling plant. For the European Thermal Paper Association (EPTA). TNO Report V5973/01.

Tollefsen K-E, Øvrevik J and Stenersen J (2004). Binding of xenoestrogens to the sex steroid-binding protein in plasma from Arctic charr (*Salvelinis alpinis* L.). Comp. Biochem. Physiol. C, **139**, 127-133.

Tominaga N, Kohra S, Iguchi T and Arizono K (2003). A multi-generation sublethal assay of phenols using the nematode *Caenorhabditis elegans*. J. Health Sci., **49**, 459-463.

Trudeau VL, Turque N, Le Méval S, Alliot C, Gallant N, Coen L, Pakdel F, Demeneix B (2005). Assessment of estrogenic endocrine-disrupting chemical actions in the brain using in vivo somatic gene transfer. Environ. Health Perspect., **113**, 329-334.

Tyl RW, Myers CB and Marr MC (2007). Draft Final Report: Two-generation reproductive toxicity evaluation of Bisphenol A (BPA; CAS No. 80-05-7) administered in the feed to CD-1® Swiss mice (modified OECD 416). RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.

Umweltbundesamt (1999, 2000). Anhang 2 - 1999 & 2000, Monitoring Data of Bisphenol A (BPA) and Metabolite 4-Hydroxyacetophenon (HAP), Ummweltbundesamt IV 2.2, Berlin.

van den Belt K, Verheyen R and Witters H (2003). Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicol. Environ. Safe., **56**, 271-281.

van der Hoeven N (1998). Power analysis for the NOEC: What is the probability to detect small toxic effects on three different species using the appropriate standardized test protocols? Ecotoxicology, **7**, 355-361.

van der Hoeven N (2005). Experiments on the effect of BPA on the snail species *Marisa cornuarietis* as described in three papers by Oehlmann: Evaluation of the applied statistics and analysis of the raw data. ECOSTAT Report 05/011. 14 October 2005.

Vethaak AD, Rijs GBJ, Schrap SM, Ruiter H, Gerritsen A and Lahr J (2002). Estrogens and xenoestrogens in the aquatic environment of the Netherlands - Occurrence, potency and biological effects. Dutch National Institute of Inland Water Management and Waste Water Treatment (RIZA) and the Dutch National Institute for Coastal and Marine Management (RIKZ). February. RIZA/RIKZ - report no. 2002.001. Supplemental data available.

Vethaak AD, Lahr J, Schrap SM, Belfroid AC, Rijs, GBJ, Gerritsen A, de Boer J, Bulder AS, Grinwis GCM, Kuiper RV, Legler J, Murk TAJ, Peijnenburg W, Verhaar HJM, de Voogt P (2005). An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of the Netherlands. Chemosphere, **59**, 511-524.

Vigano L, Mandich A, Benfenati E, Bertolotti R, Bottero S, Porazzi E and Agradi E (2006). Investigating the estrogenic risk along the river Po and its intermediate section. Archives of Environmental Contamination and Toxicology, **51**(4), 641-651.

Voutsa D, Hartmann P, Schaffner C and Giger W (2006). Benzotriazoles, alkylphenols and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. Environmental Science and Pollution Research. **13**(5), 333-341. Supplemental data provided by Dr. Giger.

Wang HY, Olmstead AW, Li H and LeBlanc GA (2005). The screening of chemicals for juvenoid-related endocrine activity using the water flea *Daphnia magna*. Aquatic Toxicology, **74**, 193-204.

Warbritton, R, V. Forbes, and N. van der Hoeven. 2007a. Bisphenol A: Effects on Fecundity, Hatchability, and Growth of Marisa cornuarietis under flow-through conditions at twenty-five degrees celsius (Phase III Definitive Toxicity Test). Unpublished report, ABC Laboratories Study Number 49820, ABC Laboratories, Columbia, MO.

Warbritton, R., V. Forbes, and N. van der Hoeven. 2007b. Bisphenol A: Effects on Fecundity of Marisa cornuarietis Under Flow-Through Conditions at Twenty-two degrees Celsius. Unpublished report, ABC Laboratories Study Number 61028, ABC Laboratories, Columbia, MO

Watanabe M, Mitani N, Ishii N and Miki K (2005). A mutation in a cuticle collagen causes hypersensitivity to the endocrine disrupting chemical, bisphenol A, in *Caenorhabditis elegans*. Mutat. Res., **570**, 71-80.

Watts MM, Pascoe D, Carroll K (2001a). Survival and precopulatory behaviour of *Gammarus pulex* (L.) exposed to two xenoestrogens. Water Research, **35**(10), 2347-2352.

Watts MM, Pascoe D and Carroll K (2001b). Chronic exposure to 17α-ethinylestradiol and bisphenol A - effects on development and reproduction in the freshwater invertebrate *Chironomus riparius* (Diptera: Chironomidae). Aquatic Toxicology, **55**, 113-124.

Watts MM, Pascoe D, Carroll K (2003). Exposure to 17α-ethinylestradiol and bisphenol A – effects on larval moulting and mouthpart structure of *Chironomus riparius*. Ecotoxicol. Environ. Safety, **54**, 207-215.

Wenzel A, Küchler T, Henschel KP, Schnaack W, Diedrich M and Müller J (1998). Konzentrationen östrogen wirkender Substanzen in Umweltmedien. Umweltbundesamt, Fraunhofer-Institut für Umweltchemie und Ökotoxikologie Schmallenberg und Bergholz-Rehbrücke, Umweltforschungsplan

Whale GF, Warden JR and Bumpus RN (1999). Bisphenol-A: Toxicity in marine sediments to *Corophium volutator*. Shell Research Report No CT.99.47028.

Wiegel S, Aulinger A, Brockmeyer R, Harms H, Loffler J, Reincke H, Schmidt R, Stachel B, von Tumpling W and Wanke A (2004). Pharmaceuticals in the river Elbe and its tributaries. Chemosphere, **57**(2), 107-126.

Wolf JC, Dietrich DR, Friedrich U, Caunter J and Brown AR (2004). Qualitative and quantitative histomorphologic assessment of fathead minnow *Pimephales promelas* gonads as an endpoint for evaluating endocrine-active compounds: A pilot methodology study. Toxicol. Pathol., **32**, 600-612.

Yamaguchi A, Ishibashi H, Kohra S, Arizono K and Tominaga N (2005). Short-term effects of endocrine-disrupting chemicals on the expression of estrogen-responsive genes in male medaka (*Oryzias latipes*). Aquat. Toxicol., **72**, 239-249.

Yang F-X, Xu Y and Wen S (2005). Endocrine-disrupting effects of nonylphenol, bisphenol A and p,p'-DDE on *Rana nigromaculata* tadpoles. Bull. Environ. Contam. Toxicol., **75**, 1168-1175.

Yim S-H, Kim HJ and Lee I-S (2003). Microbial metabolism of the environmental estrogen bisphenol-A. Arch. Pharmac. Res., **26**(10), 805-808.

Ying GG and Kookana RS (2003). Degradation of five selected endocrine-disrupting chemicals in seawater and marine sediment. Environ Sci. Technol., **37**, 1256-1260.

Ying GG and Kookana RS (2005). Sorption and degradation of estrogen-like endocrine disrupting chemicals in soil. Environ. Toxicol. Chem., **24**(10), 2640-2645.

Ying GG, Kookana RS and Dillon P (2003). Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Research, **37**, 3785-3791.

Yokota H, Tsuruda Y, Maeda M, Oshima Y, Tadokoro H, Nakazono A, Honjo T and Kobayashi K (2000). Effect of bisphenol-A on the early life stage in Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem., **19**(7), 1925-1930.

Zeng G, Zhang C, Huang G, Yu J, Wang Q, Li J, Xi B and Liu H (2006). Adsorption behaviour of bisphenol A on sediments in Xiangjiang River, south-central China. Chemosphere, **65**, 1490-1499.

Zhang C, Zeng G, Yuan L, Yu J, Li J, Huang G, Xi B and Liu H (2007). Aerobic degradation of bisphenol A by *Achromobacter xylosoxidans* strain B-16 isolated from compost leachate of municipal solid waste. Chemosphere, **68**(1), 181-190.

ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECDIN	Environmental Chemicals Data and Information Network
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives

JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
o/oo	Parts per thousand
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent

PAH	Polycyclic aromatic hydrocarbons
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SCHER	Scientific Committee on Health and Environmental Risks
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand

UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix 1 Non-summarised references

The following table lists all references retrieved from the literature search that were considered to be of low relevance for the environment update report based on the abstract. A brief reason for this decision is given in the final column. Some of these may be relevant for the human health assessment, and a few, indicated in bold highlighting, may need to be reviewed in future.

Year	Authors	Title	Reference	Notes
2007	Bannister, R., Beresford, N., May, D., Routledge, E. J., Jobling, S., and Rand-Weaver, M.	Novel estrogen receptor-related transcripts in *Marisa* cornuarietis; a freshwater snail with reported sensitivity to estrogenic chemicals.	Environmental Science and Technology. In Press. (Supplemental information in summary column)	Receptor studies with *Marisa*, not with bisphenol A.
2007	Brian, J. V., Harris, C. A., Scholze, M., Kortenkamp, A., Booy, P., Lamoree, M., Pojana, G., Jonkers, N., Marcomini, A., and Sumpter, J. P.	Evidence of estrogenic mixture effects on the reproductive performance of fish.	Environmental Science and Technology. 41(1):337-344.	Effects of mixtures
2007	Cabana, H., Jiwan, J.-L. H., Rozenberg, R., Elisashvili, V., Penninckx, M., Agathos, S. N., Jones, J. P.	Elimination of endocrine disrupting chemicals nonylphenol and bisphenol A and personal care product ingredient triclosan using enzyme preparation from the white rot fungus *Coriolopsis polyzona*.	Chemosphere. 67(4):770-778.	Clean up method.
2007	Canesi, L., Lorusso, L. C., Ciacci, C., Betti, M., Rocchi, M., Pojana, G., and Marcomini, A.	Immunomodulation of *Mytilus* hemocytes by individual estrogenic chemicals and environmentally relevant mixtures of estrogens: *In vitro* and *in vivo* studies.	Aquatic Toxicology. 81(1):36-44.	Development of screening study, similar papers included.
2007	Chen, P.-J., Rosenfeldt, E. J., Kullman, S. W., Hinton, D. E., and Linden, K. G.	Biological assessments of a mixture of endocrine disruptors at environmentally relevant concentrations in water following UV/H2O2 oxidation.	Science of the Total Environment. In Press.	Non-environmental oxidation.
2007	Chin, S. S., Lim, T. M., Chiang, K., and Fane, A. G.	Hybrid low-pressure submerged membrane photoreactor for the removal of bisphenol A.	Desalination. 202(1-3):253-261.	Analytical paper.
2007	Diano, N., Grano, V., Franconte, L., Caputo, P., Ricupito, A., Attanasio, A., Bianco, M., Bencivenga, U., Rossi, S., Manco, I., Mita, L., Del Pozzo, G., and Mita, D. G.	Non-isothermal bioreactors in enzymatic remediation of waters polluted by endocrine disruptors: BPA as a model of pollutant.	Applied Catalysis B - Environmental. 69(3-4):252-261.	Remediation.
2007	Duft, M., Schmitt, C., Bachmann, J., Brandelik, C., Schulte-Oehlmann, U., and Oehlmann, J.	Prosobranch snails as test organisms for the assessment of endocrine active chemicals – an overview and a guideline proposal for a reproduction test with the freshwater mudsnail *Potamopyrgus antipodarum*.	Ecotoxicology. In Press.	No new data
2007	Fernandez, M. P., Ikonomou, M. G., and Buchanan, I.	An assessment of estrogenic organic contaminants in Canadian wastewaters.	Science of the Total Environment. 373(1):250-269.	Waste water and contamination.
2007	Fu, K.-Y., Chen, C.-Y., and Chang, W.	Application of a yeast estrogen screen in non-biomarker species *Varicorhinus barbatulus* fish with two estrogen receptor subtypes to assess xenoestrogens.	Toxicolgy In Vitro. In Press.	Yeast assay, screening study.

Year	Authors	Title	Reference	Notes
2007	Gatidou G., Thomaidis N. S., Stasinakis A. S., Lekkas T. D.	Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography–mass spectrometry.	J Chromatography A. 1138(1-2):32-41.	Bisphenol A in wastewater.
2007	Hashimoto, S., Ueda, Y., Kurihara, R., and Shiraishi, F.	Comparison of the estrogenic activities of seawater extracts from Suruga Bay, Japan, based on chemical analysis or bioassay.	Environmental Toxicology and Chemistry. 26(2):279-286.	Bioassay on seawater extracts
2007	Hayashi, H., Nishimoto, A., Oshima, N., and Iwamuro, S.	Expression of the estrogen receptor alpha gene in the anal fin of Japanese medaka, *Oryzias latipes*, by environmental concentrations of bisphenol A.	The Journal of Toxicological Sciences. 32(1):91-96.	Estrogen receptor gene expression.
2007	Imaoka, S., Mori, T., and Kinoshita, T.	Bisphenol A causes malformation of the head region in embryos of *Xenopus laevis* and decreases the expression of the ESR-1 gene mediated by notch signalling.	Biological and Pharmaceutical Bulletin. 30(2):371-374.	Gene study
2007	Iso, T., Futami, K., Iwamoto, T., and Furuichi, Y.	Modulation of the expression of bloom helicase by estrogenic agents.	Biological and Pharmaceutical Bulletin. 30(2):266-271.	Gene study.
2007	Li, C. and Li, X. Z.	Degradation of endocrine disrupting chemicals in aqueous solution by interaction of photocatalytic oxidation and ferrate (VI) oxidation.	Water Science Technology. 55(1-2): 217-223.	Non-environmental degradation.
2007	Li, F., Li, X., Liu, C., Li, X., and Liu, T.	Effect of oxalate on photodegradation of bisphenol A at the interface of different iron oxides.	Industrial and Engineering Chemistry Research. 46(3):781-787.	Non-environmental photodegradation.
2007	Liu, Y., Deng, L., Chen, Y., Wu, F., and Deng, N.	Simultaneous photocatalytic reduction of Cr(VI) and oxidation of bisphenol A induced by Fe(III)-OH complexes in water.	Journal of Hazardous Materials. 139(2):399-402.	Paper looks at non-environmental reduction and oxidation.
2007	Mandich, A., Bottero, S., Benfenati, E., Cevasco, A., Erratico, C., Maggioni, S., Massari, A., Pedemonte, F., and Vigano, L.	*In vivo* exposure of carp to graded concentrations of bisphenol A.	General and Comparative Endocrinology. In Press.	Similar results included.
2007	Masuda, M., Yamasaki, Y., Ueno, S., and Inoue, A.	Isolation of bisphenol A-tolerant/degrading *Pseudomonas monteilii* strain N-502.	Extremophiles. In Press	Bacterial study.
2007	Oehlmann, J., Di Benedetto, P., Tillmann, M., Duft, M., Oetken, M., and Schulte-Oehlmann, U.	Endocrine disruption in prosobranch molluscs: evidence and ecological relevance.	Ecotoxicology. In Press.	Review of earlier studies
2007	Press-Kristensen, K., Ledin, A., Schmidt, J. E., and Henze, M.	Identifying model pollutants to investigate biodegradation of hazardous XOCs in WWTPs.	Science of the Total Environment. 373(1):122-130.	Similar information included
2007	Torres, R. A., Petrier, C., Combet, E., Moulet, F., and Pulgarin, C.	Bisphenol A mineralization by integrated ultrasound-UV-Iron (II) treatment.	Environmental Science and Technology. 41(1):297-302.	Non-environmental mineralization.

Year	Authors	Title	Reference	Notes
2007	Urbatzka, R., van Cauwenberge, A., Maggioni, S., Vigano, L., Mandich, A., Benfenati, E., Lutz, I., and Kloas, W.	Androgenic and antiandrogenic activities in water and sediment samples from the river Lambro, Italy, detected by yeast androgen screen and chemical analyses.	Chemosphere. 67(6):1080-1087.	Yeast screening assay.
2007	Vitrac, O., Challe, B., Leblanc, J. C., and Feigenbaum, A.	Contamination of packaged food by substances migrating from a direct-contact plastic layer: Assessment using a generic quantitative household scale methodology.	Food Additives and Contaminants. 24(1):75-94.	Paper looks at migration of plastics into food.
2007	Zhang, C., Zeng, G., Yuan,L.,Yu, J., Li, J., Huang, G., Xi, B., and Liu, H.	Aerobic degradation of bisphenol A by *Achromobacter xylosoxidans* strain B-16 isolated from compost leachate of municipal solid waste.	Chemosphere. In Press.	Similar information included.
2007	Zhou, J. L., Liu, R., Wilding, A., and Hibberd, A.	Sorption of selected endocrine disrupting chemicals to different aquatic colloids.	Environmental Science and Technology. 41(1):206-213.	Sorption to colloids.
2006	Abd-El-Aziz A. S., Okasha R. M., May L. J., Hurd J.	Synthesis of norbornenes containing cationic mono- and di(cyclopenta-dienyliron) arene complexes and their ring-opening metathesis polymerization.	J Polymer Sci Part A: Polymer Chemistry. 44(9):3053-3070.	Polymers.
2006	Aguei L., Yanez-Sedeno P., Pingarron J. M.	Preparation and characterization of a new design of carbon-felt electrode for phenolic endocrine disruptors.	Electrochimica Acta. 51(12): 2565-2571.	Analytical paper.
2006	Ahlers, J., Riedhammer, C., Vogliano, M., Ebert, R.-U., Kühne, R., and Schüürmann, G.	Acute to chronic ratios in aquatic toxicity - variation across trophic levels and relationship with chemical structure.	Environmental Toxicology and Chemistry. 25(11):2937-2945.	Ratios in aquatic toxicity.
2006	Alizadeh M., Ota F., Hosoi K., Kato M., Sakai T., Satter M. A.	Altered allergic cytokine and antibody response in mice treated with Bisphenol A.	J Med Invest. 53(1-2):70-80.	Mammalian study.
2006	Alonso-Magdalena P., Morimoto S., Ripoll C., Fuentes E., Nadal A.	The estrogenic effect of bisphenol A disrupts pancreatic □-cell function *in vivo* and induces insulin resistance.	Environ Health Perspect. 114(1):106-12.	Mammalian study
2006	Amari S., Aizawa M., Zhang J., Fukuzawa K., Mochizuki Y., Iwasawa Y., Nakata K., Chuman H., Nakano T.	VISCANA: Visualized cluster analysis of protein-ligand interaction based on the *ab initio* fragment molecular orbital method for virtual ligand screening.	J Chem Information and Modeling. 46(1):221-230.	QSAR
2006	Anahara R., Yoshida M., Toyama Y., Maekawa M., Kai M., Ishino F., Toshimori K., Mori C.	Estrogen agonists, 17□-estradiol, bisphenol A, and diethylstilbestrol, decrease cortactin expression in the mouse testis.	Arch Histol Cytol. 69(2):101-7.	Mammalian study, gene expression in mice.
2006	Ankley, G. T. and Villeneuve, D. L.	The fathead minnow in aquatic toxicology: Past, present and future.	Aquatic Toxicology. 78(1):91-102.	Review
2006	Apraiz, I., Mi, J., Bourin, S., and Cristobal, S.	Peroxisomal proteomics reveals a protein expression signature of exposure to several environmental pollutants.	Marine Environmental Research. 62(Supplement S):S38-S39.	Paper deals with proteomics.
2006	Apraiz I., Mi J., Cristobal S.	Identification of proteomic signatures of exposure to marine pollutants in mussels (*Mytilus edulis*).	Mol Cell Proteomics. 5(7):1274-85.	Proteomic study

Year	Authors	Title	Reference	Notes
2006	Awais M., Sato M., Lee X., Umezawa Y.	A fluorescent indicator to visualize activities of the androgen receptor ligands in single living cells.	Angewandte Chemie, Int Ed. 45(17):2707-2712.	Analytical chemistry paper.
2006	Arbeli, Z., Ronen, Z., and Diaz-Baez, M. C.	Reductive dehalogenation of tetrabromobisphenol-A by sediment from a contaminated ephemeral streambed and an enrichment culture.	Chemosphere. 64(9):1472-1478.	Addressed in TBBPA assessment.
2006	Auriol, M., Filali-Meknassi, Y., Adams, C. D., and Tyagi, R. D.	Natural and synthetic hormone removal using the horseradish peroxidase enzyme: Temperature and pH effects.	Water Research. 40(15):2847-2856.	Water purification.
2006	Auriol, M., Filali-Meknassi, Y., Adams, C. D., and Tyagi, R.D.	Comparative study of reactions of endocrine disruptors bisphenol A and diethylstilbestrol in electrochemical treatment and chlorination.	Water Research. 40(5):1070-1078.	Water purification
2006	Auriol, M., Filali-Meknassi, Y., Adams, C. D., and Tyagi, R.D.	Photodecomposition of bisphenol A on nanometer-sized TiO2 thin film and the associated biological toxicity to zebrafish (*Danio rerio*) during and after photocatalysis.	Water Research. 40(9):1906-1914.	Non-environmental degradation.
2006	Ballesteros O., Zafra A., Navalon A., Vilchez J. L. J	Sensitive gas chromatographic-mass spectrometric method for the determination of phthalate esters, alkylphenols, bisphenol A and their chlorinated derivatives in wastewater samples.	Chromatography A. 1121(2):154-162.	Wastewater samples.
2006	Barber L. B., Keefe S. H., Antweiler R. C., Taylor H. E., Wass R. D.	Accumulation of contaminants in fish from wastewater treatment wetlands.	E S & T. 40(2):603-611.	Analysis of water and fish from US wastewater treatment wetlands.
2006	Barsiene, J., Dedonyte, V., Rybakovas, A., Andreikenaite, L., and Andersen, O. K.	Investigation of micronuclei and other nuclear abnormalities in peripheral blood and kidney of marine fish treated with crude oil.	Aquatic Toxicology. 78(Supplement 1):S99-S104.	Mutagenicity.
2006	Barsiene J., Syvokiene J., Bjornstad A.	Induction of micronuclei and other nuclear abnormalities in mussels exposed to bisphenol A, diallyl phthalate and tetrabromodiphenyl ether-47.	Aquat Toxicol. 78 Suppl 1:S105-8.	Mutagenicity study
2006	Beck I. -C.	Estrogens in coastal surface waters - investigations in the Baltic Sea using chemical analysis and an *in vitro*-bio assay.	Available Metadata on Internet Documents, Order No. 362572 From: Metadata Internet Doc. No pp. given.	Analytical paper
2006	Beck I. -C., Bruhn R., Gandrass J.	Analysis of estrogenic activity in coastal surface waters of the Baltic Sea using the yeast estrogen screen.	Chemosphere. 63(11):1870-1878.	Analysis by yeast screening.
2006	Benigni A., Zoja C., Tomasoni S., Campana M., Corna D., Zanchi C., Gagliardini E., Garofano E., Rottoli D., Ito T., Remuzzi G.	Transcriptional regulation of nephrin gene by peroxisome proliferator-activated receptor-gamma agonist: molecular mechanism of the antiproteinuric effect of pioglitazone.	J Am Soc Nephrol. 17(6):1624-32.	Gene regulation.
2006	Berkowitz G.	Limitations of a case-control study on bisphenol A (BPA) serum levels and recurrent miscarriage.	Hum Reprod. 21(2):565-6; author reply 566-7. [No abstract available].	Human health study.

Year	Authors	Title	Reference	Notes
2006	Biau, S., Bayle, S., de Santa Barbara, P., and Roig, B.	The chick embryo: an animal model for detection of the effects of hormonal compounds.	Analytical and Bioanalytical Chemistry. In Press.	Animal model.
2006	Blazso M., Czegeny Z.	Catalytic destruction of brominated aromatic compounds studied in a catalyst microbed coupled to gas chromatography/mass spectrometry.	J Chromatogr A. May 30; [Epub ahead of print].	Non-environmental degradation
2006	Blomqvist, A., Berg, C., Holm, L., Brandt, I., Ridderstrale, Y., and Brunstrom, B.	Defective reproductive organ morphology and function in domestic rooster embryonically exposed to o.p'-DDT or ethynylestradiol.	Biology of Reproduction. 74(3):481-486.	Paper deals with o.p'-DDT and ethynylestradiol.
2006	Boas M., Feldt-Rasmussen U., Skakkebaek N. E., Main K. M.	Environmental chemicals and thyroid function.	Euro J Endocrin. 154(5):599-611.	Mammalian study.
2006	Bolognesi C., Perrone E., Roggieri P., Pampanin D. M., Sciutto A.	Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions.	Aquat Toxicol. 78 Suppl 1:S93-8.	Assesses predictive value of biomarker for marine pollution.
2006	Botolin S., McCabe L. R.	Inhibition of PPARgamma prevents type I diabetic bone marrow adiposity but not bone loss.	J Cell Physiol. Sep 13; [Epub ahead of print].	Cellular study.
2006	Brenner A., Mukmenev I., Abeliovich A., Kushmaro A.	Biodegradability of tetrabromobisphenol A and tribromophenol by activated sludge.	Ecotoxicology. 15(4):399-402.	Addressed in TBBPA assessment.
2006	Burlando B., Berti E., Viarengo A.	Effects of seawater pollutants on protein tyrosine phosphorylation in mussel tissues.	Aquat Toxicol. 78 Suppl 1:S79-85.	Biochemical study
2006	Buterin T., Koch C., Naegeli H.	Convergent transcriptional profiles induced by endogenous estrogen and distinct xenoestrogens in breast cancer cells.	Carcinogenesis. 27(8):1567-78.	Human cells.
2006	Cajaraville M. P., Ortiz- Zarragoitia M.	Specificity of the peroxisome proliferation response in mussels exposed to environmental pollutants.	Aquat Toxicol. 78 Suppl 1:S117-23.	Biomarkers. Info covered in another paper by same authors.
2006	Calafat A. M., Ye X., Silva M. J., Kuklenyik Z., Needham L. L.	Human exposure assessment to environmental chemicals using biomonitoring.	Int J Androl. 29(1):166-71; discussion 181-5. Review.	Biomonitoring for human exposure.
2006	Campbell, P. M., Fernandez, M. P., Royston, S., Smith, J. L., van Poppelen, P., Ikonomou, M. G., and Devlin, R. H.	Male coho salmon (*Oncorhynchus kisutch*) exposed to a time-course of urban sewage effluent exhibit a sporadic low incidence of sex reversal and intersex.	Water Quality Research Journal of Canada. 41(3):235-243.	Sewage effluent.
2006	Cargouet M., Bimbot M., Levi Y., Perdiz D.	Xenoestrogens modulate genotoxic (UVB) induced cellular responses in estrogen receptors positive human breast cancer cells.	Env Tox Pharma. 22(1):104-112.	Mammalian study.
2006	Chen P. J., Kullman S. W., Hinton D. E., Linden K. G.	Comparisons of polychromatic and monochromatic UV-based treatments of bisphenol-A in water via toxicity assessments.	Chemosphere. In Press.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2006	Chen P. J., Linden K. G., Hinton D. E., Kashiwada S., Rosenfeldt E. J., Kullman S. W.	Biological assessment of bisphenol A degradation in water following direct photolysis and UV advanced oxidation.	Chemosphere. 65(7):1094-102.	Water purification
2006	Chiu S. J., Chen S. H., Tsai C. T.	Effect of metal chlorides on thermal degradation of (waste) polycarbonate.	Waste Manag. 26(3):252-9.	Degradation of plastics.
2006	Choi K. J., Kim, S. G., Kim C. W., Park J. K.	Removal efficiencies of endocrine disrupting chemicals by coagulation, flocculation, ozonation, powdered/granular activated carbon adsorption, and chlorination.	Korean J Chem Eng. 23(3):399-408.	Water purification.
2006	Chu C. Y., Ponten A., Sun C. C., Jee S. H.	Concomitant contact allergy to the resins, reactive diluents and hardener of a bisphenol A/F-based epoxy resin in subway construction workers.	Contact Dermatitis. 54(3):131-9.	Health study.
2006	Colosi L. M., Huang Q., Weber W. J. Jr.	Quantitative structure-activity relationship based quantification of the impacts of enzyme-substrate binding on rates of peroxidase-mediated reactions of estrogenic phenolic chemicals.	J Am Chem Soc. 128(12):4041-4047.	Mammalian cells.
2006	Cong, L., Qin, Z-F., Jing, X.-N., Yang, L., Zhou, J.-M., and Xu, X.-B.	*Xenopus laevis* is a potential alternative model animal species to study reproductive toxicity of phytoestrogens.	Aquatic Toxicology. 77(3):250-256.	BPA not mentioned in abstract.
2006	Cui S., Liu S., Yang J., Wang X., Wang L.	Quantitative structure-activity relationship of estrogen activities of bisphenol A analogs.	Chinese Sci Bull. 51(3):287-292.	QSAR study.
2006	Daftary G. S., Taylor H. S.	Endocrine regulation of HOX genes.	Endocr Rev. 27(4):331-55.	Gene regulation study.
2006	Daidoji T., Kaino T., Iwano H., Inoue H., Kurihara R., Hashimoto S, Yokota H.	Down regulation of bisphenol A glucuronidation in carp during the winter pre-breeding season.	Aquat Toxicol. 77(4):386-92.	Biochemical study
2006	Dash C., Marcus M., Terry P. D.	Bisphenol A: Do recent studies of health effects among humans inform the long-standing debate?	Mutat Res. Jun 6; [Epub ahead of print]. No abstract available.	Human health review.
2006	Della Seta D., Minder I., Belloni V., Aloisi A. M., Dessi-Fulgheri F., Farabollini F.	Pubertal exposure to estrogenic chemicals affects behaviour in juvenile and adult male rats.	Horm Behav. 50(2):301-7.	Mammalian study
2006	Deng L., Liu Y. -X., Chen P. -Y., Wang L., Deng N. -S.	Determination of trace bisphenol A in leachate by solid phase microextraction coupled with high performance liquid chromatography.	Anal Lett. 39(2):395-404.	Analytical paper.
2006	Dhooge W., Arijs K., D'Haese I., Stuyvaert S., Versonnen B., Janssen C., Verstraete W., Comhaire F.	Experimental parameters affecting sensitivity and specificity of a yeast assay for estrogenic compounds: results of an interlaboratory validation exercise.	Anal Bioanal Chem. Aug 3; [Epub ahead of print].	Method validation.

Year	Authors	Title	Reference	Notes
2006	Dietrich D. R., O'Brien E., Hoffmann S., Balaguer P., Nicolas J. C., Seinen W., Depledge M.	Effects of BPA in snails.	Environ Health Perspect. 114(6):A340-1. No abstract available.	Comments on Oehlmann *et al*. 2006
2006	Dlubek G., Hassan E. M., Krause-Rehberg R., Pionteck J.	Free volume of an epoxy resin and its relation to structural relaxation: evidence from positron lifetime and pressure-volume-temperature experiments.	Phys Rev E Stat Nonlin Soft Matter Phys. 73(3 Pt 1): 031803.	Polymers.
2006	Dondero, F., Dagnino, A., Jonsson, H., Capri, F., Gastaldi, L., and Viarengo, A.	Assessing the occurrence of a stress syndrome in mussels (*Mytilus edulis*) using a combined biomarker/gene expression approach.	Aquatic Toxicology. 78(Supplement 1):S13-S24.	Gene expression and biomarkers.
2006	Dong, J., Li, F.-B., Lan, C.-Y., Liu, C.-S., Li, X.-M., and Luan, T.-G.	Dependence of bisphenol A photodegradation on the initial concentration of oxalate in the lepidocrocite-oxalate complex system.	Journal of Environmental Sciences-China. 18(4):777-782.	Non-environmental degradation.
2006	Duan, Z.-H. and Zhu, L.	Toxicity of bisphenol A on the growth of zebrafish embryos.	Acta Hydrobiologica Sinica. 30(6):638-642.	Toxicity of BPA to fish embryos. Few details available.
2006	Duan, Z.-H., Min, Z., and Lin, Z.	Toxicity of joint action of pentachlorophenol and bisphenol A on the growth of zebrafish (*Brachydanio rerio*) embryo.	China Environmental Science. 26(Suppl.):121-124.	Mixture toxicity.
2006	DuMond J. W. Jr., Singh K. P., Roy D.	Development of a self-proliferating leydig cell line: a hyper-sensitive E-screening model.	Oncology Reports. 16(1):73-77.	Cell line study.
2006	Endo, Y., Kimura, N., Ikeda, I., Fujimoto, K., and Kimoto, H.	Adsorption of bisphenol A by lactic acid bacteria, *Lactococcus*, strains.	Applied Microbiology and Biotechnology. In Press.	Bacterial study.
2006	Engel S. M., Levy B., Liu Z., Kaplan D., Wolff M. S.	Xenobiotic phenols in early pregnancy amniotic fluid.	Reprod Toxicol. 21(1):110-2.	Mammalian study.
2006	Fujimoto T., Kubo K., Aou S.	Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats.	Brain Res. 1068(1):49-55.	Mammalian study.
2006	Furuichi, T., Kannan, K., Suzuki, K., Tanaka, S., Giesy, J. P., and Masunaga, S.	Occurrence of estrogenic compounds in and removal by a swine farm waste treatment plant.	Environmental Science and Technology. 40(24):7896 -7902.	Water purification.
2006	Gee, D.	Late lessons from early warnings for EDSs.	Environmental Health Perspectives. 114(Supplement 1):152-160.	Review
2006	George, S. G., Diab, A., Sabine, V., and Chipman, K.	A genomic analysis of pollutant response in liver of flounders from two highly polluted European estuaries.	Marine Environmental Research. 62(Supplement S):S165-S166.	Genomic analysis
2006	Gerecke, A. C., Giger, W., Hartmann, P. C., Heeb, N. V., Kohler, H.-P. E., Schmid, P., Zennegg, M., and Kohler, M.	Anaerobic degradation of brominated flame retardants in sewage sludge.	Chemosphere. 64(2):311-317.	Addressed in TBBPA assessment.

Year	Authors	Title	Reference	Notes
2006	Gheduzzi S., Webb J. J., Miles A. W.	Mechanical characterisation of three percutaneous vertebroplasty biomaterials.	J Mater Sci Mater Med. 17(5):421-6.	Biopolymers.
2006	Gomez M. J., Martinez Bueno M. J,. Lacorte S., Fernandez-Alba A. R., Aguera A.	Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast.	Chemosphere. 66(6):993-1002.	Sewage treatment plant.
2006	Gomez M. J., Mezcua M., Martinez M. J., Fernandez-Alba A. R., Aguera A.	A new method for monitoring oestrogens, N-octylphenol, and bisphenol A in wastewater treatment plants by solid-phase extraction-gas chromatography-tandem mass spectrometry.	International Journal of Environmental Analytical Chemistry. 86(1-2):3-13.	Wastewater treatment plant.
2006	Gomiero A., Pampanin D. M., Bjornstad A., Larsen B. K., Provan F., Lyng E., Andersen O. K.	An ecotoxicoproteomic approach (SELDI-TOF mass spectrometry) to biomarker discovery in crab exposed to pollutants under laboratory conditions.	Aquat Toxicol. 78 Suppl 1:S34-41.	Biomarkers in crabs.
2006	Goodman J. E., McConnell E. E., Sipes I. G., Witorsch R. J., Slayton T. M., Yu C. J., Lewis A. S., Rhomberg L. R.	An updated weight of the evidence evaluation of reproductive and developmental effects of low doses of bisphenol A.	Crit Rev Toxicol. 36(5):387-457.	Mammalian study
2006	Gross-Sorokin, M. Y., Roast, S. D., and Brighty, G. C.	The Environment Agency's assessment of feminisation of male fish in English rivers.	Environmental Health Perspectives. 114(Supplement 1):147-151.	Review.
2006	Hahn T., Tag K., Riedel K., Uhlig S., Baronian K., Gellissen G., Kunze G.	A novel estrogen sensor based on recombinant *Arxula adeninivorans* cells.	Biosensors & Bioelectronics. 21(11):2078-2085.	Biosensors.
2006	Hamerton I., Howlin B. J., Mitchell A. L.	Developing poly(bis-benzoxazines) with improved fracture toughness. 1: Using molecular simulation to determine and predict structure-property relationships.	Reactive & Functional Polymers. 66(1):21-39.	Polymers.
2006	Han N., Liu Z., Jin L., Yue Y.	Preparation and characterization of poly(silyl ester)s containing 2,2-bis(p-dimethylsiloxy-phenyl)propane units in the polymer backbones.	App Polymer Sci. 101(3):1937-1942.	Polymers.
2006	Harbers, J. V., Huuijbregts, M. A. J., Posthuma, L., and van de Meent, D.	Estimating the impact of high-production-volume chemicals on remote ecosystems by toxic pressure calculation.	Environmental Science and Technology. 40(5):1573-1580.	Risk assessment methodology.
2006	Hastwell P. W., Chai, L. -L., Roberts K. J., Webster T. W., Harvey J. S., Rees R. W., Walmsley R. M.	High-specificity and high-sensitivity genotoxicity assessment in a human cell line: validation of the GreenScreen HC GADD45a-GFP genotoxicity assay.	Mut Res. 607(2):160-175.	Study carried out with human cells.
2006	Hattori K., Inoue M., Arai H., Tamura H. O.	A novel sulfotransferase abundantly expressed in the dauer larvae of *Caenorhabitis elegans*.	J Biochen (Tokyo). 139(3):355-62	Biochemistry.
2006	Hayden O., Podlipna D., Chen X., Krassnig S., Leidl A., Dickert F. L.	Nanolithography and subnanomolecular interactions for biomimetic sensors.	Mat Sci &Eng C: Biom and Supramolecular Systems. 26(5-7): 924-928.	Materials science.

Year	Authors	Title	Reference	Notes
2006	Hecker M., Murphy M. B., Coady K. K., Villeneuve D. L., Jones P. D., Carr J. A., Solomon K. R., Smith, E. E., Van Der Kraak G., Gross T., Du Preez L., Kendall R. J., Giesy J. P.	Terminology of gonadal anomalies in fish and amphibians resulting from chemical exposures.	Rev of Env Contam Tox. 187:103-131.	Methodology paper.
2006	Helsel, D. R.	Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it.	Chemosphere. 65(11):2434-2439.	Data analysis.
2006	Hess-Wilson J. K., Boldison J., Weaver K. E., Knudsen K. E.	Xenoestrogen action in breast cancer: impact on ER-dependent transcription and mitogenesis.	Breast Cancer Res Treat. 96(3):279-92.	Human health study.
2006	Hillen U., Jappe U., Frosch P. J., Becker D., Brasch J., Lilie M., Fuchs T., Kreft B., Pirker C., Geier J; German Contact Dermatitis Research Group.	Late reactions to the patch-test preparations para-phenylenediamine and epoxy resin: a prospective multicentre investigation of the German Contact Dermatitis Research Group.	Br J Dermatol. 154(4):665-70.	Human health study.
2006	Hiroi T., Okada K., Imaoka S., Osada M., Funae Y.	Bisphenol A binds to protein disulfide isomerase and inhibits its enzymatic and hormone-binding activities.	Endocrinology. 147(6):2773-80.	Binding activity in rats.
2006	Hjelmborg, P. S., Ghisari M., Bonefeld-Jorgensen E. C.	SPE-HPLC purification of endocrine-disrupting compounds from human serum for assessment of xenoestrogenic activity.	Anal and Bioanal Chem. 385(5):875-887.	Analytical paper.
2006	Ho S. M., Tang W. Y., Belmonte de Frausto J., Prins G. S.	Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4.	Cancer Res. 66(11):5624-32.	Human health study.
2006	Honkanen, J. O. and Kukkonen, J. V. K.	Environmental temperature changes uptake rate and bioconcentration factors of bisphenol A in tadpoles of *Rana temporaria*.	Environmental Toxicology and Chemistry. 25(10):2804-2808.	Similar information included.
2006	Honma T., Miyagawa M., Suda M., Wang R. S., Kobayashi K., Sekiguchi S.	Effects of perinatal exposure to bisphenol A on brain neurotransmitters in female rat offspring.	Ind Health. 44(3):510-24.	Mammalian study.
2006	Hoppin J. A., Ulmer R., Calafat A. M., Barr D. B., Baker S. V., Meltzer H. M., Ronningen K. S.	Impact of urine preservation methods and duration of storage on measured levels of environmental contaminants.	J Expo Sci Environ Epidemiol. 16(1):39-48.	Levels in human urine.
2006	Houtman C. J., Van Houten Y. K., Leonards P. E., Brouwer A., Lamoree M. H., Legler J.	Biological validation of a sample preparation method for ER-CALUX bioanalysis of estrogenic activity in sediment using mixtures of xeno-estrogens.	Environ Sci Technol. 40(7):2455-61.	Method for bioanalysis of estogenic activity.
2006	Hu J. Y., Chen X., Jin X., Tan X. L.	Effect of chlorination on estrogenicity in chlorinated treated effluent.	Water Sci & Tech: Water Supply. 6(2):185-191.	Water purification.
2006	Huerta G., Fomina L., Rumsh L., Zolotukhin M. G.	New polymers with N-phenylpyrrole fragments obtained by chemical modifications of diacetylene containing-polymers.	Polymer Bulletin (Heidelberg, Germany). 57(4):433-443.	Polymers.

Year	Authors	Title	Reference	Notes
2006	Hughes C.	Bisphenol A: Acute Toxicity to the Giant Ramshorn Snail, *Marisa cornuarietis*, determined under static-renewal test conditions at 22°C.	Unpublished report, ABC Laboratories Study number 50091, ABC Laboratories, Columbia, MO.	Acute tox for snails would not effect outcome. Chronic exposure only discussd in RAR.
2006	Huschek, G. and Hansen, P.-D.	Ecotoxicological classification of the Berlin river system using bioassays in respect to the European water framework directive.	Environmental Monitoring and Assessment. 121(1-3):15-31.	Bioassays and classification.
2006	Hutchinson, T. H, Shillabeer, N., Winter, M. J., and Pickford, D. B.	Acute and chronic effects of carrier solvents in aquatic organisms: A critical review.	Aquatic Toxicology. 76(1):69-92.	Review.
2006	Hutchinson, T. H., Ankley, G. T., Segner, H., and Tyler, C. R.	Screening and testing for endocrine disruption in fish - biomarkers as signposts not traffic lights in risk assessment.	Environmental Health Perspectives. 114(Suppl 1):106-114.	Review
2006	Hwang, H.-M., Green, P. G., and Young, T. M.	Tidal salt marsh sediment in California, USA. Part 1: Occurrence and sources of organic contaminants.	Chemosphere. 64(8):1383-1392.	Levels in US
2006	Iguchi, T., Watanabe, H., and Katsu, Y.	Application of ecotoxicogenomics for studying endocrine disruption in vertebrates and invertebrates.	Environmental Health Perspectives. 114(Supplement 1):101-105.	Genomic study.
2006	Ike M., Chen M. Y., Danzl E., Sei K., Fujita M.	Biodegradation of a variety of bisphenols under aerobic and anaerobic conditions.	Water Sci Technol. 53(6):153-9.	Weight of evidence is that little or no degradation occurs under anaerobic conditions.
2006	Inoue, K., Yoshida, S., Nakayama, S., Ito, R., Okanouchi, N., and Nakazawa, H.	Development of stable isotope dilution quantification liquid chromatography-mass spectrometry method for estimation of exposure levels of bisphenol A, 4-*tert*-octylphenol, 4-nonylphenol, tetrabromobisphenol A, and pentachlorophenol in indoor air.	Archives of Environmental Contamination and Toxicology. 51(4):503-508.	Analytical paper.
2006	Ioan, I., Wilson, S., Lundanes, E., and Neculai, A.	Comparison of Fenton and sono-Fenton bisphenol A degradation.	Journal of Hazardous Materials. In Press.	Non-environmental degradation.
2006	Irmak, S., Erbatur, O., and Kusvuran, E.	Degradation of alkylphenolic compounds in aqueous media by Fenton and ultraviolet induced Fenton reaction.	Fesenius Environmental Bulletin. 15(12B):1601-1606.	Non-environmental degradation.
2006	Ishibashi, H., Hirano, M., Matsumura, N., Watanabe, N., Takao, Y., and Arizono, K.	Reproductive effects and bioconcentration of 4-nonylphenol in medaka fish (*Oryzias latipes*).	Chemosphere. 65(6):1019-1026.	Paper deals with 4-nonylphenol.
2006	Iso T., Watanabe T., Iwamoto T., Shimamoto A., Furuichi Y.	DNA damage caused by bisphenol A and estradiol through estrogenic activity.	Biol Pharm Bull. 29(2):206-10.	Effects of estrogenic activity.
2006	Itoh N., Tao H., Ibusuki T.	In-tube silylation in combination with thermal desorption gas chromatography-mass spectrometry for the determination of hydroxy polycyclic aromatic hydrocarbons in water.	Analytica Chimica Acta. 555(2):201-209.	Analytical paper.

Year	Authors	Title	Reference	Notes
2006	Iwamuro S., Yamada M., Kato M., Kikuyama S.	Effects of bisphenol A on thyroid hormone-dependent up-regulation of thyroid hormone receptor alpha and beta and down-regulation of retinoid X receptor gamma in *Xenopus* tail culture.	Life Sci. Jul 20; [Epub ahead of print].	Other similar results already included
2006	Iwasaki S., Sakka M., Kimura T., Sakka K., Matsuda T., Matsui S.	Current status of estrogenlike compounds in sediments in enclosed sea areas.	ASTM Special Tech Pub, STP 1482 (Contam Sed: Evaluation and Remediation Tech), 68-78.	Review.
2006	Jaballah N., Trad H., Majdoub M., Jouini M., Roussel J., Fave J. L.	Synthesis and characterization of new blue-photoluminescent copolymer derived from bisphenol A.	J App Polymer Sci. 99(6):2997-3004.	Polymers.
2006	James, M. O., Faux, L. R., Stuchal, L. D., Sacco, J. C., and Wang, L.	Interaction of environmental chemicals with glucuronidation pathways in channel catfish.	Marine Environmental Research. 62(Supplement S):S66.	Biochemistry
2006	Jiang M., Zhang J. H., Mei S. R., Shi Y., Zou L. J., Zhu Y. X., Dai K., Lu B.	Direct enrichment and high performance liquid chromatography analysis of ultra-trace Bisphenol A in water samples with narrowly dispersible Bisphenol A imprinted polymeric microspheres column.	J Chromatogr A. 1110(1-2):27-34.	Analytical paper.
2006	Jondeau A., Dahbi L., Bani-Estivals M. -H., Chagnon M. -C.	Evaluation of the sensitivity of three sublethal cytotoxicity assays in human HepG2 cell line using water contaminants.	Toxicology. 226(2-3):218-228.	Cell line study.
2006	Jonsson H., Schiedek D., Goksoyr A., Grosvik B. E.	Expression of cytoskeletal proteins, cross-reacting with anti-CYP1A, in *Mytilus* sp. exposed to organic contaminants.	Aquat Toxicol. 78 Suppl 1:S42-8.	Gene expression.
2006	Jonsson, H., Schiedek, D., Grøsvik, B. E., and Goksøyr, A.	Protein responses in blue mussels (*Mytilus edulis*) exposed to organic pollutants: A combined CYP-antibody/proteomic approach.	Aquatic Toxicology. 78(Supplement 1):S49-S56.	Biochemistry study .
2006	Kallivretaki E., Eggen R., Neuhauss S., Alberti M., Kausch U., Segner H.	Aromatase in zebrafish: a potential target for endocrine disrupting chemicals.	Marine Env Res. 62(Suppl.):S187-S190.	Biochemistry study.
2006	Kaneko, M., Okada, R., Nakada, T., Yamamoto, K., Nakamura, M., and Kikuyama, S.	Thyroid hormone and bisphenol A act differently on the pituitary to suppress the release of thyrotropin.	Frontiers in Neuroendocrinology. 27(1):155.	Mammalian study.
2006	Kang, J. H. and Kondo, F.	Distribution and biodegradation of bisphenol A in water hyacinth.	Bull of Env Contamination and Tox. 77(4):500-507.	Water purification
2006	Kang J. H, Kondo F., Katayama Y.	Human exposure to bisphenol A.	Toxicology. 226(2-3):79-89.	Human exposure paper.
2006	Kang J. H., Kondo F.	Bisphenol A in the surface water and freshwater snail collected from rivers around a secure landfill.	Bull Environ Contam Toxicol. 76(1):113-8. No abstract available].	Levels in Japan
2006	Kang J. -H., Kondo F., Katayama Y.	Importance of control of enzymatic degradation for determination of bisphenol A from fruits and vegetables.	Analytica Chimica Acta. 555(1):114-117.	Analytical paper.
2006	Kanoh N., Kyo M., Inamori K., Ando A., Asami A., Nakao A., Osada H.	SPR Imaging of Photo-Cross-Linked Small-Molecule Arrays on Gold.	Anal Chem. 78(7):2226-2230.	Polymers.

Year	Authors	Title	Reference	Notes
2006	Kato H., Furuhashi T., Tanaka M., Katsu Y., Watanabe H., Ohta Y., Iguchi T.	Effects of bisphenol A given neonatally on reproductive functions of male rats.	Reprod Toxicol. 22(1):20-9.	Mammalian study.
2006	Kawaguchi M., Ito R., Endo N., Okanouchi N., Sakui N., Saito K., Nakazawa H.	Liquid phase microextraction with in situ derivatization for measurement of bisphenol A in river water sample by gas chromatography-mass spectrometry.	J Chromatogr A. 1110(1-2):1-5.	Analytical paper.
2006	Killinger D., Sivaprakasam V.	Water monitoring with laser fluorescence.	Optics & Photonics News. 17(1):34-39.	Analytical paper.
2006	Kim K. Y., Cheon H. G.	Antiangiogenic effect of rosiglitazone is mediated via peroxisome proliferator-activated receptor gamma-activated maxi-K channel opening in human umbilical vein endothelial cells.	J Biol Chem. 281(19):13503-12.	Mammalian study.
2006	Kim Y. J., Nicell J. A.	Impact of reaction conditions on the laccase-catalyzed conversion of bisphenol A.	Bioresource Technol. 97(12):1431-42.	Non-environmental degradation.
2006	Kim Y.-J., Nicell J. A.	Laccase-catalyzed oxidation of bisphenol A with the aid of additives.	Process Biochem. 41(5):1029-1037.	Non-environmental degradation.
2006	Kinney, C. A., Furlong, E. T., Zaugg, S. D., Burkhardt, M. R., Werner, S. L., Cahill, J. D., and Jorgensen, G. R.	Survey of organic wastewater contaminants in biosolids destined for land application.	Environmental Science & Technology. 40(23):7207-7215. (Supporting information in Summary column)	Survey of wastewater contaminants.
2006	Kitajima M., Hatanaka S. I., Hayashi S.	Mechanism of O(2)-accelerated sonolysis of bisphenol A.	Ultrasonics. Jun 5; [Epub ahead of print].	Non-environmental degradation.
2006	Kitano T., Koyanagi T., Adachi R., Sakimura N., Takamune K., Abe S. -I.	Assessment of estrogenic chemicals using an estrogen receptor beta(ERalpha) and ERbeta-mediated reporter gene assay in fish.	Marine Biol. 149(1):49-55.	*In vitro* reporter gene assay.
2006	Koh C. H., Khim J. S., Villeneuve D. L., Kannan K., Giesy J. P.	Characterization of trace organic contaminants in marine sediment from Yeongil Bay, Korea: 1. Instrumental analyses.	Environ Pollut. 142(1):39-47.	Levels in marine sediment, non-EU.
2006	Kontominas M. G., Goulas A. E., Badeka A. V., Nerantzaki A.	Migration and sensory properties of plastics-based nets used as food-contacting materials under ambient and high temperature heating conditions.	Food Add & Contam. 23(6):634-641.	Food contact migration.
2006	Korshin G. V., Kim J., Gan L.	Comparative study of reactions of endocrine disruptors bisphenol A and diethylstilbestrol in electrochemical treatment and chlorination.	Water Res. 40(5):1070-8.	Non-environmental degradation.
2006	Kricheldorf H. R., Boehme S., Schwarz G.	Competing cyclization and chain Growth in *tert*-amine-catalyzed polycondensations of bisphenol A with bisphenol A bischloroformate.	Macromolecules. 39(9):3210-3216.	Polymers.
2006	Kudo Y., Yamauchi K., Fukazawa H., Terao Y.	*In vitro* and *in vivo* analysis of the thyroid system-disrupting activities of brominated phenolic and phenol compounds in *Xenopus laevis*.	Tox Sci. 92(1):87-95.	Paper deals with brominated bisphenol A.

Year	Authors	Title	Reference	Notes
2006	Kuo S. -W., Huang C. -F., Tung Y. -C., Chang F. -C.	Effect of bisphenol A on the miscibility, phase morphology, and specific interaction in immiscible biodegradable poly(beta-caprolactone)/poly(L-lactide) blends.	J Appl Polymer Sci. 100(2): 1146-1161.	Polymers.
2006	Kuruto-Niwa R., Tateoka Y., Usuki Y., Nozawa R.	Measurement of bisphenol A concentrations in human colostrum.	Chemosphere. Aug 10; [Epub ahead of print].	Human health paper.
2006	Kwon J. -H., Liljestrand H. M., Katz L. E.	Partitioning of moderately hydrophobic endocrine disruptors between water and synthetic membrane vesicles.	Env Tox and Chem. 25(8):1984-1992.	Non-environmental partitioning.
2006	Ladewig V., Jungmann D., Koehler H. -R., Schirling M., Triebskorn R., Nagel R.	Population structure and dynamics of *Gammarus fossarum* (Amphipoda) upstream and downstream from effluents of sewage treatment plants.	Arch Env Contam and Tox. 50(3):370-383.	No specific BPA studies reported in this paper.
2006	Ladewig, V., Jungmann, D., Koehler, H.-R., Licht, O., Ludwichowski, K.-U., Schirling, M., Triebskorn, R., and Nagel, R.	Effects of bisphenol A on *Gammarus fossarum* and *Lumbriculus variegatus* in artificial indoor streams.	Toxicological and Environmental Chemistry. 88(4):649-664.	Similar information included.
2006	Landis F. A., Stephens J. S., Cooper J. A., Cicerone M. T., Lin-Gibson S.	Tissue engineering scaffolds based on photocured dimethacrylate polymers for *in vitro* optical imaging.	Biomacromolecules. 7(6):1751-7.	Polymers.
2006	Larsen B. K., Bjornstad A., Sundt R. C., Taban I. C., Pampanin D. M., Andersen O. K.	Comparison of protein expression in plasma from nonylphenol and bisphenol A-exposed Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) by use of SELDI-TOF.	Aquat Toxicol. 78 Suppl 1:S25-33.	Paper looks at protein expression.
2006	Lavado, R., Janer, G., and Porte, C.	Steroid levels and steroid metabolism in the Mussel *Mytilus edulis*: The modulating effect of dispersed crude oil and alkylphenols.	Aquatic Toxicology. 78(Supplement 1):S65-S72.	Mixture toxicity.
2006	Lee, C. J. and Rasmussen, T. J.	Occurrence of organic wastewater compounds in effluent-dominated streams in Northeastern Kansas.	Science of the Total Environment. 371(1-3):258-269.	Effluent in the USA.
2006	Lee, S.-B. and Choi, J.	Effects of bisphenol A and ethynyl estradiol exposure on enzyme activities, growth and development in the fourth instar larvae of *Chironomus riparius* (Diptera, Chironomidae).	Ecotoxicology and Environmental Safety. In Press.	May need to order in the future.
2006	Lee, Y. M., Jung, S. O., Kim, I. C., and Lee, J. S.	The hermaphroditic fish *Rivulus marmoratus* (Cyprinodontiformes, Rivulidae): A model species for molecular and environmental toxicogenomics.	Marine Environmental Research. 62(Supplement S):S178-S179.	Paper deals with nonylphenol.
2006	Lee H. -S., Sasagawa S. -I., Kato S., Fukuda R., Horiuchi H., Ohta A.	Yeast two-hybrid detection systems that are highly sensitive to a certain kind of endocrine disruptors.	Bioscience, Biotechnology, and Biochemistry. 70(2):521-524.	Bioassay.
2006	Lee J. -W., Kwon T. -O., Thiruvenkatachari R., Moon I. -S.	Adsorption and photocatalytic degradation of bisphenol A using TiO_2 and its separation by submerged hollowfiber ultrafiltration membrane.	J Env Sci. 18(1):193-200.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2006	Lee S. M., Lee S. B., Park C. H., Choi J.	Expression of heat shock protein and hemoglobin genes in *Chironomus tentans* (Diptera, chironomidae) larvae exposed to various environmental pollutants: A potential biomarker of freshwater monitoring.	Chemosphere. 65(6):1074-81.	Gene expression assay.
2006	Lee Y. M., Seo J. S., Kim I. C., Yoon Y. D., Lee J. S.	Endocrine disrupting chemicals (bisphenol A, 4-nonylphenol, 4-*tert*-octylphenol) modulate expression of two distinct cytochrome P450 aromatase genes differently in gender types of the hermaphroditic fish *Rivulus marmoratus*.	Biochem Biophys Res Commun. 345(2):894-903.	Effects of BPA on gene expression.
2006	Leung, Y. -K., Mak P., Hassan S., Ho S. -M.	Estrogen receptor (ER)-□ isoforms: a key to understanding ER-□ signaling.	Proceedings of the National Academy of Sciences of the USA. 103(35):13162-13167.	Paper deals with estrogen receptor signalling.
2006	Leusch F. D. L., van den Heuvel M. R., Chapman H. F., Gooneratne S. R., Eriksson A. M. E., Tremblay L. A.	Development of methods for extraction and *in vitro* quantification of estrogenic and androgenic activity of wastewater samples.	Comp Biochem and Phys Part C: Tox & Pharm. 143C(1):117-126.	Analytical paper.
2006	Leusch, F. D. L., Chapman, H. F., van den Heuvel, M. R., Tan, B. L. L., Gooneratne, S. R., and Tremblay, L. A.	Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand.	Ecotoxicology and Environmental Safety. 65(3):403-411.	Bioassays and STP.
2006	Li F. -B., Chen J. -J., Liu C. -S., Dong J., Liu T. -X.	Effect of iron oxides and carboxylic acids on photochemical degradation of bisphenol A.	Biology and Fertility of Soils. 42(5):409-417.	Non-environmental degradation.
2006	Li X., Zhang S., Safe S.	Activation of kinase pathways in MCF-7 cells by 17□-estradiol and structurally diverse estrogenic compounds.	J Steroid Biochem Mol Biol. 98(2-3):122-32.	Mammalian cells.
2006	Lim L. W., Takeuchi T.	On-line precolumn enrichment of bisphenol A using boronate column in microcolumn liquid chromatography.	J Chromatogr A. 1106(1-2):139-45.	Analytical paper.
2006	Lin, L, L. and Janz, D. M.	Effects of binary mixtures of xenoestrogens on gonadal development and reproduction in zebrafish.	Aquatic Toxicology. 80(4):382-395.	Mixture of xenoestrogens.
2006	Lin Y., Zeng X. G., Wu D. S., Wang X.	Study on bisphenol A induced primary cultured mesencephalic neuronal cell injury by oxidative stress.	Wei Sheng Yan Jiu. 35(4):419-22.	Cellular study.
2006	Lindblom, E., Gernaey, K. V., Henze, M., and Mikkelsen, P.	Integrated modelling of two xenobiotic organic compounds.	Water Science and Techn. 54(6-7):213-221.	Paper looks at modelling.
2006	Liu, G.-B., Dai, L., Gao, X., Li, M.-K., and Thiemann, T.	Reductive degradation of tetrabromobisphenol A (TBBPA) in aqueous medium.	Green Chemistry. 8(9):781-783.	Non-environmental degradation of TBBPA.
2006	Liu M., Hashi Y., Pan F., Yao J., Song G., Lin J. M.	Automated on-line liquid chromatography-photodiode array-mass spectrometry method with dilution line for the determination of bisphenol A and 4-octylphenol in serum.	J Chromatogr A. Aug 23; [Epub ahead of print].	Analytical paper.
2006	Liu Y., Deng L., Chen Y., Wu F., Deng N.	Simultaneous photocatalytic reduction of Cr(VI) and oxidation of bisphenol A induced by Fe(III)-OH complexes in water.	J Hazard Mater. Jun 10; [Epub ahead of print].	Non-environmental oxidation.

Year	Authors	Title	Reference	Notes
2006	Liu Y., Klep V., Luzinov I.	To patterned binary polymer brushes via capillary force lithography and surface-initiated polymerization.	J Am Chem Soc. 128(25):8106-8107.	Polymers.
2006	Lukacova V., Peng M., Tandlich R., Hinderliter A., Balaz S.	Partitioning of organic compounds in phases imitating the headgroup and core regions of phospholipid bilayers.	Langmuir. 22(4):1869-1874.	Non-environmental partitioning.
2006	Lyons, G.	Viewpoint: Policy requirements for protecting wildlife from endocrine disruptors.	Environmental Health Perspectives. 114(Suppl 1):142-146.	Policy document.
2006	Maffini M. V., Rubin B. S., Sonnenschein C., Soto A. M.	Endocrine disruptors and repro health: the case of bisphenol-A.	Mol Cell Endocrinol. 25;254-255:179-86.	Human health study.
2006	Malendowicz L. K., Trejter M., Rebuffat P., Ziolkowska A., Nussdorfer G. G., Majchrzak M.	Effects of some endocrine disruptors on the secretory and proliferative activity of the regenerating rat adrenal cortex.	Int J Mol Med. 18(1):197-200.	Mammalian study.
2006	Mao M., Liu Z., Wang T., Yu B., Wen X., Yang K., Zhao C.	Polysulfone-activated carbon hybrid particles for the removal of BPA.	Separation Science and Technology. 41(3):515-529.	Water purification.
2006	Maragou N. C., Lampi E. N., Thomaidis N. S., Koupparis M. A.	Determination of bisphenol A in milk by solid phase extraction and liquid chromatography-mass spectrometry.	J Chromatogr A. 1129(2):165-73.	Analytical paper.
2006	Marchand-Geneste N., Cazaunau M., Carpy A. J. M., Laguerre M., Porcher J. M., Devillers J.	Homology model of the rainbow trout estrogen receptor (rtERbeta) and docking of endocrine disrupting chemicals (EDCs).	SAR and QSAR in Env Res. 17(1):93-105.	Receptor modelling.
2006	Marchesini G. R., Koopal K., Meulenberg E., Haasnoot W., Irth H.	Spreeta-based biosensor assays for endocrine disruptors.	Biosens Bioelectron. Sep 11; [Epub ahead of print].	Paper deals with biosensor assays.
2006	Marchesini G. R., Meulenberg E., Haasnoot W., Mizuguchi M., Irth H.	Biosensor recognition of thyroid-disrupting chemicals using transport proteins.	Anal Chem. 78(4):1107-14.	Analytical paper.
2006	Martin-Skilton, R., Coughtrie, M. W. H., and Porte, C.	Sulfotransferase activities towards xenobiotics and estradiol in two marine fish species (*Mullus barbatus* and *Lepidorhombus boscii*): Characterization and inhibition by endocrine disrupters.	Aquatic Toxicology. 79(1):24-30.	Biochemistry paper.
2006	Martin-Skilton, R., Thibaut, R., and Porte, C.	Endocrine alteration in juvenile cod and turbot exposed to dispersed crude oil and alkylphenols.	Aquatic Toxicology. 78(Supplement 1):S57-S64.	Mixture toxicity.
2006	Maruyama H., Seki H., Matsukawa Y., Suzuki A., Inoue N.	Removal of bisphenol A and diethyl phthalate from aqueous phases by ultrasonic atomization.	Ind & Eng Chem Res. 45(18): 6383-6386.	Non-environmental degradation.
2006	Mauricio R., Diniz M., Petrovic M., Amaral L., Peres I., Barcelo D., Santana F.	A characterization of selected endocrine disruptor compounds in a Portuguese wastewater treatment plant.	Environ Monit Assess. 118(1-3):75-87.	Water purification.
2006	Mendez M. A., Suarez M. F., Cortes M. T.	Electrochemical impedance spectroscopy of diluted solutions of bisphenol A.	J Electroanal Chem. 590(2):181-189.	Analytical paper.

Year	Authors	Title	Reference	Notes
2006	Mezcua, M., Ferrer, I., Hernando, M. D., and Fernandez-Alba, A. R.	Photolysis and photocatalysis of bisphenol A: identification of degradation products by liquid chromatography with electrospray ionization/time-of-flight/mass spectrometry (LC/ESI/ToF/MS).	Food Additives and Contaminants. 23(11):1242-1251.	Analytical paper.
2006	Miska, V., Menkveld, H. W., Kuijer, L., Boersen, M., van der Graaf, J. H.	Removal of nitrogen, phosphorus and other priority (hazardous) substances from WWTP effluent.	Water Science and Technology. 54(8):189-195.	WWTP.
2006	Miyamoto K., Kotake M.	Estimation of daily bisphenol A intake of Japanese individuals with emphasis on uncertainty and variability.	Environ Sci. 13(1):15-29.	Exposure of humans in Japan.
2006	Miyatake M., Miyagawa K., Mizuo K., Narita M., Suzuki T.	Dynamic changes in dopaminergic neurotransmission induced by a low concentration of bisphenol-A in neurones and astrocytes.	J Neuroendocrinol. 18(6):434-44.	Mammalian cellular study.
2006	Mizota K., Ueda H.	Endocrine disrupting chemical atrazine causes degranulation through Gq/11 protein-coupled neurosteroid receptor in mast cells.	Toxicol Sci. 90(2):362-8.	Cellular study.
2006	Moehring G., Schaefer H. -O.	LY-CAL 85 - an activator for bisphenolic cured FKM compounds.	Gummi Fasern Kunststoffe. 59(4): 247-250.	Polymer study.
2006	Moens L. N., van der Ven K., Van Remortel P., Del-Favero J., De Coen W. M.	Expression Profiling of Endocrine-Disrupting Compounds Using a Customized Cyprinus carpio cDNA Microarray.	Toxicol Sci. 93(2):298-310.	Expression profiling using cDNA.
2006	Moors S., Diel P., Degen G. H.	Toxicokinetics of bisphenol A in pregnant DA/Han rats after single i.v. application.	Arch Toxicol. 80(10):647-55.	Mammalian study.
2006	Morohashi H., Nakanoya T., Iwata H., Yamauchi T., Tsubokawa N.	Novel contamination and gas sensor materials from amphiphilic polymer-grafted carbon black.	Polymer Journal (Tokyo, Japan). 38(6):548-553.	Polymers.
2006	Mousavi P., Wang D., Grant C. S., Oxenham W., Hauser P. J.	Effects of antioxidants on the thermal degradation of a polyol ester lubricant using GPC.	Ind & Eng Chem Res. 45(1):15-22.	BPA not mentioned in abstract.
2006	Mutou Y., Ibuki Y., Terao Y., Kojima S., Goto R.	Chemical change of chlorinated bisphenol A by ultraviolet irradiation and cytotoxicity of their products on Jurkat cells.	Env Tox and Pharm. 21(3):283-289.	Derivatives of bisphenol A.
2006	Mutou, Y., Ibuki, Y., Terao, Y., Kojima, S., and Goto, R.	Change of estrogenic activity and release of chloride ion in chlorinated bisphenol A after exposure to ultraviolet B.	Biological and Pharmaceutical Bulletin. 29(10):2116-2119.	Chlorinated bisphenol A.
2006	Nagata R., Prosnansky M., Sakakibara Y.	Electrochemical treatment of trace endocrine disrupting chemicals with a three-dimensional electrode system.	J Advanced Oxidation Technol. 9(1):97-102.	Non-environmental degradation.
2006	Nakahira A., Nishida S., Fukunishi, K.	Synthesis of magnetic activated carbons for removal of environmental endocrine disrupter using magnetic vector.	J Ceramic Soc Japan. 114(Jan.):135-137.	Water purification.
2006	Nakamura K., Itoh K., Yaoi T., Fujiwara Y., Sugimoto T., Fushiki S.	Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of bisphenol A.	J Neurosci Res. Aug 10; [Epub ahead of print].	Mammalian study.

Year	Authors	Title	Reference	Notes
2006	Nakayama K., Iwata H., Kim E. Y., Tashiro K., Tanabe S.	Gene expression profiling in common cormorant liver with an oligo array: assessing the potential toxic effects of environmental contaminants.	Environ Sci Technol. 40(3):1076-83.	Gene expression profiling.
2006	Namasivayam C., Sangeetha D.	Recycling of agricultural solid waste, coir pith: removal of anions, heavy metals, organics and dyes from water by adsorption onto $ZnCl_2$ activated coir pith carbon.	J Hazard Mater. 135(1-3):449-52.	Water treatment.
2006	Narita M., Miyagawa K., Mizuo K., Yoshida T., Suzuki T.	Prenatal and neonatal exposure to low-dose of bisphenol-A enhance the morphine-induced hyperlocomotion and rewarding effect.	Neurosci Lett. 402(3):249-52.	Mammalian study.
2006	Ndebele K., Tchounwou P. B., McMurray R. W.	Coumestrol, BPA, DDT, and TCDD modulation interleukin-2 expression in activated CD+4 Jurkat T cells.	Int J Environ Res Public Health. 1(1):3-11.	Biochemistry paper.
2006	Neamtu, M. and Frimmel, F. H.	Degradation of endocrine disrupting bisphenol A by 254 nm irradiation in different water matrices and effects on yeast cells.	Water Research. 40(20):3745-3750.	Non-environmental degradation.
2006	Nendza, M. and Wenzel, A.	Discriminating toxicant classes by mode of action. 1. (Eco) toxicity profiles.	Environmental Science and Pollution Research Int. 13(3):192-203.	Toxicity profiles
2006	Neschen S., Morino K., Rossbacher J. C., Pongratz R. L., Cline G. W., Sono S., Gillum M., Shulman G. I.	Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor-gamma-dependent mechanism in mice.	Diabetes. 55(4):924-8.	Mammalian study.
2006	Neufeld T., Biran D., Popovtzer R., Erez T., Ron E. Z., Rishpon J.	Genetically engineered pfabA pfabR bacteria: an electrochemical whole cell biosensor for detection of water toxicity.	Anal Chem. 78(14):4952-4956.	Biosensors.
2006	Nghiem L. D., Schaefer A. I.	Critical risk points of nanofiltration and reverse osmosis processes in water recycling applications.	Desalination. 187(1-3):303-312.	Water purification.
2006	Nielsen, L. and Bastrup, E.	Quantitative studies on the effects of environmental estrogens on the testis of the guppy, *Poecilia reticulata*.	Aquatic Toxicology. 80(2):140-148.	BPA is not mentioned in the abstract.
2006	Nishino T., Wedel T., Schmitt O., Schonfelder M., Hirtreiter C., Schulz T., Kuhnel W., Michna H.	The xenoestrogen bisphenol A in the Hershberger assay: androgen receptor regulation, morphometrical reactions indicate no major effects.	J Steroid Biochem Mol Biol. 98(2-3):155-63.	Mammalian study.
2006	Niwa O., Jia J., Sato Y., Kato D., Kurita R., Maruyama K., Suzuki K., Hirono S.	Electrochemical performance of angstrom level flat sputtered carbon film consisting of sp2 and sp3 mixed bonds.	J Am Chem Soc. 128(22):7144-7145.	Electrochemistry.
2006	Nomiyama, K., Tanizaki, T., Koga, T., Arizono, K., and Shinohara, R.	Oxidative degradation of BPA using TiO(2) in water, and transition of estrogenic activity in the degradation pathways.	Archives of Environmental Contamination and Toxicology. 52(1):8-15.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2006	Obata T.	Imidaprilat, an angiotensin-converting enzyme inhibitor exerts neuroprotective effect via decreasing dopamine efflux and hydroxyl radical generation induced by bisphenol A and MPP^+ in rat striatum.	Brain Res. 1071(1):250-3.	Mammalian study.
2006	Oehlmann, J., Schulte-Oehlmann, U., Oetken, M., Bachmann, J., Lutz, I., Kloas, W., and Ternes, T. A.	Effects of BPA in snails: Oehlmann *et al*. respond.	Environmental Health Perspectives. 114(6):A341-A342.	Response to comments on Oehlmann *et al*. 2006
2006	Ogawa Y., Kawamura Y., Wakui C., Mutsuga M., Nishimura T., Tanamoto K.	Estrogenic activities of chemicals related to food contact plastics and rubbers tested by the yeast two-hybrid assay.	Food Addit Contam. 23(4):422-30.	Yeast assay
2006	Ohnuma A., Conlon J. M., Kawasaki H., Iwamuro S.	Developmental and triiodothyronine-induced expression of genes encoding preprotemporins in the skin of Tago's brown frog *Rana tagoi*.	Gen Comp Endocrinol. 146(3):242-50.	Gene expression.
2006	Opitz, R., Lutz, I., Nguyen, N. H., Scanlan, T. S., and Kloas, W.	Analysis of thyroid hormone receptor beta A mRNA expression in *Xenopus laevis* tadpoles as a means to detect agonism and antagonism of thyroid hormone action.	Toxicology and Applied Pharmacology. 212(1):1-13.	Paper looks at mRNA expression.
2006	Ou J., Hu L., Hu L., Li X., Zou H.	Determination of phenolic compounds in river water with on-line coupling bisphenol A imprinted monolithic precolumn with high performance liquid chromatography.	Talanta. 69(4):1001-1006.	Analytical paper.
2006	Pacchierotti F., Ranaldi R.	Mechanisms and risk of chemically induced aneuploidy in mammalian germ cells.	Curr Pharm Des. 12(12):1489-504.	Mammalian cells.
2006	Paik M. -J., Choi Y., Kim K. -R.	Simultaneous profiling analysis of alkylphenols and amines by gas chromatography and gas chromatog-mass spectrometry.	Analytica Chimica Acta. 560(1-2):218-226.	Analytical paper.
2006	Pampanin D. M., Viarengo A., Garrigues P., Andersen O. K.	An overview of the BEEP Stavanger workshop.	Aquat Toxicol. 78 Suppl 1:S124-6.	Biomarkers.
2006	Pampanin, D. M., Viarengo, A., Garrigues, P. and Andersen, O. K.	Background for the BEEP Stavanger workshops: Biological effects on marine organisms in two common, large, laboratory experiments and in a field study. Comparison of the value (sensitivity, specificity, etc.) of core and new biomarkers.	Aquatic Toxicology. 78(Supplement 1):S1-S4.	Biomarkers
2006	Panter, G. H., Hutchinson, T. H., Hurd, K. S., Bamforth, J., Stanley, R. D., Duffell, S., Hargreaves, A., Gimeno, S., and Tyler, C. R.	Development of chronic tests for endocrine active chemicals. Part 1. An extended fish early-life stage test for oestrogenic active chemicals in the fathead minnow (*P. promelas*).	Aquatic Toxicology. 77(3):279-290.	Test method development.
2006	Park J. W., Kurosawa S., Aizawa H., Goda Y., Takai M., Ishihara K.	Piezoelectric immunosensor for bisphenol A based on signal enhancing step with 2-methacrolyl-oxyethyl phosphorylcholine polymeric nanoparticle.	Analyst. 131(1):155-62.	Analytical paper.

Year	Authors	Title	Reference	Notes
2006	Patisaul H. B., Fortino A. E., Polston E. K.	Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV.	Neurotoxicol Teratol. 28(1):111-8.	Mammalian.
2006	Pavan M., Netzeva T. I., Worth A. P.	Validation of a QSAR model for acute toxicity.	SAR and QSAR in Environ Res. 17(2):147-171.	QSAR model.
2006	Pawlowski, L.	Pathways of pollutants in the environment.	Environmental Engineering Science. 23(4):571-573.	Review
2006	Peng X., Wang Z., Yang C., Chen F., Mai B.	Simultaneous determination of endocrine-disrupting phenols and steroid estrogens in sediment by gas chromatography-mass spectrometry.	J Chromatogr A. 1116(1-2): 51-6.	Analytical paper.
2006	Peng Z., Wu F., Deng N.	Photodegradation of bisphenol A in simulated lake water containing algae, humic acid and ferric ions.	Environ Pollut. 144(3):840-6.	Photodegradation in simulated lake water.
2006	Perego C., De Angelis A., Carati A., Flego C., Millini R., Rizzo C., Bellussi G.	Amorphous aluminosilicate catalysts for hydroxyalkylation of aniline and phenol.	App Cat A: General. 307(1):128-136.	Chemical synthesis.
2006	Pettersson M., Adolfsson-Erici M., Parkkonen J., Forlin L., Asplund L.	Fish bile used to detect estrogenic substances in treated sewage water.	Sci Total Environ. 366(1):174-86.	Bioanalytical study.
2006	Politch J. A.	Bisphenol A and risk assessment.	Environ Health Perspect. 114(1):A16; author reply A16-7. [No abstract available].	Risk assessment.
2006	Potyrailo R. A., McCloskey P. J., Wroczynski R. J., Morris W. G.	High-throughput determination of quantitative structure-property relationships using a resonant multisensor system: solvent resistance of bisphenol a polycarbonate copolymers.	Anal Chem. 78(9):3090-6.	Polymers.
2006	Rafat, M., De D., Khulbe K. C., Nguyen T., Matsuura T.	Surface characterization of hollow fiber membranes in artificial kidney.	J App Polymer Sci. 101(6):4386-4400.	Polymers.
2006	Raikwar H. P., Muthian G., Rajasingh J., Johnson C. N, Bright J. J.	PPARgamma antagonists reverse the inhibition of neural antigen-specific Th(1) response and experimental allergic encephalomyelitis by Ciglitazone and 15-Deoxy-Delta(12,14)-Prostaglandin J(2).	J Neuroimmunol. 178(1-2):76-86.	Mammalian study.
2006	Ramilo G., Valverde I., Lago J., Vieites J. M., Cabado A. G.	Cytotoxic effects of BADGE (bisphenol A diglycidyl ether) and BFDGE (bisphenol F diglycidyl ether) on Caco-2 cells *in vitro*.	Arch Toxicol. Jun 17; [Epub ahead of print].	*In vitro* cellular study of BPA derivatives.
2006	Rao B. S., Pathak S. K.	Thermal and viscoelastic properties of sequentially polymerized networks composed of benzoxazine, epoxy, and phenalkamine curing agents.	J App Polymer Sci. 100(5):3956-3965.	Polymers.
2006	Rayner B. S, Duong T. T., Myers S. J., Witting P. K.	Protective effect of a synthetic anti-oxidant on neuronal cell apoptosis resulting from experimental hypoxia re-oxygenation injury.	J Neurochem. 97(1):211-21.	Neurochemistry research paper.

Year	Authors	Title	Reference	Notes
2006	Rengaraj, S. and Li, X. Z.	Photocatalytic degradation of bisphenol A as an endocrine disruptor in aqueous suspension using Ag-TiO2 catalysts.	International Journal of Environment and Pollution. 27(1-3):20-37.	Non-environmental degradation.
2006	Roepke, T. A., Chang, E. S., and Cherr, G. N.	Maternal exposure to estradiol and endocrine disrupting compounds alters the sensitivity of sea urchin embryos and the expression of an orphan steroid receptor.	Journal of Experimental Zoology Part A-Comparative Experimental Biology. 305A (10):830-841.	Bisphenol A not mentioned in the abstract.
2006	Rogalewicz R., Batko K., Voelkel A.	Identification of organic extractables from commercial resin-modified glass-ionomers using HPLC-MS.	J Environ Monit. 8(7):750-8.	Analytical paper.
2006	Roy K., Ghosh G.	QSTR with extended topochemical atom (ETA) indices. VI. Acute toxicity of benzene derivatives to tadpoles (*Rana japonica*).	J Mol Modeling. 12(3): 306-316.	QSAR study.
2006	Rubin B. S., Lenkowski J. R., Schaeberle C. M., Vandenberg L. N., Ronsheim P. M., Soto A. M.	Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A.	Endocrinology. 147(8):3681-91.	Mammalian study.
2006	Rucinski M., Ziolkowska A., Hochol A., Pucher A., Macchi C., Belloni A. S., Nussdorfer G. G., Malendowicz L. K.	Estradiol and resveratrol stimulating effect on osteocalcin, but not osteonectin and collagen-1□ gene expression in primary culture of rat calvarial osteoblast-like cells.	Int J Mol Med. 18(4):565-70.	Mammalian cellular study.
2006	Ryan B. C., Vandenbergh J. G.	Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice.	Horm Behav. 50(1):85-93.	Mammalian study.
2006	Rykowska I.	New solid-phase extraction sorbent with ketoimine groups - preparation and application to determination of bisphenol A.	Chemia Analityczna (Warsaw, Poland). 51(3):399-409.	Analytical paper.
2006	Rykowska I., Wasiak W.	Properties, threats, and methods of analysis of bisphenol A and its derivatives.	Acta Chrom. 16:7-27.	Analytical paper.
2006	Safe S., Papineni S.	The role of xenoestrogenic compounds in the development of breast cancer.	Trends in Pharm Sci. 27(8):447-454.	Human health.
2006	Sakakibara Y., Sena Y., Prosnansky M.	Direct oxidation treatment by a novel 3-dimensional electrolytic cell reactor.	Water Sci & Tech: Water Supply. 6(2):87-91.	Non-environmental degradation.
2006	Sambe H., Hoshina K., Hosoya K., Haginaka J.	Simultaneous determination of bisphenol A and its halogenated derivatives in river water by combination of isotope imprinting and liquid chromatography-mass spectrometry.	J Chromatogr A. 1134(1-2): 16-23.	Analytical paper.
2006	Sanchez-Bayo F.	Comparative acute toxicity of organic pollutants and reference values for crustaceans I. Branchiopoda, Copepoda and Ostracoda.	Env Pollution. 139(3):385-420.	Comparison of acute toxicity to planktonic crustaceans by T_{rel} and statistical analysis.

Year	Authors	Title	Reference	Notes
2006	San-Jose J., Tomas M., Juan M.	Fiber-reinforced polymer bars embedded in a resin concrete: study of both materials and their bond behavior.	Polymer Composites. 27(3):315-322.	Polymers.
2006	Savabieasfahani M., Kannan K., Astapova O., Evans N. P., Padmanabhan V.	Developmental programming: differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function.	Endocrinology. Aug 31; [Epub ahead of print].	Mammalian study.
2006	Schäfer, A. I., Nghiem, L. D., and Oschmann, N.	Bisphenol A retention in the direct ultrafiltration of greywater.	Journal of Membrane Science. 283(1-2):233-243.	Water purification.
2006	Schmidt S., Degen G. H., Seibel J., Hertrampf T., Vollmer G., Diel P.	Hormonal activity of combinations of genistein, bisphenol A and 17□-estradiol in the female Wistar rat.	Arch Toxicol. Apr 26; [Epub ahead of print].	Mammalian study.
2006	Schriks M., Vrabie C. M., Gutleb A. C., Faassen E. J., Rietjens I. M. C. M.; Murk, A. J.	T-screen to quantify functional potentiating, antagonistic and thyroid hormone-like activities of polyhalogenated aromatic hydrocarbons (PHAHs).	Tox in Vitro. 20(4):490-498.	BPA not mentioned in abstract.
2006	Schuster D., Maurer E. M., Laggner C., Nashev L. G., Wilckens T., Langer T., Odermatt A.	The discovery of new 11 □-hydroxysteroid dehydrogenase type 1 inhibitors by common feature.	J Med Chem. 49(12):3454-3466.	Enzyme study.
2006	Scott-Fordsmand, J. J. and Damgaard, C.	Uncertainty analysis of single-concentration exposure data for risk assessment - introducing the species effect distribution approach.	Environmental Toxicology and Chemistry. 25(11):3078-3081.	No new data
2006	Seeboth A., Loetzsch D., Potechius E., Vetter R.	Thermochromic effects of leuco dyes studied in polypropylene.	Chinese J Polymer Sci. 24(4): 363-368.	Polymers.
2006	Segner, H.	Comment on "Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic anvironment."	Environmental Science and Technology. 40(3):1084-1085.	General paper, not directly relevant.
2006	Seo J. S., Lee Y. M., Jung S. O., Kim I. C., Yoon Y. D., Lee J. S.	Nonylphenol modulates expression of androgen receptor and estrogen receptor genes differently in gender types of the hermaphroditic fish *Rivulus marmoratus*.	Biochem Biophys Res Commun. 346(1):213-23.	Modulation of gene expression by nonylphenol.
2006	Seo J. S., Park T. J., Lee Y. M., Park H. G., Yoon Y. D., Lee J. S.	Small heat shock protein 20 gene (Hsp20) of the intertidal copepod *Tigriopus japonicus* as a possible biomarker for exposure to endocrine disruptors.	Bull Environ Contam Toxicol. 76(4):566-72. [No abstract available].	Biomarker for exposure.
2006	Shang, E. H. H., Yu, R. M. K., and Wu, R. S. S.	Hypoxia affects sex differentiation and development, leading to a male-dominated population in zebrafish (*Danio rerio*).	Environmental Science and Technology. 40(9):3118-3122.	Hypoxia and sex differentiation.
2006	Shieh J. -Y., Lin C. -Y., Huang C. -L., Wang C. -S.	Synthesis and characterization of novel dihydrobenzoxazine resins.	J App Polymer Sci. 101(1):342-347.	Polymers.
2006	Shioda T., Chesnes J., Coser K. R., Zou L., Hur J., Dean K. L., Sonnenschein C., Soto A. M., Isselbacher K. J.	Importance of dosage standardization for interpreting transcriptomal signature profiles: evidence from studies of xenoestrogens.	Proc Natl Acad Sci USA. 103(32):12033-8.	Gene study.
2006	Shoji R., Kawakami M.	Prediction of genotoxicity of various environmental pollutants by artificial neural network simulation.	Mol Div. 10(2):101-108.	QSAR study.

Year	Authors	Title	Reference	Notes
2006	Shulin E., Wang P., Ma C., Zhang D., Deng W., Sun D.	Design for polymer vertical channel triple coupled microring resonators filter.	Proc of SPIE - Int Soc Optical Eng 6025(ICO20: Optical Comm), 60250W/1-60250W/5.	Polymers.
2006	Song M., Xu Y., Jiang Q., Lam P. K., O'Toole D. K., Giesy JP, Jiang G.	Measurement of estrogenic activity in sediments from Haihe and Dagu River, China.	Environ Int. 32(5):676-81.	*In vitro* bioassay system.
2006	Stowell C. L., Barvian K. K., Young P. C., Bigsby R. M., Verdugo D. E., Bertozzi C. R., Widlanski T. S.	A role for sulfation-desulfation in the uptake of bisphenol A into breast tumor cells.	Chem Biol. 13(8):891-7.	Uptake of BPA into breast tumor cells.
2006	Sturve, J., Hasselberg, L., Fälth, H., Celander, M., and Förlin, L.	Effects of North Sea oil and alkylphenols on biomarker responses in juvenile Atlantic cod (*Gadus morhua*).	Aquatic Toxicology. 78(Supplement 1):S73-S78.	Biomarker responses to alkylphenols.
2006	Sueiro R. A., Suarez S., Araujo M., Garrido M. J.	Study on mutagenic effects of bisphenol A diglycidyl ether (BADGE) and its derivatives in the *Escherichia coli* tryptophan reverse mutation assay.	Mutat Res. 609(1):11-6.	Mutagenic effects of BPA derivative in bacteria.
2006	Sugise, T., Hayashida, Y., Hanafusa, T., Nanjo, E., and Yamashita, I.	Monitoring of vasculogenesis inhibiting activities in sewage effluents by using medaka embryos.	Environmental Science and Technology. 40(6):2051-2055.	Sewage effluents.
2006	Sumpter, J. P. and Johnson, A. C.	Response to comment on "Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic anvironment."	Environmental Science and Technology. 40(3):1086-1087.	Not relevant.
2006	Sumpter, J. P., Johnson, A. C., Williams, R. J., Kortenkamp, A., and Scholze, M.	Modelling effects of mixtures of endocrine disrupting chemicals at the river catchment scale.	Environmental Science and Technology. 40(17):5478-5489.	Paper looks at modelling effects of EDCs.
2006	Sun C., Leong L. P., Barlow P. J., Chan S. H., Bloodworth B. C.	Single laboratory validation of a method for the determination of Bisphenol A, Bisphenol A diglycidyl ether and its derivatives in canned foods by reversed-phase liquid chromatography.	J Chromatogr A. 1129(1):145-8.	Analytical paper.
2006	Sun H., Xu L. C., Chen J. F., Song L., Wang X. R.	Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene.	Food Chem Toxicol. Jul 4; [Epub ahead of print].	Reporter gene study.
2006	Sun W. L., Ni J. R., Xu N., Sun L. Y.	Fluorescence of sediment humic substance and its effect on the sorption of selected endocrine disruptors.	Chemosphere. 66(4):700-707.	Analytical paper.
2006	Sundt R. C., Pampanin D. M., Larsen B. K., Brede C., Herzke D., Bjornstad A., Andersen O. K.	The BEEP Stavanger Workshop: Mesocosm exposures.	Aquat Toxicol. 78 Suppl 1:S5-12.	Development of biomarkers.
2006	Tabuchi Y., Takasaki I., Kondo T.	Identification of genetic networks involved in the cell injury accompanying endoplasmic reticulum stress induced by bisphenol A in testicular Sertoli cells.	Biochem Biophys Res Commun. 345(3):1044-50.	Cellular injury induced by bisphenol A.

Year	Authors	Title	Reference	Notes
2006	Takahashi O., Oishi S.	Male reproductive toxicity of four bisphenol antioxidants in mice and rats and their estrogenic effect.	Arch Toxicol. 80(4):225-41.	Mammalian study.
2006	Takai Y., Takeuchi T., Tsutsumi, O.	Gender difference and relationship with hormonal dysfunction of human exposure to endocrine disruptors.	Horumon to Rinsho. 54(3):191-195.	Human exposure.
2006	Takeda K., Kobayashi T.	Hybrid molecularly imprinted membrane for targeted bisphenol derivatives.	J Membrane Sci. 275(1-2):61-69.	Chemical synthesis.
2006	Takeuchi T., Tsutsumi O., Ikezuki Y., Kamei Y., Osuga Y., Fujiwara T., Takai Y., Momoeda M., Yano T., Taketani Y.	Elevated Serum Bisphenol A Levels under Hyperandrogenic Conditions may be Caused by Decreased UDP-glucuronosyltransferase Activity.	Endocr J. 53(4):485-91.	Mammalian study.
2006	Tanabe N., Kimoto T., Kawato S.	Rapid Ca^{2+} signaling induced by Bisphenol A in cultured rat hippocampal neurons.	Neuro Endocrinol Lett. 27(1-2):97-104.	Mammalian study.
2006	Tanaka M., Nakaya S., Katayama M., Leffers H., Nozawa S., Nakazawa R., Iwamoto T., Kobayashi S.	Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth.	Hum Exp Toxicol. 25(7):369-73.	Mammalian study.
2006	ter Veld M. G., Schouten B., Louisse J., van Es D. S., van der Saag P. T., Rietjens I. M., Murk A. J.	Estrogenic potency of food-packaging-associated plasticizers and antioxidants as detected in ER□ and ER□ reporter gene cell lines.	J Agric Food Chem. 54(12):4407-16.	Cell line study.
2006	Terasaka S., Inoue A., Tanji M., Kiyama R.	Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity.	Tox Lett. 163(2):130-141.	Human cells.
2006	Terasaki M., Kazama T., Shiraishi F., Makino M.	Identification and estrogenic characterization of impurities in commercial bisphenol A diglycidyl ether (BADGE).	Chemosphere. 65(5):873-80.	Impurities in BPA derivative.
2006	Tojo T., Tsuda K., Wada T. S., Yamazaki K.	A simple and extremely sensitive system for detecting estrogenic activity using transgenic Arabidopsis thaliana.	Ecotoxicol Environ Saf. 64(2):106-14.	Estrogen activity assay.
2006	Tominaga T., Negishi T., Hirooka H., Miyachi A., Inoue A., Hayasaka .I, Yoshikawa Y.	Toxicokinetics of bisphenol A in rats, monkeys and chimpanzees by the LC-MS/MS method.	Toxicology. 226(2-3):208-17.	Mammalian study.
2006	Tsai W. T., Hsu H. C., Su T. Y., Lin K. Y., Lin C. M.	Adsorption characteristics of bisphenol-A in aqueous solutions onto hydrophobic zeolite.	J Colloid Interface Sci. 299(2):513-9.	Water purification.
2006	Tsai W. T., Lai C. W., Su T. Y.	Adsorption of bisphenol-A from aqueous solution onto minerals and carbon adsorbents.	J Hazard Mater. 134(1-3): 169-75.	Water purification.
2006	Tsue H., Takimoto T., Kikuchi C., Yanase H., Ishibashi K., Amezawa K., Miyashita H., Miyafuji H., Tanaka S., Tamura R.	Adsorptive removal of endocrine disrupting chemicals by calix[4]crown oligomer: significant improvement of removal efficiency by oligomerization.	Chem Lett. 35(3):254-255.	Water purification.

Year	Authors	Title	Reference	Notes
2006	Tsuru N., Kikuchi M., Kawaguchi H., Shiratori S.	A quartz crystal microbalance sensor coated with MIP for "Bisphenol A" and its properties.	Thin Solid Films. 499(1-2): 380-385.	Analytical paper.
2006	Vanderford, B. J. and Snyder, S. A.	Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem massspectrometry.	Environmental Science and Technology. 40(23):7312-7320.	Analytical paper.
2006	Vanparys C., Maras M., Lenjou M., Robbens J., Van Bockstaele D., Blust R., De Coen W.	Flow cytometric cell cycle analysis allows for rapid screening of estrogenicity in MCF-7 breast cancer cells.	Tox in Vitro. 20(7):1238-1248.	Human health screening study.
2006	Velazquez A. M., Torres L. A., Diaz G., Ramirez A., Hernandez R., Santillan H., Martinez L., Martinez I., Diaz-Barriga S., Abrego V., Balboa M. A., Camacho B., Lopez-Castanares R., Duenas-Gonzalez A., Cabrera G., Angeles E.	A novel one pot, solvent-free Mannich synthesis of methylpiperidinyl phenols, methylphenylmorpholinyl phenols, and methylthiophenylmorpholinyl phenols using infrared light irradiation.	ARKIVOC (Gainesville, FL, United States) (2):150-161.	Derivatisation of phenols.
2006	Venkatachalam N., Vinu A. Anandan, S., Arabindoo, B., Murugesan V.	Visible light active photocatalytic degradation of bisphenol-A using nitrogen doped TiO_2.	J Nanosci and Nanotech. 6(8):2499-2507.	Non-environmental degradation.
2006	Vitalini D., Spina E., Rapisardi R., Scamporrino E., Mineo P.	Synthesis and matrix-assisted laser desorption/ionization time-of-flight characterization of bisphenol-A copolyformals containing nickel(II)/Schiff base, eicosane and 2-butene units in the main chain.	Rapid Commun Mass Spectrom. 20(19):2961-8.	Analytical paper.
2006	vom Saal F. S.	Bisphenol A eliminates brain and behavior sex dimorphisms in mice: how low can you go?	Endocrinology. 147(8):3679-80. No abstract available.	Mammalian study.
2006	vom Saal F. S., Welshons W. V.	Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A.	Environ Res. 100(1):50-76.	Mammalian study.
2006	Wakida S., Fujimoto K., Nagai H., Miyado T., Shibutani Y., Takeda S.	On-chip micellar electrokinetic chromatographic separation of phenolic chemicals in waters.	J Chromatogr A. 1109(2):179-82.	Analytical paper
2006	Walker C. C., Salinas K. A., Harris P. S., Wilkinson S. S., Watts J. D., Hemmer M. J.	A Proteomic (SELDI-TOF-MS) Approach to Estrogen Agonist Screening.	Toxicol Sci. 95(1):74-81.	Analytical paper.
2006	Wang G., Wu F., Zhang X., Luo M., Deng N.	Enhanced photodegradation of bisphenol A in the presence of □-cyclodextrin under UV light.	J Chem Tech and Biotech. 81(5): 805-811.	Non-environmental degradation.
2006	Wang, G., Oi, P., Xue, X., Wu, F., and Deng, N.	Photodegradation of bisphenol Z by UV irradiation in the presence of β-cyclodextrin .	Chemosphere. 67(4):762-769.	Non-environmental degradation.
2006	Wang, G., Wu, F., Zhang, X., Luo, M. and Deng, N.	Enhanced TiO2 photocatalytic degradation of bisphenol A by β-cyclodextrin in suspended solutions.	Journal of Photochemistry and Photobiology A: Chemistry. 179(1-2):49-56.	Non-environmental degradation.
2006	Wang L. Q., James M. O.	Inhibition of sulfotransferases by xenobiotics.	Curr Drug Metab. 7(1):83-104.	Biochemistry.

Year	Authors	Title	Reference	Notes
2006	Wang M. -L., Lee Z. -F.	Synthesis of novel multisite phase-transfer catalysts and their applications to the catalyzed reaction of bisphenol A and allyl bromide.	Ind & Eng Chem Res. 45(14): 4918-4926.	Paper looks at derivatisation.
2006	Wang M. -L., Lee Z. -F.	Kinetic study of synthesizing bisphenol A diallyl ether in a phase-transfer catalytic reaction.	Bulletin of the Chem Soc of Japan. 79(1):80-87.	Paper looks at derivatisation.
2006	Wang X., Zeng H., Wei Y., Lin J. -M.	A reversible fluorescence sensor based on insoluble beta-cyclodextrin polymer for direct determination of bisphenol A (BPA).	Sensors and Actuators B: Chemical. B114(2):565-572.	Analytical paper.
2006	Wang X., Zeng H., Zhao L., Lin J. -M.	Selective determination of bisphenol A (BPA) in water by a reversible fluorescence sensor using pyrene/ dimethyl beta-cyclodextrin complex.	Analytica Chimica Acta. 556(2):313-318.	Analytical paper.
2006	Wang Z. J., Li G. M., Tang W. L., Yin M.	Neuroprotective effects of stearic acid against toxicity of oxygen/glucose deprivation or glutamate on rat cortical or hippocampal slices.	Acta Pharmacol Sin. 27(2):145-50.	Mammalian study.
2006	Wang Z. J., Liang C. L., Li G. M., Yu C. Y., Yin M.	Neuroprotective effects of arachidonic acid against oxidative stress on rat hippocampal slices.	Chem Biol Interact. Aug 18; [Epub ahead of print].	Mammalian study.
2006	Welshons W. V., Nagel S. C., vom Saal F. S.	Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure.	Endocrinology. 147(6 Suppl):S56-69.	Mammalian study.
2006	Wen Y., Zhou B. S., Xu Y., Jin S. W., Feng Y. Q.	Analysis of estrogens in environmental waters using polymer monolith in-polyether ether ketone tube solid-phase microextraction combined with high-performance liquid chromatography.	J Chromatogr A. 1133(1-2): 21-28.	Analytical paper.
2006	Wilson N. K., Chuang J. C., Morgan M. K., Lordo R. A., Sheldon L. S.	An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare.	Environ Res. 103(1):9-20.	Human exposure study.
2006	Xiao Q., Li Y., Ouyang H., Xu P., Wu D.	High-performance liquid chromatographic analysis of bisphenol A and 4-nonylphenol in serum, liver and testis tissues after oral administration to rats and its application to toxicokinetic study.	J Chromatogr B Analyt Technol Biomed Life Sci. 830(2):322-9.	Mammalian study.
2006	Xie Y.	Photoelectrochemical application of nanotubular titania photoanode.	Electrochimica Acta. 51(17): 3399-3406.	Non-environmental degradation.
2006	Xie Y. B., Li X. Z.	Degradation of bisphenol A in aqueous solution by H(2)O(2)-assisted photoelectrocatalytic oxidation.	J Hazard Mater. 138(3):526-533.	Non-environmental degradation.
2006	Xie, Y. B., Li, X. Z.	Preparation and characterization of TiO_2/Ti film electrodes by anodization at low voltage for photoelectrocatalytic application.	J App Electrochem. 36(6):663-668.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2006	Yamaguchi, A., Awano, T., Oyaizu, K., and Yuasa, M.	Surface-modified mesoporous silicas as recyclable adsorbents of an endocrine disrupter, bisphenol A.	Journal of Nanoscience and Nanotechnology. 6(6):1689-1694.	Non-environmental adsorption.
2006	Yamaguchi H., Zhu J., Yu T., Sasaki K., Umetsu H., Kidachi Y., Ryoyama K.	Low-level bisphenol A increases production of glial fibrillary acidic protein in differentiating astrocyte progenitor cells through excessive STAT3 and Smad1 activation.	Toxicology. 226(2-3):131-42.	Cellular study.
2006	Yamazaki N., Washio I., Shibasaki Y., Ueda M.	Facile synthesis of aryl ether dendrimer from unprotected AB2 building blocks using thionyl chloride as an activating agent.	Organic Letters. 8(11):2321-2324.	Polymers.
2006	Yang D. H., Ju M. J., Maeda A., Hayashi K., Toko K., Lee S. W., Kunitake T.	Design of highly efficient receptor sites by combination of cyclodextrin units and molecular cavity in TiO(2) ultrathin layer.	Biosens Bioelectron. 22(3):388-92.	Analytical paper.
2006	Yang M., Kim S. Y., Chang S. S., Lee I. S., Kawamoto T.	Urinary concentrations of bisphenol A in relation to biomarkers of sensitivity and effect and endocrine-related health effects.	Environ Mol Mutagen. Jun 22; [Epub ahead of print].	Human health study.
2006	Ye X., Kuklenyik Z., Needham L. L., Calafat A. M.	Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry.	J Chromatogr B Analyt Technol Biomed Life Sci. 831(1-2):110-5.	Analytical paper.
2006	Yeo M. K., Kang M.	Photodecomposition of bisphenol A on nanometer-sized TiO_2 thin film and the associated biological toxicity to zebrafish (*Danio rerio*) during and after photocatalysis.	Water Res. 40(9):1906-14.	Non-environmental degradation.
2006	Yin H., Ito A., Bhattacharjee D., Hoshi M.	A comparative study on the protective effects of 17□-estradiol, biochanin A and bisphenol A on mammary gland differentiation and tumorigenesis in rats.	Indian J Exp Biol. 44(7):540-6.	Mammalian study.
2006	Ying G. -G.	Chromatographic analysis of endocrine disrupting chemicals in the environment.	Chrom Sci Series 93 (Chrom Anal Env (3rd Ed)), 1241-1267.	Analytical paper.
2006	Zeidan R. K., Dufaud V., Davis M. E.	Enhanced cooperative, catalytic behaviour of organic functional groups by immobilization.	J Catalysis. 239(2):200-306.	Catalysis.
2006	Zhan M. J., Yang X., Xian Q. M., Kong L. R.	Photochemical transformation of bisphenol a promoted by nitrate ions.	Bull Environ Contam Toxicol. 76(1):105-12. [No abstract available].	Non-environmental degradation.
2006	Zhan M., Yang X., Xian Q., Kong L.	Photosensitized degradation of bisphenol A involving reactive oxygen species in the presence of humic substances.	Chemosphere. 63(3):378-386.	Non-environmental degradation.
2006	Zhan M., Yang X., Xian Q., Kong L.	Photosensitized degradation of bisphenol A involving reactive oxygen species in the presence of humic substances.	Chemosphere. 63(3):378-86.	Non-environmental degradation.
2006	Zhan, M.-J., Yang, X., Xian, Q.-M., and Kong, L.-R.	Photodegradation of bisphenol A in Fe(III)-oxalate complexes solution.	Journal of Env Sciences-China. 18(4):771-776.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2006	Zhang J. -G., Wang H. -Q., Li X. -Y.	Novel hyperbranched poly(phenylene oxide)s with phenolic terminal groups: effects of reaction time and core molecules on the molecular weight and polydispersity.	Chinese J Polymer Sci. 24(4): 413-419.	Polymers.
2006	Zhang J. H., Jiang M., Zou L., Shi D., Mei S. R., Zhu Y. X., Shi Y., Dai K., Lu B.	Selective solid-phase extraction of bisphenol A using molecularly imprinted polymers and its application to biological and environmental samples.	Anal Bioanal Chem. 385(4):780-6.	Analytical paper.
2006	Zhang S., Zhang Q., Darisaw S., Ehie O., Wang G.	Simultaneous quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products (PPCPs) in Mississippi river water, in New Orleans, Louisiana, USA.	Chemosphere. 66(6):1057-1069.	Possible measured levels, non-EU.
2006	Zhang, Y., Causserand, C., Aimar, P., and Cravedi, J. P.	Removal of bisphenol A by a nanofiltration membrane in view of drinking water production.	Water Research. 40(20):3793-3799.	Water purification.
2006	Zhang Z. L., Hibberd A., Zhou J. L.	Optimisation of derivatisation for the analysis of estrogenic compounds in water by solid-phase extraction gas chromatography-mass spectrometry.	Analytica Chimica Acta. 577(1):52-61.	Analytical paper.
2006	Zhao C. Y., Zhang H. X., Zhang X. Y., Liu M. C., Hu Z. D., Fan B. T.	Application of support vector machine (SVM) for prediction toxic activity of different data sets.	Toxicology. 217(2-3):105-119.	QSAR study.
2006	Zorita, I., Ortiz-Zarragoitia, M., Soto, M., and Cajaraville, M. P.	Biomarkers in mussels from a copper site gradient (Visnes, Norway): An integrated biochemical, histochemical and histological study.	Aquatic Toxicology. 78(Supplement 1):S109-S116.	Biomarkers.
2005	Abd-El-Aziz A. S., Todd E. K., Okasha, R. M., Shipman P. O., Wood T. E.	Macromolecules containing redox-active neutral and cationic iron complexes.	Macromolecules. 38(23): 9411-9419.	Iron complexes in macromolecules.
2005	Adachi T., Yasuda K., Mori C., Yoshinaga M., Aoki N., Tsujimoto G., Tsuda K.	Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors.	Food and Chemical Toxicology. 43(5):713-719.	Mammalian study.
2005	Adriani W., Della Seta D., Dessi-Fulgheri F., Farabollini F., Laviola G.	Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to BPA.	Env Health Perspectives. 113(6):A368.	Mammalian study.
2005	Ahmad S., Ashraf S. M., Sharmin E., Nazir M., Alam M.	Studies on new polyetheramide-butylated melamine formaldehyde-based anticorrosive coatings from a sustainable resource.	Progress in Organic Coatings. 52(2):85-91.	Polymers.
2005	Alegria A., Goitiandia L.	A dielectric test of the validity of the Adam-Gibbs equation out-of-equilibrium: Polymers vs. small molecules.	J Non-Crystalline Solids. 351(33-36):2588-2592.	Polymers.
2005	Alonso-Magdalena P., Laribi O., Ropero A. B., Fuentes E., Ripoll C., Soria B., Nadal A.	Low doses of bisphenol A and diethylstilbestrol impair Ca^{2+} signals in pancreatic □-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans.	Environ Health Perspect. 113(8):969-77.	Mammalian sudy.

Year	Authors	Title	Reference	Notes
2005	Alslev, B., Korsgaard, B., and Bjerregaard, P.	Estrogenicity of butylparaben in rainbow trout *Oncorhynchus mykiss* exposed via food and water.	Aquatic Toxicology. 72(4):295-304.	Paper deals with butylparabens.
2005	Alvarez D. A., Stackelberg P. E., Petty J. D., Huckins J. N., Furlong E. T., Zaugg S. D., Meyer M. T.	Comparison of a novel passive sampler to standard water-column sampling for organic contaminants associated with wastewater effluents entering a New Jersey stream.	Chemosphere. 61(5):610-622.	Analytical paper.
2005	Andreescu S., Sadik O. A., McGee D. W.	Effect of natural and synthetic estrogens on A549 lung cancer cells: correlation of chemical structures with cytotoxic effects.	Chem Res in Toxicology. 18(3):466-474.	Study on human cells.
2005	Aoki N., Kinoshita K., Arai R., Mikuni K., Nakanishi K., Hattori K.	Preparation of insoluble chitosan beads functionalized by carboxymethylated □-cyclodextrin.	Trans Materials Res Soc of Japan. 30(4):1143-1146.	Polymers.
2005	Araki N., Ohno K., Takeyoshi M., Iida M.	Evaluation of a rapid *in vitro* androgen receptor transcriptional activation assay using AR-EcoScreen cells.	Tox in Vitro. 19(3):335-352.	Bioassay.
2005	Arrese-Igor S., Arbe A., Alegria A., Colmenero J., Frick B.	Sub-Tg dynamics in polycarbonate by neutron scattering and its relation with secondary gamma relaxation.	J Chem Phys. 123(1):014907.	Polymers.
2005	Aslanov T. A., Mamedli U. M., Guliev A. M.	Synthesis of diester disulfimides from saccharinmonocarboxylic acids and aromatic diols.	Russian J Appl Chem. 78(3):441-442.	Chemical synthesis.
2005	Ballard B. D., MacKay A. A.	Estimating the removal of anthropogenic organic chemicals from raw drinking water by coagulation flocculation.	J Env Eng (Reston, VA, USA). 131(1):108-118.	Water purification.
2005	Barontini F., Marsanich K., Petarca L., Cozzani V.	Thermal degradation and decomposition products of electronic boards containing BFRs.	Industrial & Engineering Chemistry Research. 44(12): 4186-4199.	No direct relevance.
2005	Barsiene J., Dedonyte V., Rybakovas A., Andreikenaite L., Andersen O. -K.	Induction of micronuclei in Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) after treatment with bisphenol A, diallyl phthalate and tetrabromodiphenyl ether-47.	Ekologija. 4:1-7.	*In vivo* study on genotoxic effects on marine fish.
2005	Basheer C., Jegadesan S., Valiyaveettil S., Lee H. K.	Sol-gel-coated oligomers as novel stationary phases for solid-phase microextraction.	J Chromatogr A. 1087(1-2):252-8.	Analytical paper.
2005	Basheer C., Parthiban A., Jayaraman A., Lee H. K., Valiyaveettil S.	Determination of alkylphenols and bisphenol-A. A comparative investigation of functional polymer-coated membrane microextraction and solid-phase microextraction techniques.	J Chromatogr A. 1087(1-2):274-82.	Analytical paper.
2005	Basrur, P. K.	Disrupted sex differentiation and feminization of man and domestic animals.	Environmental Research. 100(1):18-38.	Mammalian study.
2005	Bautista-Toledo I., Ferro-Garcia M. A., Rivera-Utrilla J., Moreno-Castilla C., Fernandez F. J.	Bisphenol A removal from water by activated carbon. Effects of carbon characteristics and solution chemistry.	Environ Sci Technol. 39(16):6246-50.	Non-environmental sorpion.
2005	Baydur A., Koss M. N., Sharma O. P., Dalgleish G. E., Nguyen D. V., Mullick F. G., Murakata L. A., Centeno J. A.	Microscopic pulmonary embolisation of an indwelling central venous catheter with granulomatous inflammatory response.	Eur Respir J. 26(2):351-3.	Mammalian study.

Year	Authors	Title	Reference	Notes
2005	Beinhoff M., Bozano L. D., Scott J. C., Carter K. R.	Design and synthesis of new polymeric materials for organic non-volatile electrical bi-stable storage devices: poly (biphenylmethylene)s.	Macromolecules. 38(10): 4147-4156.	Chemical synthesis.
2005	Bellona C., Drewes J. E., Xu P., Luna J., Hoppe C.	Pilot-scale study investigating the performance of reverse osmosis and nanofiltration membranes for the removal of organic micropollutants, nutrients and bulk organic carbon.	Membrane Tech Conference & Exposition Proceedings, Phoenix, AZ, United States, Mar. 6-9, 2005, 1189-1200.	Water purification.
2005	Blanco, E., Casais, M. C., Mejuto, M. C., and Cela, R.	Analysis of tetrabromobisphenol A and other phenolic compounds in water samples by non-aqueous capillary electrophoresis coupled to photodiode array ultraviolet detection.	Journal of Chromatography A. 1071(1-2):205-211.	Analytical paper that deals with TBBPA.
2005	Blustein G., Deya M. C., Romagnoli R., del Amo B.	Three generations of inorganic phosphates in solvent and water-borne paints: A synergism case.	Appl Surface Sci. 252(5): 1386-1397.	Inorganic chemistry.
2005	Braunrath R., Cichna M.	Sample preparation including sol-gel immunoaffinity chromatography for determination of BPA in canned beverages, fruits and vegetables.	J Chromatog A. 1062(2):189-198.	Analytical paper.
2005	Braunrath R., Podlipna D., Padlesak S., Cichna-Markl M.	Determination of bisphenol A in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection.	J Agric Food Chem. 53(23): 8911-7.	Analytical paper.
2005	Bravo J. C., Fernandez P., Durand J. S.	Flow injection fluorimetric determination of □-estradiol using a molecularly imprinted polymer.	Analyst. 130(10):1404-9.	Analytical paper.
2005	Brian J. V., Harris C. A., Scholze M., Backhaus T., Booy P., Lamoree M., Pojana G., Jonkers N., Runnalls T., Bonfa A., Marcomini A., Sumpter J. P.	Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals.	Environ Health Perspect. Jun; 113(6):721-8.	Mixture toxicity.
2005	Bryk S. D., Makitra R. G., Pal'chikova O. Y.	Correlation of the solubility of 4,4'-isopropylidenediphenol with solvent properties.	Russian J General Chemistry. 75(5):745-747.	Solubility study.
2005	Bureeva S., Andia-Pravdivy J., Petrov G., Igumnov M., Romanov S., Kolesnikova E., Kaplun A., Kozlov L.	Inhibition of classical pathway of complement activation with negative charged derivatives of bisphenol A and bisphenol disulphates.	Bioorganic & Medicinal Chemistry. 13(4):1045-1052.	Synthesis and investigation of BPA derivatives.
2005	Burkhardt M. R., ReVello R. C., Smith S. G., Zaugg S.	Pressurized liquid extraction using water/isopropanol coupled with solid-phase extraction cleanup for industrial and anthropogenic waste-indicator compounds in sediment.	Analytica Chimica Acta. 534(1):89-100.	Analytical paper.
2005	Byun J. -A., Heo Y., Kim Y. -O., Pyo M. -Y.	Bisphenol A-induced downregulation of murine macrophage activities *in vitro* and *ex vivo*.	Env Tox & Pharm. 19(1):19-24.	Mammalian study.
2005	Calafat A. M., Kuklenyik Z., Reidy J. A., Caudill S. P., Ekong J., Needham L. L.	Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population.	Environmental Health Perspectives. 113(4):391-395.	Levels in human urine.

Year	Authors	Title	Reference	Notes
2005	Can A., Semiz O., Cinar O.	Bisphenol-A induces cell cycle delay and alters centrosome and spindle microtubular organization in oocytes during meiosis.	Mol Hum Reprod. 11(6):389-96.	Mammalian study.
2005	Carballo, M., Aguayo, S., de la Torre, A., and Munoz, M. J.	Plasma vitellogenin levels and gonadal morphology of wild carp (*Cyprinus carpio* L.) in a receiving rivers downstream of sewage treatment plants.	Science of the Total Environment. 341(1-3):71-79.	Exposure to STP effluent
2005	Chai W., Handa Y., Suzuki M., Saito M., Kato N., Horiuchi C. A.	Biodegradation of bisphenol A by fungi.	Applied Biochemistry and Biotech. 120(3):175-182.	Paper deals with biodegradation.
2005	Chang C. -M., Chou C. -C., Lee M. -R.	Determining leaching of bisphenol A from plastic containers by solid-phase microextraction and gas chromatography-mass spectrometry.	Analytica Chimica Acta. 539(1-2):41-47.	Analytical paper.
2005	Chang S. C., Prevost N. T., Sachinvala N. D., Parikh D. V., Lambert A. A. III., Sawhney P. A., Jarrett W. A.	Preparation and characterization of macro- & monomers for azide & alkyne cycloaddition polymerization.	Polymer Preprints (American Chemical Soc, Division of Polymer Chemistry). 46(1): 737-738.	Polymers.
2005	Chaudhary, A., Harrison, L., Khan, S., Singh, S., Azua, A., Gardinali, P., and Willett, K.	Estrogen equivalents and corresponding chemical analyses of marine water samples collected along a coral reef.	44th Society of Toxicology Annual Meeting, Abstract #1588.	Non-EU levels
2005	Chetkina L. A., Belsky V. K.	Conformational features and geometry of molecules in crystals of bridging ortho, ortho-bisphenols and related compounds.	Crystallography Reports. [A review]. 50(4):571-596.	Crystal structure.
2005	Cheung K. M., Lu W. W., Luk K. D., Wong C. T., Chan D., Shen J. X., Qiu G. X., Zheng Z. M., Li C. H., Liu S. L., Chan W. K., Leong J. C.	Vertebroplasty by use of a strontium-containing bioactive bone cement.	Spine. 30(17 Suppl):S84-91.	Medical material.
2005	Chikashige Y., Chikyu Y., Miyatake, K. Watanabe M.	Poly(arylene ether) ionomers containing sulfofluorenyl groups for fuel cell applications.	Macromolecules, 38(16), 7121-7126	Polymers.
2005	Cho S. -D., Jang K. -W., Hyun J. -G., Lee S., Paik K. -W., Kim H., Kim J. -H.	Epoxy/$BaTiO_3$ composite films and pastes for high dielectric constant and low-tolerance embedded capacitors fabrication in organic substrates.	IEEE Trans on Electronics Packaging Manufacturing. 28(4):297-303.	Polymers.
2005	Choi K. J., Kim S. G., Kim C. W., Kim S. H.	Effect of polyphosphate on removal of endocrine-disrupting chemicals of nonylphenol and bisphenol-A by activated carbons.	Water Quality Research Journal of Canada. 40(4):484-490.	Water purification.
2005	Choi K. J., Kim S. G., Kim C. W., Kim S. H.	Effects of activated carbon types and service life on removal of endocrine disrupting chemicals: amitrole, nonylphenol, and bisphenol-A.	Chemosphere. 58(11):1535-1545.	Water purification.
2005	Choi M., Lee S. -J., Koo J. H., Moon H. -B., Kim G. -Y.	Occurrence and concentrations of estrogenic phenolic compounds in surface waters of rivers flowing into Masan Bay, Korea.	J of Fisheries Sci & Technol. 8(4):220-227.	Possible levels of estrogenic phenols reported.

Year	Authors	Title	Reference	Notes
2005	Choi Y. -H., Gotoh Y., Sugimoto K. -I., Song S. -M., Yanagisawa T., Endo M.	Processing and characterization of epoxy nanocomposites reinforced by cup-stacked carbon nanotubes.	Polymer. 46(25):11489-11498.	Polymers.
2005	Chu S., Haffner G. D., Letcher R. J.	Simultaneous determination of TBBPA, TCBPA, BPA and other halogenated analogues in sediment and sludge by high performance liquid chromatography-electrospray tandem mass spectrometry.	J Chromatogr A. 1097(1-2):25-32.	Analytical paper.
2005	Clara M., Strenn B., Gans O., Martinez E., Kreuzinger N., Kroiss H.	Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants.	Water Research. 39(19):4797-4807.	Wastewater levels.
2005	Clara M., Kreuzinger N., Strenn B., Gans O., Kroiss H.	The solids retention time - a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants.	Water Research. 39(1):97-106.	Paper deals with wastewater levels.
2005	Cojocariu G., Blubaugh C., Li X. Y., Malinoski J., Sybert P.	Transparent injection moldable LEXAN SLX resins.	Ann Tech Conf 63rd , Soc of Plastics Eng, 2528-2532.	Polymers.
2005	Coleman H. M., Chiang K., Amal R.	Effects of Ag and Pt on photocatalytic degradation of EDCs in water.	Chem Eng Journal (Amsterdam, Netherlands). 113(1):65-72.	Non-environmental degradation.
2005	Coleman K. P.	QSAR models of the *in vitro* steroid hormone receptor activity of alkylphenol and bisphenol A analogs.	Diss. Abstr. Int., B 2006. 66(11):5941.	QSAR study.
2005	Cornelissen E. R. Verdouw J., Gijsbertsen-Abrahamse A. J., Hofman J. A. M. H.	A nanofiltration retention model for trace contaminants in drinking water sources.	Desalination. 178(1-3):179-192.	Water purification.
2005	Costache, A.D., Pullela, P.K., Kasha, P., Tomasiewicz, H., and Sem, D.S.	Homology-modelled ligand-binding domains of zebrafish estrogen receptors alpha, beta1, and beta2: From *in silico* to *in vivo* studies of estrogen interactions in Danio rerio as a model system.	Molecular Endocrinology. 19(12): 2979-2990.	Modelling study
2005	Coulier L., Kaal E. R., Hankemeier T.	Comprehensive two-dimensional liquid chromatography and hyphenated liquid chromatography to study the degradation of poly(bisphenol A)carbonate.	J Chromatogr A. 1070(1-2):79-87.	Polycarbonate degradation
2005	Dawson R. B., Landry S. D.	Recyclability of flame retardant HIPS, PC/ABS, and PPO/HIPS used in electrical and electronic equipment.	Proc of the IEEE Int Symp on Elec & the Env, Conf Record, New Orleans, LA, USA, May 16-19, 77-82.	Polymer recycling in electronic goods.
2005	Deborde M., Rabouan S., Duguet J. P., Legube B.	Kinetics of aqueous ozone-induced oxidation of some endocrine disruptors.	Environ Sci Technol. 39(16):6086-92.	Non-environmental degradation.
2005	del Olmo M., Zafra A., Suarez B., Gonzalez-Casado A., Taoufiki J., Vilchez J. L.	Use of solid-phase microextraction followed by on-column silylation for determining chlorinated bisphenol A in human plasma by gas chromatography-mass spectrometry.	J Chromat B: Analytical Technologies in Biomedical and Life Sci. 817(2):167-172.	Analytical techniques for chlorinated bisphenol A.

Year	Authors	Title	Reference	Notes
2005	del Rio Paredes S.	Chemical contamination of the child: bioacumulation and potential effects.	Rev Esp Salud Publica. 79(2):221-8. Review.	Human exposure.
2005	Della Seta D., Minder I., Dessi-Fulgheri F., Farabollini F.	Bisphenol A exposure during pregnancy and lactation affects maternal behavior in rats.	Brain Research Bulletin. 65(3):255-260.	Mammalian study.
2005	Di Marzio W. D., Saenz M., Alberdi J., Tortorelli M., Silvana G.	Risk assessment of domestic and industrial effluents unloaded into a freshwater environment.	Ecotoxicology and Environ Safety. 61(3):380-391.	Water purification.
2005	Drewes J. E., Hemming J., Ladenburger S. J., Schauer J., Sonzogni W.	An assessment of endocrine disrupting activity changes during wastewater treatment through the use of bioassays and chemical measurements.	Water Environment Research. 77(1):12-23.	Water purification.
2005	Endo H., Nakaji-Hirabayashi T., Morokoshi S., Gemmei-Ide M., Kitano H.	Orientational effect of surface-confined cyclodextrin on the inclusion of bisphenols.	Langmuir. 21(4):1314-1321.	Sorption study.
2005	Facciolo R. M., Madeo M., Alo R., Canonaco M., Dessi-Fulgheri F.	Neurobiological effects of bisphenol A may be mediated by somatostatin subtype 3 receptors in some regions of the developing rat brain.	Toxicol Sci. 88(2):477-84.	Mammalian study.
2005	Fahlenkamp, H., Tobias, N., and Hannich, C. B.	Organic trace substances - balance of modern sewage purification plants under consideration of the water and sludge path. Organic trace substance in water and sludge stream of two large sewage purification plants.	Gewaesserschutz, Wasser, Abwasser. 198:67/1-67/20.	WWTP.
2005	Fan Y., Feng Y. -Q., Shi Z. -G. Wang J. -B.	Ordered mesoporous silica coated capillary for in-tube solid phase microextraction coupled to high performance liquid chromatography.	Analytica Chimica Acta. 543(1-2):1-8.	Analytical paper.
2005	Fan Y., Zhang M., Da S. L., Feng Y. Q.	Determination of endocrine disruptors in environmental waters using poly(acrylamide-vinylpyridine) monolithic capillary for in-tube solid-phase microextraction coupled to high-performance liquid chromato-graphy with fluorescence detection.	Analyst. 130(7):1065-9.	Analytical paper.
2005	Fan Y., Zhang M., Feng Y. -Q.	Poly(acrylamide-vinylpyridine-N,N'-methylene bisacrylamide) monolithic capillary for in-tube solid-phase microextraction coupled to high performance liquid chromatography.	J Chromatog A. 1099(1-2):84-91.	Analytical paper.
2005	Fickova M., Pravdova E., Mlynarcikova A., Scsukova S.	*In vitro* cytotoxicity of some phenol plasticizers.	Biologia (Bratislava, SK). 60 (Suppl. 17):73-76.	Cell toxicity study.
2005	Fielden M. R., Eynon B. P., Natsoulis G., Jarnagin K., Banas D., Kolaja K. L.	A gene expression signature that predicts the future onset of drug-induced renal tubular toxicity.	Toxicologic Pathology. 33(6): 675-683.	Gene expression.
2005	Freyberger A., Schmuck G.	Screening for estrogenicity and anti-estrogenicity: a critical evaluation of an MVLN cell-based transactivation assay.	Toxicology Lett. 155(1):1-13.	MVLN cell-based assay for screening estrogenicity.

Year	Authors	Title	Reference	Notes
2005	Fu A. Y., Wang D. Q., Sun Y. L.	Hexaaquacadmium(II)bis{4-[2-(4-hydroxyphenyl)-2-propyl]phenolate} dihydrate.	Acta Cryst Section E: Structure Reports Online. E61(11):m2353-m2354.	Crystal structure.
2005	Fuerhacker M., Lemmens-Gruber R., Studenik C. R.	Novel screening test to assess the potential environmental toxicity of waste water samples.	Env Tox & Pharm. 19(2): 385-388.	Screening study assessing waste water samples.
2005	Fujimoto Y., Usa K., Sakuma S.	Effects of endocrine disruptors on the formation of prostaglandin and arachidonoyl-CoA formed from arachidonic acid in rabbit kidney medulla microsomes.	Prostaglandins Leukot Essent Fatty Acids. 73(6):447-52.	Mammalian study.
2005	Fukazawa Y., Pluemsab W., Sakairi N., Furuike T.	An efficient adsorption-desorption system for hydrophobic phenolic pollutants-combined use of mono-6-amino-□-cyclodextrin and cation exchanger.	Chem Lett. 34(12):1652-1653.	Non-environmental sorption.
2005	Funakoshi, G., Ito, M., Ido, Y., and Kasuya, S.	Concentration of bisphenol A in the bottom layer water at the lower river.	8th Ann Meeting Japan Soc Endocrine Disrupters Resh, Prog and Abs, 27-29, Sept.	Levels in Japan
2005	Galina H., Lechowicz J., Walczak M.	Methods of narrowing the molecular size distribution in hyperbranched polymerization involving AB2 and B2 monomers.	J Macromolecular Sci, Part B: Physics. 44(6):925-940.	Method of polymerization.
2005	Galloway, J. M., Haggard, B. E., Meyers, M. T., and Green, W. R.	Occurrence of pharmaceuticals and other organic wastewater constituents in selected streams in Northern Arkansas, 2004.	U. S. Geological Survey Scientific Investigations Report 2005-5140.	Levels in US
2005	Ghafourian T., Cronin, M. T. D.	The impact of variable selection on the modelling of oestrogenicity.	SAR and QSAR in Env Res. 16(1-2):171-190.	QSAR study.
2005	Ghisari M., Bonefeld-Jorgensen E. C.	Impact of environmental chemicals on the thyroid hormone function in pituitary rat GH3 cells.	Mol Cell Endocrinol. 244(1-2):31-41.	Mammalian study.
2005	Glassmeyer S. T., Furlong E. T., Kolpin D. W., Cahill J. D., Zaugg S. D., Werner S. L., Meyer M. T., Kryak D. D.	Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination.	E S & T. 39(14):5157-5169.	Human exposure study.
2005	Glor M., Schwenzfeuer K.	Direct ignition tests with brush discharges.	J Electrostatics. 63(6-10): 463-468.	Ignition tests.
2005	Gomez Escalada M., Russell A. D., Maillard J. -Y., Ochs D.	Triclosan-bacteria interactions: single or multiple target sites?	Lett in Appl Microbiology. 41(6):476-481.	Bacterial study.
2005	Gomurashvili Z., Hay A. S.	Aromatic polyazines by a copper-amine catalyzed oxidative polymerization reaction.	J Macromolecular Science, Pure and Applied Chemistry. A42(2):127-138.	Polymers.
2005	Gonzales C. M., Swargam S., Billiot E. J., Billiot F.	Qualitative analysis of endocrine disrupting chemicals (EDCs) in water bodies of southeastern parts of Texas using retention time locking technique (RTL) on GC/MS.	Preprints of Ext Abstract presented at the ACS National Meeting, American Chem Soc, Division of Env Chemistry. 45(1):191-197.	Analytical paper.
2005	Gonzalez-Benito J., Esteban I.	Morphologic and kinetic study of an epoxy-poly(ethylene oxide) system. The fluorescence to predict miscibility.	Colloid and Polymer Science. 283(5):559-569.	Polymers.

Year	Authors	Title	Reference	Notes
2005	Gurer-Orhan H., Kool J., Vermeulen N. P. E., Meerman J. H. N.	A novel microplate reader-based high-throughput assay for estrogen receptor binding.	International Journal of Environmental Analytical Chemistry. 85(3):149-161.	Binding assay.
2005	Guschll P. C., Campbell P.	Rare-earth Fe-B powder coating for improvements in corrosion resistance, flux aging loss, and mechanical strength of bonded magnets.	IEEE Transactions on Magnetics. 41(10):3859-3861.	Inhibition of corrosion.
2005	Gye M. C., Kim D. H.	Bisphenol A induces hepatic vitellogenin mRNA in male *Bombina orientalis*.	Bull Environ Contam Toxicol. 75(1):1-6. [No abstract available].	mRNA transcription study
2005	Halldin, K.	Impact of endocrine disrupting chemicals on reproduction in Japanese quail.	Domestic Animal Endocrinology. 29(2):420-429.	Similar information included
2005	Halldin, K., Axelsson, J., and Brunstrom, B.	Effects of endocrine modulators on sexual differentiation and reproductive function in male Japanese quail.	Brain Research Bulletin. 65(3):211-218.	Similar information included
2005	Hanafy A. M., Sasanami T., Mori M.	Binding of xenoestrogens and phytoestrogens to estrogen receptor β of Japanese quail (*Coturnix japonica*).	J Poultry Sci. 42(3):238-244.	Binding affinity of bisphenol A to ERβ in quail.
2005	Hano, T., Oshima, Y., Oe, T., Kinoshita, M., Tanaka, M., Ozato, Y. W., and Honjo, T.,	Quantitative bio-imaging analysis for evaluation of sexual different in germ cells of olvas-GFP/ST-II YI Medaka (*Oryzias latipes*) nanoinject ovo with ethinylestradiol.	Environmental Toxicology and Chemistry. 24(1):70-77.	Bio-imaging analysis.
2005	Hashimoto S., Akatsuka Y., Kurihara R., Matsuoka S., Nakatsukuri M., Kurokawa Y., Tani Y., Kawai S.	Evaluation of the Ishikawa cell line bioassay for the detection of estrogenic substances from sediment extracts.	Env Tox and Chem. 24(7): 1587-1593.	Cell line bioassay.
2005	Hashimoto S., Horiuchi A., Yoshimoto T., Nakao M., Omura H., Kato Y., Tanaka H., Kannan K., Giesy J. P.	Horizontal and vertical distribution of estrogenic activities in sediments and waters from Tokyo Bay, Japan.	Archives of Environmental Contamination and Toxicology. 48(2):209-216.	Gene expression assay to determine estrogenic activity.
2005	Heneweer M., Muusse M., Dingemans M., de Jong P. C., van den Berg M., Sanderson J. T.	Co-culture of primary human mammary fibroblasts and MCF-7 cells as an *in vitro* breast cancer model.	Tox Sciences. 83(2):257-263.	Cellular study using an *in vitro* breast cancer model.
2005	Heravi M. M., Ahari N. Z., Oskooie H. A., Ghassemzadeh M.	Solid state S-methylation of thiols and O-methylation of phenols and naphthols with dimethyl sulfate unde microwave irradiation.	Phosphorus, Sulfur and Silicon and the Related Elements. 180(7):1701-1712.	Chemical synthesis.
2005	Hidaka H., Watanabe N., Horikoshi S.	Photocatalytic degradation of bisphenol A and its mechanism in the heterogeneous TiO_2 dispersions.	Trends in Air Pollution Research, 157-182.	Non-environmental degradation.
2005	Hirabayashi T., Sakaguchi S., Ishii Y., Davies P. W., Furstner A.	Iridium-catalyzed synthesis of vinyl ethers from alcohols and vinyl acetate.	Organic Syntheses. 82:55-58.	Synthesis of derivatives.
2005	Hofman J., Gijsbertsen A., Cornelissen E., van Dijk H.	Trace organic contaminant removal by nanofiltration: from pilot plant to full scale.	Membrane Tech Conf & Exp Proc, Phoenix, AZ, US, Mar. 6-9, 2005, 1124-1126.	Water purification.

Year	Authors	Title	Reference	Notes
2005	Hollert H., Durr M., Holtey-Weber R., Islinger M., Brack W., Farber H., Erdinger L., Braunbeck T.	Endocrine disruption of water and sediment extracts in a non-radioactive dot blot/RNAse protection-assay using isolated hepatocytes of rainbow trout.	Environ Sci Pollut Res Int. 12(6):347-60.	Hepatocytes study
2005	Holmes M. J., Hart A., Northing P., Oldring P. K., Castle L., Stott D., Smith G., Wardman O.	Dietary exposure to chemical migrants from food contact materials: a probabilistic approach.	Food Addit Contam. 22(10): 907-19.	Human exposure.
2005	Holt S., Comelli F., Costa B., Fowler C. J.	Inhibitors of fatty acid amide hydrolase reduce carrageenan-induced hind paw inflammation in pentobarbital-treated mice: comparison with indomethacin and possible involvement of cannabinoid receptors.	Br J Pharmacol. 146(3):467-76.	Mammalian study.
2005	Hong E. J., Choi K. C., Jeung E. B.	Maternal exposure to bisphenol a during late pregnancy resulted in an increase of Calbindin-D9k mRNA and protein in maternal and postnatal rat uteri.	J Reprod Dev. 51(4):499-508.	Mammalian study.
2005	Hu, L., Xu, S., Pan, C., Yuan, C., Zou, H., and Jiang, G.	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with a matrix of carbon nanotubes for the analysis of low-mass compounds in environmental samples.	Environmental Science and Technology. 39(21):8442-8447.	Analytical paper.
2005	Huang M., Jiang G., Cai Y.	Electrochemical preparation of composite polyaniline coating and its application in the determination of bisphenol A, 4-n-nonylphenol, 4-*tert*-octylphenol using direct solid phase microextraction coupled with high performance liquid chromatography.	J Sep Sci. 28(16):2218-24.	Analytical paper.
2005	Huang Q., Weber W. J. Jr.	Transformation and removal of bisphenol A from aqueous phase via peroxidase mediated oxidative coupling reactions: efficacy, products, and pathways.	Environ Sci Technol. 39(16):6029-36.	Non-environmental degradation.
2005	Huang W.	Voltammetric determination of bisphenol A using a carbon paste electrode based on the enhancement effect of cetyltrimethylammonium bromide (CTAB).	Bulletin of the Korean Chemical Society. 26(10): 1560-1564.	Analytcial paper.
2005	Huang Y., Paul D. R	Effect of physical aging on polymer thin films.	PMSE Preprints. 92:196-197.	Polymers.
2005	Hudson, R., Hemmer, M., Salinas, K., Wilkinson, S., Watts, J., Winstead, J., Harris, P., Kirkpatrick, A., and Walker, C.	High-throughput chemical screening using protein profiling of fish plasma.	44th Society of Toxicology Annual Meeting, Abstract #1598.	Screening method
2005	Huijbregts M. A. J., Rombouts L. J. A., Ragas A. M. J., van de Meent D.	Human toxicological effect and damage factors of carcinogenic and noncarcinogenic chemicals for life cycle impact assessment.	Integrated Env Assessment Management. 1(3):181-244.	Human health study.
2005	Imaizumi T., Yamashita K., Taima K., Ishikawa A., Yoshida H., Satoh K.	Effect of peroxisome proliferator-activated receptor-□ ligands on the expression of retinoic acid-inducible gene-I in endothelial cells stimulated with lipopolysaccharide.	Prostaglandins Other Lipid Mediat. 78(1-4):46-54.	Gene expression, cellular response.

Year	Authors	Title	Reference	Notes
2005	Imanaka M., Yamabe S. -I., Yamamoto J., Koezuka K., Take S., Sato A., Sasaki K.	Oxidative degradation of bisphenol A by fruit homogenates.	Journal of Food Science. 70(9):C529-C533.	Non-environmental degradation.
2005	Inoue H., Tsuruta A., Kudo S., Ishii T., Fukushima Y., Iwano H., Yokota H., Kato S.	Bisphenol A glucuronidation and excretion in liver of pregnant and nonpregnant female rats.	Drug Metabolism and Disposition.33(1):55-59.	Mammalian study.
2005	Inui, H., Sasaki, H., Kodama, S., Chua, N. H., and Ohkawa, H.	Monitoring of endocrine disruptors in transgenic plants carrying aryl hydrocarbon receptor and estrogen receptor genes.	ACS Symposium Series. 892:40-47.	Transgenic plants
2005	Irmak S., Erbatur O., Akgerman A.	Degradation of 17□-estradiol and bisphenol A in aqueous medium by using ozone and ozone/UV techniques.	J Hazard Mater. 126(1-3):54-62.	Non-environmental degradation.
2005	Ishido M.	DNA array technology in analyses for environmental factors-health interactions.	Current Topics in Pharmacology. 9(1):91-96.	DNA array technology.
2005	Ishido M., Morita M., Oka S., Masuo Y.	Alteration of gene expression of G protein-coupled receptors in endocrine disruptors-caused hyperactive rats.	Regulatory Peptides. 126(1-2):145-153.	Mammalian study.
2005	Isobe, T., Oshima, Y., Ito, S., Elnabris, K., Shimasaki, Y., and Honjo, T.	Effects of tributyltin, polychlorinated biphenyls, nonylphenol and bisphenol A on growth and generation time a free-living marine nematode (*Prochromadorella* sp.1).	8th Ann Meeting Japan Soc Endocrine Disrupters Res, Prog and Abs, 27-29, Sept.	Meeting abstract only.
2005	Ito S., Hashimoto M., Wadgaonkar B., Svizero N., Carvalho R. M., Yiu C., Rueggeberg F. A., Foulger S., Saito T., Nishitani Y., Yoshiyama M., Tay F. R., Pashley D. H.	Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity.	Biomaterials. 26(33):6449-59.	Polymers.
2005	Ito Y., Yokota H., Wang R., Yamanoshita O., Ichihara G., Wang H., Kurata Y., Takagi K., Nakajima T.	Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets.	Arch Toxicol. 79(3):147-154.	Mammalian study.
2005	Jagnytsch, O., Tooi, O., Kashiwagi, A., Oofusa, K., Yoshizato, K., Opitz, R., Lutz, I., and Kloas, W.	Bisphenol A interferes with the thyroid system of amphibians.	Environmental Research. 998(3):408.	Similar papers included.
2005	Janer, G., Lavado, R., Thibaut, W., and Porte, C.	Effects of 17β-estradiol exposure in the mussel *Mytilus galloprovincialis*: A possible regulating role for steroid acyltransferases.	Aquatic Toxicology. 75(1):32-42.	BPA not mentioned in abstract.
2005	Janer, G., Sternberg, R. M., LeBlanc, G. A., and Port, C.	Testosterone conjugating activities in invertebrates: are they targets for endocrine disruptors?	Aquatic Toxicology. 71(3):273-282.	Review-type paper
2005	Janex-Habibi, M. L., Bruchet, A., Loret, J. F., and Schlosser, O.	Endocrine disruptors: What are the issues for drinking water?	Eau, l'Industrie, les Nuisances. 283:39-42.	Drinking water
2005	Jiang J. Q., Yin Q., Pearce P., Zhou J.	A survey of endocrine disrupting chemicals in sewage and a preliminary treatment trial.	Water Sci Technol. 52(8):1-7.	Water purification.

Year	Authors	Title	Reference	Notes
2005	Jiang J. Q., Yin Q., Zhou J. L., Pearce P.	Occurrence and treatment trials of endocrine disrupting chemicals (EDCs) in wastewaters.	Chemosphere. 61(4):544-50.	Water purification.
2005	Jin H., Audus K. L.	Effect of bisphenol A on drug efflux in BeWo, a human trophoblast-like cell line.	Placenta. 19:S96-S103.	Human cell line study.
2005	Jooste, A. M., Du Preez, L., Carr, J. A., Giesy, J. P., Gross, T. S., Kendall, R. J., Smith, E. E., Van der Kraak, G. L., and Solomon, K. R.	Gonadal development of larval male *Xenopus laevis* exposed to atrazine in outdoor microcosms.	Environmental Science and Technology. 39(14):5255-5261.	Deals with atrazine.
2005	Jung Y. -W., Hong E. -J., Choi K. -C., Jeung E. -B.	Novel progestogenic activity of environmental endocrine disruptors in the upregulation of calbindin-D9k in an immature mouse model.	Tox Sci. 83(1):78-88.	Mammalian model for endocrine disruptors.
2005	Kadowaki T., Adachi T., Okamoto S., Tanaka N., Tonomura K., Tsujimoto G., Kanehisa M.	Chemical genomic study of endocrine disrupting chemicals in metabolic pathways.	Genome Informatics 2005. Int Conf, Poster & Software Demo 16th, Yokohama, Dec 19-21, P085/1-P085/2.	Genomic study of EDCs.
2005	Kamata R., Koda T., Morohoshi K., Umezu T., Morita M.	RNA constitution and estrogen-responsive gene expression in the ovariectomized rat uterus.	Anal Biochem. 341(1):131-40.	Gene expression.
2005	Kameda T., Saito M., Saito S., Umetsu Y.	Formation of Mg-Al layered double hydroxide modified by organic acid anions and its application to wastewater treatment.	EPD Cong Proc of Sess & Symp during the TMS Ann Meeting, San Fran, CA, US, Feb. 13-17, 2005, 5-12.	Water purification.
2005	Kameda T., Saito M., Umetsu Y.	Preparation of a composite material for the uptake of bisphenol A from aqueous solutions, the dodecylsulfate ion-intercalated MgAl layer-structured double hydroxide particles.	J Alloys Comp. 402(1-2):46-52.	Non-environmental sorption.
2005	Kamiya T., Yamauchi T., Hirotsuji J., Fujita M.	Ozone-based decomposition of main endocrine disruption chemicals in sewage effluent.	Ozone: Science & Eng. 27(5): 389-395.	Non-environmental degradation.
2005	Kanayama T., Kobayashi N., Mamiya S., Nakanishi T., Nishikawa J.	Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor □/retinoid X receptor pathway.	Molecular Pharmacology. 67(3):766-774.	Effects of endocrine disrupters, cell differentiation and gene expression.
2005	Kanki T., Hamasaki S., Sano N., Toyoda A., Hirano K.	Water purification in a fluidized bed photocatalytic reactor using TiO_2-coated ceramic particles.	Chem Eng J (Amsterdam, NL). 108(1-2):155-160.	Water purification.
2005	Kawaguchi M., Hayatsu Y., Nakata H., Ishii Y., Ito R., Saito K., Nakazawa H.	Molecularly imprinted solid phase extraction using stable isotope labeled compounds as template and liquid chromatography-mass spectrometry for trace analysis of bisphenol A in water sample.	Analytica Chimica Acta. 539(1-2):83-89.	Analytical paper.

Year	Authors	Title	Reference	Notes
2005	Kawaguchi M., Sakui N., Okanouchi N., Ito R., Saito K., Izumi S., Makino T., Nakazawa H.	Stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography--mass spectrometry for measurement of phenolic xenoestrogens in human urine samples.	J Chromatogr B Analyt Technol Biomed Life Sci. 820(1):49-57.	Analytical paper.
2005	Kawamoto Y., Matsuyama W., Morikawa M., Morita M., Sugimoto M., Manabe N., Morisawa S.	Disposition of bisphenol A in pregnant mice and fetuses after a single and repeated oral administration.	Tox and Env Chem. 87(2): 199-213.	Mammalian study.
2005	Kayaman-Apohan N., Demirci R., Cakir M., Gungor A.	UV-curable interpenetrating polymer networks based on acrylate/vinyl-ether functionalized urethane oligomers.	Radiation Physics and Chemistry. 73(5): 254-262.	Polymers.
2005	Kim H. S., Kang T. S., Kang I. H., Kim T. S., Moon H. J., Kim I. Y., Ki H., Park K. L., Lee B. M., Yoo S. D., Han S. Y.	Validation study of OECD rodent uterotrophic assay for the assessment of estrogenic activity in Sprague-Dawley immature female rats.	J Toxicol Environ Health A. 68(23-24):2249-62.	Mammalian study.
2005	Kim, J., Korshin, G. V., and Velichenko, A. B.	Comparative study of electrochemical degradation and ozonation of nonylphenol.	Water Research. 39(12):2527-2534.	Non-environmental degradation.
2005	Kim J. H., Kwon H., Lee S., Lee C. H.	Removal of endocrine disruptors using homogeneous metal catalyst combined with nanofiltration membrane.	Water Sci Technol. 51(6-7):381-90.	Analyical paper.
2005	Kim M. -S, Joo C. K., Lee S. M., Kim P. J., Yi J.	Development of a priority substances list for integrated environmental management.	Korean J Chem Eng. 22(2): 238-245.	Priority setting.
2005	Kirkland D., Aardema M., Henderson L., Mueller L.	Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity.	Mutation Res 588(1):70.	Mammalian study.
2005	Kirkland D., Aardema M., Henderson L., Mueller L.	Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and relative predictivity.	Mutation Res. 584(1-2):1-256.	Determination of carcinogens in rodents.
2005	Kitamura S. Kato T., Iida M., Jinno N., Suzuki T., Ohta S., Fujimoto H., Hanada H., Kashiwagi K., Kashiwagi A.	Anti-thyroid hormonal activity of tetrabromobisphenol A, a flame retardant, and related compounds: Affinity to the mammalian thyroid hormone receptor, and effect on tadpole metamorphosis.	Life Sci. 76(14):1589-1601.	Paper deals with TBBPA.
2005	Kitamura S., Suzuki T., Sanoh S., Kohta R., Jinno N., Sugihara K., Yoshihara S., Fujimoto N., Watanabe H., Ohta S.	Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds.	Tox Sciences. 84(2):249-259.	Mammalian study
2005	Kloas, W., Tooi, O., Kashiwagi, A., Oofusa, K., Yoshizato, K., Levy, G., Wuertz, S., and Lutz, I.	Endocrine disruptors act on reproductive biology and thyroid system of amphibians.	Environmental Research. 98(3):409-410.	Similar information included.

Year	Authors	Title	Reference	Notes
2005	Kobayashi K., Miyagawa M., Wang R. S., Suda M., Sekiguchi S., Honma T.	Effects of *in utero* and lactational exposure to bisphenol A on thyroid status in F1 rat offspring.	Ind Health. 43(4):685-90.	Mammalian study.
2005	Koda T., Morita M., Soya Y.	Enzyme-linked immunosorbent assay for screening estrogen receptor binding activity.	Techniques in Aquatic Tox, 277-285.	Screening assay.
2005	Koh C. -H., Khim J. S.,Villeneuve D. L., Kannan K., Johnson B. G., Giesy J. P.	Instrumental and bioanalytical measures of dioxin-like and estrogenic compounds and activities associated with sediment from the Korean coast.	Ecotoxicology and Environ Safety. 61(3):366-379.	Bioanalytical methods and instrumentation.
2005	Kong L., Wang L. -F., Zhang H. -Y.	Theoretical elucidation on mechanism and reactivity of bisphenol A derivatives as inhibitors and radical scavengers in methacrylate polymerization.	THEOCHEM. 716(1-3):27-31.	Polymers.
2005	Korn M. R.	End-functionalized poly(bisphenol A carbonate) oligomers. Part I: Synthesis and examples.	ACS Symposium Series, 898 (Advances in Polycarbonates), 39-57.	Polymers.
2005	Krakovsky I., Salmeron S. M.	Thermal transitions and structure of epoxy networks based on □, □-diamino terminated poly(propylene oxide)-block-poly(ethylene oxide)-block-poly(propylene oxide) swollen in water.	J Polymer Science Part B: Polymer Phy. 43(6):699-708.	Polymers.
2005	Kubo T., Hosoya K., Sano T., Nomachi M., Tanaka N., Kaya K.	Selective separation of brominated bisphenol A homologues using a polymer-based medium prepared by the fragment imprinting technique.	Analytica Chimica Acta. 549(1-2):45-50.	Analytical paper.
2005	Kuo S. -W., Chan S. -C., Wu H. -D., Chang F. -C.	An unusual, completely miscible, ternary hydrogen-bonded polymer blend of phenoxy, phenolic, PCL.	Macromolecules. 38(11): 4729-4736.	Polymers.
2005	Kurebayashi H., Nagatsuka S., Nemoto H., Noguchi H., Ohno Y.	Disposition of low doses of 14C-bisphenol A in male, female, pregnant, fetal, and neonatal rats.	Arch Toxicol. 79(5):243-52	Mammalian study.
2005	Kuruto-Niwa R., Nozawa R., Miyakoshi T., Shiozawa T., Terao Y.	Estrogenic activity of alkylphenols, bisphenols, and their chlorinated derivatives using a GFP expression system.	Env Tox & Pharm. 19(1):121-130.	Gene expression system to determine estogenic activity.
2005	Lahnsteiner F., Berger B., Kletzl M., Weismann T.	Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, *Salmo trutta* f. fario.	Aquat Toxicol. 75(3):213-24.	Effects of BPA on male and female trout during pre/spawning period (possible problem with reported exposure levels).
2005	Laviola G., Gioiosa L., Adriani W., Palanza P.	D-Amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors.	Brain Research Bulletin. 65(3):235-240.	Mammalian study.

Year	Authors	Title	Reference	Notes
2005	Lee H. B., Peart T. E., Svoboda M. L.	Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry.	J Chromatogr A. 1094(1-2):122-9.	Analytical paper.
2005	Lee, K. E., Sanocki, C. A., and Montz, G. R.	Physical, chemical, and biological characteristics of Sturgeon Lake, Goodhue County, Minnesota, 2003-04.	Scientific Investigations Report 2005-5182. US Department of the Interior and US Geological Survey.	Levels in US.
2005	Lee S. -M., Choi J.	HSP70 and HSC70 gene expression in Chironomus tentans (Diptera, Chironomidae) larvae exposed to various environmental pollutants: potential biomarker for environmental monitoring.	Environmental Mutagens and Carcinogens. 25(1):32-39.	Gene expression as biomarker
2005	Lee S. -M., Koo B. -W., Choi J. -W., Choi D. -H., An B. S., Jeung E. -B., Choi I. -G.	Degradation of bisphenol A by white rot fungi, Stereum hirsutum and Heterobasidium insulare, and reduction of its estrogenic activity.	Biological & Pharmaceutical Bulletin. 28(2):201-207.	Similar information already included.
2005	Lee S., Hyun J. -Gul., Park H., Kim J., Paik K. -W.	Frequency behavior of embedded epoxy/$SrTiO_3$ composite capacitor materials.	Proceedings - Electronic Components & Tech Conf, 55th (Vol. 2), 1222-1227.	Polymer/materials chemistry.
2005	Lee W. S., Takeuchi T.	Bisphenol A analog-imprinted polymers prepared by an immobilized template on a modified silica microsphere matrix.	Anal Sci. 21(9):1125-8.	Polymers
2005	Lee Y. -J., Huang J. M., Kuo S. -W., Chen J. -K., Chang F. -C.	Synthesis and characterizations of a vinyl-terminated benzoxazine monomer and its blending with polyhedral oligomeric silsesquioxane (POSS).	Polymer. 46(7):2320-2330.	Polymers.
2005	Lee Y., Yoon J., von Gunten U.	Kinetics of the oxidation of phenols and phenolic endocrine disruptors during water treatment with ferrate (Fe(VI)).	Environ Sci Technol. 39(22):8978-84.	Non-environmental degradation.
2005	Leepipatpiboon N., Sae-Khow O., Jayanta S.	Simultaneous determination of bisphenol-A-diglycidyl ether, bisphenol-F-diglycidyl ether, and their derivatives in oil-in-water and aqueous-based canned foods by high-performance liquid chromatography with fluorescence detection.	J Chromatogr A. 1073(1-2):331-9.	Chromatography.
2005	Leskinen P., Michelini E., Picard D., Karp M., Virta M.	Bioluminescent yeast assays for detecting estrogenic and androgenic activity in different matrices.	Chemosphere. 61(2):259-266.	Yeast assay.
2005	Leusch F. D. L., Chapman H. F., Koerner W., Gooneratne S. R., Tremblay L. A.	Efficacy of an advanced sewage treatment plant in southeast Queensland, Australia, to remove estrogenic chemicals.	E S & T. 39(15):5781-5786.	No mention of BPA in abstract.
2005	Li C., Li X. Z., Graham N.	A study of the preparation and reactivity of potassium ferrate.	Chemosphere. 61(4):537-43.	Non-environmental degradation.
2005	Li D., Dong M., Shim W. J., Hong S. H., Oh J. R., Yim U. H., Jeung J. H., Kanan N., Kim E. S., Cho S. R.	Seasonal and spatial distribution of nonylphenol and IBP in Saemangeum Bay, Korea.	Mar Pollut Bull. 51(8-12):966-74.	Levels in Korea.

Year	Authors	Title	Reference	Notes
2005	Li M., Daniels E. S., Dimonie V., Sudol E. D., El-Aasser M. S.	Preparation of polyurethane/acrylic hybrid nanoparticles via a mini-emulsion polymerization process.	Macromolecules. 38(10): 4183-4192.	Method for preparing nanoparticles.
2005	Li X. Z., He C., Graham N., Xiong Y.	Photoelectrocatalytic degradation of bisphenol A in aqueous solution using a Au-TiO_2/ITO film.	J Applied Electrochemistry. 35(7-8):741-750.	Non environmental degradation.
2005	Li X., Chu S., Fu S., Ma L., Liu X., Xu X.	Off-line concentration of bisphenol A and three alkylphenols by SPE then on-line concentration and rapid separation by reverse-migration micellar electrokinetic chromatography.	Chromatographia. 61(3/4): 161-166.	Analytical paper.
2005	Li X., Zhao C., Lu H., Wang Z., Na H.	Direct synthesis of sulfonated poly(ether ether ketone ketone)s (SPEEKKs) proton exchange membranes for fuel cell application.	Polymer. 46(15):5820-5827.	Polymers.
2005	Licht, O.	Effect of fenoxycarb and bisphenol A on the plant growth and benthic invertebrates in flow gutters.	Dissertation. Technische Universitat Dresden. February 2005.	Dissertation.
2005	Lill M. A., Dobler M., Vedani A.	In silico prediction of receptor-mediated environmental toxic phenomena - application to endocrine disruption.	SAR and QSAR in Env Res. 16(1-2):149-169.	QSAR study.
2005	Lill M. A., Winiger F., Vedani A., Ernst B.	Impact of induced fit on ligand binding to the androgen receptor: a multidimensional QSAR study to predict endocrine-disrupting effects of environmental chemicals.	J Medicinal Chemistry. 48(18):5666-5674.	QSAR study.
2005	Lin, B.-L., Tokai, A., and Nakanishi, J.	Approaches for establishing predicted-no-effect concentrations for population-level ecological risk assessment in the context of chemical substances management.	Environmental Science and Technology. 39(13):4833-4840.	Risk management.
2005	Liu J., Carr S., Rinaldi K., Chandler W.	Screening estrogenic oxidized by-products by combining ER binding and ultrafiltration.	Env Tox and Pharmacology. 20(2):269-278.	Screening study.
2005	Liu R., Wilding A., Hibberd A., Zhou J. L.	Partition of endocrine-disrupting chemicals between colloids and dissolved phase as determined by cross-flow ultrafiltration.	Environ Sci Technol. 39(8):2753-61.	Non-environmental sorption of EDCs.
2005	Liu Y. -L., Chou C. -I.	High performance benzoxazine monomers and polymers containing furan groups.	J Poly Sci Part A: Poly Chem. 43(21):5267-5282.	Polymers.
2005	Liu Z., Wolff M. S., Moline J.	Analysis of environmental biomarkers in urine using an electrochemical detector.	J Chrom B: Anal Technol in the Biomedical and Life Sci. 819(1):155-159.	Biomarkers in urine.
2005	Luda M. P., Euringer N., Moratti U., Zanetti M.	WEEE recycling: Pyrolysis of fire retardant model polymers.	Waste Manag (Amsterdam). 25(2):203-208.	TBBPA polymers.
2005	Lyko S., Wintgens T., Melin T.	Estrogenic trace contaminants in wastewater - possibilities of membrane bioreactor technology.	Desalination. 178(1-3):95-105.	Non-environmental degradation.
2005	MacLusky N. J., Hajszan T., Leranth C.	The environmental estrogen bisphenol a inhibits estradiol-induced hippocampal synaptogenesis.	Environ Health Perspect. 113(6):675-9.	Mammalian study.

Year	Authors	Title	Reference	Notes
2005	Majumdar S., Mitra K., Raha S.	Optimized species growth in epoxy polymerization with real-coded NSGA-II.	Polymer. 46(25):11858-11869.	Polymers.
2005	Marchesini G. R., Meulenberg E., Haasnoot W., Irth H.	Biosensor immunoassays for the detection of bisphenol A.	Analytica Chimica Acta. 528(1):37-45.	Biosensor assays.
2005	Margon V., Agarwal U. S., Peters C. J., de Wit G., Bailly C., van Kasteren J. M. N., Lemstra P. J.	Phase equilibria of binary, ternary and quaternary systems for polymerization/depolymerization of polycarbonate.	J Supercritical Fluids. 34(3): 309-321.	Polymers.
2005	Marini F., Roncaglioni A., Novic M.	Variable selection and interpretation in structure-affinity correlation modeling of estrogen receptor binders.	J Chemical Info Modeling, 45(6):1507-1519.	Binding assay.
2005	Markey C. M., Wadia P. R., Rubin B. S., Sonnenschein C., Soto A. M.	Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol A in the female mouse genital tract.	Biology of Reproduction. 72(6):1344-1351.	Mammalian study.
2005	Marks, H. and Coleman, M.	Presenting scientific theories within risk assessments.	Human and Ecological Risk Assessment. 11(2):271-287.	Risk management.
2005	Martin O. V., Lai K. M., Scrimshaw M. D., Lester J. N.	Receiver operating characteristic analysis for environmental diagnosis. A potential application to endocrine disruptor screening: *in vitro* estrogenicity bioassays.	E S & T. 39(14):5349-5355.	Bioassay.
2005	Masuda S., Terashima Y., Sano A., Kuruto R., Sugiyama Y., Shimoi K., Tanji K., Yoshioka H., Terao Y., Kinae N.	Changes in the mutagenic and estrogenic activities of bisphenol A upon treatment with nitrite.	Mutat Res. 585(1-2):137-46.	Non-environmental degradation.
2005	Masuno H., Iwanami J., Kidani T., Sakayama K., Honda K.	Bisphenol A accelerates aerminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway.	Tox Sciences. 84(2):319-327.	Cellular study.
2005	Matsumoto H., Adachi S., Suzuki Y.	Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and Its concentration changes over 6 months.	Arch Environ Contam Toxicol. 48(4):459-66.	Mammalian study.
2005	Matsumoto K., Sakai T., Torimaru A., Ishitobi S.	A surface plasmon resonance-based immunosensor for sensitive detection of bisphenol A.	J Faculty of Agriculture, Kyushu University. 50(2): 625-634.	Analytical paper.
2005	Matsuoka, M., Ishibashi, H., Takao, Y., Kohra, S., and Arizono, K.	Ecotoxicological effect and an estrogenic activity by BPA-related compounds.	8th Annual Meeting Japan Soc Endocrine Disrupters Res, Program and Abstracts, 27-29, Sept. 2005.	BPA-related substances only.
2005	Meulenberg E. P., Koopal K., Rhemrev R.	Immunoassays for alkylphenolic pollutants with endocrine disrupting activity.	Int J Env Analytical Chem. 85(12-13):871-883.	Bioassay.
2005	Michelini E., Leskinen P., Virta M., Karp M., Roda A.	A new recombinant cell-based bioluminescent assay for sensitive androgen-like compound detection.	Biosensors & Bioelectronics. 20(11):2261-2267.	Bioluminescent assay.
2005	Michizoe J., Ichinose H., Kamiya N., Maruyama T., Goto M.	Biodegradation of phenolic environmental pollutants by a surfactant-laccase complex in organic media.	J Biosci Bioeng. 99(6):642-7.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2005	Mills, L. J. and Chichester, C.	Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations?	Science of the Total Environment. 343(1-3):1-34.	Impact of EDCs on fish populations.
2005	Milnes, M. R., Bermudez, D. S., Bryan, T. A., Edwards, T. M., Gunderson, M. P., Larkin, I. L. V., Moore, B. C., and Guilletette Jr., L. J.	Contaminant-induced feminization and demasculinization of nonmammalian vertebrate males in aquatic environments.	Environmental Research. 100(1):3-17.	Review
2005	Miyazaki, S., Kitamura, S., Satoh, J., Gotoh, Y., and Hanada, H.	Effects of estrogen-like substances on amphibian metamorphosis.	8th Annual Meeting Japan Soc Endocrine Disrupters Res, Program and Abstracts, 27-29, Sept. 2005.	Meeting abstract only.
2005	Mlynarcikova A., Fickova M., Scsukova S.	Ovarian intrafollicular processes as a target for cigarette smoke components and selected environmental reproductive disruptors.	Endocr Regul. 39(1):21-32. Review.	Human health effects.
2005	Mlynarcikova A., Kolena J., Fickova M., Scsukova S.	Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate.	Mol Cell Endocrinol. 244(1-2):57-62.	Mammalian study.
2005	Modaressi K., Taylor K. E., Bewtra J. K., Biswas N.	Laccase-catalyzed removal of bisphenol-A from water: protective effect of PEG on enzyme activity.	Water Res. 39(18):4309-16.	Non-environmental degradation.
2005	Moral A., Sicilia M. D., Rubio S., Perez-Bendito D.	Determination of bisphenols in sewage based on supramolecular solid-phase extraction/liquid chromatography/fluorimetry.	J Chromatography A. 1100(1):8-14.	Deals with wastewater.
2005	Mormann W., Spitzer D.	Ammonolysis of polycarbonates with (supercritical) ammonia: an alternative for chemical recycling.	ACS Symposium Series, 898 (Advances in Polycarbonates), 244-261.	Polycarbonate recycling.
2005	Munguia-Lopez E. M., Gerardo-Lugo S., Peralta E., Bolumen S., Soto-Valdez H.	Migration of bisphenol A (BPA) from can coatings into a fatty-food simulant and tuna fish.	Food Addit Contam. 22(9):892-8.	Migration of bisphenol A into food.
2005	Munoz-de-Toro M., Markey C. M., Wadia P. R., Luque E. H., Rubin B. S., Sonnenschein C., Soto A. M.	Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice.	Endocrinology. 146(9):4138-47.	Mammalian study.
2005	Naciff J. M., Hess K. A., Overmann G. J., Torontali S. M., Carr G. J., Tiesman J. P., Foertsch L. M., Richardson B. D., Martinez J. E., Daston G. P.	Gene expression changes induced in the testis by transplacental exposure to high and low doses of 17□-ethynyl estradiol, genistein, or bisphenol A.	Toxicol Sci. 86(2):396-416.	Gene expression in testis.
2005	Nakaji-Hirabayashi T., Endo H., Kawasaki H., Gemmei-Ide M., Kitano H.	Inclusion of bisphenols by a self-assembled monolayer of thiolated calix[6]arene on a gold surface.	Environ Sci Technol. 39(14):5414-20.	Water purification.
2005	Nghiem L., Schaefer A., Elimelech M.	Nanofiltration of hormone mimicking trace organic contaminants.	Separation Sci Tech. 40(13): 2633-2649.	Water purification.

Year	Authors	Title	Reference	Notes
2005	Nikaido Y., Danbara N., Tsujita-Kyutoku M., Yuri T., Uehara N., Tsubura A.	Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice.	In Vivo. 19(3):487-94.	Mammalian study.
2005	Nishizawa H., Imanishi S., Manabe N.	Effects of exposure *in utero* to bisphenol A on the expression of aryl hydrocarbon receptor, related factors, and xenobiotic metabolizing enzymes in murine embryos.	J Reprod Dev. 51(5):593-605.	Mammalian study.
2005	Nishizawa H., Morita M., Sugimoto M., Imanishi S., Manabe N.	Effects of *in utero* exposure to bisphenol A on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos.	J Reprod and Development. 51(3):315-324.	Mammalian study.
2005	Noda S., Muroi T., Mitoma H., Takakura S., Sakamoto S., Minobe A., Yamasaki K.	Reproductive toxicity study of bisphenol A, nonylphenol, and genistein in neonatally exposed rats.	Journal of Toxicologic Pathology. 18(4):203-207.	Mammalian study.
2005	Noureldin M. B., Hasan A. K.	Global energy targets and optimal operating conditions for waste energy recovery in Bisphenol-A plant.	Appl Thermal Engineering, Volume Date 2006, 26(4): 374-381.	Waste energy recovery.
2005	Oehlmann, J., Schulte-Oehlmann, U., Bachmann, J., and Oetken, M.	Low dose effects of bisphenol A in the freshwater snail *Marisa cornuarietis*.	Environmental Research. 98(3):413.	Covered in other papers
2005	Oetken, M., Stachel, B., Pfenninger,M., and Oehlmann, J.	Impact of a flood disaster on sediment toxicity in a major river system - the Elbe flood 2002 as a case study.	Environmental Pollution. 134(1):87-95.	Mixture toxicity.
2005	Oftedal B. E., Ladstein S., Telle W., Male R.	Ligand-dependent protein interactions of the estrogen receptors using the yeast two-hybrid system.	Ann N Y Acad Sci. 1040:420-5.	Yeast hybrid assay.
2005	Ohnuma, A., Conlon, J. M., Kawasaki, H., and Iwamuro, S.	Induction by thyroid hormone of a novel antimicrobial peptide gene in the skin of *Rana tagoi* and its suppression by bisphenol A.	Zoological Science. 22(12):1500.	Gene suppression
2005	Ohsako S., Tohyama C.	Comparison of study controls.	Environ Health Perspect. 113(9):A582; author reply A582-3. [No abstract available].	Study controls.
2005	Ohtani Y., Shimada Y., Kozawa K., Shiraishi F.	Estrogen activity increased in the process of biodegradation of bisphenol A.	Zenkoku Kankyoken Kaishi. 30:18-23.	Endocrine activity of breakdown products.
2005	Okada A., Niwa Y., Katase T., Kai O.	Controlled release of estradiol-17□ and bisphenol A from a silicone tube for long-term administration in mice.	Animal Science Journal (Carlton South, Australia). 76(6):535-539.	Mammalian study.
2005	Okada K., Hiroi T., Imaoka S., Funae Y.	Inhibitory effects of environmental chemicals on protein disulfide isomerase *in vitro*.	Osaka City Med J. 51(2):51-63.	Enzyme study.
2005	Olmstead A. W., LeBlanc G. A.	Toxicity assessment of environmentally relevant pollutant mixtures using a heuristic model.	Int Env Assessment and Management. 1(2):114-122.	Mixture toxicity.
2005	Olsen C. M., Meussen-Elholm E. T., Hongslo J. K., Stenersen J., Tollefsen K. E.	Estrogenic effects of environmental chemicals: an interspecies comparison.	Comp Biochem Physiol C Toxicol Pharmacol. 141(3):267-74.	Cell line studies.

Year	Authors	Title	Reference	Notes
2005	Ooe H., Taira T., Iguchi-Ariga S. M., Ariga H.	Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1.	Toxicol Sci. 88(1):114-26.	Cellular biology.
2005	Oota S., Murakami T., Takemura K., Noto K.	Evaluation of MBR effluent characteristics for reuse purposes.	Water Sci and Tech. 51(6-7, Water Env--Membrane Technology):441-446.	Evaluation of effluents.
2005	Opitz, R., Braunbeck, T., Boegi, C., Pickford, D. B., Nentwig, G., Oehlmann, J., Tooi, O., Lutz, I., and Kloas, W.	Description and initial evaluation of a Xenopus metamorphosis assay for detection of thyroid system-disrupting activities of environmental compounds.	Environmental Toxicology and Chemistry. 24(3):653-664.	Method development
2005	Owens J. W., Chaney J. G.	Weighing the results of differing 'low dose' studies of the mouse prostate by Nagel, Cagen, and Ashby: quantification of experimental power and statistical results.	Regul Toxicol Pharmacol. 43(2):194-202.	Mammalian study.
2005	Panzica G., Mura E., Pessatti M., Viglietti-Panzica C.	Early embryonic administration of xenoestrogens alters vasotocin system and male sexual behavior of the Japanese quail.	Domest Anim Endocrinol. 29(2):436-45.	Similar information already included
2005	Park C. -K., Shin J. -S., Kim M -Y., Kim P. G.	Time serial concentration of phthalate esters and bisphenol-A contaminated from spring water container's cap and seal film.	Hangug Hwangyeong Bogeon Haghoeji. 31(6):457-466.	Human exposure.
2005	Park J. S., Lim S. H., Kim B. W.	Interferometric biosensing of DNA-damaging chemicals.	Biosens Bioelectron. May 12; [Epub ahead of print].	Analytical paper.
2005	Patel R. G., Patel M. P., Patel R. G.	3,6-Disubstituted fluorans containing 4(3H)-quinazolinon-3-yl, diethyl amino groups and their application in reversible thermochromic materials.	Dyes and Pigments.66(1):7-13.	Derivatives of bisphenol A.
2005	Paul, C., Rhind, S. M., Kyle, C. E., Scott, H., McKinnell, C., and Sharpe, R. M.	Cellular and hormonal disruption of fetal testis development in sheep reared on pasture treated with sewage sludge.	Environmental Health Perspectives. 113(11):1580-1587.	Mammalian study.
2005	Pillon A., Boussioux A. -M., Escande A., Ait-Aissa S., Gomez E., Fenet H., Ruff M., Moras D., Vignon F., Duchesne M. -J., Casellas C., Nicolas J. -C., Balaguer P.	Binding of estrogenic compounds to recombinant estrogen receptor-□: application to environmental analysis.	Env Health Perspectives. 113(3):278-284.	Paper reports a binding assay.
2005	Pinero R., Garcia J., Cocero M. J.	Chemical recycling of polycarbonate in a semi-continuous lab-plant. A green route with methanol and methanol-water mixtures.	Green Chem. 7(5):380-387.	Recycling of polycarbonate.
2005	Porrini S., Belloni V., Della Seta D., Farabollini F., Giannelli G., Dessi-Fulgheri F.	Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats.	Brain Research Bulletin. 65(3):261-266.	Mammalian study.

Year	Authors	Title	Reference	Notes
2005	Potyrailo R. A., Lemmon J. P.	Time-modulated combinatorially developed optical sensors for determination of non-volatile analytes in complex samples.	QSAR & Combinatorial Science. 24(1):7-14.	Analytical paper.
2005	Pressman E. J., Johnson B. F., Shafer S. J.	Monomers for polycarbonate manufacture: Synthesis of BPA and DPC.	ACS Symposium Series, 898 (Advances in Polycarbonates), 22-38.	Synthesis of bisphenol A.
2005	Rahman M. A., Kaneco S., Suzuki T., Katsumata H., Ohta K.	Optimized conditions for the solar photocatalytic degradation of bisphenol A in water using zinc oxide.	Ann Chim. 95(9-10):715-9. No abstract available.	Non-environmental degradation.
2005	Raikwar H. P., Muthian G., Rajasingh J., Johnson C., Bright J. J.	PPARgamma antagonists exacerbate neural antigen-specific Th1 response and experimental allergic encephalomyelitis.	J Neuroimmunol. 167(1-2):99-107.	Cellular biology study.
2005	Ranhotra H. S., Teng C. T.	Assessing the estrogenicity of environmental chemicals with a stably transfected lactoferrin gene promoter reporter in HeLa cells.	Environmental Tox and Pharmacology. 20(1):42-47.	Gene promoter assay.
2005	Rao B. S., Reddy K. R., Pathak S. K., Pasala A. R.	Benzoxazine-epoxy copolymers: Effect of molecular weight and crosslinking on thermal and viscoelastic properties.	Polymer Int. 54(10):1371-1376.	Polymers.
2005	Rasmussen K., Carstensen O., Ponten A., Gruvberger B., Isaksson M., Bruze M.	Risk of contact allergy and dermatitis at a wind turbine plant using epoxy resin-based plastics.	Int Arch of Occupational and Env Health. 78(3):211-217.	Human health study.
2005	Ravit B., Ehrenfeld J. G., Haeggblom M. M.	Salt marsh rhizosphere affects microbial biotransformation of the widespread halogenated contaminant tetrabromobisphenol-A (TBBPA).	Soil Biology & Biochemistry. 37(6):1049-1057.	Paper looks at TBBPA.
2005	Razzoli M., Valsecchi P., Palanza, P.	Chronic exposure to low doses of bisphenol A interferes with pair-bonding and exploration in female Mongolian gerbils.	Brain Research Bulletin. 65(3):249-254.	Mammalian study.
2005	Rhind, S. M., Kyle, C. E., Telfer, G., Duff, E. I., and Smith, A.	Alkyl phenols and diethylhexyl phthalate in tissues of sheep grazing pastures fertilized with sewage sludge or inorganic fertilizer.	Environmental Health Perspectives. 113(4):447-453.	Mammalian study.
2005	Rivera A., Blochowicz T., Porokhonskyy V., Rossler E. A.	Comment on "Thermal glass transition beyond the Vogel-Fulcher-Tammann behavior for glass forming diglycidylether of bisphenol A".	Phys Rev Lett. Apr 1;94(12):129603; author reply 129604. [No abstract available].	Polymers.
2005	Ronen Z., Visnovsky S., Nejidat A.	Soil extracts and co-culture assist biodegradation of 2,4,6-tribromophenol in culture and soil by an auxotrophic *Achromobacter piechaudii* strain TBPZ.	Soil Biol & Biochem. 37(9): 1640-1647.	Paper deals with the biodegradation of 2,4,6-tribromophenol.
2005	Roy R., Trono M. C., Giguere D.	Effects of linker rigidity and orientation of mannoside clusters for multivalent interactions with proteins.	ACS Symposium Series, 896 (Glycomimetics), 137-150, 1 Plate.	Paper looks at interactions with proteins.
2005	Rykowska I., Szymanski A., Wasiak W.	Determination of bisphenol-A in drinking water using new SPE sorbents with chemically bonded ketoimine groups.	Polish Journal of Food and Nutrition Sciences. 14(3): 237-241.	Analytical paper.

Year	Authors	Title	Reference	Notes
2005	Sabatini L., Barbieri A., Violante F. S.	Development and validation of a capillary high-performance liquid chromatography/electrospray tandem mass spectrometric method for the quantification of bisphenol A in air samples.	Rapid Commun Mass Spectrom. 19(23):3468-72.	Analytical paper.
2005	Sajiki, J. and Izumi, N.	Concentration of BPA and insect hormones in larvae and pupae of house fly exposed to bisphenol-A (BPA).	8th Annual Meeting Japan Soc Endocrine Disrupters Res, Program and Abstracts, 27-29, Sept. 2005.	Meeting abstract only
2005	Sakazaki H., Ido R., Ueno H., Nakamuro K.	17□-Estradiol primes elicitation of inducible nitric oxide synthase expression by lipopolysaccharide and interferon-□ in mouse macrophage cell line J774.1.	J Health Sci. 51(1):62-69.	Mammalian cell line study.
2005	Sakurai K., Sugaya N., Nakagawa T., Uchiyama T., Fujimoto Y., Takahashi K.	Simultaneous analysis of endocrine disruptors, 4-alkylphenol and bisphenol A, contained in synthetic resin products used for drug containers and household utensils.	J Health Sci. 51(5):538-548.	Possible levels in plastics.
2005	Sambe H., Hoshina K., Hosoya K., Haginaka J.	Direct injection analysis of bisphenol A in serum by combination of isotope imprinting with liquid chromatography-mass spectrometry.	Analyst (Cambridge, United Kingdom). 130(1):38-40.	Analytical paper.
2005	Sando, S. K., Furlong, E. T., Gray, J. L., Meyer, M. T., and Bartholomay, R. C.	Occurrence of organic wastewater compounds in wastewater effluent and the Big Sioux River in the upper Big Sioux River basin, South Dakota, 2003-2004.	U. S. Geological Survey Scientific Investigations Report 2005-5249.	Levels in US
2005	Sandstrom M. W., Kolpin D. W., Thurman E. M., Zaugg S. D.	Widespread detection of N,N-diethyl-m-toluamide in U.S. Streams: Comparison with concentrations of pesticides, personal care products, and other organic wastewater compounds.	Environ Tox and Chemistry. 24(5):1029-1034.	Possible levels in US waters.
2005	Sanseverino J., Gupta R. K., Layton A. C., Patterson S. S., Ripp S. A., Saidak L., Simpson M. L.; Schultz T. W., Sayler G. S.	Use of *Saccharomyces cerevisiae* BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds.	Appl Env Microbiology. 71(8):4455-4460.	Bioassay.
2005	Sasaki M., Maki J., Oshiman K., Matsumura Y., Tsuchido T.	Biodegradation of bisphenol A by cells and cell lysate from *Sphingomonas* sp. strain AO1.	Biodegradation. 16(5):449-59.	Similar studies already included.
2005	Satoh K., Nonaka R., Ohyama K. -I., Nagai F.	Androgenic and antiandrogenic effects of alkylphenols and parabens assessed using the reporter gene assay with stably transfected CHO-K1 cells (AR-EcoScreen system).	J Health Sci. 51(5):557-568.	Gene assay.
2005	Schirling, M., Jungmann, D., Ladewig, V., Nagel, R., Triebskorn, R., and Koehler, H.-R.	Endocrine effects in *Gammarus fossarum* (Amphipoda): Influence of wastewater effluents, temporal variability, and spatial aspects on natural populations.	Archives of Environmental Contamination and Toxicology. 49(1):53-61.	Not specific to bisphenol A.
2005	Schultis, T.	Detection of estrogen activity of environmental samples and pure substances by biological test systems - development and comparison of *in vitro* assays.	Stuttgarter Berichte zur Siedlungswasserwirtschaft. 181:i-xix, 1-232.	Assay development

Year	Authors	Title	Reference	Notes
2005	Schwartz-Mittelman A., Baruch A., Neufeld T., Buchner V., Rishpon J.	Electrochemical detection of xenoestrogenic and antiestrogenic compounds using a yeast two-hybrid-17-□-estradiol system.	Bioelectrochem. 65(2):149-156.	Yeast bioassay.
2005	Segner, H.	Developmental, reproductive, and demographic alterations in aquatic wildlife: Establishing causality between exposure to endocrine-active compounds (EACs) and effects	Acta Hydrochimica et Hydrobiologica. 33(1):17-26.	General paper discussing exposure to EDCs and effects.
2005	Seidlova-Wuttke D., Jarry H., Christoffel J., Rimoldi G., Wuttke W.	Effects of bisphenol-A (BPA), dibutylphtalate (DBP), benzophenone-2 (BP2), procymidone (Proc), and linurone (Lin) on fat tissue, a variety of hormones and metabolic parameters: a 3 months comparison with effects of estradiol (E2) in ovariectomized (ovx) rats.	Toxicology. 213(1-2):13-24.	Mammalian study.
2005	Shao B., Han H., Hu J., Zhao J., Wu G., Xue Y., Ma Y., Zhang S.	Determination of alkylphenol and bisphenol A in beverages using liquid chromatography/electrospray ionization tandem mass spectrometry.	Analytica Chimica Acta. 530(2):245-252.	Analytical paper.
2005	Shao J., Shi G., Jin X., Song M., Shi J., Jiang G.	Preliminary survey of estrogenic activity in part of waters in Haihe River, Tianjin.	Chinese Science Bulletin. 50(22):2565-2570.	Possible levels of EDCs in China.
2005	Shen, G., Zhang, Z., Yu, G., Li, X., Hu, H., and Li, F.	Dissolved neutral nonylphenol ethoxylates metabolites in the Haihe River and Bohai Bay, People's Republic of China.	Bulletin of Environmental Contamination and Toxicology. 75(4):827-834.	Paper discusses nonylphenol.
2005	Shen G., Yu G., Cai Z., Zhang Z.	Development of an analytical method to determine phenolic endocrine disrupting chemicals in sewage and sludge by GC/MS.	Chinese Science Bulletin. 50(23):2681-2687.	Analytical paper.
2005	Shirai M., Mitsukura K., Okamura H., Miyasaka M.	Multi-functional methacrylates bearing thermal degradation properties - Synthesis, photo- and thermal curing, and thermolysis.	J Photopolymer Sci & Tech. 18(2):199-202.	Polymers.
2005	Shoji R., Nishimura T., Vepsalainen J., Ljungberg K.	Activated carbon adsorption, activated sludge and ozonation treatments evaluated by impact intensity based on acute toxicity test.	Toxicological and Environ Chemistry. 87(1):55-65.	Water purification.
2005	Silva, E., Scholze, M., Backhaus, T., and; Kortenkamp, A.	Assessment of combinations of six mitogenic agents in the E-screen assay reveals small deviations from concentration additivity.	Environmental Research. 98(3):415.	Mixture toxicity
2005	Simoes A. M., Tallman D. E., Bierwagen G. P.	Use of ionic liquids for the electrochemical characterization of water transport in organic coatings.	Electrochemical and Solid-State Letters. 8(10):B60-B63.	Electrochemical characterization.
2005	Simoneit, B., R. T., Medeiros, P. M., and Didyk, B. M.	Combustion products of plastics as indicators for refuse burning in the atmosphere.	Environmental Science and Technology. 39(18):6961-6970.	Paper looks at plastics.

Year	Authors	Title	Reference	Notes
2005	Singh K. P., Lopez-Guerrero J. A., Llombart-Bosch A., Roy D.	Estrogen-induced mutations and its role in the development of tumorigenesis.	Horm Carcin IV, Proc Int Symp, 4th, Valencia, Spain, 21-25 June, 2003, 475-479.	Human health study.
2005	Skjevrak I., Brede C., Steffensen I. -L., Mikalsen A., Alexander J., Fjeldal P., Herikstad H.	Non-targeted multi-component analytical surveillance of plastic food contact materials: Identification of substances not included in EU positive lists and their risk assessment.	Food Add & Contaminants. 22(10):1012-1022.	Food contamination.
2005	Skretas G., Wood D. W.	A bacterial biosensor of endocrine modulators.	J Mol Biol. 349(3):464-474.	Paper describes a bacterial biosensor.
2005	Sonoki T., Kajita S., Ikeda S., Uesugi M., Tatsumi K., Katayama Y., Iimura Y.	Transgenic tobacco expressing fungal laccase promotes the detoxification of environmental pollutants.	Appl Microbiol & Biotech. 67(1):138-142.	Non-environmental degradation.
2005	Sosiak, A. and Hebben, T.	A preliminary survey of pharmaceuticals and endocrine disrupting compounds in treated municipal wastewaters and receiving rivers of Alberta.	Alberta Environment Publication Number T/773.	Survey of EDCs in wastewaters in Canada.
2005	Srividhya M., Lakshmi M. S., Reddy B. S. R.	Chemistry of siloxane amide as a new curing agent for epoxy resins: Material characterization and properties.	Macromolecular Chem and Physics. 206(24):2501-2511.	Polymers.
2005	Stuart J. D., Capulong C. P., Launer K. D., Pan X.	Analyses of phenolic endocrine disrupting chemicals in marine samples by both gas and liquid chromatography-mass spectrometry.	J Chromatogr A. 1079(1-2):136-45.	Analytical paper.
2005	Su Y. -C., Yei D. -R., Chang F. -C.	The kinetics of B-a and P-a type copolybenzoxazine via the ring opening process.	J Applied Polymer Science. 95(3):730-737.	Polymers.
2005	Sugiura-Ogasawara M., Ozaki Y., Sonta S., Makino T., Suzumori K.	Exposure to bisphenol A is associated with recurrent miscarriage.	Hum Reprod. 20(8):2325-9.	Human health.
2005	Sugiyama S., Miyoshi H., Yamauchi K.	Characteristics of a thyroid hormone responsive reporter gene transduced into a *Xenopus laevis* cell line using lentivirus vector.	Gen Comp Endocrinol. 144(3):270-9.	Cell line study.
2005	Sugiyama, S., Shimada, N., Miyoshi, H., and Yamauchi, K.	Detection of thyroid system-disrupting chemicals using *in vitro* and *in vivo* screening assays in *Xenopus laevis*.	Toxicological Sciences. 88(2):367-374.	Method development.
2005	Sumpter, J. P.	Endocrine disrupters in the aquatic environment: An overview.	Acta hydrochimica et hydrobiologica. 33(1):9-16.	Review
2005	Sumpter, J. P. and Johnson, A. C.	Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment.	Environmental Science and Technology. 39(12):4321-4332.	General paper on endocrine disruption.
2005	Sun S., Sun P., Liu D.	The study of esterifying reaction between epoxy resins and carboxyl acrylic polymers in the presence of tertiary amine.	European Polymer Journal. 41(5):913-922.	Polymers.
2005	Sun W. L., Ni J. R., O'Brien K. C., Hao P. P., Sun L. Y.	Adsorption of bisphenol A on sediments in the Yellow River.	Water, Air & Soil Pollution. 167(1-4):353-364.	Not clear what sediments the K_d values refer to.

Year	Authors	Title	Reference	Notes
2005	Suzuki S., Ishii T., Yasuhara Akio., Sakai S.	Method for the elucidation of the elemental composition of low molecular mass chemicals using exact masses of product ions and neutral losses: Application to environmental chemicals measured by liquid chromatography with hybrid quadrupole/time-of-flight mass spectrometry.	Rapid Comm in Mass Spec. 19(23):3500-3516.	Analytical paper.
2005	Sybert P., Klei S., Rosendale D., Di J., Shen D.	Weatherability and physical properties of opaque injection moldable LEXAN SLX resins.	Annual Technical Conference, Society of Plastics Engineers, 63rd, 2523-2527.	Polymers.
2005	Tai C., Jiang G., Liu J., Zhou Q., Liu J.	Rapid degradation of bisphenol A using air as the oxidant catalyzed by polynuclear phthalocyanine complexes under visible light irradiation.	J Photochem & Photobiol A: Chem. 172(3):275-282.	Non-environmental degradation.
2005	Takahashi M., Tsukamoto S., Kawaguchi A., Sakamoto A., Morikawa H.	Phytoremediators from abandoned rice field.	Plant Biotech (Tokyo, Japan). 22(2):167-170.	Non-environmental degradation.
2005	Takemura H., Ma J., Sayama K., Terao Y., Zhu B. T., Shimoi K.	*In vitro* and *in vivo* estrogenic activity of chlorinated derivatives of bisphenol A.	Toxicology. 207(2):215-221.	Paper deals with chlorinated BPA.
2005	Takao, Y., Oishi, M., Yamaguchi, H., Nagae, M., Kohra, S., and Arizono, K.	Time changes of bisphenol A concentrations in Medaka and in their eggs.	8th Annual Meeting Japan Soc Endocrine Disrupters Research, Prog and Abs, 27-29, Sept. 2005.	Meeting abstract only.
2005	Tanaka M., Ishizaka Y., Tosuji H., Kunimoto M., Hosoya N., Nishihara N., Kadono T., Kawano T., Kosaka T., Hosoya H.	A new bioassay for toxic chemicals using green paramecia, *Paramecium bursaria*.	Environmental Chemistry, 673-680.	Bioassay.
2005	Taniguchi M., Kato K., Shimauchi A., Ping X., Nakayama H., Fujita K., Tanaka T., Tarui Y., Hirasawa E.	Proposals for wastewater treatment by applying flocculating activity of cross-linked poly-gamma-glutamic acid.	J Biosci Bioeng. 99(3):245-51.	Non-environmental degradation.
2005	Teeguarden J. G., Waechter J. M. Jr., Clewell H. J., Covington T. R., Barton H. A.	Evaluation of oral and intravenous route pharmacokinetics, plasma protein binding, and uterine tissue dose metrics of bisphenol A: a physiologically based pharmacokinetic approach.	Tox Sci. 85(2):823-838.	Mammalian study.
2005	ten Cate M. G. J., Reinhoudt D. N., Crego-Calama M.	Binding of small guest molecules to multivalent receptors.	J Org Chem. 70(21):8443-8453.	Binding study.
2005	Teramoto, T., Nakajima, N., Kasai, F., Tamaoki, M., Saji, H., Aono, M., Kubo, A., Saji, H., and Kamada, H.	Glycosylation of bisphenol A by green algae.	8th Annual Meeting Japan Soc Endocrine Disrupters Research, Prog and Abs, 27-29, Sept. 2005.	Meeting abstract only.
2005	Terasaka H., Kadoma Y., Sakagami H., Fujisawa S.	Cytotoxicity and apoptosis-inducing activity of bisphenol A and hydroquinone in HL-60 cells.	Anticancer Res. 25(3B):2241-7.	Cellular study.

Year	Authors	Title	Reference	Notes
2005	Terasaki M., Shiraishi F., Nishikawa T., Edmonds J. S., Morita M., Makino M.	Estrogenic activity of impurities in industrial grade bisphenol A.	Environ Sci Technol. 39(10):3703-7.	Similar information already included.
2005	Terasaki M., Shiraishi F., Nishikawa T., Morita M., Makino M.	A practical synthesis and estrogenic activity of 5-hydroxy-1-(4'-hydroxyphenyl)-1,3,3-trimethylindan, a contaminant in industrial grade bisphenol A.	Chem Lett. 34(2):188-189.	Similar information already included.
2005	Thiruvenkatachari R., Kwon T. O., Moon I. S.	A total solution for simultaneous organic degradation and particle separation using photocatalytic oxidation and submerged microfiltration membrane hybrid process.	Korean J of Chem Eng. 22(6): 938-944.	Non-environmental degradation.
2005	Thiruvenkatachari R., Kwon T. O., Moon I. S.	Application of slurry type photocatalytic oxidation-submerged hollow fiber microfiltration hybrid system for the degradation of bisphenol A (BPA).	Science and Technology. 40(14):2871-2888.	Non-environmental degradation.
2005	Thomson B. M., Grounds P. R.	Bisphenol A in canned foods in New Zealand: an exposure assessment.	Food Addit Contam. 22(1):65-72.	Human exposure.
2005	Tilton, S. C., Foran, C. M., and Benson, W. H.	Relationship between ethinylestradiol-mediated changes in endocrine function and reproductive impairment in Japanese medaka (*Oryzias latipes*).	Environmental Toxicology and Chemistry. 24(2):352-359.	Not BPA specific.
2005	Timms B. G., Howdeshell K. L., Barton L., Bradley S., Richter C. A., vom Saal F. S.	Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra.	Proc Natl Acad Sci U S A. 102(19):7014-9.	Mammalian study.
2005	Toda M., Ogawa N., Itoh H., Hamada F.	Unique molecular recognition property of bis-pyrene-modified □-cyclodextrin dimer in collaboration with □-cyclodextrin.	Analytica Chimica Acta. 548(1-2):1-10.	Molecular recognition.
2005	Tokumoto T., Tokumoto M., Nagahama Y.	Induction and inhibition of oocyte maturation by EDCs in zebrafish.	Reprod Biol and Endocrinol 3. No pp. given.	Effects of EDCs on oocyte maturation.
2005	Trad H., Jaballah N., Majdoub M., Roudesli S., Roussel J., Fave J. L.	Synthesis of a novel luminescent copolymer based on bisphenol A.	Polymer International. 54(9):1314-1319.	Polymer synthesis.
2005	Tran T. T. M., Tran T. L., Pham H. V.	Analytical determination of relevant alkylphenols and bisphenol A of landfill leachates samples collected in Hanoi's dumping sites.	Tap Chi Phan Tich Hoa, Ly Va Sinh Hoc. 10:66-71.	Analytical paper.
2005	Trubo R.	Endocrine-disrupting chemicals probed as potential pathways to illness.	JAMA. 294(3):291-3. [No abstract available].	Human response to EDCs.
2005	Tilton, S. C., Foran, C. M., and Benson, W. H.	Relationship between ethinylestradiol-mediated changes in endocrine function and reproductive impairment in Japanese medaka (*Oryzias latipes*).	Environmental Toxicology and Chemistry. 24(2):352-359.	Not BPA specific.

Year	Authors	Title	Reference	Notes
2005	Tschmelak J., Proll G., Riedt J., Kaiser J., Kraemmer P., Barzaga L., Wilkinson J. S., Hua P., Hole J. P., Nudd., Jackson M., Abuknesha R., Barcelo D., Rodriguez-Mozaz S., Lopez de Alda M. J., Sacher F., Stien J., Slobodnik J., Oswald P., Kozmenko H., Korenkova E., Tothova L., Krascsenits Z., Gauglitz G.	Automated Water Analyser Computer Supported System (AWACSS).	Biosensors & Bioelectronics. 20(8):1509-1519.	Analytical paper.
2005	Tsue H., Takimoto T., Kikuchi C., Yanase H., Takahashi H., Amezawa K., Ishibashi K., Tanaka S., Tamura R.	Adsorptive removal of bisphenol A by calix[4]crown derivatives: Significant contribution of hydrogen bonding interaction to the control of adsorption behavior.	Chem Lett. 34(7):1030-1031.	Water purification.
2005	Tsuruta Y., Inoue H., Fukunaga K., Munemura S., Ozaki M., Ohta M., Matsuura F.	Determination of bisphenol-A in water by semi-micro column high-performance liquid chromatography using 2-methoxy-4-(2-phthalimidinyl)phenylsulfonylchloride as a fluorescent labeling reagent.	Anal Sci. 21(6):697-9.	Analytical paper.
2005	Tsutsumi O.	Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction.	J Steroid Biochem Mol Biol. 93(2-5):325-30.	Human health.
2005	Turan N., Waring R. H., Ramsden D. B.	The effect of plasticisers on "sulphate supply" enzymes.	Molecular and Cellular Endocrin. 244(1-2):15-19.	Paper looks at plasticizers.
2005	Turner S. R., King B., Ponasik J., Adams V., Connell G.	Amorphous copolyesters containing monomers derived from bisphenols.	High Performance Polymers. 17(3):361-376.	Polymers.
2005	Ueda, T., Imaoka, T., and Yoshimura, T.	Leaching load of bisphenol A from the plastic sheet by rainfall.	8th Annual Meeting Japan Soc Endocrine Disrupters Research, Prog and Abs, 27-29, Sept. 2005.	Meeting abstract only.
2005	Ueki T., Nishijima S., Izumi Y.	Designing of epoxy resin systems for cryogenic use.	Cryogenics. 45(2):141-148.	Polymers.
2005	Ulrich S., Wachtershauser A., Loitsch S., von Knethen A., Brune B., Stein J.	Activation of PPARgamma is not involved in butyrate-induced epithelial cell differentiation.	Exp Cell Res. 310(1):196-204.	Cellular study.
2005	Urase T., Kagawa C., Kikuta T.	Factors affecting removal of pharmaceutical substances and estrogens in membrane separation bioreactors.	Desalination. 178(1-3):107-113.	Non-environmental degradation.
2005	Urase T., Kikuta T.	Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process.	Water Res. 39(7):1289-300.	Not easy to relate to wwtp or environment.
2005	Vedani A., Dobler M., Lill M. A.	Combining protein modeling and 6D-QSAR. Simulating the binding of structurally diverse ligands to the estrogen receptor.	J Med Chem. 48(11):3700-3703.	Binding modelling.
2005	Verslycke, T. A., Vethaak, A. D., Arijs, K., and Janssen, C. R.	Flame retardants, surfactants and organotins in sediment and mysid shrimp of the Scheldt estuary (The Netherlands).	Environmental Pollution. 136(1):19-31.	Paper looks at flame retardants.

Year	Authors	Title	Reference	Notes
2005	Verstegen E. J. K., Kloosterboer J. G., Lub J.	Synthesis and photopolymerization of oxetanes derived from BPA.	J Applied Polymer Science. 98(4):1697-1707.	Polymers.
2005	Vethaak A.D., Lahr J., Schrap S. M., Belfroid A. C., Rijs G.B.J., Gerristen A., de Boer J., Bulder A. S., Grinwis G. C. M., Kuiper R. V., Legler J., Murk T. A. J., Peijnenburg W., Verhaar H. J. M., de Voogt P.	An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of the Netherlands.	Chemosphere. 59, 511-524.	Field study in NL. Mixed exposure, not useable in assessment.
2005	Viglietti-Panzica, C., Montoncello, B., Mura, E., Pessatti, M., and Panzica, G.	Organizational effects of diethylstilbestrol on brain vasotocin and sexual behavior in male quail.	Brain Research Bulletin. 65(3):225-233.	Not BPA
2005	Voelkel W., Bittner N., Dekant W.	Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry.	Drug Metab Dispos. 33(11):1748-57.	Analytical paper.
2005	Vogel, J. R., Barber, L. B., Furlong, E. T., Coplen, T. B., Verstraeten, I. M., and Meyer, M. T.	Occurrence of selected pharmaceutical and non-pharmaceutical compounds and stable hydrogen and oxygen isotope ratios in a riverbank filtration study, Platte River, Nebraska, 2002 to 2005, volume 2.	U. S. Geological Survey Data Series 141.	Levels in US
2005	Vogel, J. R., Verstraeten, I. M., Coplen, T. B., Furlong, E. T., Meyer, M. T., and Barber, L. B.	Occurrence of selected pharmaceutical and non-pharmaceutical compounds and stable hydrogen and oxygen isotope ratios in a riverbank filtration study, Platte River, Nebraska, 2001 to 2003, volume 1.	U. S. Geological Survey Data Series 117. (Supplemental section in Critical Evaluation column)	Levels in US
2005	vom Saal F. S., Hughes C.	An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment.	Environ Health Perspect. 113(8):926-33. Review.	Literature review of mammalian studies.
2005	vom Saal F. S., Nagel S. C., Timms B. G., Welshons W. V.	Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public.	Toxicology. 212(2-3):244-52, author reply 253-4. [No abstract available].	Paper looks at human response to bisphenol A.
2005	vom Saal F. S., Richter C. A., Mao J., Welshons W. V.	Commercial animal feed: variability in estrogenic activity and effects on body weight in mice.	Birth Defects Res A Clin Mol Teratol. 73(7):474-5. [No abstract available].	Mammalian study.
2005	vom Saal F. S., Richter C. A., Ruhlen R. R., Nagel S. C., Timms B. G., Welshons W. V.	The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A.	Birth Defects Research, Part A: Clinical and Molecular Teratology. 73(3):140-145.	Mammalian study.
2005	Vranova D.	Quantification of soy isoflavones in meat products by HPLC.	Scripta Medica Facultatis Medicae Universitatis Brunensis Masarykianae. 78(4):235-242.	Analytical methodology.
2005	Wagner, M. and Nicell, J. A.	Evaluation of horseradish peroxidase for the treatment of estrogenic alkylphenols.	Water Quality Research Journal of Canada. 40(2):145-154.	Non-environmental degradation.

Year	Authors	Title	Reference	Notes
2005	Walsh D. E., Dockery P., Doolan C. M.	Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [Ca^{2+}] in human breast cancer cells.	Molecular and Cellular Endocrin. 230(1-2):23-30.	Human cells, genomic study.
2005	Wan L., Luo Y., Hu Y., Huang F., Du L.	Synthesis and characterization of a novel polymer containing 1,2,3-triazole.	Pol Preprints (Am Chem Soc, Division Polymer Chem). 46(2):1014-1015.	Polymer synthesis.
2005	Wang S., Wei X., Du L., Zhuang H.	Determination of bisphenol A using a flow injection inhibitory chemiluminescence method.	Luminescence. 20(1):46-50.	Analytical paper.
2005	Wang X. Q., Cui S., Liu S., Yin D., Wang L.	Holographic QSAR of environmental estrogens.	Science in China, Series B: Chemistry. 48(2):156-161.	QSAR study.
2005	Wang X., Liu N., Cao H., Liu W., Chen C., Zhang W., Wei Y.	Chiral crystal structure of racemic binaphthyl Poly(ether ketone) macrocycles.	Macromolecular Rapid Comm. 26(7):554-557.	Polymer.
2005	Wang Y., Hu W., Cao Z., Fu X., Zhu T.	Occurrence of endocrine-disrupting compounds in reclaimed water from Tianjin, China.	Anal Bioanal Chem. 383(5):857-63.	Analytical.paper.
2005	Warbritton R.	BPA: Acute Toxicity Test (96 hours) with the Giant Ramshorn Snail, *Marisa cornuarietis*, determined under static renewal test conditions.	Unpublished report, ABC Laboratories Study Number 50017, ABC Laboratories, Columbia, MO.	Acute tox to snails would not affect outcome. RAR discusses chronic exposures only.
2005	Watabe Y., Hosoya K., Tanaka N., Kondo T., Morita M., Kubo T.	LC/MS determination of BPA in river water using a surface-modified molecularly-imprinted polymer as an on-line pretreatment device.	Analytical and Bioanalytical Chemistry. 381(6):1193-1198.	Analytical paper.
2005	Watabe Y., Hosoya K., Tanaka N., Kubo T., Kondo T., Morita M.	Novel surface modified molecularly imprinted polymer focused on the removal of interference in environmental water samples for chromatographic determination.	J Chromatogr A. 1073(1-2): 363-70.	Chromatography.
2005	Watabe Y., Hosoya K., Tanaka N., Kubo T., Kondo T., Morita M.	Shielded molecularly imprinted polymers prepared with a selective surface modification.	J Polymer Science, Part A: Polymer Chemistry. 43(10): 2048-2060.	Polymers.
2005	Watanabe E., Eun H., Baba K., Arao T., Endo S., Ueji M., Ishii Y.	Synthesis of haptens for development of antibodies to alkylphenols and evaluation and optimization of a selected antibody for ELISA development.	J Agric Food Chem. 53(19): 7395-403.	Bioanalytical paper.
2005	Watanabe, M., Mitani, N., Ishii, N., and Miki, K.	Erratum: A mutation in a cuticle collagen causes hypersensitivity to the endocrine disrupting chemical, bisphenol A, in Caenorhabditis elegans.	Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 578(1-2):436.	Correction to paper already included.
2005	Watanabe, N., Ishibashi, H., Miyazaki, H., Hirano, M., Chihiro, K., Takao, Y., Nishimura T., Arizono, K.	Combination effects of estradiol-17ß, 4-t-octylphenol and bisphenol A on the reproduction of medaka.	8th Annual Meeting Japan Soc Endocrine Disrupters Research, Prog and Abs, 27-29, Sept. 2005.	Mixture toxicity
2005	Watermann B. T., Daehne B., Sievers S., Dannenberg R., Overbeke J. C., Klijnstra J. W., Heemken O.	Bioassays and selected chemical analysis of biocide-free antifouling coatings.	Chemosphere. 60(11):1530-41.	No BPA effects reported.

Year	Authors	Title	Reference	Notes
2005	Watson C. S., Bulayeva N. N., Wozniak A. L., Finnerty C. C.	Signaling from the membrane via membrane estrogen receptor-alpha: estrogens, xenoestrogens, and phytoestrogens.	Steroids. 70(5-7):364-71.	Genomic steroid signalling mechanisms.
2005	Webb L. J., Miles K. K., Auyeung D. J., Kessler F. K., Ritter J. K.	Analysis of substrate specificities and tissue expression of rat UDP-glucuronosyl transferases UGT1A7 and UGT1A8.	Drug Metabolism and Disposition. 33(1):77-82.	Mammalian study.
2005	Weber S., Leuschner P., Kaempfer P., Dott W., Hollender J.	Degradation of estradiol and ethinyl estradiol by activated sludge and by a defined mixed culture.	Appl Microbiol & Biotech. 67(1):106-112.	Non-environmental degradation.
2005	Weltje L., vom Saal F. S., Oehlmann J.	Reproductive stimulation by low doses of xenoestrogens contrasts with the view of hormesis as an adaptive response.	Hum Exp Toxicol. 24(9): 431-7.	Effects of xenoestrogens on human reproduction.
2005	Westerhoff, P., Yoon, Y., Snyder, S., and Wert, E.	Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes.	Environmental Science and Technology. 39(17):6649-6663.	Water purification.
2005	Wetherill Y. B., Fisher N. L., Staubach A., Danielsen M., de Vere White R. W., Knudsen K. E.	Xenoestrogen action in prostate cancer: Pleiotropic effects dependent on androgen receptor status.	Cancer Res. 65(1):54-65.	Human health study.
2005	Wong K. O., Leo L. W., Seah H. L.	Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles.	Food Addit Contam. 22(3):280-8.	Human exposure.
2005	Wozniak A. L., Bulayeva N. N., Watson C. S.	Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-□-mediated Ca^{2+}fluxes and prolactin release in GH3/B6 pituitary tumor cells.	Environmental Health Perspectives. 113(4):431-439.	Cell assay.
2005	Xie, J.	Analysis of Xenopus laevis claudius (Xcla) tight junction genes in development.	Developmental Biology. 283(2):692-693.	Gene assay.
2005	Xu J. -z., Jiang N. -z., Zhang J., Jiang R. -s.	Synthesis of bisphenols carrying long hydrocarbon side chains.	Chem Research in Chinese Universities. 21(1):65-68.	Paper looks at derivatives of BPA.
2005	Xu J., Wong C. P.	Dielectric behavior of ultrahigh-k carbon black composites for embedded capacitor applications.	Proceedings - Electronic Comp & Tech Conf 55th (Vol. 2), 1864-1869.	Polymers.
2005	Xu L. C., Sun H., Chen J. F., Bian Q., Qian J., Song L., Wang X. R.	Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol *in vitro*.	Toxicology. 216(2-3):197-203.	Paper focuses on transcriptional activities of BPA.
2005	Yamashita U., Sugiura T., Yoshida Y., Kuroda E.	Effect of endocrine disrupters on macrophage functions *in vitro*.	Journal of UOEH. 27(1):1-10.	Macrophge functions.
2005	Yamazaki N., Washio I., Shibasaki Y., Ueda M.	Facile synthesis of poly(phenylene-ether) dendrimers from unprotected AB2-building block using thionyl chloride as a condensing agent.	Polymer Preprints (Am Chemical Society, Division of Polymer Chemistry). 46(1): 645-646.	Polymers.

Year	Authors	Title	Reference	Notes
2005	Yan Y., Tao X., Sun Y., Yu W., Wang C., Xu G., Yang J., Zhao X., Jiang M.	Synthesis, structure and nonlinear optical properties of a two-photon photopolymerization initiator.	Journal of Materials Science. 40(3):597-600.	Polymers
2005	Yanagihara N., Toyohira Y., Ueno S., Tsutsui M., Utsunomiya K., Liu M., Tanaka K.	Stimulation of catecholamine synthesis by environmental estrogenic pollutants.	Endocrinology. 146(1):265-272.	Paper discusses catecholamine synthesis.
2005	Yang C. -P., Chen R. -S., Chen K. -H.	Organosoluble and light-colored fluorinated polyimides based on 2,2-bis[4-(4-amino-2-Trifluoromethyl phenoxy)phenyl]propane and aromatic dianhydrides.	J Applied Polymer Science. 95(4):922-935.	Polymers.
2005	Yang F. -X., Xu Y., Pfister G., Henkelmann B., Schramm K. -W.	Nonylphenol, bisphenol-A and DDTs in Lake Donghu, China.	Fresenius Environmental Bulletin. 14(3):173-180.	Possible levels in China.
2005	Yang K. L., Zongbin M. M., Zhang X., Zhao C., Nishi N.	Molecularly imprinted polyether-sulfone microspheres for the binding and recognition of bisphenol A.	Analytica Chimica Acta. 546(1):30-36.	Analytical paper.
2005	Yasuda S., Wu P. -S., Okabe M., Tachibana H., Yamada K.	Tissue-specific distribution of genistein, daidzein and bisphenol A in male Sprague-Dawley rats after intragastric administration.	Food Science and Technology Research. 11(2):187-193.	Mammalian study.
2005	Ye X., Kuklenyik Z., Needham L. L., Calafat A. M.	Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzo phenone in humans by online solid phase extraction- high performance liquid chromatography-tandem mass spectrometry.	Anal Bioanal Chem. 383(4):638-44.	Analytical paper.
2005	Ye X., Kuklenyik Z., Needham L. L., Calafat A. M.	Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine.	Anal Chem. 77(16):5407-13.	Analytical paper.
2005	Yoshino T., Kato F., Takeyama H., Nakai M., Yakabe Y., Matsunaga T.	Development of a novel method for screening of estrogenic compounds using nano-sized bacterial magnetic particles displaying estrogen receptor.	Analytica Chimica Acta. 532(2):105-111.	Analytical paper.
2005	Yoshioka T., Sugawara K., Mizoguchi T., Okuwaki A.	Chemical recycling of polycarbonate to raw materials by thermal decomposition with calcium hydroxide/steam.	Chem Letters. 4(3):282-283.	Paper looks at polycarbonate recycling.
2005	Zandi-Zand R., Ershad-Langroudi A., Rahimi A.	Organic-inorganic hybrid coatings for corrosion protection of 1050 aluminum alloy.	J Non-Crystalline Solids. 351(14&15):1307-1311.	Paper looks at polymers.
2005	Zeng Z., Shan T., Tong Y., Lam S. H., Gong Z.	Development of estrogen-responsive transgenic medaka for environmental monitoring of endocrine disrupters.	Environ Sci Technol. 39(22):9001-8.	Development of fish strain for monitoring.
2005	Zhan M., Yang X., Xian Q., Kong L.	Photosenstized degradation of bisphenol A involving reactive oxygen species in the presence of humic substances.	Chemosphere, 63, 378-386	Possible small effect on regional PECs only

Year	Authors	Title	Reference	Notes
2005	Zhao C. Y., Zhang R. S., Zhang H. X., Xue C. X., Liu H. X., Liu M. C., Hu, Z. D., Fan B. T.	QSAR study of natural, synthetic and environmental endocrine disrupting compounds for binding to the androgen receptor.	SAR and QSAR in Env Res. 16(4):349-367.	QSAR study.
2005	Zhao Y., Foryst-Ludwig A., Bruemmer D., Culman J., Bader M., Unger T., Kintscher U.	Angiotensin II induces peroxisome proliferator-activated receptor gamma in PC12W cells via angiotensin type 2 receptor activation.	J Neurochem. 94(5):1395-401.	Cellular study.
2005	Zhou X., Andrienko D., Delle Site L., Kremer K.	Dynamic surface decoupling in a sheared polymer melt.	Europhysics Letters. 70(2): 264-270.	Polymers.
2005	Zhou X., Andrienko D., Delle Site L., Kremer K.	Dynamic surface decoupling in a sheared polymer melt.	Los Alamos Nat Lab, Pre Arch, Condensed Matter 1-7, arXiv:cond-mat/0502575	Polymers.
2005	Zoeller R. T.	Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals?	Mol Cell Endocrinol. 242(1-2):10-5. Review.	Mammalian study.
2005	Zoeller R. T., Bansal R., Parris C.	Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist *in vitro*, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain.	Endocrinology. 146(2):607-612.	Mammalian study.
2005	Zsarnovszky A., Le H. H., Wang H. S., Belcher S. M.	Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A.	Endocrinology. 146(12):5388-96.	Mammalian study.
2004	Benijts T., Lambert W., De Leenheer A.	Analysis of multiple endocrine disruptors in environmental waters via wide-spectrum solid-phase extraction and dual-polarity ionization LC-Ion Trap-MS/MS.	Analytical Chemistry. 76(3):704-711.	Analytical paper.
2004	Chin Y-P., Miller P. L.,Cawley K., Weavers L. K.	Photosensitized degrdadation of bisphenol A by dissolved organic matter.	Environ. Sci. Technol., 38, 5888-5894	Possible small effect on regional PECs only
2004	Eriksson J., Rahm S., Green N., Bergman A., Jakobsson E.	Photochemical transformation of tetrabromobpisheol A and related phenols in water.	Chemosphere, 63, 117-126	Not environmental conditions
2004	Hernando M. D., Mezcua M., Gomez M. J., Malato O., Aguera A., Fernandez-Alba A. R.	Comparative study of analytical methods involving gas chromatography-mass spectrometry after derivatization and gas chromatography-tandem mass spectrometry for the determination of selected endocrine disrupting compounds in wastewaters.	J Chromatography A. 1047(1):129-135.	Wastewater.
2004	Jacobsen B. N., Kjersgaard D., Winther-Nielsen M., Gustavson K.	Combined chemical analyses and biomonitoring at Avedoere wastewater treatment plant in 2002.	Water Science and Technology. 50(5):37-43.	Wastewater treatment plant.

Year	Authors	Title	Reference	Notes
2004	Sone K., Hinago M., Kitayama A., Morokuma J., Ueno N., Watanabe H., Iguchi T.	Effects of 17β-estradiol, nonylphenol, and bisphenol-A on developing *Xenopus laevis* embryos.	Gen. Comp. Endocrinol. 138:228-236.	Covered elsewhere.
2004	Stehmann A., Meesters R. J. W., Schroeder H. Fr.	Mass spectrometric analytical methods for the determination of endocrine disrupting chemicals (EDCs).	Water Science and Tech. 50(5):165-171.	Paper looks at analytical methods.
2003	Arbeli Z., Ronen Z.	Enrichment of a microbial culture capable of reductive debromination of the flame retardant tetrabromobisphenol-A, and identification of the intermediate metabolites produced in the process.	Biodegradation. 14:385-395.	TBBPA reference.
2003	Arizono K., Ura K., Tominaga N., Kai T., Kohara Y., Iguchi T.	*C. elegans* as a tool for environmental toxicology.	First Toxicogenomics International Forum Tokyo, Japan October 31 - November 01, 2001. Toxicogenomics. 129-134.	*C elegans*, plate exposure so not relevant for assessment.
2003	Fuerhacker M.	Bisphenol A emission factors from industrial sources and elimination rates in a sewage treatment plant.	Water Science and Technology. 47(10):117-122.	Paper deals with sewage treatment plants.
2003	Furuya M., Sasaki F., Hassanin A. M., Kuwahara S., Tsukamoto Y.	Effects of bisphenol-A on the growth of comb and testes of male chicken.	The Canadian Journal of Veterinary Research. 67(1):68-71.	Injection, not relevant route of exposure (chicken).
2003	Ishihara K., Nakajima N.	Improvement of marine environmental pollution using eco-system: decomposition and recovery of endocrine disrupting chemicals by marine phyto- and zooplanktons.	J Molecular Catalysis B: Enzymatic. 23:419-424.	Sorption study, does not effect the conclusions.
2003	Ohtani Y., Shimada Y., Shiraishi F., Kozawa K.	Variation of estrogenic activities during the bio-degradation of bisphenol A.	Kankyo Kagaku. 13:1027-1031.	Endocrine activity of breakdown products.
2003	Sashihara K., Yamashita T., Takagi T., Nakanishi T., Furuse M.	Effects of Intrayolk Injection of Bisphenol A on Hatchability and Sex Ratio in Chickens.	Journal of Applied Animal Research. 24:113-122.	More recent study by same authors in update (chicken).
2002	Nieminen P., Lindstrom-Seppa P., Juntunen M., Asikainen J., Mustonen A., Karonen S., Mussalo-Rauhamaa H., Kukkonen J.V.K.	In vivo effects of bisphenol A on the polecat (*Mustela putorius*).	Journal of Toxicology and Environmental Health, Part A., 65(13), 933-945.	Limited secondary poisoning section to standard org. Would not affect assessment.

Year	Authors	Title	Reference	Notes
2002	Nieminen P., Lindstrom-Seppa P., Mustonen A., Mussalo-Rauhamaa H., Kukkonen J.V.K.	Bisphenol A Affects Endocrine Physiology and Biotransformation Enzyme Activities of the Field Vole (*Microtus agrestis*).	General and Comparative Endocrinology. 126:183-189.	Limited secondary poisoning section to standard organsisms. Not relevant route of exposure, injection.
2002	Staples C. A., Woodburn K., Caspers N., Hall A. T., Klečka G. M.	A weight of evidence approach to the aquatic hazard assessment of bisphenol A.	Hum. Ecol. Risk Assess. 8:1083-1105.	Review paper, no new data reported.
2001	Ishibashi H., Tachibana K., Tsuchimoto M., Soyano K., Ishibashi Y., Nagae M., Kohra S., Takao Y., Tominaga N., Arizono K.	In Vivo Testing System for Determining the Estrogenic Activity of Endocrine-Disrupting Chemicals (EDCs) in Goldfish (*Carassius auratus*).	Journal of Health Science. 47(2):213-218.	VTG goldfish. Nothing significantly different to what is already included.
2001	Kishida M., McLellan M., Miranda J. A., Callard G.V.	Estrogen and xenoestrogens upregulate the brain aromatase isoform (P450aromB) and perturb markers of early development in zebrafish (*Danio rerio*).	Comparative Biochemistry and Physiology. 129B:261-268.	Not significantly different to what is already included.
2001	Spengler P., Korner W., Metzger J. W.	Substances with estrogenic activity in effluents of sewage treatment plants in south western Germany. 1. Chemical analysis.	Env Tox and Chem. 20(10):2133-2141.	Sewage treatment plants.
2000	Bolz U., Koerner Q., Hagenmaier H.	Development and validation of a GC/MS method for determination of phenolic xenoestrogens in aquatic systems.	Chemosphere. 40(9-11):929-935.	Analytical paper.
2000	Fuerhacker M., Scharf S., Weber H.	Bisphenol A: emissions from point sources.	Chemosphere. 41(5):751-756.	Covered elsewhere.
2000	Koerner W., Bolz U., Sussmuth W., Hiller G., Schuller W., Hanf V., Hagenmaier H.	Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany.	Chemosphere. 40(9-11):1131-1142.	Wastewater treatment.
2000	Ronen Z., Abeliovich A.	Anaerobic-aerobic process for microbial degradation of tetrabromobisphenol A.	Applied Environ Microbiol. 66:2372-2378.	TBBPA reference.
2000	Weltin D.	Part 1. Mobility and fate of endocrine disrupters in soil. Lysimeter and run-off studies.	Final Report. Contract no ENV4-CT97-0473.	Sorption study, does not affect the conclusions.
1999	Caunter J. E., Evans M. R., Sumpter J., Sohoni A.	Bisphenol A: Effect on the embryo-larval developmental stage of the fathead minnow (*Pimephales promelas*).	Unpublished report, Brixham Environmental Laboratories, Brixham, UK.	Range finder for study not included in RAR, would not change outcome.

Year	Authors	Title	Reference	Notes
1999	Celius T., Haugen T.B., Grotmol T., Walther B.T.	A sensitive zonagenetic assay for rapid in Vitro assessment of estrogenic potency of xenobiotics and mycotoxins.	Environmental Health Perspectives. 107(1):63-68.	Not significantly different to what is already included.
1999	Islinger M., Pawlowski S., Hollert H., Volkl A., Braunbeck T.	Measurement of vitellogenin-mRNA expression in primary cultures of rainbow trout hepatocytes in a non-radioactive dot blot/RNAse protection-assay.	The Science of the Total Environment. 233:109-122.	Not significantly different to what is already included.
1999	Koerner W., Spengler P., Bolz U., Hagenmaier H., Metzger J.	Monitoring of estrogenic substances in sewage plant effluents by biological and chemical analysis.	Organohalogen Compounds. 42:29-32.	Wastewater.
1999	Lutz I., Kloas W.	Amphibians as a model to study endocrine disruptors: I. Environmental pollution and estrogen receptor binding.	The Science of the Total Environment. 225: 49-57.	Not significantly different to what is already included.
1989	Bayer AG.	Biodegradation study.	Unpublished report by Bayer AG.	Biodegradation, does not affect conclusions.
1989	Bayer AG.	Acute toxicity for *Brachydanio rerio*.	Unpublished report by Bayer AG, 114A/89F.	RAR has value from Bayer for *B. rerio*, but not same value. Would not affect assessment.
1984	Alexander H. C.	Analysis of Bisphenol A (p,p') in Lagoon Feed and 303 Outfall.	Unpublished report of the Dow Chemical Company.	Biotreatment pond, unusual treatment, not useable in the assessment.

UPDATED RISK ASSESSMENT OF 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A)

CAS Number: 80-05-7
EINECS Number: 201-245-8

FINAL APPROVED VERSION AWAITING PUBLICATION

(to be read in conjunction with published EU RAR of BPA, 2003)

April 2008

Introduction

A risk assessment of 4,4'-isopropylidenediphenol (Bisphenol-A, BPA) produced in accordance with Council Regulation (EEC) 793/93[1] was published in 2003[2]. In relation to human health, conclusion (i) "There is need for further information and/or testing" was reached for developmental toxicity. Further research was needed to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development at low doses. The information requirements were a 2-generation study in mice according to OECD 416 (with some specific modifications).

This 2-generation study has now been submitted to the Rapporteur (UK) for evaluation. The UK has updated the original risk assessment, reviewing the requested study and the new data on human exposure and effects of BPA that have become available since the original risk assessment report was completed.

The format of the report is broadly in line with that of the original risk assessment. Significant new information is summarised in this updated risk assessment and a comment is added to indicate how this affects the findings from the original risk assessment.

Rapporteur: United Kingdom

Contact (human health): Health & Safety Executive
Chemicals Assessment Schemes Unit
Redgrave Court, Merton Road
Bootle, Merseyside L20 7HS
Tel: +44 151 951 3791
Fax: +44 151 951 4889
E-mail: ukesrhh@hse.gsi.gov.uk

Contact (environment): Environment Agency
Chemicals Assessment Unit
Isis House, Howbery Park, Wallingford, Oxfordshire, OX10 8BD
Fax: + 44 1491 828 556
E-mail: ukesrenv@environment-agency.gov.uk

[1] O.J. No. L 084, 05/04/1993 p. 0001 - 0075
[2] European Union Risk Assessment Report: 4,4'-isopropylidenediphenol (BPA) – 3rd Priority List, Volume 37. European Commission Joint Research Centre, EUR 20843 EN.

CONTENTS

TABLES

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No: 80-05-7
EINECS No: 201-245-8
IUPAC name: 2,2-bis(4-hydroxyphenyl)propane (also known as Bisphenol-A, or BPA)

0.1 HUMAN HEALTH

0.1.1 Human health (toxicity)

0.1.1.1 Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached in relation to repeated dose systemic effects and for reproductive toxicity during the manufacture of BPA and the manufacture of epoxy resins. In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact with high concentrations (>30%) of BPA.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached in relation to repeated dose systemic effects and reproductive toxicity for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, thermal paper manufacture and manufacture of tin plating additive. This conclusion also applies in relation to eye and respiratory tract irritation and repeated dose local effects in the respiratory tract for all scenarios.

0.1.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached for all consumer scenarios in relation to all endpoints.

0.1.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached for both local and regional exposure scenarios in relation to all endpoints.

0.1.1.4 Combined exposure

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This conclusion is reached for combined exposure scenarios in relation to all endpoints.

0.1.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached because there are no risks from physico-chemical properties arising from the use of BPA.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 Occupational exposure

4.1.1.1.1 Summary of original risk assessment report

The total number of persons occupationally exposed to BPA is not known, but due to its widespread use in epoxy resins and polycarbonate it is expected to be thousands. However, the exposure is likely to be negligible in many cases as the residual BPA in epoxy resins and polycarbonate is low.

Most of the data used in this assessment have been supplied by industry, either directly or through trade organisations. The HSE has no BPA exposure data on its NEDB (National Exposure Database) and no data were available from any of the other competent authorities. There is little data available from published papers although two were found relating to (i) use of epoxy resin-based paint and (ii) the use of epoxy resin-based powder paints.

The occupational exposure to BPA is discussed in 10 scenarios:

- manufacture of BPA
- manufacture of PC
- manufacture of articles from PC
- manufacture of epoxy resins and moderated epoxy resins
- use of BPA in PVC manufacture
- manufacture of liquid epoxy paints, lacquers and powder coatings
- use of epoxy resin-based powder coatings, paints and lacquers
- manufacture of thermal papers
- manufacture of tin-plating additive
- manufacture of tetrabrominated flame retardants (TBBA)

Some uses of BPA have been identified but not discussed in the following sections as these uses do not apply in the European Union or because information on some of the minor uses was not available. These include tyre manufacturing, brake fluid manufacturing, polyols/polyurethane manufacturing and polyamide processing.

In a number of instances, companies supplying information stated that personal protective equipment and/or respiratory protective equipment was used. However, unless stated otherwise in the text, details of the type were not provided.

BPA Manufacture

The industry from which the highest inhalation exposures were reported was the BPA manufacturing industry with 8hr TWAs ranging from “none detected” to 23.3 mg/m^3.

Reasonable worst case scenarios were estimated using the 90th percentile. This was calculated where there is sufficient data. Where insufficient data were available, professional judgement was used to estimate the 90th percentile. A reasonable worst case 8hr TWA for BPA manufacturing was estimated at 5 mg/m^3.

Short term exposures varied considerably, ranging from "none detected" to 43.6mg/m^3. Generally, short term exposures rarely exceeded 10 mg/m^3.

Dermal exposure ranged from 0 to 5 mg/cm^2/day (EASE estimation). Bag filling and maintenance activities gave rise to the highest estimates for dermal exposure. A reasonable worst case scenario was decided as 5 mg/cm^2/day.

The inhalation exposure results on which this assessment was based can be found summarised in **Tables 4.1** (8hr TWAs) and **4.2** (short term exposures). The BPA manufacturing process is largely an enclosed system with breaches for product sampling, product bagging and tanker/silo filling and some maintenance activities. Product sampling is a short term activity typically lasting about 3 to 5 minutes, and may be carried out once or twice per shift. There were no short term sample results available so EASE was used to estimate exposures during this activity giving a three minute exposure range of 0 to 5 mg/m^3 and a short term exposure level of 0 to 1 mg/m^3. Short term results for bagging gave results of 14 and 15 mg/m^3, although these results were reported not to reflect the current occupational exposure. Data provided by SPI (USA) gave short term task-specific results between none detected and 0.96 mg/m^3. A reasonable worst case scenario for short term exposures was agreed as 10 mg/m^3.

8hr TWA exposures for operators varied widely, both in the way they were sampled and analysed, and in the range of the results reported. Many operators measured total inhalable particulate or respirable dust, with some samples being analysed specifically for BPA. The results ranged from none detected (nd) to 23.3 mg/m^3 8hr TWA. Product bagging and tanker/silo filling were reported to be full shift activities. Exposures for these activities were generally below 5 mg/m^3. All the EASE results predicted exposure ranges below 5 mg/m^3 for the above activities. The highest results were obtained where maintenance activities or cleaning was carried out during the sampling period, although information regarding the types of tasks carried out were not available. Sampling results for more recent maintenance activities (1998-2000) ranged from less than 0.05 to 0.62 mg/m^3. A reasonable worst case scenario for 8hr TWA for manufacturing activity was agreed as 5 mg/m^3.*Polycarbonate manufacture*

It was reported that there was little or no opportunity for exposure to BPA during the manufacture of polycarbonate, as the BPA entered the plant as a solution and was piped directly into a closed system. However, four respirable dust samples for PC dust had been collected in 1990-1991, although they were not analysed for BPA. Further dust sampling was undertaken from 1993 to 1996. These were for total inhalable particulate (TIP) and were not analysed for BPA. These results ranged from 0.1 to 1.1 mg/m^3. The 90th percentile for these figures was 1.0 mg/m^3. It was reported by industry that there is a maximum of 100 ppm residual BPA in the PC polymer. Taking this into account the reported results range from 7x10^{-7} to 1.1x10^{-4} mg/m^3, 8hr TWA with a 90th percentile of 1x10^{-4} mg/m^3, 8hr TWA. In 2000, the same company took a personal sample to confirm that there was no exposure to BPA in the PC manufacturing plant. The sample was analysed for BPA. The result was less than 1x10^{-3} mg/m^3, 8hr TWA. EASE modelling resulted in a range of 0 to 1x10^{-4} mg/m^3, 8hr

TWA. A reasonable worst case scenario for this activity was agreed as $1x10^{-3}$ mg/m^3, 8hr TWA.

There is reported to be no opportunity for exposure to BPA during the manufacture of articles from polycarbonate, due to the stability of the polymer, and the retention of any residual BPA within the polymer matrix. As the manufacturing process does not use any higher temperatures than those used for extrusion in the PC manufacturing industry, the same results were used to represent exposure in the manufacture of articles from PC. The reasonable worst case scenario was agreed as $1x10^{-3}$ mg/m^3, 8hr TWA. A number of responses from companies manufacturing epoxy resins and modified epoxy resins highlighted the charging of vessels with BPA prills or flakes as the main source of exposure in this industry. Short term exposures during this activity ranged from 0.32 to 17.5 mg/m^3, with 8hr TWAs of up 1.2 mg/m^3. A reasonable worst case scenario was agreed as an 8hr TWA of 0.7 mg/m^3. A reasonable worst case scenario for short term exposure was agreed as 11 mg/m^3.

PVC Manufacture

The use of BPA in PVC manufacture is being phased out. As handling of BPA is considered to be similar to industries such as thermal paper manufacturing, the EASE data for that scenario were used to generate data for PVC manufacturing. A reasonable worst case scenario was estimated to be 0.1 mg/m^3 8hr TWA. A short term reasonable worst case exposure was estimated to be 1 mg/m^3.

Manufacture of epoxy resins

Manufacture of liquid epoxy resin-based paints is not reported to be a source of significant exposure to BPA given the very low (10 ppm) quantity of residual BPA in the uncured epoxy resin, most of which would be retained within the resin matrix.

Use of epoxy resin-based paints

The residual amount of BPA in epoxy resins for powder paints is reported to be about 300 ppm. Calculations made using this figure and total inhalable particulate exposure measurements from the HSEs NEDB, gave an estimated exposure of up to 0.02 mg/m^3, 8hr TWA. Industry supplied data for personal exposure across all activities ranging from 0.3 to 10 mg/m^3, 8hr TWA for total inhalable particulate. This was calculated to give a range of personal exposures to BPA of $9x10^{-5}$ to $3x10^{-3}$ mg/m^3. Given that the amount of residual BPA in powder paints is likely to be lower than that calculated, a reasonable worst case scenario of 0.01 mg/m^3 8hr TWA was estimated. A short term reasonable worst case estimate of 0.3 mg/m^3 was made based on data from SPI

Exposure to total inhalable particulate during the use of powder paints has been reported to be across a higher range than for manufacturing. The percentage of BPA in the coating powder is up to 40%. The estimated range of 8hr TWAs is up to 0.02 mg/m^3. Actual measured exposure results were reported in a NIOSH paper. The range of 8hr TWAs reported was 0.003 to 1.063 mg/m^3. A reasonable worst case scenario for an 8hr TWA was estimated to be 0.5 mg/m^3 for spraying coating powders and 0.005 mg/m^3 for dip-painting.

Manufacture of thermal papers

Thermal paper manufacturers reported only one exposure result for BPA, which was lower than the limit of detection for an hour-long sample. An 8hr TWA calculated from this result gave a figure of less than 0.25 mg/m^3. Enough information was available to allow EASE estimations to be made. The estimated range predicted was 0 to 0.04 mg/m^3. A reasonable worst case scenario for an 8hr TWA for this industry was estimated to be 0.1 mg/m^3. A reasonable worst case scenario for short term exposure would be 4 mg/m^3.

Manufacture of tin plating additives

Small quantities of BPA are used in the manufacture of tin plating additives. No exposure data were available but sufficient information was supplied to allow an EASE prediction to be made. This gave an exposure range of 0.02 to 0.05 mg/m^3 8hr TWA, with the only source of exposure identified being the charging of the reactor vessel with BPA. A reasonable worst case scenario would be an 8hr TWA of 0.05 mg/m^3.

Manufacture of TBBA

One company was manufacturing TBBA using BPA. No exposure data were available, but EASE was used to estimate exposure during the packaging process. This gave an estimated exposure range of $6x10^{-6}$ to $1.5x10^{-5}$ mg/m^3 8hr TWA.

In summary, 8hr TWAs rarely exceeded 5 mg/m^3 in BPA manufacturing facilities, and rarely exceeded 0.5 mg/m^3 in the other industries discussed. Short term exposures could reach as high as 43.6 mg/m^3, but were more usually less than 10 mg/m^3.

Table 4.1 Summary table of occupational inhalation exposure data (8hr TWA) from the 2003 published RAR

Work activities	No of samples	Type of sample	Range 8hr TWA (mg/m^3)	Mean 8hr TWA (mg/m^3)	90th percentile 8hr TWA (mg/m^3)	RWC exposure inhal. BPA (mg/m^3)	Source
BPA manufacturing							
Sampling and filling (1988-1992)	24	resp. part.	0.04 to 5.01	0.59	1.23		Industry
Filling big bags (1998)	3	inhal. BPA	0.21 to 1.79	0.81	1.61		Industry
Filling silo tankers (1998)	3	inhal. BPA	less than 0.5 to 1.61	0.89			Industry
Various (1998)	8	inhal. BPA	0.13 to 0.62	0.3			
Various (1997)	8	TIP	less than 0.1 to 0.9	0.38	1.79		
Various (1993-1996)	15	TIP	less than 0.1 to 6	0.94			Industry
Filling (1988-1992)	4	TIP	0.42 to 1.79	1.1			
Packaging	9	TIP	0.002 to 7.5	1.1	n/a		Industry
Reworking	8	TIP	0.002 to 23.3	7.9	n/a		Industry
Plant operator	12	TIP	less than 0.1 to 0.8	0.3	n/a		Industry
Plant operator	13	*BPA	0.02 to 2.13	0.61	2.12		Industry
Maintenance operator	2	*BPA	0.04 to 2.08	1.06			Industry
Plant operator	7	inhal. BPA	0.21 to 1.04	not known	n/a		Industry
Maintenance	3	inhal. BPA	0.52 to 1.35	not known	n/a		Industry
Maintenance (1998-2000)	8	*BPA	Less than 0.05 to 0.62		n/a		Industry
Charging big bags	5	inhal. BPA	0.02 to 0.93	0.35	n/a		Industry
Various	not known	*BPA	nd to 2.6	not known	n/a		SPI (USA)
Operator incl. sampling	n/a	inhal. BPA	0 to 0.03	n/a	n/a		EASE
Product silo filling	n/a	inhal. BPA	0 to 1	n/a	n/a		EASE
Bag filling	n/a	inhal. BPA	0 to 5	n/a	n/a	5	EASE
PC manufacturing							
Plant operator	4	resp. part (BPA)	0.07 to 0.27 ($7x10^{-7}$ to $2.7x10^{-5}$)	0.2 ($2x10^{-5}$)	n/a	$1x10^{-3}$	Industry
Plant operator	16	TIP (BPA)	0.1 to 1.1 ($1x10^{-5}$ to $1.1x10^{-4}$)	0.43 ($4.3x10^{-5}$)	$1x10^{-4}$		Industry

Work activities	No of samples	Type of sample	Range 8hr TWA (mg/m^3)	Mean 8hr TWA (mg/m^3)	90th percentile 8hr TWA (mg/m^3)	RWC exposure inhal. BPA (mg/m^3)	Source
Plant operator	Not known	Resp. part (BPA)	Not known	Less than 0.1 (less than $1x10^{-5}$)	Not known		Industry
Plant operator	1	Inhal. BPA	n/a	Less than $1x10^{-3}$	n/a		Industry
Plant operator	n/a	Inhal. BPA	0 to 1x10-4	n/a	n/a		EASE
Manufacture of articles from PC							
Plant operator	n/a	Inhal. BPA	0 to 1x10-4	n/a	n/a	$1x10^{-3}$	EASE /Industry
Epoxy resin manufacturing							
Charging reactors	not known	various	less than 0.01 to 1.09	not known			Industry
Various	96	*BPA	less than 0.1 to 2.8	0.24	0.7		SPI (USA)
Container unloading	n/a	inhal. BPA	0 to 0.25	n/a		0. 7	EASE
Use of BPA in PVC manufacture							
Charging reactors	n/a	Inhal. BPA	0 to 0.04	n/a	n/a	0.1	EASE
Manufacture of epoxy resin-based paints, lacquers, and coating powders							
Coating powders manufacturing							
Various	28	inhal. BPA	$3.3x10^{-4}$ to 0.02 (calc)	0.01	0.008	0.01	HSE
Various	210	Inhal. BPA	$9x10^{-5}$ to $3x10^{-3}$	Not known	Not known		Industry
Use of epoxy resin-based paints, lacquers and coating powders							
Coating powders use							
Spraying, loading, cleaning	53	inhal. BPA	$2.4x10^{-5}$ to 0.02 (calc)	$1.6x10^{-3}$	0.005		HSE
Spray painters	6	inhal. BPA	0.173 to 1.063	0.6			NIOSH
Spray painters	n/a	Inhal. BPA	$6x10^{-4}$ to $6x10^{-3}$	n/a	n/a	0.5	EASE
Dip painters	2	inhal. BPA	0.004 to 0.005	0.0045		0.005	NIOSH
Dip/spray painters	7	resp. BPA	0.003 to 0.131	0.04			NIOSH
Dip painters	n/a	Inhal. BPA	$6x10^{-4}$ to $6x10^{-3}$	n/a	n/a		EASE

Work activities	No of samples	Type of sample	Range 8hr TWA (mg/m^3)	Mean 8hr TWA (mg/m^3)	90th percentile 8hr TWA (mg/m^3)	RWC exposure inhal. BPA (mg/m^3)	Source
Thermal paper manufacturing							
Charging reactor	1	inhal.BPA	less than 0.25	less than 0.25		0.1	Industry
Charging reactor	n/a	inhal. BPA	0 to 0.04	n/a			EASE
Manufacture of tin plating additive							
Manufacture of tin plating additive - charging vessel	n/a	inhal. BPA	0.02 to 0.05	n/a		0.05	EASE
Manufacture of TBBA							
Packaging final product	n/a	inhal. BPA	$6x10^{-6}$ to $1.5x10^{-5}$	n/a		$1.5x10^{-5}$	EASE

TIP = total inhalable particulate
Inhal. Part = inhalable particulate
Inhal. BPA = inhalable BPA
Resp. part. = respirable particulate
(BPA) = calculated BPA concentration in particulate

Table 4.2 Summary table of short term, task specific occupational inhalation exposures to BPA from the 2003 published RAR

Work activities	No of samples	Range (mg/m^3)	Mean (mg/m^3)	RWC exposure (mg/m^3)	Source
Bagging machine operator 1990	2	14 to 15	14.5	10	Industry
BPA manufacturing Various	15	nd to 0.96	not known		SPI (USA)
PC manufacturing Connecting BPA chargepoint	6	nd to less than 0.64	0.29	0.5	SPI (USA)
Epoxy resin manufacture- charging reactor	12	0.32 to 17.5 (inhalable dust)	1.52	11	Industry
Epoxy resin manufacture various	68	nd to 43.6	1.81		SPI (USA)
Manufacture of coating powders	2	Nd to 0.3	0.15	0.3	SPI (USA)
Use of BPA in PVC manufacture – charging reactors	n/a	0 to 1	n/a	1	EASE

Work activities	No of samples	Range (mg/m^3)	Mean (mg/m^3)	RWC exposure (mg/m^3)	Source
Thermal paper manufacture charging reactor	1	less than 4	less than 4	4	Industry

The results of dermal exposure predictions can be found in **Table 4.3**.

Dermal exposure to BPA can occur during manufacturing and use of BPA. During manufacturing, operators can come into contact during product sampling and during bag filling and other filling operations. Using the EASE model, dermal exposure during sampling was estimated to be in the range 0 to 0.1 mg/cm^2/day. Exposure is likely to be towards the lower end of the range as the activity takes less than five minutes to complete. It is estimated that 420 cm^2 of skin may be exposed during this activity.

Filling operations are full shift activities, so the potential for dermal exposure is greater. The EASE estimation gave a range of 1-5 mg/cm^2/day. The operators are reported to wear personal protective equipment, including gloves. PPE, properly selected and worn will significantly reduce exposure. A reasonable worst case exposure would be 1 mg/cm^2/day. It is estimated that 420 cm^2 of skin may be exposed during this activity.

The only potential for dermal exposure during PC manufacturing was during the bagging of PC granules. The EASE estimation gave a range of $1x10^{-5}$ to $1x10^{-4}$ mg/cm^2/day.

The same exposure range was used to estimate exposure during the manufacture of articles from PC, when loading PC granules from the big bags to the extruder.

The main source of exposure identified during epoxy resin manufacturing was the charging of reactors. The EASE estimation gave a range of 0.1 to 1 mg/cm^2/day. The use of PPE during this task was reported. PPE, properly selected and worn will significantly reduce exposure.

Estimations of dermal exposure during two maintenance activities were carried out using EASE as an illustration of the potential dermal exposures during general maintenance activities. The EASE prediction gave a range of 0.1 to 1 mg/cm^2/day for both activities.

For PVC manufacturing, a reasonable worst case scenario of 0.1 mg/cm^2/day was estimated for dermal exposure using EASE data. It was estimated that an area of skin equivalent to 420 cm^2 may be exposed during this activity.

EASE was used to predict dermal exposures during the manufacture and use of epoxy resin-based powder coatings. Although controls are generally poorer in these industries, the potential for exposure is lower due to the small amount of residual BPA in the epoxy resin (approximately 300 ppm). The range of dermal exposure predicted using EASE during epoxy resin-based powder coating manufacture was 3×10^{-4} to 1.5×10^{-3} mg/cm^2/day. The figure of $1.5x10^{-3}$ mg/cm^2/day was taken to be a reasonable worst case dermal exposure. The range estimated using EASE for powder coating application was 6×10^{-4} to 1.8×10^{-3} mg/cm^2/day given a maximum BPA content of 120 ppm. Charging reactors was the only activity identified by the thermal paper manufacturers and the tinplating additive manufacturers where the potential for dermal exposure arises. This activity takes about 5 to 10 minutes per shift. EASE was used to estimate a range of dermal exposure. The range predicted was 0 to 0.1 mg/cm^2/day.

Dermal exposure during bag filling of TBBA was estimated using EASE. The range predicted, taking into account the fact that there is only 3 ppm BPA in the final product, is $3x10^{-7}$ mg/cm^2/day to $3x10^{-6}$ mg/cm^2/day.

In summary, dermal exposure was estimated to be highest during filling operations during BPA manufacture, which is a full shift activity. The estimated range of exposures were the same for charging reactors and maintenance activities during epoxy resin manufacturing, but these tasks were shorter lived, so exposures are likely to be lower. The lowest dermal exposure range predicted was for PC manufacturing, which has a very low percentage of residual BPA.

Table 4.3 Summary table of estimated dermal exposures using EASE from the 2003 published RAR

Work activities	Extent of area of dermal contamination	Range of dermal exposures (mg/cm²/day)	RWC for dermal exposure (mg/cm²/day)
BPA manufacturing			
Product sampling	420	0 to 0.1	0.1
Bag filling/other filling operations	420	1 to 5	1
Manufacture of PC			
Bag filling of PC granules	420	$1x10^{-5}$ to $1x10^{-4}$	$1x10^{-4}$
Manufacture of articles from PC			
Loading PC granules from big bags	420	$1x10^{-5}$ to $1x10^{-4}$	$1x10^{-4}$
Epoxy resin manufacturing			
Charging reactors	420	0.1 to 1	1
Maintenance - changing filter socks	840	0.1 to 1	1
Maintenance - emptying weigh vessel	840	0.1 to 1	1
Use of BPA in PVC manufacture			
Charging reactors	420	0 to 0.1	0.1
Manufacture of coating powders			
Manufacturing	1300	$3x10^{-4}$ to $1.5x10^{-3}$	$1.5x10^{-3}$
Use of coating powders			
Powder coating	1300	$6x\ 10^{-4}$ to $1.8x10^{-3}$	$1.8x10^{-3}$
Thermal paper manufacturing			
Charging reactors	420	0 to 0.1	0.1
Manufacture of tin-plating additive			
Charging reactor	420	0 to 0.1	0.1
Manufacture of TBBA			
Bag filling	420	$3x10^{-7}$ to $3x10^{-6}$	$3x10^{-6}$

Occupational exposure limits

Table 4.4 Occupational exposure limits for bisphenol-A (from the 2003 published RAR)

Country	8-hour TWA exposure (mg/m^3)	Source
Germany	5 (inhalable)	List of MAK and BAT values 1997
Holland	5 (respirable)	The National MAC-list 1999
USA*	5	Proposed WEEL - AIHA

* This information was provided by personal communication and no information was available with respect to whether the limit would be for inhalable or respirable dust.

These limits are provided for information and not as an indication of the level of control of

exposure achieved in practice in workplaces in these countries.

4.1.1.1.2 Updated information

Some applications of BPA have been discontinued in the EU. The uses of BPA as an inhibitor in PVC polymerisation and in the manufacture of TBBA have ceased. Therefore these scenarios are not considered further.

New information and exposure data have been provided by industry for BPA manufacture and epoxy resin manufacture.

New Industry Data for BPA Manufacture

Inhalation exposure

In 2007 Plastics Europe provided HSE with some new exposure data (Tables 4.5 and 4.6) and information on control measures. The data cover all companies (A, B, C and D) that manufacture PBA in the EU and are believed to be representative of all the sites that undertake this process.

Table 4.5 Industry inhalation exposure data for BPA manufacture

Industry inhalation exposure data for BPA manufacture (8hr-TWA)Company/ Site	**Date**	**Parameter**	**Job**	**Number of samples**	**Range 8 hr TWA (mg/m^3)**	**Mean 8hr TWA (mg/m^3)**
C/1	2000	Dust		14	1.36-3.45	2.18
C/1	2001	Dust		15	0.35-0.43	0.39
C/1	2002	Dust		12	0.27-2.48	1.13
C/1	2003	Dust		20	<0.1-0.8	0.24
C/1	2004	Dust		16	<0.1-3.9	1.34
C/1	2005*	Dust		15	<0.1-1.1	0.53
C/1	2006*	Dust		8	<0.01-0.1	<0.1
B/1	2003 - 2004	Total Dust	Bisphenol A (general operations)	6	0-3.6**	0.8
B/1	2003 and 2007	Total Dust (mostly BPA)	Bagging	2	0-0.33	0.17
B/1	2003 and 2007	Total Dust (mostly BPA)	Line Openings	2	0-2.9	1.7
A/1	Jan 07	BPA dust (respirable)	Shift foreman		<0.0002	

A/1	Jan 07	BPA dust (respirable)	Shift personnel		0.0010	
A/1	Jan 07	BPA dust (respirable)	Lab assistant		<0.0006	
A/1	Jan 07	BPA dust (respirable)	Lab personnel		<0.0006	
A/1	Jan 07	BPA dust (respirable)	PCT personnel		<0.0002	
A/1	Jan 07	BPA dust (respirable)	Sampling personnel		0.0182	
A/1	Jan 07	BPA dust (respirable)	Maintenance foreman		0.0050	
A/1	Jan 07	BPA dust (respirable)	Safeguard		0.0050	
A/2	Jul 07	BPA dust (respirable)	Sampling personnel		0.1322	
A/2	Jul 07	BPA dust (respirable)	Tank truck filler		0.0762	
A/2	Jul 07	BPA dust (respirable)	Big bag filler		1.0810	
A/2	Aug 07	BPA dust (respirable)	Big bag filler		1.0317	
A/2	Aug 07	BPA dust (respirable)	Big bag filler		1.1502	

* Modifications to bagging facilities in 2005 led to further reductions in exposure

** The determination was for total dust measured by personal samplers on workers in a variety of occupations. The upper end of the range is believed to be an outlier in a data set which includes three determinations below the limit of detection.Company D also stated that they considered the data given above to be representative for their workplace.The newer data from Company A, which measured BPA dust, indicate that exposures for a range of jobs within the plant can be controlled to less than 1.1 mg/m^3 (8hr-TWA). The results from Company C are harder to interpret as they have measured 'dust' with no indication of how much of that dust is BPA. Based on these newer data it is proposed that the RWC for 8hr TWA inhalation exposures for BPA manufacture should be 3 mg/m^3.

Plastics Europe also provided data from one company (B) on short term exposures. This is given in Table 4.6.

Table 4.6 Industry short term inhalation exposure data for BPA manufacture

Work Activities	**No of Samples**	**Sample Analysis**	**Range (15 min TWA)**	**Mean (15 min TWA)**
Loading of tankers (2004)	2	Total dust	0.2 – 0.5	0.4

		(mostly BPA)		
Loading of tankers (2007) Reduced dust levels due to improvement of ventilation system)	3	Total dust (mostly BPA)	not known	< 0.5
Line Openings 2005 – 2007	4	Total dust (mostly BPA)	0 – 0.5	0.38
Sampling	4	Total Dust (mostly BPA)	0 – 0.83	0.2
Bagging of Big Bags 2005-2007	3	Total Dust (mostly BPA)	0.31 – 0.5	0.43

Although these data are from a much more recent time period that those quoted in the original RAR, they are only from one company (B). The short term data given in the RAR were also only from one company, but a different one (C). In the original RAR there was a general lack of contextual data, particularly sampling period. Therefore it is difficult to know how comparable the data are. We also have no information as to how representative these data are for the industry as a whole.

Given this uncertainty, professional judgement has been used to determine the short term inhalation RWC. This was also done in the original RAR, and there it was agreed that the RWC should be 10 mg/m^3. Following consideration of this new data, it is now proposed that the short term inhalation RWC should be 6 mg/m^3.

*Dermal Exposure*The RAR used EASE predictions to determine dermal exposure and no sampling data have been provided by industry to use instead of the EASE predictions. However, industry have provided information on control measures used to allow the mitigating effect of PPE to be taken into account when determining the RWC dermal exposure for BPA manufacture. The use of PPE can be taken into account if two conditions are met:

- PPE is used regularly by the great majority (90%) of workers in the majority (90%) of facilities making or using the chemical and;
- the PPE used is appropriate and fit for purpose.

Information on PPE as well as on training and supervision of workers has been provided by all companies manufacturing BPA. In general a set of measures are in place at the six BPA manufacturing plants in Europe:

- every employee is informed and trained with respect to the work and the associated hazards in both spoken and written forms;
- there is detailed written information on the following aspects of production available to every trained employee on;
    - general safety instructions for the plant,
    - instructions on the use of PPE
    - permit to work used for modifications and maintenance,
    - handling of dangerous chemicals,
    - substance and task specific information and instructions,
    - action plans in the event of accidents/spillages
    - rules for the disposal of chemicals.

The standard PPE is:

- working shoes;
- working suit with long sleeved jacket and long leg trousers;
- safety helmet;
- safety goggles and
- protective gloves – type (nitrile butadiene rubber coated cotton , latex, leather) varies depending on the company and the task.

All of this information taken together gives confidence that the mitigating effects of PPE can be taken into account when determining the RWC dermal exposures for BPA manufacture. Therefore following the guidance given in the TGD exposures values have been reduced by 90%. Modified values are given in Table 4.7

Table 4.7 Modified dermal exposure values for BPA manufacture

Work activities	Extent of area of dermal contamination (cm^2)	RWC for dermal exposure ($mg/cm^2/day$)	Modified RWC for dermal exposure ($mg/cm^2/day$)
BPA manufacture			
Product sampling	420	0.1	0.01
Bag filling/other filling operations	420	1	0.1

New Industry Information for Epoxy Resin Manufacture

There are 15 sites across the EU which manufacture epoxy resins. Industry has provided information on risk management measures from 5 of these. The RAR identifies charging of reactors as a potential source of dermal exposure and a predicted exposure was estimated. Information from five companies indicate that on their sites this exposure is likely to be negligible as either the BPA is manufactured on site and charged via a closed system or where delivered by tanker, unloading is done under a nitrogen blanket. Charging at some sites is done from hoppers within a closed system. However, some sites charge from bags (of varying sizes). Although some information is available on control measures from these sites,information is not available from all sites manufacturing epoxy resins, so it is not possible to say how representative of the industry they are. Therefore it is not possible to modify the RWC values given in the RAR.

4.1.1.1.3 Impact of new information

Some applications of BPA have been discontinued in the EU. The uses of BPA as an inhibitor in PVC polymerisation and in the manufacture of TBBA have ceased. Therefore these scenarios are not considered further.

Industry has supplied new information which has allowed the modification of RWC exposure values for BPA manufacture only.

For inhalation exposures the revised RWCs proposed are 3mg/m^3 8hr-TWA and 6 mg/m^3, short term. For dermal exposures during BPA manufacture the RWC for product sampling is 0.01 mg/cm^2/day over 420 cm^2 and for bag filling it is 0.1 mg/cm^2/day over 420 cm^2.

Occupational exposure limits

In 2004 the EU DG Employment Scientific Committee on Occupational Exposure Limits (SCOEL) recommended an Indicative Occupational Exposure Limit Value (IOELV) for BPA of 10 mg/m^3 (8h – TWA). The value is expected to be included in a forthcoming IOELV Directive. The justification for the value is as it follows (SCOEL SUM 113, May 2004):

"In relation to establishing a recommended occupational exposure limit (OEL), SCOEL began by considering the available data relating to inhalation exposure. In rats exposed daily to airborne BPA for 13 weeks there was a clear NOAEL of 10 mg/m^3, with mild olfactory epithelium inflammation at 50 and 150 mg/m^3. There was no evidence of systemic toxicity in this study; if it is assumed that all of the inhaled BPA was retained and absorbed, exposure to 150 mg/m^3, a level at which no systemic effects were observed, would equate to a body burden in the rat of about 34 mg/kg/day.

If one then considers the surrounding toxicological evidence, most of which arises from oral dosing studies in rodents, there are no findings that preclude the recommendation of a health-based occupational exposure limit. In long-term repeated oral dosing studies NOAELs of 74 mg/kg/day in rats and 80 mg/kg/day in dogs have emerged; in mice liver toxicity was seen at 120 mg/kg/day, the lowest dose level used. The use of these results to make predictions of dose-response characteristics for inhalation exposure, via route-to-route extrapolation, is hampered by the knowledge that following oral dosing there is extensive first-pass metabolism of BPA absorbed and transported directly to the liver. Nevertheless, the available data from oral dosing studies support the contention that no systemic toxicity arises in experimental animals with inhalation exposures of 10 mg/m^3.

Dramatically contrasting results have been reported in different laboratories conducting standard and non-standard developmental toxicity studies in rats and mice. This has been an area of much dispute, centred on the alleged endocrine-modulating potency of BPA. Although a 2-generation study is being conducted in an attempt to clarify the situation, the judgement of SCOEL was to regard the 50 mg/kg/day NOAEL established in a standard multigeneration study in rats, as the most appropriate reference point for OEL considerations. Set against the analysis above, this suggests no concern for reproductive toxicity in experimental animals with exposures in the region of 10 mg/m^3.

Returning to the repeated inhalation NOAEL of 10 mg/m^3 in rats, with mild nasal olfactory epithelium inflammation arising at 50 mg/m^3, and considering extrapolation of these findings to humans, one would expect that humans could be less sensitive than rats to this effect, based on what is understood of general differences in inhaled particle deposition between the two species. SCOEL threby arrived at a conclusion that repeated inhalation exposure to 10 mg/m^3 BPA (as inhalable dust) would pose no concern for local or systemic toxicity and therefore recommended an 8h TWA OEL at this level. In humans inhaling 10 m^3 of air, if it assumed that all of the inhaled BPA would be retained and absorbed (a worst-case assumption), this would result in a body burden of just a little over 1 mg/kg/day.

There is no toxicological basis for recommending an additional specific short-term OEL; nor are "Sk" or "Sen" notations appropriate."

4.1.1.2 Consumer exposure

As indicated in section 2.1 of the updated environment report, the EU usage of BPA is estimated to be approximately 1,149,870 tonnes/year. The largest quantities are used in the production of polycarbonates and epoxy resins, which have many applications in consumer goods, such as food contact containers, adhesives and protective coatings. A description of the major uses of BPA is given in section 2.2.2.

In these consumer applications, BPA is contained within or generated from a polymer matrix. Potential consumer exposure can therefore arise only under conditions where residual monomer in the polymer matrix becomes available for exposure or where breakdown of the polymer occurs, to generate additional monomer which is available for exposure. Under certain conditions, for example, at elevated temperature or extreme pH, hydrolysis of the polymer may occur, resulting in the regeneration of BPA from the polymer and thus increasing the amount of BPA which may be available for exposure. The products that are likely to have the potential for the highest exposure of consumers to BPA are those that are used in applications which involve direct contact with foodstuff. These include food and beverage containers which have epoxy resin internal coatings, and polycarbonate tableware and bottles, such as those used for infant formula milk. Exposure to BPA arising from use of these products is determined by the migration of BPA from the polymer into the food with which it is in contact, under the particular conditions of use. Migration of BPA from these products into food or beverages stored in them may occur if conditions are created which allow hydrolysis of the polymer during food or beverage storage or if there is residual monomer in the polymer. Consumption of the food or beverage will then result in ingestion of BPA. Inhalation and dermal exposure is considered to be negligible.

Other relatively minor sources of consumer exposure to BPA that are considered in this consumer exposure assessment arise from its use in dental fissure sealants and in epoxy-based surface coatings and adhesives. The use of BPA in dental fissure sealants will result in oral exposure. For epoxy-based surface coatings and adhesives, the main route of exposure is dermal.

Other uses of BPA, such as in printing inks and thermal paper, are considered to result in negligible potential for consumer exposure in comparison with the other sources considered and therefore will not be addressed further in this assessment.

4.1.1.2.1 Summary of original risk assessment report

<u>BPA polycarbonate - food contact applications</u>

There are many applications of BPA polycarbonates which involve direct contact with food. These include returnable beverage bottles, infant feeding bottles, tableware such as plates and mugs and food-storage containers. These main uses will be considered in this exposure assessment. A number of studies have been conducted which investigate the potential consumer exposure to BPA as a result of using these products. These studies have addressed the potential for exposure to residual BPA contained within the polycarbonate and have also explored the conditions which are necessary to initiate hydrolysis of the polymer to generate BPA which is then available for migration.

There is relatively limited good quality information on the levels of BPA in food and drink resulting from migration from polycarbonate tableware. A small number of studies have been conducted to measure BPA concentrations in the contents of polycarbonate infant feeding bottles. Two of these studies have measured levels of up to about 50 ppb (50 μg/l; 0.05 mg/kg assuming a density of 1 g/ml) BPA in the food simulant contents of used bottles, in tests which represent realistic worst case exposure conditions. This value will be used as the basis for calculating consumer exposure for this scenario.

Using these values, estimates of daily ingestion of BPA can be calculated. Table 4.5 shows the estimates of daily ingestion for infants, arising from the use of polycarbonate feeding bottles. Estimates are derived for infants aged 1-2 months and 4-6 months. The estimates for daily intake of milk are taken from MAFF (1998).

Table 4.8 Estimates of infant ingestion of BPA from the use of polycarbonate feeding bottles (from the 2003 published RAR)

Age of baby	Daily intake of milk (l)	Concentration of BPA in milk (μg/l)	Daily ingestion of BPA (μg/day)
1 – 2 months	0.699	50	35
4 – 6 months	0.983	50	50

These values of 35 μg/day (0.035 mg/day) for a 1-2 month baby and 50 μg/day (0.05 mg/day) for a 4-6 month baby will be taken forward to the risk characterisation.

In relation to polycarbonate tableware and food storage containers, a number of well-reported studies have found no detectable levels of BPA in the food or drink contents of the tableware. Where detectable migration levels have been reported, the data derive from reports of limited detail and reliability and in studies in which food simulants have been used; migration into actual foodstuffs has not been detected. The highest reported level of BPA in food simulants detected as a result of migration from polycarbonate tableware is 5 ppb (5 μg/kg; 5×10^{-3} mg/kg). Although there is some uncertainty about the reliability of this value, it will be used as the basis of calculating consumer exposure for this scenario.

For exposure arising from the use of polycarbonate tableware, the most realistic scenario is considered to be that of a young child, for whom the total daily food and drink intake may be taken from polycarbonate tableware. The total daily intake of food and drink for a young child (1.5-4.5 years) is estimated to be 2 kg. This value is based on UK data for the consumption of solid and liquid food by young children, and represents the 97.5th percentile consumption (HMSO, 1995). Therefore, assuming that the concentration of BPA in the foodstuff is 5 μg/kg (5×10^{-3} mg/kg), total daily ingestion of BPA is 10 μg (0.01mg/day). This value will be taken forward to the risk characterisation.

<u>BPA epoxy resins - food contact applications</u>

BPA based epoxy resins are formulated with curing agents to yield high-performance crosslinked coatings. Heat cured epoxy coatings are, due to their favourable properties such as toughness, adhesion and chemical resistance, used as protective linings for metal sanitary cans to maintain the quality of canned food and beverages.

Epoxy resins with differing molecular weights have different applications. High molecular weight epoxy resins are used in heat cured protective interior coatings for food and beverage containers; liquid and low molecular weight epoxy resins are typically used in ambient cured industrial protective coatings, adhesives, floorings or fillers. The majority of exterior coating applications are industrial and therefore negligible consumer exposure is expected. However, there are some consumer applications for these products and therefore these scenarios will be addressed in this exposure assessment.

BPA epoxy resins are used as binders in protective linings in food and beverage cans and in wine storage vats. Migration levels from epoxy coatings are governed by a variety of parameters such as coating composition, coating weight, curing conditions, sterilisation time and temperature and type of foodstuff. Carbonated soft drinks are the predominant type of beverage distributed in cans. These cans are typically filled at room temperature, and stored at or below room temperature. Canned foods are mostly sterilised at high temperatures, up to 135°C. The sterilisation time will vary, with shorter residence times for higher temperatures. Typically, sterilisation at 120°C is performed for 90 minutes. The canned foods are subsequently stored at room temperature.

Approximate coating weights for typical beverage cans are 250 mg/330 ml (1.06 mg/cm^2) for a tinplate can and 125 mg/330 ml for an aluminium can; for food cans, coating weight may vary between 0.4 and 2.5 mg/cm^2 (Nehring Institute, 1998).

A number of studies which have investigated migration of BPA from epoxy resin coated cans and a single study of migration into wine vats, are available, and have been considered in this exposure assessment.

Three studies provide consistent evidence for migration of BPA from epoxy resin linings of food cans into the can contents. Two of these studied migration under conditions which represent the sterilisation process which would normally occur. Migration of BPA in these studies results in levels of up to about 70-90 ppb (70-90 μg/kg) in the can contents, from studies using fatty foods or simulants which mimic fatty foods. As migration is likely to be greatest into fatty foods, these results are considered to be representative of realistic worst case conditions. Rounding this up, a value of 100 ppb (100 μg/kg; 0.1 mg/kg) BPA in the contents of a typical food can will be used in the calculation of total daily ingestion of BPA in this scenario. This value will be taken forward to the risk characterisation.

A study into the migration of BPA from epoxy resins used as coating materials for wine vats (Larroque *et al.,*1989) calculated that based on a level of BPA migration of 100 mg/kg resin, for a 1500 l vat lined with 10 kg resin, the amount of BPA in the wine will be 650 ppb (650 μg/l). No other information is available on this exposure scenario. Given the conditions of the single study available (newly applied resin, with extended contact time), it is likely that the level of migration and resultant estimated levels of BPA in the wine contents of the vat will be over-estimated, although it is not known to what extent. The value of 650 ppb (650 μg/l) wine will be taken forward to the risk characterisation as a very worst-case estimate for this scenario.

Table 4.9 provides the estimates of daily ingestion of BPA, as a result of food contact applications of epoxy-resins. Intake for adults is based on consumption of one bottle (0.75 l) of wine per day and consumption of all other food and drink from canned sources. Based on UK data, the estimate of total daily food and drink consumption for an adult is 4.5 kg (of

which 2 kg is expected to be water); this represents the 97.5th percentile of consumption (HMSO, 1990). Of the 2.5 kg of food consumed daily, it is assumed that 1 kg is from canned food (recommendation by the SCF). A combined adult intake for consumption of wine and all other food is also given.

Intake is also calculated for infants aged 6-12 months, for whom a high quantity of food may come from canned products. Intake is calculated for young children, in the age group 1.5-4.5 years. This age group has been chosen to represent the group with the highest potential food intake per kg bodyweight. In calculating BPA intake for infants, estimated intake of canned food is based on UK survey data, which indicated that the 97.5th percentile daily consumption of canned foods of the type which could contain a source of BPA, for this age group (including baby foods) is 0.375 kg (FSA, 2001; HMSO, 1992). In calculating intake for young children, there is no reliable information on canned food intake. The only information available is an estimated daily intake of food and drink, again based on 97.5 percentile values obtained from UK data (HMSO, 1995). Therefore, for the purposes of risk characterisation, a value of 2 kg for total intake is assumed. It should however be noted that as this intake includes drink and assumes that all food could come from sources resulting in BPA exposure, it will result in an overestimate of actual intake, although the degree of overestimation is unknown.

Table 4.9 Estimates of daily ingestion of BPA from food contact applications of epoxy-resins (from the 2003 published RAR)

Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of BPA in wine (μg/l) or food (μg/kg)	Daily ingestion of BPA (μg/day)
Wine	0.75	650	500
Canned food (infant 6-12 months)	0.375	100	40
Canned food (young child 1.5-4.5 years)	2	100	200
Canned food (adult)	1.0	100	100
Canned food + wine (adult)	0.75 l wine 1.0 kg food	650 μg/l wine 100 μg/kg food	600

The value of 500 μg/day (0.5 mg/day) for ingestion of BPA resulting from consumption of wine will be carried forward to the risk characterisation.

The values of 100 μg/day (0.1 mg/day) for an adult, 200 μg/day (0.2 mg/day) for a young child and 40 μg/day (0.04 mg/day) for an infant, for ingestion of BPA resulting from the consumption of canned food will be carried forward to the risk characterisation as a worst case scenario. In addition, a combined adult intake of 600 μg/day (0.6 mg/day), for consumption of wine in addition to food, will be carried forward.

BPA epoxy resins - marine antifouling paints

Marine antifouling paints are used in the consumer sector for the protection and decoration of yachts and boats. The paints are applied by brush or roller. In the UK these paints are

typically applied once per year. There are some measured data on consumer exposure arising from the brush application of these products.

Calculations of BPA exposure as a result of brush application of antifouling paints are based on a paint containing 40% epoxy-resin and a residual level of 10 ppm BPA in the resin. Exposure occurs via the inhalation and dermal routes. Although exposure to BPA vapour would be low in these applications (because of low vapour pressure), exposure to BPA in an aerosol is possible. Values of 3×10^{-4} μg (3×10^{-7} mg) for inhalation exposure and 29 μg (0.03 mg) for dermal exposure to BPA per event, resulting from brush application of paint without protective clothing will be taken forward to the risk characterisation.

BPA epoxy resins - wood varnish

There are no data on consumer exposure arising from the application of wood varnish. However, measured data are available for the professional application of wood preservatives. Given that the application methods are similar, these data are likely to be representative of the exposure arising from the application of wood varnish and therefore have been used to derive consumer exposure estimates for this scenario. As before, estimates of exposure to BPA are calculated on the basis of its content in the product; resin content is 40% w/w, with a residual level of BPA in the resin of 10 ppm. Exposure occurs via the inhalation and dermal routes. The amount of wood varnish used per event ranges from 1.0-8.5 litre, with a median value of 4 litre. Values of 0.02 μg (2×10^{-5} mg) for inhalation and 3.6 μg (0.0036 mg) for dermal exposure to BPA per event, for brush application without the use of protective clothing and gloves, will be carried forward to the risk characterisation.

BPA epoxy resins - wood fillers

BPA is present in some wood fillers sold for consumer use. Information provided by industry indicates that a typical product on the market contains approximately 20% of epoxy resin with a residual BPA content of 10 ppm. Exposure occurs to the hands only. A value of 9 μg (0.009 mg) BPA per event, resulting from the handling of wood filler without gloves, will be taken forward to the risk characterisation.

BPA epoxy resins - adhesives

Epoxy resin based adhesives are available to consumers. These adhesives are sold in '2-pack' systems. Potential dermal exposure to residual BPA in the epoxy resin can therefore arise from consumer use of these 2-pack products. In 2-pack adhesives, residual BPA content is less than 1 ppm. Based on a residual level of 1 ppm BPA in the adhesive, dermal exposure to BPA arising from the use of adhesives is calculated to be 0.014 mg per event.

Dental fissure sealant

BPA is a component of restorative materials such as fissure sealant, used in dentistry. It is not an active ingredient in any dental sealant or composite, but derivatives of BPA used in dentistry include bis-glycidyldimethacrylate (bis-GMA) and BPA-dimethyl acrylate (bis-DMA). BPA may be present as an impurity in these substances, or may be formed as a result of degradation. It has been demonstrated that BPA can be released from sealants which contain bis-DMA but not those containing bis-GMA. Sealants consist of an organic resin matrix, whereas resin based composites (or fillings) consist of an organic resin matrix with an inorganic filler. According to information from the British Dental Association, filled

composites would result in substantially less exposure than sealants, possibly because they contain proportionally less resin. Most sealants contain only bis-GMA.

Consumer exposure occurs during the polymerisation process following application of the resin. The resin matrix is initially present as a fluid monomer that is converted into a rigid polymer by a free radical initiated addition. Once applied to tooth cavities, composites and sealants are polymerised *in-situ*; the polymerisation reaction may be initiated chemically or by photo-initiation using UV or visible light. The degree of formation of oligomers into polymers varies depending on the composition of the resin and its distance from the tooth surface. Conversion of 60-75% is expected with most common composites. Lower levels of conversion may be associated with greater migration of free components from the composites.

A number of studies have been conducted looking at the release of BPA from commercially available dental sealants under a variety of exposure conditions. The information suggests that release of BPA is most likely only under conditions where degradation of the parent monomer (bis-DMA or bis-GMA) could occur. The data also suggest that degradation of bis-GMA does not occur and therefore only those sealants which contain bis-DMA are likely to release BPA.

Three studies have shown the release of BPA into the saliva of humans following placement of dental sealant. The results of these three studies provide somewhat different estimates of BPA concentration in saliva measured 1 hour post treatment (5.8 - 105.6 ppb, 3-31 ppm or 0.3-2.8 ppm). However, it appears possible that the higher estimates of BPA concentration in saliva may overestimate the actual concentrations which could be expected to arise following dental treatment, as a result of interference in the analytical method used to determine BPA.

Given the uncertainties surrounding the reliability of the higher estimates of BPA concentration in saliva, the concentration of BPA in saliva following dental treatment is considered more likely to be in the range 0.3-3 ppm. This concentration of saliva was measured at 1 hour post treatment. When saliva samples were analysed for BPA concentration at time points later than 1 hour post treatment, in two studies, no measurable levels were detected. This suggests that any exposure to BPA as a result of dental treatment will be an acute event.

4.1.1.2.2 Updated information

BPA polycarbonate – tableware and food storage containers

Since the finalisation of the RAR, the European Food Standard Authority (EFSA) has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the migration of BPA into foods and food simulants have been evaluated.

Recent studies suggest that migration of BPA from polycarbonate tableware and food storage containers may increase when receptacles are used for heating or cooking foods, for example in the case of microwave heating (Nerin *et al.*, 2003). However, quantitative data to estimate BPA migration under these conditions are not available. Therefore, the value of 5 μg/kg already identified in the original RAR is still valid. Based on a daily adult consumption of 3 kg of food or beverages, a potential dietary exposure of 15 μg/day (0.25 μg/kg bw/day; bw = 60 kg) from polycarbonate tableware and food storage containers can be derived for an adult.

For a young child (1.5 – 4.5 years), based on a daily consumption of 2 kg of food or beverages, a potential dietary exposure of 10 µg/day (0.9 µg/kg bw/day; bw = 11 kg) from polycarbonate tableware and food storage containers can be derived.

BPA polycarbonate – infant feeding bottles

Since the finalisation of the RAR, the European Food Standard Authority (EFSA) has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the migration of BPA from polycarbonate infant feeding bottles have been evaluated.

In a recent study by Wong et al. (2005), 30 new commercial plastic baby milk bottles available on the market in Singapore were cut into pieces and tested for migration into 10% ethanol at 70°C or corn oil at 100°C. After 240 h incubation, BPA migration into 10% ethanol was detected in 21 samples whereas BPA migration into corn oil was detected in 12 samples. BPA concentration values calculated from this study are much higher than those based on other migration studies but the test conditions were so far removed from any normal conditions of use of baby bottles that they were not considered by EFSA to assess potential exposure to BPA.

Brede *et al.* (2003) subjected polycarbonate baby feeding bottles to repeated washing/boiling/brushing. When 12 different bottles from the Norwegian market were tested by filling them with hot water (100°C) for 1 hour, the mean BPA level from new bottles was 0.23 µg/l (range of 0.11 to 0.43 µg/l) while the mean level from bottles subjected to simulated repeated use was 8.4 µg/l (ranging from 3.7 to 17 µg/l) after 51 dishwasher cycles and 6.7 µg/l (ranging from 2.5 to 15 µg/l) after 169 dishwasher cycles. While all 12 bottles released higher levels of BPA after 51 cycles compared to new, there was no trend between 51 and 169 cycles. Migration from 5 of the bottles remained the same, it decreased significantly for 5 bottles, and it increased significantly for 2 bottles. The authors commented that the effects seen could be due to depolymerisation of the polycarbonate.

In another study by Tan and Mustafa (2003), BPA leaching was measured in 30 new baby feeding bottles collected on the Malaysian market and in 100 baby feeding bottles used for more than 3 months collected from Malaysian families. The authors noted that most of the bottles collected in families were used or passed from one child to the next as long as they were not cracked or rendered useless. Mean BPA leaching from the new bottles filled with water was 0.18 ng/cm^2 (ranging from non detectable to 1.34 ng/cm^2) at 80°C. Mean BPA leaching from the used bottles filled with water was 3.37 ng/cm^2 (ranging from 0.11 to 25.51 ng/cm^2) at 80°C. EFSA noted that based on these data and considering a contact area of 172 cm^2 (for a standard feeding bottle filled to the 200 ml mark), BPA leaching would range from non-detectable to 1.15 µg/l in new bottles and from 0.1 to 21.9 µg/l in used bottles.

In a study performed in the UK (CSL, 2004), migration from samples of two different brands of polycarbonate feeding bottles was measured. The migration tests were performed on the virgin bottle (after sterilization), and after 20 and 50 cycles of dishwashing and bottle-brushing. One hour contact at 70°C was applied with 10% ethanol to simulate infant formulae and 3% acetic acid to simulate acidic fruit juice. As prescribed by Directive 93/8/EEC for repeated use articles, three successive contacts were performed. No BPA migration was detected in the new bottles. Migration was observed at the first contact with concentrations varying from non-detectable (<1.1 µg/l) to 4.5 µg/l in 10% ethanol and from non-detectable (<0.3 µg/l) to 0.7 µg/l in acetic acid for washed bottles. The dishwasher reagents (detergent, rinse aid and salt) were

tested and found not to be the source of the elevated migration levels. In all but one sample, migration was not observed at the second and third contact.

To summarise the above reported data, EFSA noted that BPA migrates from polycarbonate feeding bottles and that migration can increase with repeated use of the bottle due to the cleaning treatments (dishwashing, sterilization, brushing, etc). In recent years, the decrease in the limit of detection has allowed determination of BPA leaching that was not detected previously. The migration levels observed vary according to studies in relation to varying experimental conditions (temperature, time of contact, migrant). The degree of leaching may vary according to the bottle brand (due to varying manufacturing process and raw material); new studies made available recently suggest that it may also vary according to the age of the bottle, probably in relation to the number and type of cleaning treatment performed at household level. Infants are likely to be fed everyday using bottles of the same brand cleaned in the same way. For this reason an estimate of average BPA migration values would not capture the exposure of infants who are fed every day with bottles leaching more BPA than the average. EFSA noted that migration testing by 3 successive contacts, as prescribed by Directive 93/8/EEC, did not enable the identification of an increased release of BPA whereas cycles of dishwashing and, even more, domestic use for 3 months or more did.

EFSA noted that according to the two migration studies conducted since 2003 under conditions mimicking realistic conditions of use, levels of BPA migration in used polycarbonate bottles were respectively up to 22 μg/l (Tan & Mustafa, 2003) and up to 14 μg/l (Brede, 2003). These upper values were lower than the upper value of 50 μg/l identified in the EU RAR. Although based on a limited number of bottles, the studies used in the RAR mimicked realistic conditions of treatment of commercially available used bottles and were of sufficient quality from an analytical point of view. The concentration value of 50 μg/l of infant formulae in used bottles was therefore used by EFSA as the basis to calculate a conservative estimate of exposure in infants.

Therefore, despite this new data, the original estimates from the RAR of 35 μg/day (8 μg/kg bw/day; bw = 4.5 kg) for a 1-2 month baby and 50 μg/day (7 μg/kg bw/day; bw = 7 kg) for a 4-6 month baby for infant ingestion of BPA from the use of polycarbonate feeding bottles, are still valid.

EFSA also considered a scenario in which powdered infant formulae may be packed in food cans with epoxy-phenolic resins used as internal surface. Kuo and Ding (2004) determined the content of BPA in 6 brands of canned powdered infant formulae and follow up formulae available on the market in Taiwan. BPA was detected in all samples at concentrations ranging from 45 to 113 μg/kg. Based on a reconstitution ratio of 135 g/l of liquid formula and on a concentration value of 100 μg BPA/kg, the above mentioned 1-2 and 4-6 month infant consuming 155 and 140 ml/kg bw/day of reconstituted infant formulae respectively would consume 21 and 19 g/kg bw of powdered infant formulae, leading to a potential exposure of up to 2.1 and 1.9 μg BPA/kg bw/day. EFSA noted that this potential source of exposure is quantified on the basis of a very limited number of samples from a non-EU market. These figures will not be used in the risk characterisation as in the EU infant formulae are not packed in food cans.

In the case of breastfed infants, BPA in human milk occurs as a consequence of exposure of the mother through oral and dermal routes. In a study by Sun et al. (2004), twenty-three human milk samples of healthy lactating women living in Japan were analysed for BPA. BPA was

detected in all samples (limit of detection 0.11 µg/l) with values in the range from 0.28 to 0.97 µg/l. The mean value was 0.61 µg/l. Considering the consumption of 174 ml/kg bw of human milk per day in infants exclusively breastfed, EFSA calculated a potential dietary exposure of 0.1 µg/kg bw/day at the mean and 0.2 µg/kg bw/day at the highest BPA concentration observed. EFSA noted that this estimate is based on a limited number of samples of human milk collected in Japan and may not be representative of the EU situation.

BPA epoxy resins - food contact applications

Since the finalisation of the RAR, the European Food Standard Authority (EFSA) has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the migration of BPA into foods and food simulants have been evaluated (see Table 4.10).

Table 4.10 BPA concentrations in canned commercial products according to recent published studies (from EFSA, 2006)

Reference/Country	Type of product	LOD/LOQ (µg/kg)	Number of products analysed	Percent samples above LOQ	Percent samples above 100 µg/kg	Minimum (µg/kg)	Maximum (µg/kg)	Average concentration of values above LOQ (µg/kg) (1)
FSA (2000)/UK	Beverages	2/7	11	0%	0%	<2	<7	-
	Foods	2/7	46 (2)	78%	0%	7	70	23
Goodson et al (2004)/UK	Foods	2 (4)	10 (3)	100%	0%	9	91	40
Braunrath et al (2005)/Austria	Beverages	0.1 – 0.9 (4)	7	86%	0%	0.1	3.4	1.1
	Vegetables	1.1 – 7.4 (4)	6	100%	0%	8.5	35	23.9
	Fruits	1.2 – 5.4 (4)	4	100%	0%	5	24	10.5
	Fat-containing products	0.2 – 9.3 (4)	9	100%	0%	4.8	17.6	10.7
Horie et al (1999)/Japan	Beverages	<1/1	80	n.a.	n.a.	<1	212	18
Imanaka et al (2001) (6)/Japan	Meat	<1/1	8	100%	n.a.	17	602	n.r.
	Vegetables	<1/1	14	100%	n.a.	2	25	n.r.
Yoshida et al (2001)/Japan	Foods	10 (7)	12	50%	0%	<10	95	44
Kang &Kondo (2003)/Japan	Dairy products	1 (4)	3	100%	0%	21	43	31
Thomson & Grounds (2005)/New Zealand	Food	10-20 (7)	79	32%	2%	<10	191	34
	Beverages	10 (7)	4	0%	0%			-

n.r. not reported ; n.a. not available
(1) Calculated by EFSAI.
(2) Two high values from a meat product were excluded since they related to a technology no longer in use in the EU (Association of Plastics Manufacturers in Europe, 2006a).
(3) In this study, ten retail food cans were analysed for BPA before studying the effect of heating and storing.
(4) LOD only.
(5) Average of all samples, including undetectable levels which were given a value of zero.
(6) Abstract only available.
(7) LOQ only.

According to EFSA (2006), in both food and beverages, concentrations of BPA above 100 µg/kg were rarely observed. Three surveys performed on the EU market covered a limited number of products: 18 beverages with concentrations in the range of non-detectable to 3 µg BPA/kg and 65 solid foods with concentrations in the range of 5 to 91 µg BPA/kg. BPA concentrations above 100 µg/kg were not observed in these surveys. In other surveys, the highest observed concentration in canned beverages was 212 µg/kg in a coffee sold in Japan (Horie *et al.*, 1999). The highest observed concentrations of BPA in canned foods were

reported in Japan for meat products: up to 602 µg BPA/kg in corned beef and 212 µg BPA/kg in chicken (Imanaka *et al*., 2001). In New Zealand, individual samples of tuna, corned beef and coconut cream reached up to 191 µg BPA/kg (Thomson & Grounds, 2005).

A number of migration studies of BPA from epoxy resin linings into the can contents have also been performed. BPA migration from cans of beverages was assessed by Kang and Kondo (2002) in Japan. BPA migration into water, decaffeinated and non-decaffeinated coffee averaged 14 µg BPA/l (range 9 to 31 µg /l), 66 µg BPA/l (range 33 to 107 µg/l) and 84 µg BPA/l (range 50 to 134 µg/l), respectively.

BPA migration into water was assessed for 9 different food cans differing in shape, size and material, used for packaging of fruits and juice in Japan (Takao et al., 2002). All cans were filled with bottled spring water and sealed with a seamer. Cans were either not heated or heat-treated for 30 minutes. Low levels of BPA migration (less than 2 µg/l) were found in all unheated cans. When the heat treatment was performed, the migration was up to 5 µg BPA/l at 80°C and up to 30 µg BPA/l at 100°C.

In a study by Goodson et al. (2004), experiments were conducted to investigate the effects of different storage conditions and damage (experimentally produced denting) to cans on the migration of BPA into foods by filling epoxy-phenolic coated cans with four foods (soup, minced beef, evaporated milk and carrots) and one food simulant (10% ethanol). Filled cans of each food type or simulant were then sealed and processed before storage at three different temperatures. It was found that 80–100% of the total BPA present in the coating had migrated to foods or simulant directly after heat processing. The level was not changed during extended storage (up to 9 months) or in damaged cans or if canned foods were then heated in the can to make ready to eat. This indicates that most migration occurs during the can retorting step.

EFSA noted that migration values vary according to a number of factors (heating time, temperature, food or simulant). Each consumer is likely to consume a variety of canned foods and beverages that will not always have the same BPA concentrations. On this basis, single high migration values observed were not considered in the assessment of chronic dietary exposure.

EFSA considered whether to use a migration value of 100 µg BPA/kg both for canned solid food and for canned beverages, but concluded that this would not be representative of beverages and would provide an overly conservative assessment of chronic dietary exposure for adults and children with a varied diet. EFSA noted that in the 3 surveys conducted in the EU in canned commercial products, BPA in canned beverages was always less than 7 µg BPA/kg and that the average concentration of BPA in solid foods in which it was quantified was up to 40 µg BPA/kg. Although limited, these data were used to develop an exposure scenario considering 10 µg BPA/kg as the value for canned beverages and 50 µg BPA/kg as the value for canned solid foods. This scenario was used by EFSA to provide a conservative assessment of exposure to BPA through canned products in adults and children consuming a variety of products. These estimates, which are slightly lower than that (100 µg/kg) identified in the original RAR, will be used to calculate revised adult dietary intakes of BPA from canned food and beverages to be taken forward to the risk characterisation.

A different scenario was considered for infants since they tend to consume a limited number of commercial products and may be more likely to consume the same products which may

have a high BPA concentration. Thus, for infants aged 0-6 months and 6-12 months, a value of 100 μg BPA/kg canned foods and beverages was used. This value is the same as that identified in the original RAR.

BPA was recently determined in samples of wine available on the Austrian market and sourced from vats (steel, wood and plastic), glass bottles and carton packages (Brenn-Struckhofova & Cichna-Markl, 2006). Reported storage time varied from 0.25 to 11 months. In 13 of the 59 wine samples, the BPA concentration was below the LOQ of 0.2 μg/l. Mean BPA concentrations for all wine samples above the LOQ was 0.58 μg/l. In seven samples, BPA levels ranged from 0.2 to 0.5 μg/l. Only in one sample (stored 10.5 months in a steel vat) was a significantly higher BPA level of 2.1 μg/l found. The mean and median for all wine samples with BPA concentrations above the LOQ were 0.58 and 0.40 μg/l respectively. These values are far lower than previously published BPA levels (650 μg/l) derived from migration experiments using wine simulants (Larroque et al.,1989 – see original RAR). EFSA noted that even though this survey is limited and further information on the possible deterioration of epoxy resins in wine vats used for many years would be desirable, potential residues of BPA in wine appear to be in the same range as those found in canned beverages (within 10 μg/l). Therefore, rather than the very conservative estimate of 650 μg/l, the more realistic value of 10 μg/l will be used to calculate the adult dietary intake of BPA from wine to take forward to the risk characterisation.

The Association of Plastics Manufacturers in Europe (2006) confirmed that epoxy resins, in which BPA is used as an accelerator in amine-based hardeners, may be used for tanks holding alcoholic beverages and that these are multiple-use applications with containers continuously filled, emptied and refilled over long time periods in use – sometimes many years. On the other hand the Association stated that these are relatively minor applications for such products and that surface-to-volume ratios are extremely small.

Table 4.8 provides the estimates of daily ingestion of BPA, as a result of food contact applications of epoxy-resins. Intake for adults is based on consumption of one bottle (0.75 l) of wine per day and consumption of 1 kg of canned food and 2 litres of canned beverages, including wine (recommendation by EFSA). A combined adult intake for consumption of canned beverages and canned food is also given.

Intake is also calculated for infants aged 6-12 months, for whom a high quantity of food may come from canned products. Intake is calculated for young children, in the age group 1.5-4.5 years. This age group has been chosen to represent the group with the highest potential food intake per kg bodyweight. In calculating BPA intake for infants, estimated intake of canned food is based on UK survey data, which indicated that the 97.5th percentile daily consumption of canned foods of the type which could contain a source of BPA, for this age group (including baby foods) is 0.375 kg (FSA, 2001; HMSO, 1992). In calculating intake for young children, there is no reliable information on canned food intake. The only information available is an estimated daily intake of food and drink, again based on 97.5 percentile values obtained from UK data (HMSO, 1995). Therefore, for the purposes of risk characterisation, a value of 2 kg for total intake is assumed.

Table 4.11 Estimates of daily ingestion of BPA from food contact applications of epoxy-resins.

Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of BPA in wine (μg/l) or food (μg/kg)	Daily ingestion of BPA (μg/day)

Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of BPA in wine (μg/l) or food (μg/kg)	Daily ingestion of BPA (μg/day)
Wine	0.75	10	7.5
Canned beverages (adult)	2	10	20
Canned food (adult)	1.0	50	50
Canned food + beverages (adult)	1 kg food 2 l beverages (including wine)	50 μg/kg food 10 μg/l beverages (including wine)	70
Canned food and beverages (infant 6-12 months)	0.375	100	37.5
Canned food and beverages (young child 1.5-4.5 years)	2	50	100

For an adult the worst-case dietary intake of 70 μg/day BPA (1.25 μg/kg bw/day; bw = 60 kg) resulting from consumption of canned food and canned beverages (including wine) will be carried forward to the risk characterisation. For a 6-12 month infant and for a young child, dietary intakes of 37.5 and 100 μg/day BPA (4.3 μg/kg bw/day; bw = 8.7 kg and 9 μg/kg bw/day; bw = 11 kg) resulting from consumption of canned food and canned beverages will be carried forward to the risk characterisation.

Another potential source of BPA exposure is migration from polycarbonate tableware and storage containers. Potential dietary exposures of 15 μg/day (0.25 μg/kg bw/day; bw = 60 kg) and 10 μg/day (0.9 μg/kg bw/day; bw = 11 kg) were derived for an adult and a young child (1.5 – 4.5 years) respectively for this scenario.

Therefore, the overall potential dietary exposure to BPA in the adult population would be 1.50 (1.25 + 0.25) μg/kg bw/day and in young children 9.9 (9 + 0.9) μg/kg bw/day.

BPA epoxy resins - marine antifouling paints

There in no significant new information on this consumer use.

BPA epoxy resins - wood varnish

There in no significant new information on this consumer use.

BPA epoxy resins - wood fillers

There in no significant new information on this consumer use.

BPA epoxy resins – adhesives

There in no significant new information on this consumer use.

Dental fissure sealant

There in no significant new information on this consumer use.

4.1.1.2.3 Impact of new information

New information on levels of BPA in polycarbonate tableware and food storage containers, polycarbonate infant feeding bottles and food contact applications of epoxy resins has led to slight changes in the exposure estimates taken forward to the risk characterisation. These are summarised in the table below and compared to the values of the original RAR.

Table 4.12 Exposure estimates taken forward to the risk characterisation for the different oral consumer scenarios

Source of exposure	Daily ingestion of BPA (mg/day)	Estimated body burden (mg/kg/day)	Daily ingestion of BPA (mg/day) from orginal RAR	Estimated body burden (mg/kg/day) from original RAR
Infant feeding bottles (1-2 month baby)	0.035	0.008	0.035	0.008
Infant feeding bottles (4-6 month baby)	0.050	0.007	0.050	0.007
Canned food and beverages (infant 6-12 months)	0.0375	0.0043	0.040	0.0046
Canned food and beverages (young child 1.5-4.5 years)	0.100	0.009	0.200	0.018
Canned food (adult)	0.050	8×10^{-4}	0.100	1.6×10^{-3}
Canned beverages (adult)	0.020	3×10^{-4}	n.a.	n.a.
Wine (adult)	0.010	1.7×10^{-4}	0.500	8.3×10^{-3}
Canned food and beverages including wine (adult)	0.070	0.00125	0.600	0.01
Polycarbonate tableware (young child, 1.5-4.5 years)	0.010	9×10^{-4}	0.010	9×10^{-4}
Polycarbonate tableware (adult)	0.015	2.5×10^{-4}	n.a.	n.a.
Canned food and beverages + polycarbonate tableware (young child, 1.5-4.5 years)	0.110	0.01	0.210	0.019
Canned food and beverages + polycarbonate tableware (adult)	0.085	0.00150	0.600	0.01

n.a. not available

4.1.1.3 Humans exposed via the environment

4.1.1.3.1 Summary of original risk assessment report

Table 3.9 from the environment section of R325_0207_env_hh has been repeated here (Table 4.13) and gives the predicted environmental exposures to BPA and the daily human doses arising from releases from production, processing and manufacture of BPA, epoxy resins, PVC and thermal paper, and for releases at the regional level.

It can be seen that the daily human intake via the environment based upon typical human consumption and inhalation rates at the regional level is 1.78×10^{-5} mg/kg/day and the highest local exposure (use as an inhibitor in PVC production) is 0.059 mg/kg/day. These two figures were taken forward to the risk characterisation.

Table 4.13 Concentrations for indirect exposure of humans via the environment (from the 2003 published RAR)

	Concentration in drinking water (mg/l)	Concentration in wet fish (mg/kg)	Concentration in plant roots (mg/kg)	Concentration in plant leaves (mg/kg)	Concentration in milk (mg/kg wet weight)	Concentration in meat (mg/kg wet weight)	Concentration in air (mg/m^3)	Total daily intake (mg/kg day)
Site specific								
BPA production	3.93×10^{-4}	0.027	1.49×10^{-3}	1.96	2.64×10^{-3}	8.35×10^{-3}	3.61×10^{-4}	0.0338
Epoxy resin production	0.012	0.074	0.3	0.065	4.85×10^{-5}	1.53×10^{-4}	2.08×10^{-10}	3.22×10^{-3}
Thermal paper production	1.88×10^{-3}	0.127	$1.4x10^{-4}$	3.14×10^{-5}	2.11×10^{-6}	6.67×10^{-6}	2.08×10^{-10}	2.65×10^{-4}
Generic scenarios								
Phenoplast cast resin processing	1.29×10^{-3}	0.0875	0.013	2.81×10^{-3}	$2.98x10^{-6}$	9.42×10^{-6}	2.08×10^{-10}	3×10^{-4}
Thermal paper recycling	0.187	12.6	2.03	0.441	4.45×10^{-4}	1.41×10^{-3}	2.08×10^{-10}	0.0448
PVC – Inhibitor during production process	0.227	15.4	2.97	0.643	6.01×10^{-4}	1.9×10^{-3}	2.08×10^{-10}	0.0591
PVC – Anti-oxidant during processing	2.19×10^{-4}	0.0148	1.51×10^{-3}	3.28×10^{-4}	4.45×10^{-7}	$1.41x10^{-6}$	2.08×10^{-10}	4.46×10^{-5}
PVC – Preparation of additive packages	8.8×10^{-3}	0.595	0.114	0.0246	2.3×10^{-5}	7.3×10^{-5}	2.08×10^{-10}	2.27×10^{-3}
PVC – Anti-oxidant in plasticiser production	0.0014	0.0964	0.0173	0.00374	3.63×10^{-6}	1.15×10^{-5}	2.08×10^{-10}	3.58×10^{-4}
PVC – Plasticiser use	1.88×10^{-4}	0.0127	1.1×10^{-3}	2.39×10^{-4}	3.62×10^{-7}	$1.15x10^{-6}$	2.08×10^{-10}	3.64×10^{-5}
Regional	1.14×10^{-4}	7.74×10^{-3}	1.96×10^{-4}	4.37×10^{-5}	1.85×10^{-7}	5.86×10^{-7}	2.08×10^{-10}	1.78×10^{-5}

4.1.1.3.2 Updated information

Overall environmental exposure to BPA was recently assessed in Japan (Miyamoto and Kotake, 2006). Exposure levels from different possible sources (atmosphere, water, food, tableware, toys, etc.) were estimated and aggregated in different age classes. Children aged 1–6 years had the highest average estimated level of exposure (1.2 µg/kg bw/day) due to relatively high dietary consumption per unit body weight and the use of polycarbonate tableware for this age class. Daily BPA exposure was also estimated, based on 24 h urines collected in 58 adult subjects. The 95% confidence intervals for average daily exposure were estimated to be 0.028–0.049 µg/kg/day for adult males and 0.034–0.059 µg/kg bw/day for adult females. The 95% confidence intervals for high-exposure (95th percentile) were estimated to be 0.037–0.064 µg/kg bw/day for adult males and 0.043–0.075 µg/kg/day for adult females. EFSA (2006) considered these data but since no details were available in relation to the characteristics of the diet of subjects from which urines were collected, EFSA agreed that the relevance of this information to the EU situation remained uncertain. The authors of the survey pointed out that in Japan, in recent years, industries voluntarily reduced the amount of BPA used as an additive in the production of PVC, thermal paper manufacturers voluntarily substituted BPA used as a developing agent and polycarbonate tableware and polycarbonate feeding bottles were substituted with non-polycarbonate articles (with less than 6% of feeding bottles currently being made with polycarbonate).

Because of changes in the environmental emissions of BPA (see updated environmental RAR; draft of May 2007; R325_0705_env), the predicted daily human doses for the local and regional scenarios have been subject to revision. For the production of PVC additive packages containing BPA, section 3.1.2.2 of R325_0705_env indicates that industry has carried out two sampling exercises at sites operating this process. As data are now available for all sites, specific rather than generic calculations have been carried out in estimating human exposure for this local scenario. For washing of polycarbonate bottles, figures for human exposure via the environment have now been added. As indicated in section 3.1.2.1 of R325_0705_env, the use of BPA as an inhibitor in PVC production ceased voluntarily in the EU in 2003. Therefore, as there are no longer any emissions from this application, it is not considered further.

Revised figures of human exposure via the environment (see table 4.14) have all been calculated with EUSES by using typical human consumption and inhalation rates. It can be seen that the daily human intake via the environment at the regional level is 9.1×10^{-6} [it was 1.78×10^{-5} in the original RAR] mg/kg/day and the highest local exposure (BPA production) is 0.041 [it was 0.06 for PVC production in the original RAR] mg/kg/day. These two figures will be taken forward to the risk characterisation.

It should be pointed out that although there is a large amount of measured data of BPA concentrations in surface water (see section 3.1.4.5.3 of R325_0705_env) and some data on measured levels in sewage sludge (see section 3.1.4.6.3 of R325_0705_env), these cannot be related to any of the local scenarios. Therefore the risk assessment will be based on the calculated values. The measured levels in surface water show that BPA is present at concentrations below 1 ug/l. Comparing the measured and calculated surface water levels, these are in quite good agreement in general - certainly the higher end of measured levels are of a similar order to the calculated values. It should be noted however that the drinking water concentrations included in Table 4.11 assume no removal of BPA from surface water in the production of drinking water (the default assumption based on BPA's properties). There are a small number of studies which have measured BPA in water before and after treatment to

produce drinking water, and these show a significant reduction in concentration. They also show lower levels of BPA in drinking water than those calculated. The levels at a treatment plant in Spain were all below the quantification limit of 6.5 ng/l (Rodriguez-Mozaz et al., 2004). Wenzel et al. (2003) summarised levels in drinking water in Europe; from three studies these were 0.5 – 2.0 ng/l at one site and <8.8 or <11 ng/l at two others. Although these cannot be related to specific scenarios, they indicate that the actual concentrations in drinking water are likely to be much lower than those calculated in this assessment.

Table 4.14 Concentrations for indirect exposure of humans via the environment estimated using EUSES

	Concentration in drinking water (mg/l)	Concentration in wet fish (mg/kg)	Concentration in plant roots (mg/kg)	Concentration in plant leaves (mg/kg)	Concentration in milk (mg/kg wet weight)	Concentration in meat (mg/kg wet weight)	Concentration in air (mg/m^3)	Total daily intake (mg/kg day)
Site-specific								
BPA production	$7.1x10^{-5}$	$2.2x10^{-3}$	$1.8x10^{-3}$	2.4	$3.2x10^{-3}$	0.01	$4.4x10^{-4}$	0.041
Epoxy resin production	$8.2x10^{-4}$	0.056	$8.7x10^{-3}$	$1.9x10^{-3}$	$2.0x10^{-6}$	$6.2x10^{-6}$	$1.6x10^{-10}$	$1.9x10^{-4}$
Thermal paper production	$8.0x10^{-4}$	0.055	$1.4x10^{-4}$	$3.1x10^{-5}$	$9.2x10^{-7}$	$2.9x10^{-6}$	$1.6x10^{-10}$	$1.1x10^{-4}$
PVC – preparation of additive packages	$2.3x10^{-4}$	0.016	$2.3x10^{-3}$	$5.1x10^{-4}$	$5.6x10^{-7}$	$1.8x10^{-6}$	$1.6x10^{-10}$	$5.4x10^{-5}$
Generic scenarios								
Phenoplast cast resin processing	$1.0x10^{-3}$	0.069	0.013	$2.8x10^{-3}$	$2.7x10^{-6}$	$8.5x10^{-6}$	$1.6x10^{-10}$	$2.6x10^{-4}$
Polycarbonate bottle washing	$3.4x10^{-5}$	$2.2x10^{-3}$	$1.4x10^{-4}$	$3.1x10^{-5}$	$7.8x10^{-8}$	$2.5x10^{-7}$	$1.6x10^{-10}$	$5.8x10^{-6}$
PVC – Anti-oxidant during processing	$1.4x10^{-4}$	$9.3x10^{-3}$	$1.5x10^{-4}$	$3.3x10^{-4}$	$3.5x10^{-7}$	$1.1x10^{-7}$	$1.6x10^{-10}$	$3.3x10^{-5}$
PVC – Anti-oxidant in plasticiser production	$2.8x10^{-4}$	0.019	$3.4x10^{-3}$	$7.3x10^{-4}$	$7.3x10^{-7}$	$2.3x10^{-6}$	$1.6x10^{-10}$	$7.0x10^{-5}$
PVC – Plasticiser use	$1.1x10^{-4}$	$7.2x10^{-3}$	$1.1x10^{-3}$	$2.4x10^{-4}$	$2.7x10^{-7}$	$8.6x10^{-7}$	$1.6x10^{-10}$	$2.5x10^{-5}$
Thermal paper recycling – deinking	b: $3.8x10^{-5}$	$2.2x10^{-3}$	$9.7x10^{-4}$	$2.1x10^{-4}$	$1.8x10^{-7}$	$5.8x10^{-7}$	$1.6x10^{-10}$	$1.4x10^{-5}$
	p: 0.016	$2.2x10^{-3}$	0.41	0.089	$6.6x10^{-5}$	$2.1x10^{-4}$	$1.6x10^{-10}$	$4.2x10^{-3}$
	c: 0.014	$2.2x10^{-3}$	0.35	0.075	$5.6x10^{-5}$	$1.8x10^{-7}$	$1.6x10^{-10}$	$3.6x10^{-3}$
Thermal paper recycling – no deinking	b: $4.8x10^{-5}$	$2.3x10^{-3}$	$1.2x10^{-3}$	$2.6x10^{-4}$	$2.2x10^{-7}$	$7.0x10^{-7}$	$1.6x10^{-10}$	$1.6x10^{-5}$
	p: $9.0x10^{-4}$	$2.3x10^{-3}$	0.029	$4.9x10^{-3}$	$3.7x10^{-6}$	$1.2x10^{-5}$	$1.6x10^{-10}$	$2.4x10^{-4}$

	c: $7.7x10^{-4}$	$2.3x10^{-3}$	0.019	$4.2x10^{-3}$	$3.2x10^{-6}$	$1.0x10^{-5}$	$1.6x10^{-10}$	$2.0x10^{-4}$
Regional	$3.2x10^{-5}$	$2.2x10^{-3}$	$4.9x10^{-4}$	$1.1x10^{-4}$	$1.8x10^{-7}$	$5.8x10^{-7}$	$1.6x10^{-10}$	$9.1x10^{-6}$

Notes for thermal paper recycling: b = biological sludge only; p = paper sludge only; c = combined sludges

Levels of BPA in human blood and excretion of BPA and BPA-metabolites in unintentionally exposed humans

Since the finalisation of the RAR, EFSA has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the concentration of BPA in human fluids have been evaluated (see Tables 4.15 and 4.16).

A number of methods to quantitate low concentrations of BPA in biological samples have been developed. These methods were applied to determine BPA concentrations in blood or urine samples from human subjects without intentional exposures to BPA. The analytical methods applied include ELISAs, single trace chromatographic separations such as HPLC with fluorescence detection (both with and without fluorophore derivatisation), and HPLC with electrochemical detection. Recently, results from studies using more specific methods for BPA-quantitation based on mass spectrometry using both single and triple quadrupol instruments were published.

Moreover, the studies used widely different sample workup procedures. These included simple dilution of aqueous samples with polar organic solvents, extraction of BPA into ethyl acetate or ether, and solid phase extractions. Some studies included treatment with glucuronidase and/or sulphatase to cleave the expected major metabolites of BPA, or applied specific methods to quantitate BPA-glucuronide. The results of the many studies available for evaluation are summarised in Tables 4.9 and 4.10.

The studies on BPA blood levels in humans without intentional exposure to BPA report concentrations of up to 10 µg/l blood (Fung et al., 2000; Fukata et

Table 4.15. Reported plasma or blood concentrations of bisphenol A in human subjects without known specific exposures to BPA (from EFSA, 2006)

Reference and sampling region	**Analytical method, sample workup**	**No. of samples analyzed**	**Concentration ranges reported**	**Comments**
(Yamada *et al.* 2002) Japan	ELISA (EcoAssay Bisphenol A kit from Otsuka pharmaceutics, Tokyo, Japan), solid phase extraction,	248 samples of maternal serum and amniotic fluid	0.64 to 6.63 µg BPA/l in maternal serum (90th percentile) < LOD of 0.81 µg BPA/l in amniotic fluid	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody, LOD of 0.2 µg BPA/l, Background below LOD
(Ikezuki *et al.* 2002) Japan	ELISA (EcoAssay Bisphenol A kit from Otsuka pharmaceutics, Tokyo, Japan)	Blood samples from 13 healthy pre-menopausal women, 37 women with early pregnancy, 37 late pregnancy, 32 umbilical cord blood samples, 36 ovarian follicular fluid samples	2.0 ± 0.8 µg BPA/l (non-pregnant) 1.5 ± 1.2 µg BPA/l (early pregnancy); 2.4 ± 0.8 µg BPA/l (follicular fluid); 2.2 ± 1.8 µg BPA/l (fetal serum; 8.3 ± 8.9 µg BPA/l (amniotic fluid)	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody, LOD of 0.5 µg BPA/l
(Takeuchi and Tsutsumi 2002) Japan	ELISA (EcoAssay Bisphenol A kit from Otsuka pharmaceutics, Tokyo, Japan), solid phase extraction	14 healthy women, 16 women with polycystic ovary syndrome (PCOS) and 11 healthy men	0.64 ± 0.1 µg BPA/l (normal women) 1.49 ± 0.11 µg BPA/l (healthy men); 1.04 ± 0.1 µg BPA/l (women with PCOS)	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody, LOD not given, very small standard deviations
(Inoue *et al.* 2001) Japan	LC/MS, electrospray ionization, glucuronidase treatment, solid phase extraction	Only 3 blood samples analyzed	0.1 to 1 µg BPA/l	Very limited number of samples analyzed
(Ohkuma *et al.* 2002)	competitive ELISA	100 samples analyzed, no details about sample workup	Many < 0.3 µg BPA/l, up to 1 µg BPA/l (no details given)	Some results with antibody confirmed by GC/MS determination of BPA, no assessment of BPA-glucuronide
(Fung *et al.* 2000) Japan	HPLC with fluorescence detection solid phase extraction	Serum samples from 18 men and 22 women after application of dental sealant containing BPA	None above detection limit of 5 ppb (5 µg BPA/l)	No assessment of BPA-glucuronide
(Schonfelder *et al.* 2002b) Germany	GC/MS after derivatisation by silylation, solvent extraction with ethylacetate	37 maternal and fetal plasma samples, and placenta tissue levels	Median BPA conc. in maternal plasma 3.1 µg BPA/l (range from 0.3 to 18.9 µg BPA/l); 2.3 µg BPA/l in fetal plasma (range from 0.2 to 9.2 µg BPA/l); median of 12.7 µg BPA/l placenta tissue (range	Glucuronidase cleavage not applied; contradictory statements and results presented regarding background of BPA in blanks; LOQ 0.1 µg BPA/l

			from 1 to 104.9 µg BPA/kg)	
(Takeuchi *et al.* 2004) Japan	ELISA (no source specified, presumably EcoAssay Bisphenol A kit)	73 blood samples analyzed from women with different endocrine status	1.17 ± 0.16 µg BPA/l to 0.71 ± 0.09 µg BPA/l in obese resp. non-obese women (mean ± SD)	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody
(Volkel *et al.* 2005) Germany	LC/MS/MS with and without glucuronidase treatment	Randomly collected blood samples from 7 males and 12 females	All samples below LOD of 0.5 µg BPA/l	LOD 0.5 µg BPA/l, no background after method adjustment
(Fukata *et al*. 2006) Japan	ELISA with three different kits, same samples analyzed by HPLC with electrochemical detection	Randomly collected from 21 male and 31 female subjects, age 22 – 51 years	Two of the ELISA kits indicated BPA concentrations of 0.66 + 0.29 resp. 0.71 + 0.49 µg/l, LC with electrochemical detection all samples < LOD	LC with electrochemical detetction had LOD of 0.2 µg/l
(Sajiki *et al.*, 1999) Japan	HPLC with electrochemical detector, solid phase extraction	Randomly collected blood samples from 12 adult women and nine adult men	Average BPA-concentrations of 0.33+0.54 µg/l in females (range from 0 - 1.6 µg/l) and 0.59+0.21 µg/l in males (range 0.38-1 µg/l)	No glucuronidase treatment, LOD of 0.2 µg/l, use of glass vessels for sampling

Table 4.16. Reported urine concentrations of BPA in human subjects without known specific exposures to BPA (from EFSA, 2006)

Reference and sampling region	**Analytical method sample workup**	**No. of samples analyzed**	**Concentration ranges reported**	**Comments**
al. 2004) Japan	GC/MS/MS, glucuronidase treatment, solvent extraction followed by solid phase extraction	Samples from 5 health adults, on 5 consecutive days, in addition, 24h urine samples from 36 male subjects	< 0.58 to 13 µg BPA/day (median of 1.3 µg/day) in five subjects observed over 5 days; < 0.21 µg BPA/day to 14 µg BPA/day for the 36 other subjects (median of 1.2 µg BPA/day)	Detection limit 0.38 µg BPA/l of urine, no information on contamination of solvents or leaching of BPA
(Matsumoto *et al.* 2003) Japan	HPLC with fluorescence detection, glucuronidase treatment, solvent extraction	Morning spot urine from 46 male and 4 female students,	Up to 30 µg BPA/g creatinine/app. 18 µg BPA/l, 39 % of samples collected in 1999 were below LOD of 1.7 µg BPA/g creatinine (1 µg BPA/l)	Single trace method, no information on contamination
(Hanaoka *et al.* 2002) Japan	HPLC with electrochemical detection, glucuronidase	Spot urine samples from 42 individuals without intentional	BPA > 1 µmol/mol creatinine in controls (< app. 1.2 µg BPA/l), no further details	Single trace method, no information on contamination.

	treatment followed by protein precipitation,	BPA-exposure, 42 males exposed to BADGE		
(Kim *et al.* 2003) Korea	HPLC with fluorescence detection, separate assessments with and without glucuronidase treatment, solvent extraction	Spot urine samples collected from 15 healthy men and 15 healthy women	BPA from 0.28 to 2.36 µg/l (mean 0.58 + 0.14) in males and 0.068 – 1.65 µg/l (mean 0.56 + 0.1) in females; BPA glucuronide from 0.16 to 11.67 µg/l (mean 2.34 + 0.85) in males and < LOD to 4.34 (mean 1.0 + 0.34) in females; BPAsulphate from LOD to 1.03 µg/l (mean 0.49 + 0.27) in males and < LOD to 3.4 (mean 1.2 + 0.32) in females	Single trace method, no information on contamination
(Ye *et al.* 2005) United States	LC/MS/MS with column switching, glucuronidase treatment	30 spot urine samples from adults	Mean conc. of 3.5 µg BPA/l, (95 percentile of 11.5 µg BPA/l)	LOD of 0.4 µg/l, reagent blank gives response of app. 0.1 µg BPA/l
(Yang *et al.* 2003) Korea	HPLC with fluorescence detection; glucuronidase treatment, solvent extraction	Morning spot urine from 34 adult males and 39 adult females	Geometric mean of 8.91 + 8.32 µg BPA/l	LOD of 0.34 µg/l, no background
(Kawaguchi *et al.* 2005) Japan	GC/MS with EI ionization, thermal desorption; glucuronidase treatment, sorptive extraction after derivatisation	Urine samples from 5 health subjects, no further information	Range from < LOD to 5.41 µg BPA/l	LOD 0.1 µg/l, reagent background not detailed
(Mao *et al.* 2004) China	HPLC after fluorophore derivatisation with p-nitrobenzoyl chloride; acid hydrolysis to cleave conjugates followed by solid phase extraction	10 healthy male and 10 healthy female subjects, no information on urine collection	Range from < LOD to 3.95 mg BPA/l, mean 1.22 + 1.38 mg BPA/l;	LOD 2.7 µg BPA/l, but poorly resolved peak for BPA, peak assignment on retention time only, very high concentrations of endogenous hormones indicted by assay suggest systematic error in evaluation
(Kuklenyik *et al.* 2003) United States	GC/MS with chemical ionization and electrophore derivatisation, negative ion detection; glucuronidase treatment and extractive derivatisation	30 urine samples from individuals painting houses and 6 unexposed individuals used as controls	Quantitative evaluation of BPA levels in urine of unexposed controls not detailed, based on graphic presentation estimated as below 2 µg/l	LOD of 0.1 µg BPA/l, no information on BPA-contamination of blanks
(Calafat *et al.* 2005) United States	GC/MS with chemical ionization and electrophore derivatisation, negative ion detection;	Spot urine samples from 394 adults in the US, collected at different times of the day	Geometric mean of 1.21 µg BPA/l for urban and of 1.56 µg BPA/l for rural residents	LOD of 0.1 µg BPA/l; no information on BPA-contamination of blanks

	glucuronidase treatment and extractive derivatisation			
(Ouchi and Watanabe 2002) Japan	HPLC with electrochemical detector and column switching, determination with and without pretreatment with glucuronidase	Morning spot urine samples from 48 female students	BPA below LOD except for one sample with 0.2 µg BPA/l; BPA-glucuronide detected in all samples with concentrations from 0.2 to 19.1 µg BPA-gluc/l (median of 1.2 µg/l)	Background of 0.26 µg BPAglucuronide/l
(Volkel *et al.* 2005) Germany	LC/MS/MS with and without glucuronidase treatment	Randomly collected urine samples from 7 males and 12 females without known BPAexposure	All samples below LOD of 1.14 µg BPA/l LOD 1.14 µg BPA/l	Background below LOD after method adjustment
(Ye *et al.* 2005) United States	LC/MS/MS with column switching, separate analysis for free BPA without enzymatic hydrolysis, after glucuronidase and after sulfatase treatment	Randomly collected urine samples from 30 adult individuals without known BPA-exposure	Means for free BPA were < LOD, for BPAglucuronide 3.1 µg/l, BPA-sulphate 0.5 µg/l	LOD 0.3 µg BPA/l
(Fukuta *et al*. 2006) Japan	HPLC with electrochemical detection for total and free BPA; BPA-glucuronide concentrations in some samples confirmed by LC/MS-MS	Randomly collected urine samples from 21 male and 31 female subjects, age 22 – 51 years	2 samples showed free BPA (0.24 and 0.35 µg/l); mean of total BPA was 1.92 + 1.99 µg/l. ELISA kits gave total BPA concentrations of 15.9 + 9.9; 16.7 + 19.5; and 18.6 + 23.7 µg/l	LOD 0.5 µg BPA/l for HPLC

al., 2006; Ikezuki et al., 2002; Inoue et al., 2001; Ohkuma et al., 2002; Schonfelder et al., 2002b; Takeuchi and Tsutsumi 2002; Takeuchi et al., 2004; Volkel et al., 2005; Yamada et al,. 2002). The studies reporting detection of BPA in human blood in concentrations higher than 1 µg/l have usually determined BPA, without prior enzymatic cleavage of BPA glucuronide. Based on toxicokinetics of BPA in humans, BPA glucuronide is expected to be present in higher concentrations as compared to BPA (Teeguarden et al., 2005; Volkel et al., 2002). The fate of BPA glucuronide under the conditions of the diverse sample processing conditions and a possible cross-reactivity of the antibodies with BPA glucuronide is not reported, leaving the possibility that reported BPA levels actually reflect BPA glucuronide levels.

EFSA also noted that blood levels for BPA in unintentionally exposed human subjects (reported as up to 10 µg/l) are higher than the peak BPA concentrations determined in blood of monkeys (5 nM, app. 1.1 µg/l) after oral administration of a dose of 100 µg/kg bw or in blood of humans given oral doses of 60 – 80 µg/kg bw. In these human subjects, free BPA in plasma was not detected even within a short time after doses much higher than the doses of BPA received by the general population from the diet. Furthermore, these reported concentrations of BPA in blood of unintentionally exposed human subjects of up to 10 µg/l are orders of magnitude above the maximal concentrations of BPA predicted in blood by PBPK models on the basis of human BPA toxicokinetics after oral administration (see below, app. 40 pmol/l or 9 ng/l) (Filser et al., 2003; Teeguarden et al., 2005). Based on the PBPK model, these maximal blood levels will be reached after oral uptake of BPA at a daily dose of 1 µg/kg bw and after simulation of a dietary exposure pattern (Filser et al., 2003; Teeguarden et al., 2005).

A number of other confounders have also been reported. Regarding the use of ELISA to quantify BPA, the cross-reactivity of the antibodies to other constituents in serum is unknown and may result in an overestimation of BPA concentrations. Attempts to confirm BPA concentrations indicated by ELISA using instrumental analytics have failed, and consistently indicate a large overestimation of BPA concentrations by ELISA (Inoue *et al*., 2002; Tominaga, *et al.,* 2006). In addition, studies have reported contamination of reagents with BPA or leaching of BPA from the materials for sample collection, storage and processing (Sajiki et al., 1999; Sajiki, 2001). The background may interfere with the analytical quantitation of BPA in low concentrations, suggesting higher BPA concentrations than actually present. Due to all these confounders, the reported analytical results on BPA blood concentrations most probably considerably overestimate real blood concentrations actually present.

A recent paper (Fukata *et al*., 2006) compares LC/MS/MS, LC/ECD, and three commercially available ELISA kits for measurement of BPA in 52 matched human urine and serum samples. The LC/MS/MS method, which positively identifies BPA and BPA-monoglucuronide, correlates very well with the LC/ECD method. However, the three ELISA kits not only have poor correlation with the reliable LC-based methods, but they also have poor correlation with each other. From this set of data, it can be concluded that the ELISA kits not only produce inaccurately high values for BPA, but they are not apparently measuring BPA at all.

Considering the evidence as a whole, EFSA concluded that the validity of the reported high blood levels of BPA in unintentionally exposed human subjects is questionable.

The studies on human urinary concentrations of BPA metabolites show peak levels of 15 µg/l and confirm that BPA is mainly present as BPA glucuronide in urine. The more recent studies

analyzing BPA concentrations in human urine often applied sensitive and selective mass spectrometry and are considered useful to assess daily exposures to BPA in humans. While spot urine samples may not be totally appropriate, due to the dietary exposure pattern and the rapid excretion, the BPA concentrations in spot urine samples and in 24 h pooled urine samples correlate reasonably well. The cumulative daily human exposures can be derived from urinary excretion of BPA and/or BPA metabolites since orally administered BPA is almost completely recovered in urine within 24 h after an oral exposure (Volkel et al., 2002). Mean urinary (total) BPA concentrations in the USA and in Japan are reported to range from 1.2 to 3.5 µg/l, while samples from a cohort in Germany did not contain detectable concentrations of BPA with a detection limit of 1.1 µg/l (Volkel et al., 2005).

A study in the USA, quantifying BPA in the urine of 394 subjects from the general population, detected BPA in 95% of the urine samples in concentrations up to 5.18 µg/l, 95th percentile) (Calafat et al., 2005).

Supplementary information supporting that exposure to BPA is in the above-mentioned range is available from the CDC biomonitoring program. BPA was included in the most recent CDC biomonitoring program, which is a part of the broader National Health and Nutrition Examination Survey (NHANES). The BPA biomonitoring raw data is now available to the public on the CDC website (http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/nhanes03_04.htm). Total BPA (after hydrolysis of conjugates) was measured in urine samples from 2,517 individuals (age 6-85 years). Initial preliminary analysis of these data indicate that this study is in agreement with previous CDC studies; overall weighted statistics of these BPA concentrations in urine have produced a median of 2.8 ng/ml, a geometric mean of 2.6 ng/ml, a 5th percentile of ND (0.36 ng/ml LOD) and a 95th percentile of 16 ng/ml (detection frequency: 93.5%).

Based on a total urine volume of 2 litres excreted over 24 h, the available data give an estimate of an average daily dietary exposure of BPA of up to 7 µg/adult and upper range dietary exposures up to 10 µg per adult (0.16 µg/kg bw for a 60 kg person). A recent Japanese assessment of BPA exposure of the general population used urinary excretion data and estimated (95 % confidence interval) daily BPA exposure as 0.037 to 0.064 µg/kg bw/day for male and 0.043 – 0.075 µg/kg bw/day for female adults (Miyamoto and Kotake, 2006).

EFSA (2006) noted that exposure assessed from urinary excretion measured in groups of subjects from the general population in the USA, Japan and Korea could be used to assess the order of magnitude of overall average BPA exposure. The discrepancy between the levels of exposure estimated through biomarkers and the levels of exposure assessed by combining food consumption data with BPA concentration in the diet is likely to be due to the highly conservative assumptions performed in the latter, which are aimed at assessing exposure in the most exposed population groups.

4.1.1.4 Combined exposure

4.1.1.4.1 Summary of original risk assessment report

The worst case combined exposure would be someone exposed via the environment near to a PVC production plant, and who is also exposed via food contact materials as described in section 4.1.1.2.

The exposures for these component parts are presented below. The maximum combined exposure from these sources is 0.009 and 0.069 mg/kg/day for the regional and local scenarios respectively.

Table 4.17 Components of combined exposure (from the 2003 published RAR)

Source of exposure		Exposure (mg/kg/day)
As a consumer (oral exposure via food and wine)		9×10^{-3}
Indirect exposure via the environment:		
Regional		1.78×10^{-5}
Local		0.06
Total :	Regional	9×10^{-3}
	Local	0.069

The value of 9×10^{-3} for consumer exposure is based on an adult consumer receiving exposure via canned food and wine. The values of 1.78×10^{-5} and 0.06 for regional and local environmental exposure respectively, have been taken from Table 4.10. The main route of exposure from environmental sources is the oral route. The average body weight of 70 kg has been assumed.

4.1.1.4.2 Updated information

The worst case combined exposure would be for someone exposed via the environment near to a BPA production plant, and who is also exposed via food contact materials as described in section 4.1.1.2.

The exposures for these component parts are presented below. The maximum combined exposure from these sources is 1.45×10^{-3} [it was 9×10^{-3} in the original RAR] and 0.043 [it was 0.069 in the original RAR] mg/kg/day for the regional and local scenarios respectively.

Table 4.18 Components of combined exposure

Source of exposure		Exposure (mg/kg bw/day)
As a consumer (oral exposure from canned food and canned beverages and from polycarbonate tableware and storage containers)		1.45×10^{-3}
Indirect exposure via the environment:		
Regional		9.1×10^{-6}
Local		0.041
Total :	Regional	1.45×10^{-3}

Source of exposure	Exposure (mg/kg bw/day)
Local	0.043

The value of 1.45 x 10^{-3} for consumer exposure is based on an adult consumer receiving exposure via canned food and canned beverages and from polycarbonate tableware and storage containers. The values of 9.1 x 10^{-6} and 0.041 for regional and local environmental exposure respectively, have been taken from Table 4.11. The main route of exposure from environmental sources is the oral route. Average body weights of 60 and 70 kg have been assumed for consumers and humans exposed via the environment.

4.1.2 Effects assessment: Hazard identification and dose (concentration) - response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Summary of original risk assessment report

The limited data available in humans, from a single study, indicates that BPA does not accumulate in endometrium or body fat (the only tissues tested). In experimental animals, toxicokinetic data are available from three oral studies in a single species, the rat and from an *in vitro* dermal absorption study, using human skin. These studies provide the basis for a general understanding of the main features of the toxicokinetic profile. Following oral administration, absorption from the gastrointestinal tract is rapid and extensive, although it is not possible to reliably quantify the extent of absorption. Following dermal exposure, the available data suggest that there is limited absorption, in the region of about 10% of the applied dose. BPA was removed rapidly from the blood, and metabolism data indicate extensive first pass metabolism following absorption from the gastrointestinal tract. A clear sex difference was observed in the clearance of parent compound from the blood. In females parent compound was present in the blood at much later sampling times. There are no data available to explain why this sex difference was observed. In view of this first pass metabolism, the bioavailability of unconjugated BPA is probably limited following oral exposure, at no more than 10-20% of the administered dose. Limited data are available for the distribution of BPA following oral administration: an *in vivo* DNA adduct study shows that BPA reaches the liver, an *in vivo* micronucleus study suggests that BPA or a metabolite reaches the bone marrow, a limited toxicokinetic study suggests that BPA or a metabolite reaches the testes, and a repeat dose study in pregnant rats suggests that BPA reaches the liver of both the dam and fetus. However, because of first pass metabolism, it is likely that the distribution and bioavailability of unconjugated BPA is limited following oral exposure. There is also evidence of enterohepatic circulation occurring.

The major metabolic pathway in rats involves glucuronide conjugation; limited sulphate conjugation may also occur. Approximately 10% and 20% of the administered dose was recovered in the urine as the glucuronide metabolite in males and females, respectively. There are no data available to explain why this sex difference was observed. Comparative *in vitro* studies of metabolism suggest some quantitative differences in the rate of metabolism between rats, mice and humans. In general, human liver samples show slower rates of glucuronidation compared with either rats or mice. Estimates of overall liver metabolic capacity suggest that human liver may have greater metabolic capacity than either rats or

mice and that capacity is lowest in the mouse. However, these estimates are based on limited kinetic data and are therefore of uncertain reliability. *In vitro* data in rats also indicate that fetuses do not metabolise BPA as extensively as immature and adult animals. In addition, data from cell free systems and *in vivo* studies on the interaction of BPA with DNA, supported by a chemical photodecomposition study, suggest that limited oxidation of BPA to bisphenol O-quinone by cytochrome P450 may occur.

The major route of excretion is via the faeces with the urinary route being of secondary importance: over 7 days post dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post dose. A sex difference was also observed in elimination, with females excreting approximately twice as much radioactivity in the urine (24-28%) than males (14-16%). Again, there are no data available to explain why this sex difference was observed. In addition, a strain difference was observed in elimination, with female F344 rats excreting approximately twice as much radioactivity in the urine than female CD rats. Data from a number of studies suggest limited excretion of BPA in the milk. However, the data do not allow a reliable quantitative determination to be made.

The first pass metabolism and extensive and rapid elimination of BPA suggest that the potential for transfer to the foetus and bioaccumulation may be limited. This is supported by data from toxicokinetic studies in pregnant rats that suggest limited distribution of BPA to the foetus, but no evidence for accumulation, and results from a repeat dose study in pregnant rats which show limited distribution to the fetal liver, with no evidence to indicate accumulation in the liver, the only organ tested.

There are no data on the toxicokinetics of BPA following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

4.1.2.1.2 Updated information

A number of recent publications addressing the toxicokinetics of BPA in primates and in pregnant and non-pregnant rodents of different ages have become available since the original RAR was finalised.

Studies in humans and non-human primates

A study in human volunteers investigated the toxicokinetics of a low dose of BPA given orally (Volkel *et al*, 2002). In the first half of the study, designed to provide information on the metabolic fate of BPA, a single oral dose of 5 mg deuterium-labelled BPA was given to three healthy adults of each sex; mean body weight was 67 kg (range 57 – 82 kg). Samples of blood were taken at intervals of 4 hours, and urine produced during 6-hour periods was collected. These samples were assayed for BPA and its metabolites by HPLC with mass spectrometry; the assay limit of detection for free BPA was 6nM for urine and10nM for blood samples. Measurements of individual metabolites were confirmed by gas chromatography, and by enzymatic hydrolysis of samples. In the second half of the study,

designed to investigate the toxicokinetics of BPA in plasma in more detail, the same dose of deuterium-labelled BPA was given orally to four male subjects, including one from the first half of the study. The mean body weight was 78 kg (range 63 – 92 kg). Samples of blood were taken at intervals of 40 minutes and urine produced over 6-hour periods was collected for assay of BPA and BPA metabolites as described above.

In both parts of the study BPA-glucuronide was the only metabolite detected in samples of plasma and urine. Neither free BPA nor other BPA metabolites were found.

The first part of the study showed that the dose of deuterium-labelled BPA administered orally was completely recovered in urine as BPA-glucuronide. The urinary concentration of this metabolite fell rapidly from a peak at 6 hours after dosing with a terminal half-life ($t_{1/2}$) of 5 hours. The concentration of BPA-glucuronide in the plasma also fell rapidly after dosing. There were no apparent sex differences in the profiles of BPA-glucuronide in either plasma or urine.

In the second part of the study it was found that in plasma the C_{max} of BPA-glucuronide was approximately 800nM and occurred 80 minutes after dosing. The plasma concentration of BPA-glucuronide declined biexponentially with a half-life of 89 min, and the clearance from the plasma (0.13 litres/minute) was found to be similar to that reported for plasma creatinine, signifying that BPA-glucuronide probably enters the urine by filtration, with no renal secretion or reabsorption. The apparent distribution volume (37 litres) was similar to the total body water volume, suggesting that BPA-glucuronide distributes throughout the extra- and intracellular aqueous media and does not bind to plasma proteins.

In summary, this study found that in humans low doses of BPA (in the range 54 to 88 µg/kg bw) were absorbed rapidly and completely from the GI tract after oral administration. BPA was efficiently conjugated to the glucuronide by first-pass-metabolism, with free BPA and other metabolites not being detected in plasma from 40 minutes after dosing onwards. The BPA-glucuronide formed was cleared from the circulation within 24 hours by urinary excretion. No evidence of enterohepatic recirculation was found.

The same authors also carried out a study in which BPA and BPA-glucuronide were measured by a sensitive technique (high performance liquid chromatography/tandem mass spectrometry) in plasma and urine of human volunteers (3 males and 3 females) given a single oral dose of 25 µg (~ 0.35 µg/kg bw) BPA and in human subjects (7 males and 12 females) without intentional exposure to BPA (Volkel et al., 2005).

In urine samples from human volunteers administered BPA, BPA was below the limit of detection (LOD = 2.5 pmol/ml urine) with the exception of two samples (~ 1 µg/l) in which BPA was less than 2% of the administered dose; 75-85% of the administered dose was recovered as BPA-glucuronide within 5 hours after application. An elimination half-life of 4 hours was calculated. In urine or blood samples from human subjects without intentional exposure to BPA, BPA concentrations were always below the LOD of ~ 1.14 µg/l; the concentration of BPA-glucuronide in urine was always below the LOQ of 65 pmol/ml. Due to the rapid and complete elimination of BPA in humans as BPA-glucuronide in urine, the concentration of BPA determined in the urine of the 19 subjects without intentional exposure to BPA investigated in this study suggests that human exposure to BPA in these subjects is less than 2.3 µg/person/day (0.04 µg/kg bw/day; bw = 60 kg) based on a volume of 2 litres of urine excreted.

A GLP-compliant study investigated the toxicokinetics of a low dose of ^{14}C-labelled BPA given orally or intravenously to cynomolgus monkeys (Kurebayashi *et al*, 2002). Three males and three females were administered 0.1 mg/kg ^{14}C-labelled BPA intravenously, and then two weeks later they were given the same dose orally by gavage. Blood samples were taken 5 minutes after dosing and at selected intervals for up to one week after dosing. Urine, including cage-washings, and faeces were collected over 12 or 24 hour periods up to one week after dosing. Metabolites were separated and quantitated using HPLC, and the nature of metabolites was investigated by enzymatic hydrolysis.

Following intravenous (iv) administration, most of the radioactivity (79 – 86%) was excreted in the urine, with only 2% being excreted in the faeces. Plasma radioactivity declined with a terminal half-life of approximately 14 hours. The proportion of blood radioactivity bound to plasma proteins was relatively high (94%). The total body clearance and distribution volume were calculated to be 4.5 ml/min/kg body weight and 1.6-1.8 litres/kg body weight respectively.

Following oral dosing, as after iv dosing, most of the administered dose (82-85%) was excreted in urine, with a high proportion appearing in the urine within 12 hours of administration, suggesting that absorption from the GI tract was rapid and relatively complete. Only a small proportion of the dose of BPA administered (2-3%) was excreted in the faeces. Plasma radioactivity rose to a maximum value between 0.25 and 2 hours after dosing, and subsequently fell with a terminal half-life of approximately 10 hours. No radioactivity was detected in plasma 72 hours after dosing. The terminal elimination half-life of ^{4}C-BPA-derived radioactivity in plasma following iv dosing was larger (13.5 hours in males and 14.7 hours in females) than that following oral dosing (9.6 hours in males and 9.8 hours in females). It has been suggested by the authors that this might have been due to deposition of the lipophilic free BPA in adipose tissue after iv dosing, in contrast to first-pass metabolism after oral dosing.

HPLC analysis recognised 5 radioactive species in samples of urine and plasma following both iv and oral dosing: free BPA, BPA-glucuronide, BPA-diglucuronide and two unidentified minor metabolites. In urine samples, free BPA was found at low levels (between 0.0 and 1.5% of the radioactivity detected) following both iv and oral dosing. The largest fraction of the radioactivity detected was due to BPA-glucuronide (73-81% of urinary radioactivity), BPA-diglucuronide and other BPA metabolites, suggested by the study authors to be BPA-sulphate and 5-hydroxy BPA.

After oral dosing, the major radioactive species in plasma was found to be BPA-glucuronide (up to 95-100% of plasma radioactivity) with unconjugated BPA representing only up to 1.4% of the total plasma radioactivity. After iv dosing, most (between 57 and 82%) of the plasma radioactivity was due to BPA-glucuronide, with unconjungated BPA representing 27-29% of the total plasma radioactivity. It was noted that after iv dosing the plasma levels of unconjugated BPA declined more rapidly in females than in males with a fast phase terminal half-life of 0.4 hours in females and 0.6 hours in males. Since there were no significant sex differences in urinary excretion, this difference was thought to be related to female animals having a higher adipose tissue content than males, suggesting that circulating free BPA can partition into the adipose tissue.

In summary, this study in cynomolgus monkeys found that a low oral dose of BPA was extensively absorbed from the GI tract (up to 85% of the administered dose), subsequently undergoing rapid first-pass metabolism to BPA-glucuronide and a slower conversion to BPA-diglucuronide. Both metabolites were excreted into the urine, and, consistent with the data in humans but in contrast to rats, the urine was a much more important route of elimination of

BPA-derived radioactivity than the faeces. This study also suggested that there could be preferential distribution of lipophilic unconjugated BPA to adipose tissue after iv dosing, in contrast to first-pass metabolism after oral dosing.

EFSA (2006) noted that rapid elimination from blood and extensive first-pass metabolism of orally administered BPA in humans and primates is also indicated by results of a recent study on the blood toxicokinetics of BPA in chimpanzees and monkeys (Tominaga et al., 2006).

Overall, the results on the toxicokinetics of BPA in humans and primates show a rapid first-pass biotransformation and elimination of orally administered BPA and indicate that, after oral uptake, only low levels of unmodified BPA may reach the systemic circulation. The data also show that enterohepatic recirculation of BPA does not occur in humans and primates.

Studies in rodents

A GLP-compliant study (Domoradzki et al, 2003) was recently conducted with the aim of comparing the metabolism and toxicokinetics of BPA (BPA) in pregnant rats at different stages of gestation and in non-pregnant rats. The study also investigated the distribution of BPA or its metabolites to the embryo/foetal tissues. The experimental study design consisted of three parts.

In the first part which was conducted to evaluate any impact of stage of pregnancy on BPA metabolism, ^{14}C-labelled BPA was administered via oral gavage in corn oil at 10 mg/kg to groups of 4 animals/gestational stage of pregnant Sprague-Dawley (SD) rats on gestational days (gd) 6, 14 and 17, and to non-pregnant, 11 week-old female SD rats. Rats were placed in Roth-type metabolism cages immediately post-dosing to allow for the separate collection of urine and faeces up to 96 hours post-dosing.

Blood samples were collected at the expected free, unconjugated BPA T_{max} time point (15 minutes post administration) and at additional selected time points over the 96-hour period post administration. Levels of total plasma radioactivity for each time point and amounts of free, unconjugated BPA and BPA-glucuronide metabolite were quantified in pooled plasma samples by liquid scintillation counting or by HPLC with radiochemical detection. Levels of BPA, BPA-glucuronide and other metabolites were also quantified in pooled excreta samples from selected groups and collection intervals. Specific structural identification of free, unconjugated BPA and BPA-glucuronide was confirmed by LCMS for selected plasma, urine and faecal samples. At sacrifice (96 hours post-dosing), selected tissues were collected and analysed for radioactivity, levels of free, unconjugated BPA and metabolites. The tissues analysed included maternal blood, brain, fat, liver, kidneys, ovaries, uterus, skin, placenta, embryo/foetus (6 individual embryos/foetuses and 1 pooled embryo/foetus sample for each gd 6, gd 14 and gd 17 dam) and remaining carcass. A final cage wash was collected and mass balance of radioactivity determined.

In the second part which was conducted to study further the distribution of BPA and BPA-glucuronide to the embryo/foetal tissue, ^{14}C-BPA was administered via oral gavage at 10 mg/kg in corn oil to groups of 5 animals/sacrifice time point/gestational stage of pregnant SD rats on gd 11 (embryonic stage), 13 (foetal stage) and 16 (foetal stage). Rats were then sacrificed at one of three different time points. Sacrifice time point 1 was 15 minutes post-dosing, the anticipated peak plasma radioactivity time point in the maternal animals. Sacrifice time point 2 was 12 hours post-dosing, the time of the secondary maximum of plasma

radioactivity in maternal animals and sacrifice time 3 was 96 hours post-dosing, when BPA was anticipated to be non quantifiable in the maternal blood of most animals. The timing of the three sacrifices meant that the experiment covered stages in development from embryo to foetus to perinatal. Samples of maternal blood, embryos/foetuses and yolk sacs/placentas collected at each of the three sacrifice points for each of the three gestational stages were analysed for radioactivity, free, unconjugated BPA and/or metabolite profile, including quantitation of BPA monoglucoronide. In addition, from gd 20 (96 hours post dosing of gd 16 rats) sacrificed dams, samples of foetal blood, plasma, liver and brain were also collected for analysis.

In the third part, 5 pregnant SD rats were dosed by gavage with 0 or 10 mg/kg ^{14}C-BPA in corn oil on gd 16, using a radioactivity level of approximately 500uCi per rat. This higher specific activity of ^{14}C-BPA was employed to improve the limit of detection of analytes. Fifteen minutes post-dosing, all animals were sacrificed and maternal blood, placentas and foetuses were collected for analysis. The levels of radioactivity, free, unconjugated BPA and BPA-glucuronide were determined in these samples.

Part 1 - There were no signs of treatment-related toxicity observed and no deaths occurred during part 1 of the study. At 96 h post-dosing, the faeces were the major elimination route of ^{14}C-BPA-derived radioactivity for all 3 gestational groups and for the non-pregnant animals, accounting for 65-78% of the administered dose. The mean percentage of the administered dose that was eliminated via the urine was also similar for all groups, ranging from 14-22%. The ^{14}C-BPA-derived radioactivity in tissues (brain, fat, liver, kidney, ovary, uterus and skin) and remaining carcass was 1-6% of the administered dose across all groups, and the embryo/foetus samples accounted for <0.1% of the administered dose in the gd 17 group and were non-quantifiable in the gd 14 and gd 6 groups. In general, liver and kidney had the highest concentrations of tissue radioactivity. Brain did not contain quantifiable levels of radioactivity, and levels in fat were only occasionally quantifiable in individual animals. Placentas (only available from the last two gestational groups as the placenta is not formed yet on gd 6) had quantifiable radioactivity only in the last gestational group, representing 0.01% of the administered dose. These data showed that the pregnancy status had no effect on the overall disposition of orally administered ^{14}C-BPA-derived radioactivity in SD female rats. Generally, the concentration of ^{14}C-BPA-derived radioactivity quantified in embryo/foetus and placenta samples was equal to or less than the levels found in other tissues analysed for radioactivity, such as liver or skin or ovaries. This suggests that orally administered ^{14}C-BPA-derived radioactivity does not preferentially accumulate in the embryo/foetal or placental compartment of pregnant SD rats.
Plasma ^{14}C-BPA-derived radioactivity was quantifiable from 15 minutes post-dosing through to 48 hours post-dosing for all groups. Plasma radioactivity in all groups was highest at 15 minutes post-dosing but reached a secondary maximum between 12 and 24 hours post-dosing. The pattern of a primary peak and a secondary peak in total plasma radioactivity, which was reproducible across all groups, is considered to be evidence of enterohepatic recirculation of ^{14}C-BPA-derived radioactivity in pregnant and non-pregnant SD rats. By 72 hours post-dosing, radioactivity was not quantifiable in the plasma of non-pregnant rats or from gd 14 dams. ^{14}C-activity was not quantifiable in the plasma from gd 17 dams by 96 hours post-dosing, while plasma from gd 6 dams still contained quantifiable levels of radioactivity at terminal sacrifice (96 hour).

Unchanged BPA was only quantifiable at a few time points (15 and 45 minutes post-dosing) in most groups with typical concentrations close to the levels of detection. Only the late stage pregnancy group (gd 17-21) had low but quantifiable levels of free, unconjugated BPA in plasma for the first 12 hours post-dosing.

BPA-glucuronide represented the largest fraction of quantifiable plasma radioactivity for all groups, up to 100% in some cases. Two BPA-glucuronide maximum occurred; these correlated with the peaks of plasma ^{14}C-BPA-derived radioactivity observed at 15 minutes and 12-24 hours post-dosing, clearly indicating that BPA-glucuronide is the predominant form of ^{14}C-BPA-derived radioactivity in the systemic circulation, and that enterohepatic circulation is a major factor in the metabolism of BPA. The BPA-glucuronide concentration-time profiles were very similar to those for plasma radioactivity, with BPA-glucuronide reaching non quantifiable levels at the same time, by either 72 or 96 hours post-dosing (non-pregnant, gd 14 and gd 17) or remaining quantifiable at terminal sacrifice (gd 6). Two other metabolites of BPA were observed in plasma but both were minor and were not structurally identified.

Up to nine separate peaks were identified in the urine samples, with the majority of the urinary radioactivity identified as BPA-glucuronide (0.3 – 5% of the administered dose and 62-70% of the radioactivity recovered from the urine over the 96-hour collection period for all groups). Approximately 19 to 23% of the radioactivity recovered from the urine over the 96-hours collection period was determined to be BPA for all groups. No marked differences were observed between the urinary metabolite profiles in non-pregnant and pregnant animals.

Up to seven peaks were identified in faecal samples with the majority of the faecal radioactivity identified as free, unconjugated BPA (83-89% of the radioactivity recovered from the faeces over the 96-hour collection period for all groups). Approximately 2 to 3% of the radioactivity recovered from the faeces over the 96-hour collection period was found to be BPA-glucuronide for all groups. No marked differences were observed between the faecal metabolite profiles in non-pregnant and pregnant animals.

Part 2 - The mean percent of the administered dose recovered in maternal blood for animals dosed with 10 mg/kg at gd 11,13 and 16 were in the same range (0.11 – 0.25%) as observed before in other studies conducted with SD rats. In general, BPA was non-detectable in maternal plasma from all groups. The average peak maternal plasma concentrations of BPA-glucuronide at 15 minutes post-dosing were similar for the animals dosed on gd 11 or 13, but were 1.7-2 times higher in rats dosed on gd 16. By 12 hours post-dosing, the concentration of BPA-glucuronide was about 7- to 11-fold less than that seen at 15 minutes post-dosing for all groups. At 96 hour post-dosing there was insufficient radioactivity for analysis.
There was no apparent selective affinity of either placenta or embryo/foetus for ^{14}C-BPA-derived radioactivity. Analysis of the yolk sacs and embryos from gd 11 animals indicated that BPA was non-detectable at all times post-dosing. BPA-glucuronide was detected only at 15 minutes post-dosing in pooled samples of yolk sac, but not in pooled embryos. For gd 13 and 16 animals, BPA and BPA-glucuronide were detected in yolk sac/placenta at 15 minutes and 12 hours post-dosing. BPA was detected in embryonic tissue only at 15 minutes post-dosing from gd 13 animals. BPA and BPA-glucuronide were detected only in pooled samples of foetal tissue from gd 16 animals at 15 minutes post-dosing.

Part 3 - Maternal plasma, placental and foetal concentrations of free, unconjugated BPA and BPA-glucuronide were confirmed in a separate experiment (part 3 of the study) where gd 16 animals received a higher specific activity of BPA. At 15 minutes post-dosing, the percentage of the administered dose recovered in maternal plasma, placenta (combined value per dam) and foetus (combined value per dam) ranged from 0.12 – 0.24%, 0.05 – 0.13% and 0.005 – 0.020%, respectively. These data show that the distribution of the total ^{14}C-BPA-derived material to the placenta was only about ¼ of that found in the maternal plasma and that the distribution of the total ^{14}C-BPA-derived material to the foetal tissue was about 1/10 of that found in the placenta.

The concentrations of BPA detected in maternal plasma, placenta and foetus at 15 minutes post-dosing were 0.064, 0.0953 (1.5 times the concentration in maternal plasma) and 0.0176 (18% of the concentration in the placenta) µg/g plasma-tissue respectively, and the concentrations of BPA-glucuronide measured in maternal plasma, placenta and foetus at 15 minutes post-dosing were 1.699, 0.3421 (20% of the concentration in maternal plasma) and 0.0130 (4% of the concentration in the placenta) µg/g plasma-tissue respectively. These values were in agreement with those obtained in part 2 of the study where a lower specific activity was used. It can be seen that BPA-glucuronide in maternal plasma distributes to placenta and foetus only to a limited degree, 20% to placenta and 4% to the foetal tissue, and that the distribution of BPA-glucuronide corresponds to the distribution of ^{14}C-BPA radioactivity from maternal plasma to placental and foetal tissue. BPA from maternal plasma appears to be better distributed to placental tissue, and to a reduced degree to foetal tissue. Accordingly, the ratio of BPA-glucuronide to BPA is altered in these tissues as compared to plasma, the ratios being 27, 4 and 0.7 for maternal plasma, placental tissue and foetal tissue, respectively. This decrease in the ratio of BPA-glucuronide to BPA concentrations in the placenta as compared with the maternal plasma may have been a result of the ample glucuronidase activity found in the rat placenta which could hydrolyse some of the BPA-glucuronide that is distributed from maternal plasma to placental tissue back to BPA, rather than a result of increased distribution of free, unconjugated BPA from maternal plasma. It is also reported in the literature that UDP-glucuronosyltransferase is not fully developed in foetal liver; therefore, conversion of BPA to BPA-glucuronide may be saturated in the foetus after exposure to 10 mg/kg BPA. This would provide an explanation for the decreased ratio of BPA-glucuronide to BPA concentrations observed in the foetal tissue as compared with the placental tissue after exposure to a high dose of BPA.

Overall, these findings clearly demonstrate that very low levels of BPA are distributed to the foetus following a relatively high oral dose of BPA (10 mg/kg). However, the distribution of BPA to the foetus (or placenta) did not alter the overall pharmacokinetic fate of BPA in pregnant rats as compared to non-pregnant rats. Therefore, the rates of transfer in and out of the foetus were apparently not the rate-limiting processes in determining the overall elimination half-life of BPA from the maternal-foetal “unit”.

The disposition of oral doses of BPA in pregnant SD rats was similar to that found in non-pregnant rats. ^{14}C-BPA-derived radioactivity in plasma was quantifiable through 48 hours post-dosing. The time course of radioactivity in plasma demonstrated two peaks of radioactivity over the first 24 hours post-dosing in both pregnant and non-pregnant animals. This pattern of a primary and a secondary peak in total plasma radioactivity was clear evidence that enterohepatic recirculation of ^{14}C-BPA-derived radioactivity occurred in both pregnant and non-pregnant SD rats.

Free, unconjugated BPA in plasma was quantifiable only at a few time points in most groups, if at all, and when detected, was very close to the level of quantitation. In contrast to the level of unchanged BPA, BPA-glucuronide represented the largest fraction of quantifiable plasma radioactivity, up to 100%, for all groups. Plasma concentrations of BPA-glucuronide also demonstrated two peaks with time in both pregnant and non-pregnant animals. These data again offer clear evidence of enterohepatic recirculation as a major factor in the metabolism of BPA in both pregnant and non-pregnant SD rats. Clearly, BPA-glucuronide is the predominant form that is circulating and systemically available.

In summary, the tissue distribution, metabolism, or the rates or routes of excretion of BPA, or the plasma concentration-time profiles of BPA-glucuronide did not appear to be altered at any stage of gestation investigated as compared to non-pregnant rats. In addition, no selective

affinity of either yolk sac/placenta or embryo/foetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in this study. Maternal and embryo/foetal exposure to free, unconjugated BPA did occur following oral administration, but systemic levels were found to be low due to extensive first pass metabolism.

The same research group also carried out a GLP-compliant study investigating the toxicokinetics of BPA in neonatal rats (Domoradzki *et al*, 2004). In this study, a single dose of ^{14}C-labelled BPA was administered by oral gavage to neonatal rats at post-natal day (pnd) 4, 7, and 21, and also to 11-week old adult rats for reference.

Neonatal Sprague-Dawley animals of both sexes were dosed with ^{14}C-BPA at either 1 or 10 mg/kg, and at each of 8 time points between 15 minutes (the time point expected for the peak plasma concentration of free, unconjugated BPA) and 24 hours after dosing, animals (3 per age/sex/dose) were anaesthetised and sacrificed to provide samples of blood plasma, brain, kidney, liver, carcass/skin and testes or uterus and ovaries.

Groups of male and female adult animals of the same strain (4 animals/sex) were dosed at 10 mg/kg and housed in metabolism cages to enable collection and analysis of urine and faeces produced over 12-hour or 24-hour time periods. Samples of blood were collected from these adult animals by in-dwelling jugular vein cannula at 11 time points between 15 minutes and 96 hours after dosing for provision of plasma. At the end of this 96-hour time period these animals were sacrificed, and samples of blood, brain, carcass, fat, kidneys, liver, skin and testes or uterus and ovaries were collected.

Samples of plasma, urine and faeces from individual animals taken at these sampling times were assayed for total ^{14}C-BPA-derived radioactivity by liquid scintillation counting, and the mass balance of radioactivity determined. As in the previous study, samples of plasma taken from animals of the same age at the same time-point were pooled, and the concentrations of radiolabelled compounds in these pooled samples were measured by HPLC with radiochemical detection. The HPLC peaks produced by free, unconjugated BPA and BPA-glucuronide were identified by mass spectrometry/electrospray ionisation.

During the study there were no deaths, and no signs of treatment-related toxicity were observed. At the dose of 10 mg/kg, ^{14}C-BPA-derived radioactivity in the plasma of animals of all ages rose to a peak value within an hour of administering BPA, and then declined over the next few hours. In neonatal animals after the initial peak, plasma radioactivity declined over the subsequent 24 hours, and mean half-lives were in the region of 4 to 10 hours. In adult animals plasma radioactivity fell during the 6 hours after dosing, but over the next 18 hours rose slightly before falling to low levels at 96 hours. The highest peak levels of ^{14}C-BPA-derived radioactivity were found in the plasma of pnd 4 neonates, where peak values were between 20 and 100 times higher than in adults. In neonates at pnd 7 and pnd 21, peak values were between 10 and 20 times higher than at 11 weeks. The reason for the much higher peak values of ^{14}C-BPA-derived radioactivity in the plasma of young neonates than in adult animals was not discussed by the study authors, but it is likely to reflect an immaturity in the development of hepatic excretory functions in neonatal rats. In adult females plasma ^{14}C-BPA-derived radioactivity fell more rapidly than in adult males, and the calculated half-life ($t_{1/2}$) in females was 15 hours, compared with 22 hours in males. In neonates at pnd 7 and pnd 21 this sex difference in the decline of plasma radioactivity was less marked, and was not seen at pnd 4. At the 1 mg/kg dose of BPA, radioactivity was not detected in the plasma of adult animals at any time point, but the kinetics of plasma radioactivity in neonatal animals followed the profile seen at the higher dose.

Following administration of BPA at 10 mg/kg, HPLC showed the presence in the plasma of at least 13 different radiolabelled compounds. Of these, the two compounds present in the largest amounts were peaks 6 and 8, which were identified as BPA-glucuronide and free, unconjugated BPA respectively. The other radiolabelled compounds detected in the plasma were not characterised. In general, fewer individual radiolabelled compounds were detected in the plasma of older animals. The number of radioactive peaks detected in the plasma at pnd 4 was 12 in male animals and 11 in female animals, whereas at pnd 7 in male and female animals 7 and 8 different peaks respectively were detected. In animals at pnd 21 and at 11 weeks only 3 or 4 different radioactive peaks were detected. The study authors concluded that this reflected the conversion of BPA to a larger number of metabolites in younger neonates than in older animals and indicated saturation of the BPA glucuronidation pathway. However, since the total concentration of radiolabelled compounds appearing in the plasma was found to be considerably higher at pnd 4 and at pnd 7 than at pnd 21 and at 11 weeks, an alternative explanation for the larger number of peaks in younger neonates might be that in the plasma of animals at pnd 21 and at 11 weeks some minor metabolites had been present below the limit of detection. These data also indicate a dose dependency in the metabolism and pharmacokinetics of BPA administered to neonates with nearly complete metabolism of BPA to BPA-glucuronide (94-100% of the plasma radioactivity) at a dose of 1 mg/kg and with up to 13 different plasma metabolites observed at the 10 mg/kg dose.

At 10 mg/kg ^{14}C-BPA, the concentration of free, unconjugated BPA in the plasma of all animals rose to a peak within 2 hours of dosing, and subsequently fell over the period from 2 to 24 hours after dosing. Peak plasma concentrations of free, unconjugated BPA were highest in pnd 4 neonates, and lowest in adults; the difference between the peak values at the two ages was over 150 times. The reason for the much higher peak values of free, unconjugated BPA in the plasma of young neonates than in adult animals was not discussed by the study authors, but it is likely to be partly due to increased activity of the glucuronidation pathway in adults. In neonates, free, unconjugated BPA in the plasma fell with a half-life in the region of 4 to 17 hours. After the administration of 1 mg/kg ^{14}C-BPA, the kinetics of free, unconjugated BPA in animals at pnd 4 and 7 tended to follow the pattern seen at the higher dose. Free, unconjugated BPA was not detected in the plasma of animals at pnd 21 or at 11 weeks after administration of the lower dose.

BPA-glucuronide was detected in the plasma of animals of all ages at all time-points during the 24 hours after dosing with 10 mg/kg ^{14}C-BPA. In neonates, plasma BPA-glucuronide peaked within 2 hours after dosing and then fell over the period between 2 and 24 hours after dosing. The decay followed first–order kinetics, with a half-life in the region of 4 to 10 hours. In adult animals the plasma concentration of BPA-glucuronide fell during the one-hour period after dosing, but rose to a second peak between 12 and 24 hours after dosing. After administration of 1 mg/kg ^{14}C-BPA, BPA-glucuronide was not detected in the plasma of adult animals. In neonatal animals, the kinetics of BPA-glucuronide followed the pattern seen at the higher dose. These profiles of BPA-glucuronide in the plasma suggest that in adults, but not in neonates, BPA undergoes enterohepatic circulation. In neonates, there were no sex differences in the kinetics of BPA-glucuronide, but the plasma concentration of BPA-glucuronide fell more rapidly in adult females than in adult males, with calculated half-lives of 11 and 22 hours respectively. These data indicate that the half-lives for the elimination of BPA-glucuronide in plasma were more rapid in neonatal animals than in adults, likely due to reduced microflora β-glucuronidase activity and an absence of enterohepatic recirculation in neonates. As it had already been found for BPA-derived radioactivity and for free, unconjugated BPA, the peak concentration of BPA-glucuronide in the plasma was higher in neonates than in adults; the difference was between 9-fold and 22-fold. As for total ^{14}C-BPA-

derived radioactivity, the reason for the higher peak values of BPA-glucuronide in the plasma of neonates than in adult animals was not discussed by the study authors, but it is likely to reflect poorly-developed excretory functions.

The ratio of BPA-glucuronide to free, unconjugated BPA in the plasma was calculated by comparing the areas under the curve (AUC) for the plots of BPA-glucuronide concentration and free, unconjugated BPA concentration against time, although in adult animals given 10 mg/kg ^{14}C-BPA, and in adult and pnd 21 animals given 1 mg/kg ^{14}C-BPA, the ratio could not be determined. For the 10 mg/kg dose of ^{14}C-BPA, the AUC ratio of BPA-glucuronide to free, unconjugated BPA was found to be in the region of 3 to 7 at pnd 4, between 31 and 36 at pnd 7 and in the region of 55 to 56 at pnd 21. For the 1mg/kg dose, in animals at both pnd 4 and pnd 7, this ratio was found to be in the region of 45 to 96. The fact that for the pnd 4 animals the AUC ratio of BPA-glucuronide to free, unconjugated BPA was found to be an order of magnitude lower at the high dose than at the lower dose, suggests that the glucuronidation pathway may have been subject to substrate saturation at the higher dose. On the other hand, with the pnd 7 animals, the AUC ratios at the two dose levels were similar, suggesting that substrate saturation of the pathway had not occurred.

In relation to BPA excretion, this study did not investigate this kinetic parameter in neonates but only in adult animals. In adult rats dosed with 10 mg/kg ^{14}C-BPA, the major route of excretion was in the faeces. During the 96-hour period after dosing 75% of the radioactivity administered appeared in the faeces. The rate of excretion appeared to be higher in females than in males; in the 24 hours after dosing 41% of the administered dose appeared in the faeces of females, compared with 24% in males. During the 96 hours after dosing 15% of the administered dose was excreted in the urine of both males and females. The initial rate of urinary excretion of radioactivity also appeared to be higher in females than in males; in the 12 hours after dosing 4.1% of the administered dose was excreted in the urine of females compared with 1.5% in males. Ninety-six hours after giving ^{14}C-BPA at 10 mg/kg, the proportion of the administered dose which remained in the tissues and carcass of adult animals was 4%. In this study, the contributions of individual radiolabelled compounds to ^{14}C-BPA-derived radioactivity in excreta were not investigated. However, historical data were presented which showed that in previous studies of adult rats given the same oral dose, most of the faecal radioactivity was found to be in the form of free, unconjugated BPA, while most of the urinary radioactivity was in the form of BPA-glucuronide.

At sacrifice, in animals of all ages, the concentration of ^{14}C-BPA-derived radioactivity expressed per unit tissue weight was lowest in the brain, slightly higher in the testes and tended to be higher still in the kidney and liver. In neonatal animals, radioactivity was found to be highest in the skin/carcass. In the tissues of adult animals, the skin, carcass and fat were sampled separately; the carcass was found to contain the highest concentration of radioactivity, with the skin and fat containing only low concentrations.

The kinetics of ^{14}C-BPA-derived radioactivity in individual tissues tended to follow those of plasma radioactivity, with tissue concentrations peaking within 2 hours of dosing and subsequently falling with a half-life of several hours. In general in animals at pnd 4 and pnd 7 the concentrations of radioactivity in the tissues tended to be higher, and half-lives tended to be longer, than in animals at pnd 21. The tissue/plasma ratio of ^{14}C-BPA-derived radioactivity in all groups of neonatal animals was lowest in the brain where the ratio was less than 0.25. In the testes, the ratio was also low, being less than 0.6 in all groups of neonatal animals. The tissue/plasma ratio tended to be in the region of 1 in liver and kidney, and between 1 and 3 in the skin/carcass across all groups of neonatal animals. Regarding the ovaries and uterus, the tissue/plasma ratio tended to be in the region of 1, although in pnd 4

animals at 15 minutes, 1.5 hours or 3 hours after treatment with 10 mg/kg ^{14}C-BPA, the tissue/plasma ratio was between 3 and 5. However this finding of high radioactivity levels in ovaries and uterus was not reproduced in pnd 4 animals given 1 mg/kg ^{14}C-BPA.

In summary, this study found that BPA was rapidly absorbed from the GI tract and metabolised to BPA-glucuronide in both neonatal and adult rats. The capacity of the glucuronidation pathway was more prone to substrate saturation at pnd 4 than at pnd 7, suggesting an age-dependent increase in metabolic capacity consistent with an age-dependent development of glucuronyl transferases. The plasma concentrations of free, unconjugated BPA and BPA-glucuronide peaked within 2 hours of dosing, and peak concentrations were several times higher in the youngest neonates than in adults, suggesting that neonates have poorly-developed excretory functions. In adult animals, but not in neonates, plasma levels of BPA-glucuronide underwent a secondary rise between 12 and 24 hours after dosing, suggesting that BPA was subject to enterohepatic circulation. There were no sex differences in the kinetics of BPA-glucuronide in neonates, but in adult animals BPA-glucuronide was removed from the circulation more rapidly in females than in males, probably due to faster excretion. A number of minor metabolites of BPA were detected in plasma but not identified. In general, fewer metabolites were detected in older animals reflecting the conversion of BPA to a larger number of metabolites in younger neonates than in older animals and indicating a likely saturation of the glucuronidation pathway in the younger animals. In both neonatal and adult animals, ^{14}C-BPA-derived radioactivity tended to concentrate in the skin/carcass, but concentrations in the brain and testes were found to be lower than in the plasma.

Another recent study investigated the contribution of biliary excretion to the elimination of oral and intravenous doses of BPA in rats (Kurebayashi *et al*, 2003). In the first part of the study, adult male Fischer-344 rats (3 per dosing schedule) were given a single, low dose of ^{14}C-labelled BPA (0.1 mg/kg) administered either by oral gavage or intravenously. In addition, for each of these dosing schedules, 6 animals (3 males and 3 females) had the bile duct cannulated, and bile was collected over 2-hour periods till 6 hours after dosing. In the second part of the study, designed to characterise in detail BPA metabolites, 3 adult male rats of the same strain were given a single high dose (100 mg/kg) of deuterium-labelled BPA or unlabelled BPA by oral gavage. For this high dose experiment, another 3 male animals had the bile duct cannulated for collection of bile over the 18-hour period after dosing. Animals were housed individually in metabolic cages for the collection of faeces and urine produced over the first and second 24-hour periods after dosing.

Blood samples were taken at 9 selected time points between 15 minutes and 48 hours after dosing. Total ^{14}C-BPA-derived radioactivity was measured in samples of blood, plasma and excreta from low dose rats by scintillation counting. In addition, samples were assayed for free, unconjugated BPA and individual BPA metabolites by HPLC with scintillation counting, mass spectrometry/electrospray ionisation or NMR spectroscopy. BPA metabolites isolated by HPLC were subjected to enzymatic hydrolysis with glucuronidase or sulphatase to confirm their composition.

In the male rats given a low dose of BPA by intravenous administration, blood levels of ^{14}C-BPA-derived radioactivity initially fell rapidly with an elimination half-life of 0.6 hours. This phase was followed by a second longer phase with a terminal elimination half-life of 40 hours. The clearance from the circulation was 8 ml/min/kg body weight. The distribution volume (27 litres/kg bw) was high, suggesting substantial binding to plasma proteins/and or tissues. In fact, partition measurements showed that the proportion of ^{14}C-BPA-derived radioactivity in the blood bound to plasma proteins was relatively high (95%). In the male animals given the low dose of BPA orally, ^{14}C-BPA-derived radioactivity in the blood

initially rose to a peak 0.4 hours after dosing, and subsequently fell with a terminal elimination half-life of 45 hours. An analysis of the AUCs for blood ^{14}C-BPA-derived radioactivity over the 48-hour period after dosing showed an oral bioavailability of 86%. During the 48-hour period after oral or intravenous dosing, most (78-82%) of the ^{14}C-BPA-derived radioactivity was found to be eliminated in the faeces, with a relatively small proportion (10-12%) appearing in the urine.

Measurements in the rats with cannulated bile ducts showed that a sizeable fraction (45-66%) of the administered radioactivity was excreted into the bile within 6 hours of oral or intravenous dosing. The proportion of the administered dose excreted was slightly higher in males than in females. HPLC analysis of the bile produced during this period following oral or intravenous administration suggested that 84-88% of biliary radioactivity was in the form of BPA-glucuronide. The nature of the other radiolabelled species in the bile was not reported.

In experiments where rats were given a high dose of unlabelled BPA by the oral route, 41% of the administered dose was excreted in the bile during the 18-hour period after dosing. HPLC analysis revealed that most of this (>99%) was in the form of BPA-glucuronide. Free, unconjugated BPA was present in trace amounts and BPA-sulphate was not detected. Analysis of the excreta produced within 72 hours of dosing revealed that most (61%) of the administered dose was eliminated from the body in the faeces, with only 8% of appearing in the urine. HPLC analysis revealed that the faeces contained only free, unconjugated BPA, with BPA glucuronide and BPA-sulphate not being detected. The urine contained mostly (82%) BPA-glucuronide, with free, unconjugated BPA and BPA-sulphate making minor contributions (14% and 4% respectively).

In summary, in this study the toxicokinetics and fate of BPA were found to be similar when free, unconjugated BPA was given at a low dose either intravenously or orally, and at a high dose orally. After oral administration BPA was absorbed rapidly and efficiently from the GI tract (up to 86% of the administered dose). A high proportion of the dose (45-66%) was rapidly excreted in the bile in the form of BPA-glucuronide, with the rate of biliary excretion tending to be higher in males than females. With all three dosing schedules most (78-82%) of the administered dose was eliminated from the body in the faeces, with only a small proportion of the dose appearing in the urine (10-12%). In the low dose experiments the chemical identity of this form was not investigated, but in the high dose experiments most of the faecal radioactivity was found to be in the form of free, unconjugated BPA. Since BPA has a high oral bioavailability, the free, unconjugated BPA in the faeces is more likely to be derived from BPA glucuronide excreted in the bile and hydrolysed to free BPA in the GI tract, rather than representing unabsorbed free BPA which might have passed along the GI tract into the faeces unchanged.

EFSA (2006) noted that most of the studies examining the toxicokinetics of BPA in rodents used rats as experimental models. In contrast to primates, in adult rats several studies using oral doses of BPA ranging from 20 μg/kg bw to 100 mg/kg bw have confirmed that BPA-glucuronide formed in the liver and the intestinal wall after oral administration undergoes enterohepatic recirculation after cleavage of the glucuronide back to BPA and most of the dose is slowly excreted with faeces (Kurebayashi *et al.*, 2005; Sakamoto *et al.*, 2002). Urinary excretion of BPA and its metabolites in rats is strain-specific and accounts for 10 to 40 % of applied dose; the major metabolite present is BPA-glucuronide, but a small percentage of the applied dose is recovered in urine as parent BPA. In faeces of rats dosed orally with ^{14}C-BPA, the majority of the BPA-derived radioactivity was attributed to the

parent compound. Other BPA metabolites, such as a diglucuronide or a mixed sulphate/glucuronide represent minor identified BPA metabolites in rats.

EFSA (2006) commented that, in summary, these results, in combination with results from previous publications (Pottenger *et al.,* 2000; Snyder *et al.,* 2000), confirm that BPA in rats is mainly metabolised to BPA glucuronide and excreted from the liver with the bile. This is in contrast to the situation in humans where BPA-glucuronide is excreted through the urine because the threshold for biliary elimination (MW of 350 D) is lower in rats than primates (MW of 500 D). Thus, BPA in rodents is subject to enterohepatic recirculation irrespective of dose and route of administration, resulting in slow elimination with apparent terminal elimination half-lives between 19 and 78 h (Domoradzki *et al.*, 2004; Kurebayashi *et al.*, 2003; Kurebayashi *et al.*, 2005; Pottenger *et al.*, 2000).

No study on the disposition and biotransformation of BPA in mice after oral administration was identified. One study addressed the biotransformation of a low dose (20 µg/kg bw) of ^{3}H-BPA in pregnant CD-1 mice (Zalko *et al.*, 2003) after subcutaneous (sc) injection. BPA was extensively metabolised in CD-1 mice. Identified metabolites included BPA-glucuronide as major metabolite, but also several double conjugates, and conjugated methoxylated derivatives. Fetal radioactivity (more than 4% of the administered radioactivity 24 hours after administration of BPA) was associated with free BPA, BPA-glucuronide, and a disaccharide conjugate. While these data suggest a more intensive biotransformation of BPA in CD-1 mice and a higher contribution of metabolic oxidation to this biotransformation, a quantitative comparison of differences in rat and mouse biotransformation of BPA is not possible due to differences in study design, route of exposure and periods of observation after BPA administration. Furthermore, formation of metabolites attributable to a metabolic oxidation of BPA has not been observed in studies in rats or in primates *in vivo*.

In studies in which subcellular fractions from the liver of both mice and rats were incubated with BPA, a number of BPA-metabolites were reported to be formed by oxidative biotransformation. These were identified as 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, isopropyl-hydroxyphenol, a BPA glutathione conjugate, glutathionyl-phenol, glutathionyl 4-isopropylphenol, and BPA dimers (Jaeg *et al.*, 2004; Yoshihara *et al.*, 2004). One of the BPA metabolites formed by S-9 catalyzed oxidation, 4-methyl-2,4-bis(phydroyphenyl)pent-1-ene, has an approximately two orders of magnitude greater affinity to the oestrogen receptor alpha as compared to BPA (Yoshihara *et al.*, 2004). However, there is no evidence that these metabolites are formed to a larger extent in rats or in primates *in vivo* or in intact hepatocytes from rodents or humans (Pritchett *et al.*, 2002), likely due to an effective glucuronidation of BPA (Domoradzki *et al.*, 2003; Kurebayashi *et al.*, 2003; Kurebayashi *et al.*, 2002; Kurebayashi *et al.*, 2005; Pottenger *et al.*, 2000; Snyder *et al.*, 2000; Volkel *et al.*, 2005; Volkel *et al.*, 2002).

PBPK and PBPK modelling

In the EFSA (2006) opinion, some consideration is given to the available PBPK and PBPD models for BPA.

Blood concentrations of BPA after human dietary exposures were estimated using two

physiologically based toxicokinetic models developed for BPA. These models successfully predicted experimental BPA toxicokinetics in humans (Volkel *et al.*, 2002; Teeguarden *et al.*, 2005). Experimentally determined partition coefficients, plasma protein binding, binding of BPA to the oestrogen receptor alpha and its oestrogenic activity in competition with oestradiol, and the kinetics of BPA elimination by glucuronidation after oral administration in humans were incorporated into a physiological toxicokinetic-toxicodynamic model to predict age dependently the concentrations of free (non-protein bound) BPA in blood and other tissues. When simulating a daily dietary uptake of 1 µg BPA/kg bw separated into three meals, peak concentrations of free (not bound to plasma proteins) BPA in blood were predicted as 3 pmol/l in a one year old child and as 3.7 pmol/l in 50 year old adults. Normalised for the oestrogenic activity of endogenous 17-ß-oestradiol, the highest increase in the oestrogenic activity induced by this dose of BPA was calculated to be 0.22% (Filser *et al.*, 2003) for 11-year old boys (lowest circulating 17-β-oestradiol levels).

Computational modeling of the possible effects of plasma protein binding of oestradiol and BPA, incorporating affinities of oestradiol and BPA to different binding proteins and physiologic concentrations of these proteins in rodents and in male and female humans, predicts that unless very high concentrations (> 100 nM) of BPA are reached in blood, oestradiol binding to the receptor will always dominate. Therefore, under realistic blood concentrations expected in humans from oral exposure to BPA from diet in the range of up to 0.05 nM, only a very small fraction of the oestrogen receptor will be occupied by BPA. Occupancy of the oestrogen receptor by BPA is predicted to be further decreased when the rapid elimination of BPA is incorporated into the modelling (Teeguarden and Barton, 2004; Teeguarden *et al.*, 2005).

4.1.2.1.3 Impact of new information and summary of toxicokinetics

The new information on the toxicokinetics of BPA in humans and in pregnant and non-pregnant rodents of different ages provides an important contribution to our knowledge of the kinetic properties of BPA. However, the most significant impact of the new information for risk assessment purposes arises from the studies in humans. These studies have demonstrated that at comparable exposure levels the blood concentrations of free BPA in humans are much lower than those in rodents, indicating that there are important quantitative differences in the fate of BPA between humans and rodents.

The toxicokinetics of BPA have been well studied in rats both *in vivo* and *in vitro*, and have been investigated to a lesser extent in mice and cynomolgus monkeys. Two studies have investigated the toxicokinetics and fate of an oral dose of labelled BPA in human volunteers.

Absorption
In the species studied (rats, mice, monkeys, humans), the available evidence suggests that following oral administration, BPA is rapidly and extensively absorbed from the gastrointestinal tract. Analysis of plasma AUC values suggests that the extent of absorption from the GI tract is up to 86% in rats and up to 85% in monkeys. The only relevant human studies suggest that, on the basis of the recovery of labelled BPA-glucuronide from the urine, a relatively low dose of BPA (54-88 µg/kg) was completely absorbed after oral dosing.

An *in vitro* dermal absorption study using human skin found limited absorption of BPA at millimolar concentrations; the extent of absorption was in the region of 10% of the applied dose.

There are no data on the toxicokinetics of BPA following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and the high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first-pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

Metabolism

The available data indicate that BPA is subject to extensive first-pass metabolism following absorption from the gastrointestinal tract.

In all species studied, the major metabolic pathway involves conjugation of BPA to BPA-glucuronide. Studies conducted in rats suggest that in neonates the glucuronidation pathway is more susceptible to saturation than in adults indicating an age-dependent increase in metabolic capacity. *In vitro* studies with microsomal preparations also suggest species differences, with the rank order for the metabolic clearance rate per unit weight of tissue being mice > rats > humans. When the total clearance rates for the whole liver were calculated, the rank order was reversed (humans > rats > mice).

In addition to the glucuronidation pathway, *in vivo* and *in vitro* studies suggest that in the rat, BPA may be subject to limited oxididation to bisphenol O-quinone by cytochrome P450, and also to conjugation to BPA-sulphate and 5-hydroxy-BPA.

A study in pregnant mice given subcutaneous doses of BPA also found that glucuronidation was the major pathway for the metabolism of BPA, although dehydrated, sulphated and methoxylated conjugates of BPA were also produced. Some minor metabolites were double conjugates, such as a double conjugate of BPA with glucuronide and N-acetyl galactosamine which was found in the intestine, placenta, amniotic fluid and foetal tissue. A study in cynomolgus monkeys showed that BPA-glucuronide was the major metabolite, although there was evidence for production of a minor metabolite, possibly BPA-sulphate or 5-hydroxy-BPA. Studies conducted in humans provide evidence for the glucuronidation of BPA in man; some studies also found evidence for the sulphation of BPA.

Distribution

Most studies investigating the distribution of BPA measured tissue radioactivity levels after giving labelled BPA to experimental animals. An oral dosing study in rats found that the tissue concentrations of BPA-derived-radioactivity were highest in the liver, kidney and carcass, and lowest in the brain and testes, and there were no large differences between adult and neonatal animals. A number of studies in rats suggest that BPA metabolites and especially free BPA have a limited distribution to the embryo/foetal or placental compartments following oral administration. No selective affinity of either yolk sac/placenta or embryo/foetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in a recent study in rats after oral dosing. However, maternal and embryo/foetal

exposure to free BPA did occur, but systemic levels were found to be low due to extensive first-pass metabolism.

Regarding the distribution of free, unconjugated BPA to tissues after oral dosing, since free BPA is removed rapidly from the blood after absorption by first pass metabolism, it has been suggested that in animals the availability of free BPA to extrahepatic tissues is likely to be limited following oral exposure. In adult rats it has been estimated that no more than 5-10% of the administered dose of free BPA is available to the tissues, although this figure may be higher in neonates. In humans, the systemic availability of free BPA is very low as enterohepatic recirculation of BPA does not occur.

In summary, there are differences between humans and rodents in the distribution of BPA. After oral administration, BPA is rapidly metabolised in the gut wall and the liver to BPA-glucuronide. This metabolite is devoid of endocrine activity. In humans, the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion. Due to the rapid biotransformation and excretion ($t_{1/2}$ = 5 hours) and plasma protein binding, peak free BPA concentrations in humans after oral exposure that are available for estrogen receptor binding are very low. In contrast, BPA glucuronide is eliminated in bile in rodents and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic recirculation results in slow excretion ($t_{1/2}$ = 15-22 hours) and increased systemic availability of free BPA in rodents.

This conclusion is supported by the observation that in urine of rats dosed orally with BPA, a part of the dose was excreted as free BPA in urine (1 -4 % of applied dose, whereas BPA-glucuronide in urine accounted for 20-40 % of applied dose). In both of the human studies and the monkey study free BPA was below the limit of detection in all urine and blood samples (equivalent to a ratio of free BPA to BPA-glucuronide of < 0.5 %). Since free BPA found in urine is translocated from blood to urine in the kidney, these observations of higher free BPA levels in urine of rats compared with primates further support the existence of species differences in blood levels of free BPA between rodents and humans with higher AUCs for free BPA in rats.

Excretion

The major route of elimination in the rat is via the faeces. The available data indicate that the percentage of the administered dose recovered in the faeces is in the range 50% to 83%. Urinary excretion is of secondary importance in the rat, with 13% to 42% of the administered dose being recovered in the urine. Over 7 days post-dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post-dosing. A sex difference was also observed for urinary elimination, with females excreting approximately twice as much radioactivity (24-28%) than males (14-16%). A study in female SD rats found that excretion was not affected by pregnancy at 3 different stages of gestation. Data from a number of studies suggest limited excretion of BPA in the milk. However, the data do not allow a reliable quantitative determination to be made.

Following oral administration to rats, a high proportion of the administered dose (45-66%) was rapidly excreted in the bile in the form of BPA-glucuronide, with the rate of biliary excretion tending to be higher in males than females. Most of the faecal radioactivity was found to be in the form of free BPA. Since BPA has a high oral bioavailability in the rat, the free BPA found in the faeces is more likely to be derived from BPA-glucuronide excreted in the bile and hydrolysed to free BPA in the gastrointestinal tract rather than representing unabsorbed BPA which might have passed along the gastrointestinal tract into the faeces

unchanged. Most of the urinary radioactivity was found to be in the form of BPA-glucuronide (82%) with free BPA and BPA-sulphate making minor contributions (14% and 4% respectively).

In contrast to the findings in rodents, in cynomolgus monkeys given BPA orally most of the administered dose (82–85%) was recovered in the urine, with only 2-3% of the dose being recovered in the faeces. In two studies in human volunteers given a low dose of BPA orally, the administered dose was completely recovered in the urine as BPA-glucuronide. No free BPA was detected and no gender differences in the kinetics of BPA-glucuronide in plasma and urine were reported.

4.1.2.2 Acute toxicity

4.1.2.2.1 Summary of original risk assessment report

No useful information is available on the effects of single exposure to BPA in humans. Oral LD_{50} values beyond 2,000 mg/kg are indicated in the rat and mouse, and dermal LD_{50} values above 2,000 mg/kg are evident in the rabbit. Few details exist of the toxic signs observed or of target organs. For inhalation, a 6-hour exposure to 170 mg/m^3 (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed. These data indicate that BPA is of low acute toxicity by all routes of exposure relevant to human health.

4.1.2.2.2 Updated information

There in no significant new information on the acute toxicity of BPA.

4.1.2.3 Irritation

4.1.2.3.1 Summary of original risk assessment report

Limited human anecdotal information of uncertain reliability is available from written industry correspondence suggesting that workers handling BPA have in the past experienced skin, eye and respiratory tract irritation. It cannot be determined whether the reported skin reactions were related to skin sensitisation or irritation. However, a recent well conducted animal study clearly shows that BPA is not a skin irritant. A recent well conducted animal study shows that BPA is an eye irritant; effects persisted until the end of the study (day 28 postinstillation) in 1 of 3 rabbits. Overall, taking into account the animal and human evidence, BPA has the potential to cause serious damage to the eyes.

Slight and transient nasal tract epithelial damage was observed in rats exposed to BPA dust at 170 mg/m^3 for 6 hours. Slight local inflammatory effects in the upper respiratory tract were observed in rats exposed to 50 mg/m^3 and 150 mg/m^3 of BPA in 2 and 13 week repeat inhalation studies, but were not observed at 10 mg/m^3 in the same studies. Increased duration of exposure did not increase the severity of the response at 50 and 150 mg/m^3. Taken together with anecdotal human evidence, these data suggest BPA has a limited respiratory irritation potential.

4.1.2.3.2 Updated information

There in no significant new information on the irritation of BPA.

4.1.2.4 Corrosivity

The data available and summarised above show that BPA is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Summary of original risk assessment report

There are several reports of patients with dermatitis responding to BPA in patch tests. However, it is unclear whether BPA or related epoxy resins were the underlying cause of the hypersensitive state. Anecdotal information indicates skin inflammation in workers handling BPA, although given the uncertain reliability of this information no conclusions can be drawn from it. In animals, a skin sensitisation test performed to current regulatory standards is not available. The available studies are negative, but the test reports lack detail and no reliable justifications were given for the choice of concentrations used. In the study using the highest challenge concentration, 50% in a guinea pig closed-patch test, a sensitisation rate of 12.5% was obtained. It is possible that the concentrations used in all the available studies were not maximised and a greater response might have been obtained with higher induction and challenge concentrations. Based on the findings from the most robust study, BPA may possess a skin sensitisation potential, albeit a limited one. BPA in the presence of UV light can also elicit skin responses in humans, and reproducible positive results for photosensitisation have been obtained in mouse ear swelling tests. Mechanistic studies in mice have suggested this is an immune-mediated process. Therefore, examination of the available human and experimental animal studies leaves the picture somewhat unclear as to whether one or more of the following are properties of BPA; (1) orthodox skin sensitisation (2) photosensitisation (3) BPA eliciting a response in people previously skin sensitised to another substance (e.g. epoxy resins).

Overall, it is clear that skin reactions can be a potential consequence of repeated skin exposure in humans. Thus, taking all of these data available into account, BPA is considered capable of producing skin sensitisation responses in humans. There are no data from which to evaluate the potential of BPA to be a respiratory sensitiser.

4.1.2.5.2 Updated information

<u>Animal data</u>

In a GLP-compliant, modified Local Lymph Node Assay (LLNA) recently submitted by industry (Vohr, 2002), the skin sensitisation potential of BPA was investigated according to a slight modification of the OECD TG 429. This modification involved measurement of cell proliferation in the draining lymph nodes by cell count determinations instead of radioactive labelling, and measurement of ear swelling and ear weight (due to increased vascular

permeability in the associated inflammatory irritative response) as indicators of acute skin irritation reactions of the test substance (Homey et al., 1998). Groups of 6 female NMRI mice were treated epicutaneously on to the dorsal part of both ears with 25 µl of BPA in DAE 433 (40% dimethyhlacetamide, 30% acetone, 30% ethanol) at concentrations of 0, 3, 10 or 30%. No information is provided in the study report on how these concentrations were selected and why 30% was chosen as the highest concentration. DAE 433 was the vehicle of choice because of its photostability which made it suitable for the subsequent photo-LLNA (see below). This treatment was repeated on three consecutive days. One day after the last application, the animals were sacrificed and the auricular lymph nodes removed for cell count determinations. Ear swelling and ear weights were also measured. Stimulation indices for cell counts were then calculated by dividing the number of cell counts of the treated lymph nodes by the number of cell counts obtained from the control (vehicle) animals.

No systemic toxicity and no local irritation were observed at any of the concentrations tested. No dose-related or statistically significant increase in the stimulation indices was observed at any of the concentrations tested (stimulation indices of 1.25, 1.35 and 1.24 were obtained at 3, 10 and 30% respectively). No increase above controls was also noted for the ear swelling and the ear weight in any of the treated animals. The positive control, alpha hexyl cinnamic aldehyde (HCA) applied at concentrations of 3, 10 and 30% in DAE 433 produced the appropriate response. Although the positive level (stimulation index ≥1.3) was reached at a concentration of 10% BPA, this was neither dose-dependent nor statistically significant.

Overall, under the conditions of this LLNA, BPA did not show either a skin sensitisation or a skin irritation potential up to a concentration of 30%.

In a GLP-compliant LLNA (Vohr, 2003) modified to test for photoreactivity, 0, 3, 10 or 30% BPA in DAE 433 was applied to the dorsal part of both ears (25 µl/ear) of groups of 6 female NMRI mice on 3 consecutive days. The animals were irradiated with UV-light immediately after application (20J UV-A/cm^2). A control group of 6 mice were treated with 30% BPA without exposure to UV-light. Again, no information is provided in the study report on how these concentrations were selected and why 30% was chosen as the highest concentration. On day 1 and 4 of the study, the ear swelling of the animals was measured using a spring-loaded micrometer, and mean ear swelling calculated. On day 4 of the study, the ear weight of the animals was also measured. These two parameters, ear swelling and ear weight were used as indicators of unspecific irritation reactions by the test substance. The mice were sacrificed on the fourth day and the auricular lymph nodes excised and transferred to sterile physiological saline. The lymph nodes were weighed and cell counts per ml determined. The stimulation indices were calculated by dividing the absolute weight or cell counts of the treated lymph nodes by the vehicle ones.

A "positive level" was considered by the author to be a stimulation index ≥ 1.3. In this study, both the weight and cell count indices were less than 1 in all dose groups and hence were considered to be negative. A "positive level" for ear swelling was stated to be a 2×10^{-2} mm increase compared to the vehicle UV-A irradiated animals, and in this study the changes in ear swelling were less than this value and hence considered to be negative. The positive control, 8-methoxypsoralen applied at concentrations of 0, 0.1 and 0.3% in DAE 433 with UV-A irradiation and at 0.3% without UV-A irradiation produced the appropriate response.

Overall, under the conditions of this photo-LLNA, BPA did not show either a skin photo-sensitisation or a skin photo-irritation potential up to a concentration of 30%.

Human data

Medical surveillance information obtained from 5 out of the 6 BPA manufacture plants present in the EU was recently provided by industry (PlasticsEurope, 2007). During BPA manufacture, workers may be exposed to phenol, acetone and BPA. As phenol and acetone are not skin sensitisers, the assessment of the potential skin sensitising activity of BPA is not confounded by exposure to other chemicals in these factories. In company A, no cases of skin sensitisation were identified among 110 workers examined since 1991 (site 1) and among 190 workers examined since 1984 (site 2). In company B, no cases of dermatitis were identified among 500 workers examined since 1976, and in company C no cases were identified among 75 workers. Employees are examined every 1 to 3 years by a physician or a nurse. During examination, the condition of the skin is checked. Workers are also asked to report any work-related health problem.

4.1.2.5.3 Impact of new information and summary of sensitisation

A recent LLNA study has shown that BPA does not possess skin sensitisation potential. However, in this study the concentration of BPA was not maximised. Therefore, there remains some uncertainty as to whether high concentrations (> 30%) of BPA can still exert skin sensitising activity. Similarly, a recent photo-LLNA has shown that BPA does not possess skin photo-sensitisation potential. However, again, in this study the concentration of BPA employed was not maximised. Although there are sporadic reports showing that BPA in the presence of UV light can elicit skin responses in humans, comprehensive medical surveillance data obtained from BPA manufacture plants has shown that no cases of skin sensitisation have been identified among approximately 875 employees examined for several years. Due to the nature of these data, although it can be concluded that the risk of skin sensitisation is low under the exposure conditions experienced by these workers, a potential skin sensitisation hazard cannot be completely excluded.

Overall the new information does not confirm the previously reported evidence of a skin sensitisation potential of BPA. While the data do not exclude a skin sensitising activity of BPA at high concentrations (> 30%), there is no evidence that this is a concern for workers in current BPA manufacturing plants (such workers are believed to represent the group most likely to be exposed to BPA dust).

There are no data from which to evaluate the potential of BPA to be a respiratory sensitiser. However, based on the lack of reports of cases of respiratory sensitisation, there are no grounds for concern for this endpoint.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Summary of original risk assessment report

No useful information on the effects of repeated exposure to BPA in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in

rats following exposure to 50 mg/m^3 (6 hours/day, 5 days/week for 13 weeks). There was no significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m^3. A NOAEL of 10 mg/m^3 was identified in rats in this 13-week study.

Dietary studies in rats produced a decrease in body weight gain and minor changes in the weights of several organs at higher doses probably of no toxicological significance, especially given the absence of other related pathological findings. However, in one study in male rats, reductions in the weight of several reproductive organs and testicular toxicity were seen following dietary exposure to 235 mg/kg for 44 days. A NOAEL was not established from this study. Although these effects on the reproductive organs have not been seen in any other robust repeated dose toxicity study in rats or mice (including a 2-year study in F344 rats), the severity of effects was generally dose-related and therefore cannot be disregarded. The only other finding was an inconsistent observation of caecal enlargement in some 90-day studies. The caecal enlargement was observed at 25 mg/kg and above and was without any associated histological abnormalities. In addition, it was not observed in a 2-year study at doses up to about 140 mg/kg or a multigeneration study at doses up to 500 mg/kg/day. Consequently, this is not regarded as a toxicologically significant observation of relevance to humans. A NOAEL of 74 mg/kg has been established for rats from a 2-year study based on marginal effects on bodyweight gain at the next dose level of 148 mg/kg. Chronic inflammation of the liver was seen from 50 mg/kg in a 3-generation study, but with no convincing dose-response relationship. These liver effects in rats were thus considered to be background variation and not treatment-related. Renal tubule degeneration of the kidney was also seen in this 3-generation study in females at 500 mg/kg but not at 50 mg/kg.

Dietary studies in mice indicated that the liver is a target organ in this species, with changes being observed in the size and nucleation state of hepatocytes in 2-year and 90-day studies. The incidence and severity of these treatment-related multinuclear giant hepatocytes was greater in males than in females, and it was not possible to identify a no effect level for males. The effect was observed at all dose levels used in males from 120 mg/kg. In females, a no-effect level of 650 mg/kg was identified for these cellular changes in the 2-year study. The only other findings in mice were significant reductions in body weight gain at dose levels of approximately 650 mg/kg/day and above. Thus, LOAELs of 120 mg/kg in males for multinuclear giant hepatocytes and 650 mg/kg in females for a reduction in body weight gain of unknown magnitude were identified in a 2-year study.

In a 90-day dietary study in dogs, a no effect level of approximately 80 mg/kg was identified, with increases in relative liver weight being the only other finding observed at approximately 270 mg/kg: in the absence of histopathology this finding is of doubtful toxicological significance.

There are no animal data available for repeated dermal exposure.

4.1.2.6.2 Updated information

Information on repeated dose toxicity can be derived from a well conducted and reported 13 week rangefinding study for a 2-generation study (Tyl *et al.* 2005) and the subsequent 2-generation study (Tyl *et al.*, 2007, see Section 4.1.2.9.2 for full details of this study, including information on findings in the reproductive organs) in mice.

In the rangefinding study, groups of 10 male and 10 female CD-1 mice were exposed to BPA in the diet at concentrations of 0, 500, 2000, 2500 or 3500 ppm, continuously for 13 weeks. The resulting average BPA intakes were, respectively, 0, 74, 298, 373 and 541 mg/kg/day for

males and 0, 100, 370, 487 and 728 mg/kg/day for females. Clinical signs, bodyweights and food consumption were monitored throughout the study. At the terminal necropsy the kidneys and liver were removed, weighed, and processed for histopathological examination. There were no treatment-related clinical signs of toxicity, mortality or effects on bodyweight gain. For males only, relative liver weight was significantly increased at 2000, 2500 and 3500 ppm, although the differences did not follow a dose-related pattern. Histological examination of the liver revealed a dose related increase in the incidence of centrilobular hepatocyte hypertrophy in all BPA-exposed groups of males. In females the incidence of hepatocyte degeneration or necrosis was increased at 2500 and 3500 ppm. In males only relative kidney weight was significantly increased at 500, 2000, 2500 and 3500 ppm, although the differences did not follow a dose-related pattern. The only histopathological findings in the kidney that were considered to be treatment relation were an increased incidence of nephropathy at 3500 ppm. This study confirmed that the liver and kidney are targets for BPA toxicity.

In the subsequent 2-generation study (Tyl et al., 2007), eight groups of 28 male and 28 female CD-1 mice (F_0 generation) were exposed to BPA in the diet at concentrations of 0, 0.018, 0.18, 1.8, 30, 300 or 3500 ppm, which resulted in a BPA intake close to the target doses of 0, 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg/day, respectively. For the BPA exposed F_0 and F_1 parental/retained animals there were no treatment-related mortalities or clinical signs of toxicity. Evidence of general toxicity was observed in 300 ppm and 3500 ppm groups. At 300 ppm, this evidence was limited to an increased incidence of centrilobular hepatocyte hypertrophy of minimal to mild severity in F_0 males (40% vs. 11% in controls) and females (10% vs. 2%) and F_1 parental/retained males (30% vs. 10%). There were no increases in liver weight at this dose level. At 3500 ppm, bodyweight gain was reduced among the F_1 parental/retained males; at termination mean bodyweights of the parental and retained males were 4% and 10%, respectively, less than the vehicle controls. Kidney weights were increased in F_0 males and in F_1 parental/retained males. Histological examination of the kidney revealed an increased incidence of minimal to mild nephropathy in the F_0 males and F_1 parental/retained animals at 3500 ppm. Absolute liver weights were significantly increased in F_0 males (by 18%) and females (by 20%) and in F_1 parental males (by 17%) at 3500 ppm. Histological examination of the liver revealed an increased incidence of minimal to mild centrilobular hypertrophy in the F_0 males (100% vs. 11% in controls) and females (60% vs. 2%) and F_1 parental/retained males (65% vs. 10% in controls) and parental females (70% vs. 4%) at 3500 ppm. The increased incidence of centrilobular hypertrophy at 300 ppm (50 mg/kg/day) was not accompanied by an increase in the group mean liver weight, suggesting that the liver changes seen at this dose level were minor and without toxicological significance. Therefore, the study NOAEL for general toxicity can be set at 50 mg/kg/day on the basis of the observation of toxicologically significant effects on bodyweight gain, kidney and liver at the next highest dose level of 600 mg/kg/day (3500 ppm).

4.1.2.6.3 Impact of new information and summary of repeated dose toxicity

A recent oral 2-generation study in mice has confirmed that the repeated dose toxicity of BPA involves effects on bodyweight gain, liver and kidney. A NOAEL of 50 mg/kg/day was identified from this study. No useful information on the effects of repeated exposure to BPA in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in rats following exposure to 50 mg/m^3 (6 hours/day, 5 days/week for 13 weeks). There was no

significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m^3. A NOAEL of 10 mg/m^3 was identified in rats in this 13-week study.

Oral studies in rats and mice have shown that the repeated dose toxicity of BPA involve effects on bodyweight gain, liver and kidney. A NOAEL of 50 mg/kg/day has been identified in a recent 2-generation study in mice for these effects. This NOAEL rather than the original LOAEL of 120 mg/kg/day for liver effects from the published report is taken forward to the risk characterisation.

There are no animal data available for repeated dermal exposure.

4.1.2.7 Mutagenicity

4.1.2.7.1 Summary of original risk assessment report

No human data regarding mutagenicity are available. However, BPA appears to have demonstrated aneugenic potential *in vitro,* positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems there is information that shows BPA disrupts microtubule formation. BPA has been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. However, some deficiencies in the conduct of these studies have been noted and the negative results cannot be taken as entirely conclusive. BPA does not appear to be anuegenic *in vivo*, since a recently conducted, standard mouse bone marrow micronucleus test has given a negative result. BPA was negative in a briefly reported dominant lethal study in rats but, given the limited details provided, this is not regarded as an adequate negative result. The only other data in somatic cells *in vivo* are from a ^{32}P-postlabelling assay, which showed that BPA is capable of producing DNA adduct spots in rat liver following oral administration. These adduct spots were not characterised fully.

Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies, it does not appear that BPA has significant mutagenic potential *in vivo*. Any aneugenic potential of BPA seems to be limited to *in vitro* test systems and is not of concern. The relevance of the finding that BPA can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health.

4.1.2.7.2 Updated information

In vivo studies

A recent study (Hunt et al., 2003) has investigated the effects of short-term, low-dose exposure to BPA on the meiotic processes of female mice during the final stages of oocyte growth. The investigation was triggered by the sudden, spontaneous increase (from 1-2% to 40%) in the background rate of meiotic disturbance in oocytes from female mice, observed as misalignment of chromosomes on the metaphase spindle (termed ‘congression failure’) of the first meiosis (MI). Coincident with this was an increase (from 0.5-1% to 5.8% determined by pooling together incidence values from control animals of different ages, strains/genotypes and derived from different breeding stocks) in the background level of aneuploidy

(hyperploidy, i.e. cells with >20 chromosomes). According to the authors, these observations were associated with the accidental damage to caging material by the inadvertent use of harsh alkaline detergents. Subsequent investigations led the authors to postulate that the changes could be due to exposure to BPA leaching from the damaged cages and water bottles. It should be noted that these data cannot be unambiguously associated with BPA itself. While release of BPA is one possibility, other degradation products or oxidation products could also be involved. No information was provided in the paper on the active and inert ingredients present in the detergents that were used.

The study focused primarily on the first discovery of this change in the levels of congression failure and hyperploidy, and the subsequent investigations to identify the exposure source producing the effect. Details of experiments conducted with BPA itself are limited.

Groups (size not stated) of juvenile (20- to 22-day-old), sexually immature (prepuberal) female C57BL/6 mice were treated with oral gavage doses of 0, 20, 40 or 100 μg/kg bw/day BPA in corn oil for 6-8 days. The animals were then sacrificed and germinal vesicle (GV)-stage oocytes (meiotically competent oocytes) were liberated from antral follicles (they contain a large number of oocytes). These oocytes were then cultured overnight, and only those exhibiting a polar body the following morning, i.e. metaphase II (MII)-arrested oocytes were embedded in fibrin clots, fixed and prepared (immunostained with tubulin antibodies to visualise the spindle and counterstained with DAPI to visualise the chromosomes) for analysis (46-255 oocytes were examined per group). MII-arrested oocytes were preferred to MI oocytes (those analysed when the change in congression failure levels was first observed) since this static arrest phase alleviates the problem of variation due to differences in cell cycle rate.

A dose-related increase in congression failure was observed among the treated animals. Congression failure was seen in 2/115 (1.7%), 10/172 (5.8%), 19/255 (7.5%) and 5/46 (10.9%) oocytes at 0, 20, 40 and 100 μg/kg bw/day respectively. It is unclear from the study report whether or not this increase was statistically significant at all three exposures or whether the dose-response showed a statistically significant trend. Furthermore, to determine the shortest exposure that produced detectable effects, an additional set of experiments using a dose of BPA of 0 or 20 μg/kg bw/day for 3, 5 or 7 days prior to oocyte analysis was conducted. Again, the number of animals per group was not stated. A total of 67, 138 and 234 oocytes in treated animals and 61, 70 and 140 oocytes in controls were examined on days 3, 5 and 7 respectively. All three exposures resulted in increased levels of defects of the alignment of the chromosomes on the meiotic spindle, although only the 7-day treatment was statistically significantly elevated above control values. Percentages (values taken from a graph) of congression failure of 1.7, 3 and 2% were obtained from the three different control groups, and of 3.2, 5.4 and 8.5% from the 3-, 5- and 7-day treatment groups, respectively.

The dose levels of BPA employed in the experiments (20-100 μg/kg bw) were selected based on the levels of BPA measured by GC-MS in the water from damaged bottles. However, despite this attempt to mimic the exposure dose resulting from damaged caging materials (up to ~ 70 μg/kg bw), the congression failure levels measured in the BPA dosing studies were lower (up to 10.9%) than those obtained using damaged cages and damaged bottles together (41.4%), damaged bottles only (26.9%) or even damaged cages only (8.7-20.1%). It has been speculated by the authors that the explanation for this discrepancy was the lower bioavailability of BPA following single bolus dosing (the dosing regime employed in the BPA exposure studies), due to rapid first-pass elimination, in comparison to that obtained following continuous exposure via drinking water (the dosing regime that was believed to be operative in those experiments using damaged caging materials). However, this hypothesis is

not consistent with the known toxicokinetics and metabolism of BPA, and therefore this discrepancy raises some uncertainties as to the cause of the original observed changes in background levels of congression failure. Also, it is unclear why BPA was not administered in drinking water. This method of administration would have been more directly relevant to the original observations. Further uncertainties arise as a result of the observation that the level of hyperploidy in oocytes of control mice did not return to the typical background level (0.5-1%) after elimination of all damaged caging materials (2%).

The principal finding of this study was an increase in congression failure, a misalignment of chromosomes during the metaphase stages of meiosis. Experiments to demonstrate that BPA, following congression failure, actually induced aneuploidy (hyperploidy) were not conducted. While one might expect that a misalignment of chromosomes during meiotic metaphase would be associated with improper chromosome segregation, this has not been conclusively demonstrated for meiotic cells. The alignment of chromosomes at metaphase is a dynamic process. Chromosomes that appear to be mis-aligned at one point can quickly become properly aligned and segregate properly. As a result, chromosome misalignment or aberrant congression represent cellular effects that may eventually lead to aneuploidy but are not considered definitive genotoxic effects. Similar studies of chromosome alignment during mitosis have provided examples of cases where, under conditions where high frequencies of abnormal metaphases were induced, relatively few cells exhibited lagging chromosomes during anaphase or telophase (Schuler, et al., 1999).

The hyperploid results, which represent a more important and established genotoxic endpoint, were generated only for control animals before, during and after the inadvertent use of damaged cages and water bottles, by pooling incidence values from animals of different ages, strains/genotypes and derived from different breeding stocks. It is questionable whether it is appropriate to pool and compare the hyperploid results from these groups. For example, since the incidence of aneuploidy increases with age in many cell types, it would not seem appropriate to combine or compare the incidence in the 8-12 month old mice with those that are 4 weeks of age.

Normally, the statistical analysis of *in vivo* mutagenicity studies compares the frequency of abnormal events per animal. In this study, however, the number of oocytes and aberrant oocytes appears to have been pooled from several animals and analysed without accounting for animal-to-animal variability. This approach is not ideal as it may mask animal to animal variability and give erroneous results.

Historical background frequency for congression failure in meiosis II was not provided. In the absence of these data, the true biological significance of the results obtained with BPA treatment (congression failure in meiosis II) cannot be assessed.

Overall, there are several methodological weaknesses in this study, namely lack of direct evidence that BPA induces aneuploidy, a non-standard, non-validated methodology, discrepancies between the original observations and the findings with BPA, absence of standardisation in the number of oocytes examined, inappropriate pooling of data, problems with the statistical evaluation, small sample size, lack of historical control data, along with reporting inadequacies.

Furthermore, these results appear to differ in significant ways from what is known of the mutagenic and toxicological profile of BPA. For example, BPA has been shown in *in vitro* studies to interfere with chromosome segregation in mitotic cells (Ochi, 1999; Parry, et al., 2002; Tsutsui, et al., 2000; Tsutsui, et al., 1998) at concentrations ranging from 20 to 150μM. However, given the very low doses of BPA administered in this study and the efficient

conjugation of BPA during first-pass metabolism, one would expect the plasma concentrations achieved in these experiments to be much lower than those that have shown effects *in vitro*.

BPA is negative in standard bone marrow micronucleus tests in mice, so it would be unlikely to induce micronuclei (possibly by an aneugenic effect) in germ cells.

Lastly, if BPA significantly affected chromosome segregation in 10% of the affected oocytes, one would expect that it would also affect reproduction with decreases in litter size being seen in the treated animals. However, no effects on litter size have been seen in a comprehensive multi-generation study in mice covering a wide array of doses (10^{-3} to 10^{+2} mg/kg/day), lower, equal, and substantially higher than those used in this investigation.

Overall, therefore, in view of a number of weaknesses and flaws identified in the study along with the reporting inadequacies and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis.

A follow up study was published by the Hunt lab (Susiarjo *et al.*, 2007). This publication used a different study design but suffers from similar weaknesses.

Noting the Hunt et al (2003) data, Attia et al (2004) reported on a series of preliminary experiments that have examined the potential aneugenic activity of BPA in germ cells *in vivo*. The study information is currently available only as an abstract. These studies showed no increase in hyperploidy in mouse spermatocytes or oocytes. The authors concluded that the aneuploidy predicted by Hunt et al (2003) could not be confirmed in these other studies and that further investigations were being conducted.

CERHR (Center for the Evaluation of Risks to Human Reproduction) interim draft (CERHR, 2007) states that the Attia study is now available as a draft-publication (Pacchierotti *et al.*, 2007). In this publication the potential aneugenic effects of BPA were investigated in mouse somatic and germ cells. C57Bl/6 female mice were superovulated using pregnant mare serum and hCG and subsequently gavaged with BPA at 0.2 or 20 mg/kg bw. Metaphase II oocytes were collected after 17 hours and evaluated using C-banding. Additional female mice were gavaged with BPA at 0.04 mg/kg bw/day for 7 days or were given BPA in drinking water at a concentration of 0.4 mg/l for 7 weeks. These mice were superovulated at the end of the 7-day or 7-week treatment period and housed overnight with untreated males. Females without vaginal plugs were killed for evaluation of oocytes by C-banding. Females with vaginal plugs were treated with colchicine to prevent the first embryonic cleavage, and zygotes were collected the next morning for evaluation by C-banding. There were no effects of BPA on induction of aneuploidy. There was a statistically significant increase in premature centromere separation in the group treated for 7 weeks, but there was no effect of BPA treatment on the proportion of zygotes with structural or numeric chromosome changes.

4.1.2.7.3 Impact of new information and summary of mutagenicity

The new information on the mutagenicity of BPA deals with the effects of short-term, low-dose exposure to BPA on the meiotic processes of female mice during the final stages of oocyte growth. These new data have shown that BPA produces an increase in congression

failure, a misalignment of chromosomes during the metaphase stages of meiosis II. However, in view of several methodological weaknesses and flaws identified in the study along with the reporting inadequacies, and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis. Furthermore, these findings have not been confirmed in more recent publications.

Therefore, the original conclusion from the published assessment that BPA has no significant mutagenic potential *in vivo*, is still valid.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Summary of original risk assessment report

There are no human data contributing to the assessment of whether or not BPA is carcinogenic. In animals, a dietary carcinogenicity study in two species is available; F344 rats and $B6C3F_1$ mice. A small increased incidence of leukaemias was seen in male and female F344 rats along with increases in the frequency of mammary gland fibroadenomas in male rats. These increases were not statistically significant, were slight and in a strain prone to these tumours. An increased incidence in benign Leydig cell tumours seen in male rats was within historical control limits. In mice, a small increased incidence in lymphomas was observed in males, but was not statistically significant and there was no dose-related trend. No increased incidence in any tumour type was observed in female mice. Overall, all of these tumour findings in rats and mice are not considered toxicologically significant. Consequently, it is concluded that BPA was not carcinogenic in this study in both species. No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, BPA did not exhibit properties that raise concern for potential carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m^3 in a 13 week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m^3. Taking into account all of the animal data available the evidence suggests that BPA does not have carcinogenic potential.

4.1.2.8.2 Updated information

The effects of transplacental and lactational exposure to BPA on the development of prostate cancer in rats were assessed using a model for reproductive organ carcinogenicity (Ichihara *et al*., 2003). Modulating effects of BPA on prostate cancer incidence in male offspring exposed transplacentally and lactationally to BPA were investigated in female F344 rats (~8–15 dams/group) administered 0, 0.05, 7.5, 30, and 120 mg BPA/kg/day by gavage during pregnancy and lactation. Dam body weight and food intake were monitored during the study. Gestation duration and implantation sites were evaluated. Pups were counted and sexed at birth. Litters were randomly culled to 8 pups on PND 4, and pups were weaned on PND 21. At 5 weeks of age, 21 male rats/group were injected subcutaneously with 50 mg/kg bw 3,2-dimethyl-4-aminobiphenyl (DMAB) 10 times at 2-week intervals. An additional 12 rats/group in the 0, 0.05, 7.5, and 120 mg/kg bw/day BPA groups were injected with corn oil during the same time period. Surviving male offspring were killed and necropsied at 65 weeks of age. Blood was collected for analysis of serum testosterone levels in 5 rats/group. Reproductive organs were examined for gross abnormalities, weighed, and fixed in 10% buffered formalin. A histopathological examination of the prostate was conducted.

Body weights of dams in the 120 mg/kg bw/day group were significantly lower than control values from GD 14 to 20. There were no consistent or dose-related effects on dam body weights during lactation. Exposure to BPA had no effect on gestation duration or number of implantation sites. In pups exposed to BPA, there were no differences in number of live births, sex ratio, external anomalies, or body weights during the lactation period. Exposure to BPA had no effect on weights of prostate, testis, or epididymis. Without DMAB treatment, incidences of prostatic intraepithelial neoplasia (PIN), carcinoma, and atypical hyperplasia were not increased by exposure to BPA, and there were no increases in tumours of the non-reproductive organs. No effect was observed on serum testosterone levels. This screening study showed that exposure of rat dams up to 120 mg/kg bw/day BPA during the gestation and lactation periods did not predispose their offspring to prostate cancer development later on in life (65 weeks of age). However, it is noted that the sample sizes were inadequate for the assessment of the cancer endpoint.

In another study on the prostate gland, the effect of short-term neonatal exposure to BPA on susceptibility of Sprague Dawley rats to prostate cancer were investigated (Ho *et al.*, 2006). On PND 1, 3, and 5 (day of birth = PND 0), 20–30 male pups/group were subcutaneously injected with corn oil vehicle, BPA at 0.1 μg/pup (0.010 mg/kg bw), or estradiol benzoate (EB) at 0.001 μg/pup (0.1 μg/kg bw) or 25 μg/pup (2500 μg/kg bw). Pups were weaned on PND 21. At PND 90, half of the rats from each treatment group were implanted with Silastic capsules containing 17β-estradiol (E) and testosterone (T) and the other half were implanted with empty capsules; the capsules were left in place for 16 weeks. The treatment was designed to result in a serum E level of ~75 pg/ml and T level of ~3 ng/ml, levels reported to induce prostatic intraepithelial neoplasia (PIN) in 33% of Sprague Dawley rats. Rats were killed at 28 weeks (PND 200; 6-7 months) of age. Prostates were removed, and histopathological evaluations were conducted on each lobe. Immunohistochemistry techniques were used to measure proliferation. Apoptosis was measured using the TUNEL technique. In addition, PCR techniques were used to study methylation pattern and expression changes in prostate cell signaling proteins on PND 10, 90 (before adult E+T treatment), and 200.

The study authors stated that similar responses were observed in each of the 3 prostate lobes; and thus results were presented only for dorsal prostate. In animals that did not receive E+T in adulthood, BPA exposure had no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index. In animals treated with E+T in adulthood, BPA exposure resulted in a statistically significant increased incidence and severity of PIN (100 [10/10] vs. 40% [4/10] incidence in controls). In the BPA/E+T group compared to the E+T group, the proliferation index was increased and the apoptosis index was decreased in regions where PIN was observed.

In the investigation of a molecular basis for increased susceptibility to PIN, neonatal exposure to estrogenic (either BPA or EB) compounds altered methylation patterns in several cell signaling genes. Phosphodiesterase type 4 (PDE4), an enzyme involved in cyclic AMP breakdown, was selected for further investigation. Neonatal BPA exposure resulted in hypomethylation of the PDE4 gene and increased expression of that gene at 90 and 200 days of age, with or without E+T exposure in adulthood. Similar responses in PDE4 gene methylation and expression were observed with exposure to the low and high EB doses.

Overall, this study showed that short-term neonatal exposure of male rats to 10 μg/kg bw BPA by subcutaneous administration had no effect on the prostate gland later on in life (at 6-7 months of age). This is consistent with the findings by Ichihara *et al.* (2003). The

observation of hypomethylation of the cell signalling gene, PDE4, in the BPA-treated animals, in itself does not represent an adverse effect. However, when in adulthood the animals were given implants of E and T for 16 weeks, BPA exposure appeared to increase prostate gland susceptibility to hormonally-induced histopathological lesions (PIN). Although the study authors claim that PIN is a precancerous lesion leading to prostate cancer, as the animals were sacrificed at 6-7 months of age, this could not be verified and, hence, the toxicological significance of PIN in animals remains unknown. It is also noted that no information was provided on the background variation of PIN in this strain of rats and on the experimental variation of E+T-induced PIN. Overall, therefore, due to the small sample sizes, use of a single dose level (and hence no dose-response information) and lack of information on the background variation of PIN and E+T-induced PIN, it is difficult to establish whether the increased incidence of E+T-induced prostrate lesions was a real, treatment-related effect. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures. The kinetics of BPA following subcutaneous administration, including the extent of absorption and its rapid metabolism in the liver to the endocrine inactive conjugate, BPA-glucuronide, are likely to differ from the kinetics of BPA by relevant routes of exposure.

Effects of maternal exposure to BPA on uterine carcinogenesis were studied in offspring of Donryu rats (a strain of rats with a high rate of spontaneous and ENNG-induced uterine tumours) administered BPA (0, 0.006 and 6 mg/kg bw/day, n = 12, 15 and 19/group respectively) daily by gavage from GD 2 to PND 21 (Yoshida *et al*., 2004). After delivery, offspring were sexed, weighed, and examined for external abnormalities on PND 1 and then weighed weekly through PND 21. Litters were adjusted to 8–10 pups at PND 4 or 6 (day of birth = PND 0). Dams were weighed, and observed during the study and killed following weaning of litters on PND 21. Implantation sites were examined and organs including uterus, vagina, and ovaries were examined histologically. All female offspring were examined for vaginal opening, and following vaginal opening, vaginal smears were taken for the remainder of the study. Three to 5 offspring/group from different litters were killed on PND 10, 14, 21, or 28 and at 8 weeks of age. At most time periods, uteri were weighed and examined histopathologically to determine development of uterine glands. Ovaries and vagina were also examined histologically. ERα was determined using an immunohistochemical method. Serum was collected for measurement of FSH and LH by RIA. Four offspring/group from different litters were killed at 8 weeks of age on the morning of estrus to examine ovulation by counting ova in oviducts. At 11 weeks of age, 35-36 animals/group were injected in the uterine horn with N-ethyl-N′-nitro-nitrosoguanidine (ENNG) to initiate uterine carcinogenesis. At ~24 weeks following cancer initiation (~15 months of age), the 24–30 surviving animals/group were killed and uteri were examined histologically to determine the presence of tumors and other lesions.

In dams exposed to BPA, there were no clinical signs of toxicity or effects on body weight, implantation sites, or gestation length. BPA exposure had no effect on litter size, pup body weight at birth and through PND 21, external abnormalities in pups or age of vaginal opening. In uteri of BPA-exposed offspring, there were no effects on weight, gland development, ERα, or cell proliferation. BPA exposure had no effect on ovulation, estrous cyclicity, or serum FSH or LH levels. There were no effects on ovarian histopathology following BPA treatment. The incidence of uterine preneoplastic or neoplastic lesions induced by ENNG was not increased by BPA exposure. Overall, transplacental and lactational exposure of rats to BPA did not exert an influence on uterine development and maturation and on ENNG-induced uterine carcinogenesis up to 15 months of age. However, it is noted that the sample sizes were inadequate for the assessment of the cancer endpoint.

Takashima *et al.* (2001) examined the effect of BPA exposure during development on multi-organ carcinogenicity (including thyroid and lungs) induced by N-nitrosobis (2-hydroxypropyl)amine (BHP). Female Wistar rats (12/group) were fed a diet containing 0 or 10,000 ppm BPA for 10 weeks prior to mating, and through mating, gestation and lactation. Intakes of BPA were reported to be about 400-600 mg/kg bw/day. The rats were mated to untreated males and GD 0 was defined as the day of the vaginal plug. Endpoints associated with pregnancy, delivery, and nursing were evaluated. Dam body weight and food intake were measured. Offspring were not culled and were weaned at 3 weeks of age. Dams were killed following weaning of offspring. Serum levels of thyroid hormones were measured in 2–4 dams/group. Implantation sites were evaluated. Weights of several organs, including ovary, were measured. The organs were fixed and processed for histopathological evaluation. Offspring (n = 32–50/group) were evaluated for body weight gain, preputial separation, and vaginal opening. Beginning at 5 weeks of age and continuing for 12 weeks, offspring in each group were subdivided into 2 groups (n = 17–21/group/sex) that received either undosed tap water or tap water containing 2000 ppm BHP. Offspring were killed at 25 weeks of age. Serum thyroid hormone levels were measured. Organs, including testis, ovary, and uterus were weighed. In 5–19 offspring/sex/group, histopathological examinations were conducted in organs targeted by BHP (lungs, thyroid, esophagus, liver, and thymus).

Dam body weight was lower in the BPA group compared to the control group during the gestation period and at weaning. Food intake and maternal serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone were unaffected by BPA exposure. There were no changes in weights or histopathological alterations of maternal organs, including uterus and ovary. BPA had no significant effect on mating, fertility, duration of gestation, live-born pups, implantation loss, or offspring viability through PND 21. In pups from dams exposed to BPA compared to pups from control dams, body weights were higher (by 11%) in females at 3 days of age and lower in males and females at 10 days and 2 weeks of age (16–22% decreases in males and 12–19% decreases in females). Prenatal and postnatal exposure to BPA did not affect preputial separation or vaginal opening. No effects were observed on thyroid hormones and offspring organ weights. Prenatal and postnatal BPA exposure was not associated with significant differences in the development of BHP-induced neoplasms in the offspring. The results of this study indicate that oral exposure to 400-600 mg/kg bw/day BPA during development does not exert promoting effects on BHP-induced thyroid, lung, liver, thymus and esophagus carcinogenesis in rats. However, it is noted that the sample sizes were inadequate for the assessment of the cancer endpoint.

The effects of prenatal BPA exposure on susceptibility to mammary tumorus induced by N-nitroso-N-methylurea (NMU) were examined in Wistar rats (Durando *et al*., 2007). On GD 8–23 (GD 1 = day of vaginal sperm), 11–14 dams/group were subcutaneously dosed with 0 or 0.025 mg/kg bw/day BPA. Pups were delivered on GD 23 and weaned on PND 21. During the study, body weights and day of vaginal opening were monitored. Offspring were killed before puberty (PND 30), after puberty (PND 50), or in adulthood (PND 110 and 180). In mammary gland stroma and epithelium, proliferation was assessed by BrdU incorporation and apoptotic cells were identified by the TUNEL method. Morphometric analyses were conducted in sectioned mammary glands. Mast cells were identified by immunostaining for proteinase. At least 6 offspring/group/time point were evaluated. No littermates were used in the evaluation at any given time point. Additional offspring were examined for responsiveness to chemically-induced mammary preneoplastic or neoplastic lesions. On PND 50, the established carcinogen N-nitroso-N-methylurea (NMU) was administered

intraperitoneally to 10–16 offspring from the vehicle control group at 25 or 50 mg/kg bw and to 21 offspring from the BPA group at 25 mg/kg bw. Based on findings from a pilot study, 25 mg/kg bw NMU was considered a subcarcinogenic dose and 50 mg/kg bw NMU was considered a positive control. During the study, rats were palpated for tumours. Rats that received 50 mg/kg bw NMU were killed on PND 180 and rats that received 25 mg/kg bw NMU were killed on PND 110 or PND 180. Whole-mounted mammary glands were examined for tumours. Immunostaining was conducted to identify cytokeratin 8 (an epithelial marker) and p63 (a myoepithelial marker). Data were statistically analyzed using the Mann-Whitney *U* test. It was not clear if the litter or offspring were considered the statistical unit.

BPA exposure did not affect successful pregnancies, dam weight gain, pregnancy duration, number of pups/litter, or percent females/litter. Anogenital distance on PND 1 or 5 and postnatal body weights were unaffected in pups exposed to BPA. Vaginal opening was accelerated in pups from the BPA group (mean 34 days of age compared to 39 days of age in controls). On PND 50, the BrdU/apoptosis ratio was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma of BPA-exposed animals; the effects were not observed on PND 30 or PND 110. Significantly increased percentages of hyperplastic ducts, density of stromal nuclei, and numbers of mast cells were observed in the BPA group on PND 110 and PND 180. Exposure to BPA resulted in formation of a dense stroma layer around mammary epithelial structures and replacement of normal adipose tissue with a fibroblastic stroma. In rats exposed to 25 mg/kg bw NMU on PND 50, incidence of hyperplastic lesions on PND 180 was significantly higher in the group with prenatal BPA exposure compared to controls (mean incidence of 35.5% compared to 15.7% in controls). Although statistical significance was not achieved, exposure to 25 mg/kg bw NMU resulted in tumors in 2 of 15 rats in the prenatal BPA group and 0 of 10 rats in the prenatal control group on PND 180. Cytokeratin 8 immunostaining revealed no invasion by stromal epithelial cells. The study authors concluded that in rats prenatal exposure to a low dose (0.025 mg/kg bw/day) of BPA perturbs mammary gland histoarchitecture and increases its carcinogenic susceptibility to a chemical carcinogen (NMU) administered 50 days after the end of BPA exposure. However, due to the small sample size, lack of clarity on statistical analysis and use of a single dose level, it is difficult to establish whether the effects reported were due to chance or were real, treatment-related effects. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures.

In a similar study, Murray *et al.* (2007) examined the effect of prenatal BPA exposure on in situ induction of mammary tumors in rats. From GD 9 (GD 1 = day of vaginal sperm) through PND 1 (PND 0 = day of birth) Wistar-Furth rat dams received subcutaneous injections of 0, 0.0025, 0.025, 0.250, or 1 mg/kg bw/day BPA. Number of dams treated was not reported. Based on a limited amount of information provided on the number of offspring examined, it appears that ≤ 6 dams/group were treated. Pup viability was assessed on PND 1. On PND 2 pups were sexed and litters were culled to 8 pups. Anogenital distance was measured on PND 4. Litters were weighed during the lactation period. Female offspring were monitored for body weight and vaginal opening in the post-weaning period. Female offspring were killed on PND 50 or PND 95. Mammary glands were collected and whole-mounted or sectioned for histopathological examination. Morphometric analyses were conducted to examine possible presence of preneoplastic lesions. Mammary glands were examined for ER-α and Ki-67 protein by an immunohistochemistry technique. One female/litter was included in the histological examinations. Apparently, ≤6 offspring/group were examined

histopathologically. The number of offspring examined for the other endpoints was not reported. It was not clear if dams or offspring were considered the statistical unit.

BPA exposure did not affect offspring viability, sex ratio, age at vaginal opening, or female anogenital distance. Anogenital distance was reduced on PND 4 in males from the 0.250 mg/kg bw/day group. Percent hyperplastic ducts was increased in all dose groups on PND 50; the study authors noted that the effect on PND 50 was quantitatively similar in all dose groups (i.e. 3–4-fold increase). Cribriform structures classified as carcinomas-in-situ were observed in the 0.25 and 1 mg/kg bw/day groups. The incidence of these structures in the controls and lower dose groups were not reported. Although the study authors classified the cribriform structures as carcinoma in situ because of their hallmarks, it is difficult to establish whether or not these histopathological findings are clear neoplastic lesions of the mammary gland. The study authors concluded that fetal BPA exposure at dose levels of 0.250 and 1 mg/kg bw/day in rats is able to induce development of preneoplastic and neoplastic mammary lesions. However, again, due to the small sample size, lack of clarity on the statistical analysis, absence of a dose-response relationship and uncertainty about the incidence of the cribriform-like lesions in the controls it is difficult to establish whether the effects reported were due to chance or were real, treatment-related effects. In addition, because of the uncertainty about the significance of the cribriform structures, it is unclear whether real neoplasia actually occurred. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures.

4.1.2.8.3 Impact of new information and summary of carcinogenicity

BPA has not shown any significant carcinogenic activity in two standard oral cancer bioassays in rats and mice.

The new information principally concerns the potential promoting effects of prenatal and/or neonatal exposure of rats to BPA on the carcinogenesis induced by established carcinogens/initiators in specific organs (prostrate, uterus, thyroid, lungs, liver, thymus, esophagus, liver and mammary gland). One single study (Murray *et al*., 2007) examined the potential full carcinogenic activity of prenatal exposure to BPA on the mammary gland. Three studies were conducted by the oral route of exposure and three by subcutaneous administration. Although not conclusive, the studies involving oral administration showed that BPA does not exert promoting activity up to relatively high levels of exposure on DMAB-induced prostate cancer (up to 120 mg/kg bw/day), ENNG-induced uterus cancer (up to 6 mg/kg bw/day) and BHP-induced thyroid, lung, liver, thymus and esophagus cancer (up to 400-600 mg/kg bw/day). The studies involving subcutaneous administration showed that BPA at relatively low doses (in the μg/kg bw/day range) does increase the incidence of E+T-induced preneoplastic and neoplastic lesions of the prostate and the incidence of NMU-induced hyperplastic lesions of the mammary gland and does induce hyperplastic and cribriform lesions of the mammary gland. However, these studies had several limitations and methodological weaknesses which make difficult to establish whether the reported findings were real, treatment-related effects. Furthermore, because of the subcutaneous route of administration, it is questionable whether they are relevant to normal routes of exposures.

Overall, there is only one new study in which the full carcinogenic potential of BPA on the mammary gland has been examined in a prenatal model. Although this study claims that prenatal exposure to BPA induces preneoplastic and neoplastic lesions of the mammary

gland, its validity is hampered by serious methodological limitations. It is also noted that these findings are inconsistent with the absence of preneoplastic lesions of the mammary gland in the offspring of several standard multi-generation studies in rats and mice.

Regarding the other new studies, it can be concluded that prenatal and/or neonatal exposure to BPA does not exert promoting activity on the carcinogenesis induced by established carcinogens/initiators in specific organs.

Overall, therefore, the new information on the potential carcinogenic and/or promoting effects of BPA in prenatal and neonatal rat models supports the original conclusion from the published report that BPA does not possess any significant carcinogenic potential.

4.1.2.9 Reproductive toxicity

4.1.2.9.1 Summary of original risk assessment report

No human data are available. BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with BPA glucuronide *in vitro.* The available data also indicate that there is a marked strain difference in the response to BPA in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories. Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of BPA on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. Although no effect on fertility was seen in the rat two-generation study, low dose levels were employed (0.2-200 μg/kg/day). In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse, it is prudent to assume that BPA may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg.

The continuous breeding study in the mouse provides some evidence that BPA can cause adverse effects on fertility. In the F_0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter

tested at each dose level from the F_1 generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F_1 generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F_0 generation) there was no effect on epididymal weight. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern. Although no effects were seen in the 2-generation rat study, it is not considered suitable for use in the risk characterisation due to the low dose levels employed (0.2-200 μg/kg/day). However, these data combined with that for the multigeneration study does provide a comprehensive dose-response range for evaluating effects on fertility in the rat. In addition, comparing the rat and mouse data it can be seen that similar toxicological profiles were observed for effects on fertility; effects were seen in both species at approximately the same dose level (i.e. reductions in litter size at 500 mg/kg/day in the rat and at 600 mg/kg/day in the mouse). Consequently, it is considered that the NOAEL of 50 mg/kg/day identified in the rat multigeneration study is also likely to produce no adverse effects in mice for which there is only a LOAEL of 300 mg/kg/day (for a small but statistically significant decrease in epididymal weight in F_1 males only). Therefore, the NOAEL of 50 mg/kg/day identified from the multigeneration study will be used for risk characterisation purposes, in relation to effects on fertility.

No evidence that BPA is a developmental toxicant was observed in standard development studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F_1-F_3). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg.

However, additionally, some studies have investigated the potential of BPA to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at dose levels in the range 2 – 50 μg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. It is noted that in contrast to the studies showing effects on the male reproductive tract, the studies that did not find an effect of BPA also did not show any effects of DES. Furthermore, no functional changes in reproductive parameters or reproductive organ development were observed in a recent rat two-generation study using similar dose levels. The reasons for the differences in these results are unclear. Recent evidence from one study suggests that there are differences in the sensitivity of different mice strains to the effects of oestrogens, which may be related to the selection of strains for large litter size. This difference in sensitivity may in part explain some of the differences in the current database, although the relevance of these rodent strain differences in relation to human health remains unclear.

Overall, in standard developmental studies in rodents, there is no convincing evidence that BPA is a developmental toxicant. However, the available and apparently conflicting data from studies conducted using low doses (in the μg/kg range) do raise uncertainties. Overall,

the majority of EU member states felt that the studies reporting effects at low doses could not be dismissed. However, the member states disagreed on how these studies should be used, if at all, in the risk characterisation for this endpoint. The disagreements were based on differing views about the uncertainties surrounding the reproducibility of the findings and their biological significance, if any, to human health.

This issue was referred to the Competent Authorities in June 2001. It was agreed unanimously by the Competent Authorities that further work was required to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development at low doses. In addition, it was agreed that a provisional NOAEL of 50 mg/kg/day for developmental effects, derived from the rat multi-generation study, should be used in the risk characterisation in the interim, whilst awaiting the outcome of further testing, with the aim of identifying those scenarios which are clearly of concern irrespective of the outcome of the further testing.

4.1.2.9.2 Updated information

Member States required that a 2-generation study in the mouse involving exposure to low (µg/kg bw/day range) and high (mg/kg bw/day range) doses of BPA be conducted. This study has now become available (Tyl *et al.* 2007) and is summarised below.

In addition to this comprehensive 2-generation study, a large number of studies investigating the reproductive toxicity of BPA have become available since the finalisation of the RAR. Among these studies, several (approximately 40-50) have investigated the same standard reproductive and developmental endpoints as those examined by Tyl *et al*. (2007). These studies have been performed on a range of animal species and strains, at different life stages, over a wide array of doses, using a variety of exposure routes, for varying exposure durations, and have investigated a large assortment of endpoints (for a detailed review see Gray *et al*., 2004; Goodman *et al*., 2006; EC SCF, 2002; and EFSA, 2006). The majority of these studies have reported only small changes (unrelated to dose) in organ weight, tissue architecture, receptor expression or hormone levels of unknown pathophysiological consequences. Some have found no effect, but, overall, no consistent, reproducible, adverse effects have been observed. Furthermore the results from these studies have been in contrast to the results of investigations conducted according to internationally recognised guidelines and in compliance with GLP, including the recent 2-generation study in the mouse by Tyl *et al*. (2007). As we consider this investigation by Tyl *et al*. (2007) as the gold-standard, definitive study of the reproductive toxicity of BPA (for the endpoints examined), all the other recent publications investigating the same standard reproductive and developmental endpoints have not been evaluated in detail in this report.

However, there are also numerous recent studies which have investigated the potential developmental neurotoxicity of BPA. As these endpoints were not examined by Tyl *et al.* (2007), these publications have been considered in detail in this evaluation.

Additionally, one study investigated the effects of neonatal exposure to BPA on the morphology of the reproductive tract at 18 months of age in female mice (Newbold *et al*. 2007). Because the potential for the expression of developmental effects in old-age has not been assessed in the standard reproductive toxicity studies, an appraisal of this study is included below.

One relevant human study has been published since the finalisation of the RAR and is included in this update (Sugiura-Ogasawara *et al.* 2005). This is an investigation of the possible association between BPA exposure and recurrent miscarriage.

2-generation study in mice

The effects of BPA on fertility and reproductive performance in mice have been investigated in a two-generation study, conducted in compliance with GLP (Tyl *et al*, 2007). The study design and interpretation of the results were supervised by a Steering Group, that was chaired by a representative of the European Chemicals Bureau and included experts from several EU Member States. The overall design of this study was based on OECD Test Guideline 416, enhanced by incorporation of a second vehicle control group, a positive control group, a total of 6 exposure levels of BPA, the retention of additional F_1 male offspring for organ weight and other assessments, and extending histopathological examinations to all treatment groups. This study was conducted in response to an ESR risk assessment conclusion that further research is needed to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development of the male reproductive tract at low doses (0.002- 0.05 mg/kg/day) in mice.

Eight groups of 28 male and 28 female CD-1 mice (F_0 generation) were exposed to BPA in the diet (Purina Certified Ground Rodent Diet®, No. 5002) at concentrations of 0 (2 vehicle control groups), 0.018, 0.18, 1.8, 30, 300 or 3500 ppm, which resulted in a BPA intake close to the target doses of 0, 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg/day, respectively. CD-1 strain mice were used as there have been claims that this strain is specifically sensitive to low doses of BPA. Two vehicle control groups were used to better characterise the natural variability in mice of the parameters evaluated in the study. The exposure levels were selected as a range that would make possible a comprehensive assessment of the dose-response relationship for reproductive toxicity. The lowest BPA dietary concentration was selected to provide a BPA intake of about 0.003 mg/kg/day, close to that at which effects on the development of the male reproductive system have been reported by Nagel *et al*. 1997 and vom Saal *et al*. 1998. The next two higher concentrations of 0.18 and 1.8 ppm were selected as 10-fold incremental increases. Concentrations of 30 and 300 ppm were selected to produce intakes that matched the NOAEL and LOAEL, respectively, for general parental toxicity in a 3-generation dietary study in the rat (Tyl *et al*. 2002). The highest concentration of 3500 ppm was selected as an exposure level that would cause mild general parental toxicity, based on the results of a 13-week rangefinding study (Tyl *et al*. 2005). The phytoestrogen content of the batches of diet used were: genistein 177-213 ppm, daidzein 173-181 ppm, glycitein 39-55 ppm and total isoflavones 390-449 ppm.

The positive control group of 28 male and 28 female mice was exposed to 17β-oestradiol (E2) in the diet at a concentration of 0.5 ppm (resulting in an E2 intake of about 0.08 mg/kg/day), to confirm the sensitivity of the mouse model to a potent endogenous oestrogen. This exposure level was selected as one that would result in effects on oestrogen sensitive reproductive parameters, based on the findings of a E2 rangefinding (Tyl *et al*. 2004a) and 2-generation study (Tyl *et al.* 2004b, 2006 - this study is briefly summarised below).

Exposure of the F_0 generation commenced at 6 weeks of age and continued throughout an 8 week pre-breed exposure period, a 2 week mating period (each male was paired with a female from the same exposure group) and gestation. Exposure of F_0 females to BPA continued throughout lactation until weaning on post-natal day (pnd) 21. At weaning, 28 male and 28

female F_1 animals were selected from each exposure group for retention and were similarly exposed for a pre-breed, mating gestation and lactation period. An additional one F_1 male from each litter was retained (termed 'F_1 retained males') with continued exposure for 3 months until sacrifice concurrently with the parental F_1 males. The remaining F_1 animals were sacrificed at weaning. Parental males were sacrificed at the end of their respective delivery period and parental females were sacrificed at weaning of their litters. The study was terminated with the sacrifice at weaning of the F_2 generation.

For the parental F_0 and F_1 generation and retained F_1 males, clinical signs of toxicity, body weights and food consumption were recorded. Oestrous cycles were monitored in the last 3 weeks of the pre-breed exposure period and during the mating period for both the F_0 and F_1 parental animals. A necropsy was conducted on all adult animals in which reproductive (including the prostate) and other selected organs were removed and weighed. Histology of selected tissues was conducted on all vehicle control animals and on 10 parental males and females from each of the F_0 and F_1 BPA and E2 groups. Histology was also conducted on all vehicle control and E2 F_1 retained males and on 10 randomly selected F_1 retained males from each BPA group. For all parental and retained males, epididymal sperm number, motility and morphology were assessed, testicular homogenisation-resistant spermatid head count was recorded, and daily sperm production and efficiency of daily sperm production was calculated. For all parental females the number of ovarian primordial follicles was counted. Parameters assessed in the young offspring included litter size, body weight, survival, gross appearance, anogenital distance (on PND 0 and 21), vaginal patency and preputial separation. For offspring killed at weaning a gross necropsy was conducted on all; selected organs were removed and weighed from two randomly selected pups/sex/litter and histopathology was conducted on the reproductive organs of one pup/sex/litter and on all selected organs from the other selected pup/litter.

All statistical comparisons to each BPA group and the E2 positive control group values were made against the pooled values for the two vehicle control groups.

For the BPA exposed F_0 and F_1 parental/retained animals there were no treatment-related mortalities or clinical signs of toxicity. Evidence of general toxicity was observed in 300 ppm and 3500 ppm groups. At 300 ppm, this evidence was limited to an increased incidence of centrilobular hepatocyte hypertrophy of minimal to mild severity in F_0 males (40% vs 11% in controls) and females (10% vs 2%) and F_1 parental/retained males (30% vs 10%). There were no increases in liver weight at this dose level. At 3500 ppm, bodyweight gain was reduced among the F_1 parental/retained males; at termination mean bodyweights of the parental and retained males were 4% and 10%, respectively, less than the vehicle controls. Kidney weights were increased in F_0 males and in F_1 parental/retained males. Histological examination of the kidney revealed an increased incidence of minimal to mild nephropathy in the F_0 males and F_1 parental/retained animals at 3500 ppm. Absolute liver weights were significantly increased in F_0 males (by 18%) and females (by 20%) and in F_1 parental males (by 17%) at 3500 ppm. Histological examination of the liver revealed an increased incidence of minimal to mild centrilobular hypertrophy and minimal to mild nephropathy in the F_0 males (100% vs 11% in controls) and females (60% vs 2%) and F_1 parental/retained males (65% vs 10% in controls) and parental females (70% vs 4%) at 3500 ppm. The increased incidence of centrilobular hypertrophy at 300 ppm (50 mg/kg/day) was not accompanied by an increase in the group mean liver weight, suggesting that the liver changes seen at this dose level were minor and without toxicological significance. Therefore, the study NOAEL for general toxicity can be set at 50 mg/kg/day on the basis of the observation of toxicologically significant effects on bodyweight, kidney and liver at the next highest dose level of 600 mg/kg/day (3500 ppm).

Concerning the reproduction system, there were no effects on F_0 or F_1 adult reproductive organ weights (F_1 prostate weights are further discussed below), sperm parameters, ovarian primordial follicle count, oestrous cyclicity or histopathology. There were no effects on F_0 or F_1 mating performance or fertility. However, gestational length was statistically significantly increased for both the F_0 and F_1 generations at 3500 ppm (19.3 days vs. 19.0 days for the vehicle controls, both generations), although the health implications of this marginal difference are questionable.

There were no statistically significant differences and no treatment related changes in prostate weight in either the F_1 parental or retained males. Prostate weights appeared slightly increased at 0.018 ppm in the F_1 parental males but because this difference was not also seen in the F_1 retained males, and statistical significance was not achieved, this was considered not to be treatment related.

F_1 and F_2 litter size, pup survival at birth and during lactation were not affected by BPA exposure. There were no treatment-related malformations or clinical signs during lactation. However, several effects on the offspring were apparent at 3500 ppm only. F_1 pup bodyweights were significantly less than vehicle controls on pnd 7, 14 and 21, but F_2 pup bodyweights were not affected. Absolute and bodyweight- or brain-related testes and spleen weights were reduced in the F_1 and F_2 males sacrificed at weaning. The effects on testes weight correlated with an increased incidence of hypoplasia of the seminiferous tubules at 3500 ppm (F_1: 12% vs. 1% in vehicle control; F_2: 35% vs. 4% in vehicle control). Similar effects, however, were not seen in the F_1 parental and retained males. Also, acquisition of preputial separation was slightly delayed in the F_1 parental (by 2 days, compared with negative control, adjusted for bodyweight at time of acquisition) and F_1 retained (by 1.8 days) males at 3500 ppm. Additionally, at 3500 ppm there was a slightly increased incidence of undescended testes observed at weaning sacrifice (a condition with a high and variable background rate in CD-1 mice) of the F_1 and F_2 generations, but there were no indications in adult males of a permanent effect on testes descent. Anogenital distance (AGD) was significantly reduced in F_1 pups on pnd 21 at 300 and 3500 ppm, although this was considered unlikely to be treatment-related because the effect was not also seen on pnd 0 in the F_1 generation, on pnd 0 or 21 in the F_2 offspring; in addition the fact that AGD was not consistently affected in E2 two-generation studies (Tyl *et al*. 2006, Biegel *et al*, 1998) shows that AGD is not an oestrogen regulated endpoint.

In the E2 positive control group no general toxicity was observed in the F_0 and F_1 parental animals. Reproductive toxicity was expressed as changes in a number of parameters, demonstrating the sensitivity of the mouse model to an oestrogenic substance. F_0 and F_1 gestational length was increased. There was a reduced number of live F_1 litters born, reduced F_1 litter sizes and F_1 fertility was reduced. Onset of puberty (vaginal patency) was accelerated in the F_1 females, preputial separation was delayed in F_1 males, female reproductive organ weights in adults and offspring of all generations were increased. Testes and epididymal weights were decreased and the incidence of seminiferous tubule hypoplasia of the testes was increased among F_1 and F_2 weanlings. Anogenital distance was reduced in male offspring of the F_1 and F_2 generations, although this is considered not to be an oestrogen regulated endpoint. Finally, there was an increased incidence of vaginal epithelial keratinization and bilateral luminal dilatation of the uterus in F_1 and F_2 weanlings. Overall, with the exception of reduced anogenital distance in male offspring, the findings with the E2 positive control group were consistent with those from the E2 2-generation study (Tyl *et al*, 2004b, 2006, briefly summarised below).

To conclude, BPA caused effects on pregnancy and the offspring (observed as a slightly increased duration of gestation, reduced pup bodyweight during lactation, a slight increase in the incidence of undescended testes at weaning, seminiferous tubule hypoplasia in offspring at weaning, and delayed acquisition of preputial separation), occurring only at the highest dietary concentration 3500 ppm (intake approximately 600 mg/kg/day), an exposure level that also caused mild parental toxicity. Fertility was not affected by BPA exposure. There was no evidence of an adverse effect on the development of the male reproductive tract at low doses of BPA. Overall, the study NOAEL for both general and reproductive toxicity is 50 mg/kg/day.

The design of the E2 2-generation study (Tyl *et al.* 2004b, 2006) was based on OECD Test Guideline 416. Six groups of 25 male and 25 female CD-1 mice (F_0 generation) were exposed to E2 in the diet (Purina Certified Ground Rodent Diet®, No. 5002) at concentrations of 0, 0.001, 0.005, 0.05, 0.15 or 0.5 ppm, which resulted in E2 intakes of about 0.2, 1.0, 10, 30 or 100 µg/kg/day, respectively. The exposure concentrations were selected as a range likely to include both effect and no-effect levels for E2 reproductive toxicity, based on the results of a rangefinding study (Tyl *et al.* 2004a). The F_0 and F_1 exposure periods, mating and sacrifice schedules and experimental observations were essentially the same as for the BPA 2-generation study (Tyl *et al.* 2007). The study was terminated with the sacrifice at weaning of the F_2 generation.

There were no treatment-related mortalities or clinical signs of toxicity among F_0 and F_1 parental/retained animals. Evidence of general toxicity in the parental/retained animals was limited to significantly reduced bodyweight gain during gestation and food consumption during lactation for F_0 females at 0.5 ppm. Reproductive effects were seen at the three highest exposure levels. At 0.5 and 0.15 ppm, F_0, F_1 and F_2 uterus weights were increased, F_1 and F_2 weanling testes and epididymides weights were deceased, F_1 and F_2 litter size was reduced, the timing of vaginal patency was accelerated in F_1 females and the timing of preputial separation was delayed in F_1 males. At 0.05 ppm, uterus weights were increased in F_1 and F_2 generations. No general or reproductive toxicity was observed at 0.005 or 0.001 ppm. Overall, the study NOAEL for reproductive toxicity in this E2 study is 0.005 ppm (about 1 µg/kg/day) and the study LOAEL is 0.05 ppm (approx. 10 µg/kg/day).

Developmental neurotoxicity endpoints

The effect of prenatal and perinatal exposure to BPA on neurological development has been investigated in a large number of recent studies. Many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. Thirty-one studies conducted using the oral route and three studies using the subcutaneous route of exposure are summarised below. The impact of these recent studies on the hazard assessment for BPA is assessed using a weight of evidence approach that focuses on the reliability and consistency of the evidence. This overall assessment, which is presented after the individual study summaries, draws attention to a low level of confidence in the reliability of the studies and a lack of consistency in the results, such that no firm conclusions can be drawn.

The literature search also located several developmental neurotoxicity studies in which BPA was administered by intracisternal injection. These studies have been omitted because this route is clearly of no relevance to the risk assessment for human health.

To ensure transparency with respect to any connections between investigations, the study summaries have been grouped according to the investigating team.

Studies by collaborating researchers, mainly from Universities of Florence, Siena, Rome, Calabria and Parma, Italy

The effects of prenatal and post natal exposure to BPA at maternal dose levels of 0.01 - 0.4 mg/kg/day on a range of behaviours and on receptor expression in the brain were investigated in a series of 12 studies conducted by the Italian collaborating researchers.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in male and female Sprague-Dawley rats (Farabollini *et al.* 1999). Dosing was by the oral route, using a micropipette. A group of 11 Sprague-Dawley females was fed BPA dissolved in arachis oil at 0.04 mg/kg/day from 10 days prior to mating to the day of weaning (pnd 21) of their offspring. A second group of 11 females received the vehicle from 10 days prior to mating to gd 13, then 0.4 mg/kg/day BPA from gd 14 to pnd 6 and then the vehicle only until pnd 21. A control group received the vehicle from 10 days prior to mating to pnd 21. Twelve-15 pups/gender/group were selected for behavioural testing, conducted between pnd 85 and 87. The order of testing with respect to treatment group was counterbalanced to avoid confounding due to circadian rhythm and variations in testing conditions. Activity during a 5 min session in a holeboard box was recorded, immediately followed by a 5 min session in an elevated-plus maze. These tests are thought to provide measures of 'anxiety' and locomotion. The statistical analysis was probably conducted using the individual pup as the experimental unit. This is a weakness in the study design, which is present in many of the other BPA developmental neurotoxicity studies.

There were no signs of maternal toxicity or foetal malformations (data not presented). In the holeboard test, the frequency and duration of head dipping for females was significantly reduced (2-4-fold, in comparison with controls) in both BPA groups, with the effects being more marked at 0.4 mg/kg/day. Among males, the only head dipping parameter affected was the frequency, which was reduced at 0.4 mg/kg/day. Reduced head dipping frequency is thought to indicate an increased state of anxiety. No other holeboard parameters were affected by BPA treatment. Among females, the proportion of time spent in the centre of the maze was significantly reduced at both 0.04 and 0.4 mg/kg/day and the frequency of self grooming was increased at 0.04 mg/kg/day. For males in both BPA exposed groups, the percentage of entries that were to open arms and the frequency of stretched posture (stretching the body forward without moving the paws) was significantly increased. A relatively higher proportion of entries to open arms is thought to indicate a decreased state of anxiety, so the results of maze test for males appear in conflict with the holeboard findings.

The effect of prenatal or neonatal exposure to BPA on social and sexual behaviour was investigated in the Sprague-Dawley rat (Farabollini *et al*. 2002). Groups of 7 females were administered 0.04 mg/kg/day BPA via the oral route, by feeding from a micropipette, either during gestation or the lactation period. A group of 13 control females received the arachis oil vehicle throughout gestation and lactation. On pnd 2 the offspring were culled to a standard litter size of 4/sex and cross-fostered to establish the following groups of 12 pups/sex:

(1) Prenatal exposure group: born to BPA treated mothers and nursed by vehicle control mothers
(2) Postnatal exposure group: born to control mothers but fostered to BPA treated dams

(3) Control group: born to and nursed by mothers exposed to only the vehicle

Behavioural testing commenced at about 14 weeks of age when the response of each rat to the introduction of an unfamiliar ('intruder') rat of the same sex and bodyweight was assessed over a 15 min period. The results for females not in dioestrus were excluded from the analysis. At 15 weeks, each rat was placed between two cages, one containing a sexually mature male and the other a sexually responsive female, and the time spent ('sexual preference') in the area adjacent to each cage was recorded. In a second phase of this test, each male was given access to a sexually responsive female and its sexual performance was scored. At 16 weeks, the sexual behaviour of each female, when paired with a mature male, was examined. On the day of the test, the stage of the oestrous cycle was determined. Behaviour was video recorded for later scoring by an observer blinded to treatment group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably based on the individual pup, and did not take account of the litter of origin.

In the intruder test, the proportion of prenatally exposed males displaying defensive behaviour was greater (9 vs. 4, out of 12) and displaying ambivalent behaviour was less (3 vs. 8) in comparison with the control males. Also, the ratio of defensive/antagonistic was increased in this BPA group (by 280%). For the BPA postnatal males, and both BPA female groups, intruder test behaviour was similar to the control animals. There were no differences in sexual preference behaviour between the control and BPA exposed groups of either sex. In the sexual performance testing of males, there were statistically significant differences for several parameters in the BPA groups in comparison with the controls; in the post natal group the mean number of intromissions required for ejaculation was increased (15 vs. 11 in the controls) and in the prenatal group the mean latency to first intromission (120 vs. 40 sec) and duration of female genital sniffing (40 vs. 15 sec) were increased. However, the numbers of mounts, latency to ejaculation and time between ejaculation and the next mount were similar for all groups. For the females, the results of the prenatal and post natal BPA groups were combined; for BPA females in pro-oestrus the time taken to enter the arena occupied by the male was reduced (20 vs. 120 sec) and the mean number of displays of lordosis was increased (12 vs. 4).

The effect of prenatal and neonatal exposure to BPA on the behavioural response to pain of offspring was investigated (Aloisi *et al*. 2002). Groups of 7 Sprague Dawley female rats were administered 0.04 mg/kg/day BPA via the oral route, by feeding from a micropipette, during either gestation or the lactation period. As a control group 13 females received the peanut oil vehicle throughout gestation and lactation. Within 48 h of birth the offspring were cross-fostered to form the following groups:

(1) Prenatal exposure group: 11 males and 9 females born to BPA exposed mothers and nursed by vehicle control mothers
(2) Postnatal exposure group: born to vehicle control mothers but fostered to BPA exposed mothers
(3) Control group: 16 males and 11 females, born to and nursed by mothers exposed to only the vehicle

At 22 weeks of age, each pup received a subcutaneous injection of either formalin (half the animals in each group) or saline in a hind paw. Each animal was then observed in an open field for 60 mins. Behaviours such as licking, flexing and jerking of the paw were recorded.

The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis did not take account of the litter of origin.

The frequency of paw jerking was significantly reduced 40-60 min after the injection in the males of the post-natal BPA exposure group. Duration of paw flexing was increased during the first 30 mins in males and females from the pre-natal BPA exposure group. The statistical analysis of the other behaviours observed in the open field, such as duration of exploration activity or rearing, revealed no effect of BPA exposure. Overall, only slight inter-group differences in the pain response were seen in this study.

The effect of prenatal and neonatal exposure to BPA during pregnancy and lactation on play behaviour was investigated in the Sprague-Dawley rat (Dessi-Fulgheri *et al.* 2002). Dosing was by the oral route, using a micropipette. A group of 11 females was fed BPA dissolved in arachis oil at 0.04 mg/kg/day from 10 days prior to mating through to the day of weaning (pnd 21) of their offspring. A second group of 11 females received the vehicle from 10 days prior to mating to gd 13, 0.4 mg/kg/day BPA from gd 14 to pnd 6 and then the vehicle until pnd 21. A control group received the vehicle from 10 days prior to mating to pnd 21. For behavioural testing, either 12 or 15 pups of each sex/group were randomly chosen at weaning from different litters and housed by group in cages containing 3 males and 3 females. Behavioural observations were made at three ages, pnd 35, 45, and 55. Six cage mates were transferred to an arena and observed for 6 minutes; scoring of the various social and non-social behaviours was conducted by an observer blinded to treatment group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. Presumably the individual pup was taken as the experimental unit.

The results for the three ages were pooled for analysis. Eight types of behaviour were identified by principal component analysis, and statistically significant differences between the control and treated groups were reported for four of these. The frequency of play directed towards females was significantly increased in the females of the BPA 0.04 mg/kg/day group. Behaviours considered to represent low-intensity mating elements were significantly decreased in both sexes of the BPA 0.4 mg/kg/day group. Sociosexual exploration was significantly decreased in males of both BPA groups and females at 0.4 mg/kg/day. The frequency of social interest behaviour was significantly decreased in both sexes at BPA 0.4 mg/kg/day, but increased among 0.04 mg/kg/day males.

The effect of prenatal and neonatal exposure to BPA on the somatostatin receptor subtype 2 (sst_2) in the limbic regions of the brain was investigated in the Sprague-Dawley rat (Facciolo *et al.* 2002). Females were fed BPA dissolved in arachis oil via the oral route at 0.04 or 0.4 mg/kg/day from an unspecified time point prior to mating to the day of weaning (pnd 23) of their offspring. A vehicle control group was included. The number of females in each group was not specified, although the report indicated that a total of 32 females were used. At birth, all litters were culled to 8 pups and one pup/litter was cross-fostered to another litter of the same dose group. Receptor binding was assessed using ^{125}I-Tyr^0-somatostatin-14 as a ligand in animals killed on pnd 10 or 23 (4-6 animals/group/age). At the same ages, interactions of sst_2 with α-containing γ-aminobutyric acid (GABA) receptors, using the agonists zolpidem and Ro 15-4513, were examined in 12–13 rats/group. Results were reported for only the 0.40 mg/kg day because higher affinity was obtained for receptor ligand binding. The statistical analysis was probably conducted using the individual pup as the experimental unit, and did not take account of the litter of origin.

At pnd 10, significantly lower sst_2 levels of the low-affinity state receptor were found in the gyrus dentate of the hippocampus and basomedial nucleus of the amygdala. Significantly

higher sst_2 levels were observed only for the high-affinity state, in the periventricular nucleus of the hypothalamus. A similar trend was seen at pnd 23, with the exception that there were much lower levels of the high-affinity sst_2 receptor subtype in the amygdala nucleus and ventromedial hypothalamic nucleus. These differences were potentiated when the binding activity of sst_2 was measured in the presence of two selective agonists (zolpidem and Ro 15-4513) specific for the α-containing GABA type A ($\alpha GABA_A$) receptor complex. The authors consider that these results provide evidence for a possible role of a sst_2 subtype α-containing GABA type A receptor system in the promotion of oestrogen-like activities of BPA.

In a subsequent study, the effect of prenatal and neonatal exposure to BPA on the expression of somatostatin receptor subtype 3 (sst_3) mRNA receptors in the limbic regions of the brain was assessed (Facciolo *et al.* 2005). Dosing was by the oral route, using a micropipette. Groups of 12 Sprague-Dawley females were fed BPA dissolved in arachis oil at 0.04 or 0.4 mg/kg/day from 8 days prior to mating to the day of weaning (pnd 23) of their offspring. A vehicle control group of 8 females was included. At birth, one female pup/litter was cross-fostered to another litter of the same BPA dose group, but the report does not state that this procedure was also conducted in the control group. On pnd 7 and at 55 days of age, 4 females/group/age were killed. Brains were sectioned and a ^{32}S-labeled probe was used in an in situ hybridization method to measure sst_3 mRNA expression. The effects of $\alpha GABA_A$ receptor subunits on expression of sst_3 mRNA was examined by incubating the brain sections in 1 nM–100 μM of $\alpha GABA_A$ receptor agonists (zolpidem, flunitrazepam, RY 080, and RO 15-4513). Additional brain sections from high-dose rats were used to determine interactions between sst_3 with α1 and α5 subunits with or without addition of 5–500 nM zolpidem or RY 080.

In the BPA 0.4 mg/kg/day group killed at 55 days of age, sst_3 mRNA levels in level V of the frontoparietal cortex were reduced in comparison with the control group. In the BPA 0.4 mg/kg/day group killed on pnd 7, sst_3 mRNA levels were reduced in the hypothalamic periventricular nuclei and increased in the ventromedial nuclei. The expression of sst_3 mRNA showed more marked upregulation and downregulation in the presence of agonists specific for $\alpha GABA_A$ receptors. According to the authors, these results suggest that BPA exposure has an influence on cross-talking mechanisms that are implicated in the plasticity of neural circuits.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in the Sprague-Dawley rat (Adriani *et al.* 2003). Groups of 9 mated females were dosed by the oral route, using a micropipette, with 0 (arachis oil vehicle control) or 0.04 mg/kg/day BPA from the day of mating to the day of weaning of their offspring. One male and one female from each litter were retained and subjected to the following series of tests: 'novelty preference' behaviour at pnd 30-45; 'impulsivity' at pnd >70; open field activity following amphetamine treatment at pnd >70. Testing of the experimental groups was reported to have been counterbalanced across time for the novelty preference test, but it was not stated if this was done for the impulsivity or open field testing.

Novelty preference was tested in an opaque Plexiglas box, subdivided into two compartments that were connected via a closable door. One compartment had a wide-mesh floor and the other a narrow mesh floor. Activity in the apparatus was monitored from video recordings by counting the number of times imaginary lines were crossed. During a familiarisation phase (days 1-3 of the testing schedule) each rat was placed in one compartment for 20 minutes on each day. On day 4 each animal was placed in the familiar compartment for 5 minutes and then the connecting door was opened and the rat was able to explore the whole apparatus for

a 24 minute period. Time spent in each compartment and activity rate (number of lines crossed per minute) were recorded.

Impulsivity testing was conducted during a food-deprivation period. Each animal was placed in box containing two nose-poking holes for 30 minute daily sessions. Nose-poking was detected by a photocell. One hole was termed the 'immediate and small (IAS)' hole, poking of which triggered the release of one food pellet. The other was a 'large and delayed (LAD) hole' that triggered the release of five food pellets. For a 1 week training period, when food pellet release was immediate, a chamber light was turned on for one second after nose poking and for 25 seconds after food delivery, during which time nose poking had no consequences. At the start of a 1 week testing session a 10 second delay was introduced for the LAD hole between nose-poking and food delivery. This delay was progressively increased to 100 seconds during the week. The light was turned on during the delay, during which time nose-poking (termed 'inadequate responding') had no consequence. The percentage choice between the LAD and IAS holes and frequency of inadequate responding was recorded.

The open field response to amphetamine investigation was conducted 1 week after the impulsivity testing. Animals were observed for 30 minutes in an open field, 15 minutes after receiving a subcutaneous injection of either saline (4/sex/group) or d-amphetamine (1 mg/kg, 4/sex/group). Rearing, grooming, and number of lines crossed were recorded.

Graphical representation of the novelty preference test results for day 4 show that males from the control and treated groups spent a similar proportion of time in the novel compartment, whereas for BPA treated females there was a marked, statistically significant, reduction in the proportion of time spent in the novel compartment. The analysis of the activity while in the novel compartment showed statistically significantly increased counts for both males and females of the BPA treated group, particularly towards the end of the 24 minute session.

In the impulsivity test, both treated and control animals of both sexes developed a preference for the LAD hole during the training sessions. During the testing session preference progressively shifted towards the IAS hole, although the BPA group retained a consistently higher preference for the LAD hole compared to controls throughout the testing week. There were no differences in preference between the males and females. Concerning the pattern of inadequate responding, as the length of delay was increased the frequency of inadequate poking in the LAD hole progressively decreased and that for the IAS hole progressively increased. For BPA treated males the progressive increase in inadequate responding at the IAS hole was less marked than for the controls and very similar to the behaviour shown by the female groups. For females, the pattern of inadequate responding for the control and treated groups was similar. The authors' interpretation of these findings was that the BPA group was exhibiting reduced impulsivity.

The open field behaviour for saline treated animals of the control and BPA groups was similar. But for males only of the BPA treated groups there was a partial inhibition of an amphetamine-stimulated increase in rearing and number of lines crossed.

The effect of prenatal and neonatal exposure to BPA on play behaviour of female offspring was investigated in the Sprague-Dawley rat (Porrini *et al.* 2005). Dosing was by the oral route, using a micropipette. A group of 12 females was fed BPA at 0.04 mg/kg/day from mating through to the day of weaning (pnd 21) of their offspring. A second group of 10 females received the peanut oil vehicle for the same period. Cross-fostering between mothers of the same treatment group was conducted on pnd 2. At weaning, the pups were housed by treatment group in cages containing 3 male and female sibling pairs. Play behaviour was assessed in six cage mates placed together in an arena for 6 min on pnd 35, 45 and 55, as

described by Dessi-Fulgheri *et al.* (2002). The results for 18 females from each exposure group were analysed. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably conducted using the individual pup as the experimental unit, and litter of origin was not taken into account.

Six types of behaviour were identified by principal component analysis, and statistically significant differences between the control and the BPA group were reported for three of these. The score for social and non-social exploration in the BPA group was significantly increased (by 34%) at 35 days and (by ~25%) at 45 days, in comparison with controls. Play with males was significantly decreased (by a factor of 2) at 45 days. Duration of grooming behaviour was reduced (by a factor of 2) on day 45.

The effect of BPA exposure after weaning on the behaviour of juvenile males was investigated in the Sprague-Dawley rat (Della Seta *et al.* 2006). Groups of 26 males were dosed via the oral route, by feeding from a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.04 mg/kg/day from pnd 23 to 30. Another group of 26 males were similarly exposed to 0.0004 mg/kg/day ethinyl oestradiol. The behaviour of 12 males from each group was examined on pnd 45 and at pnd >90. The report did not state whether the order of testing was counterbalanced with respect to treatment group.
At the younger age, the response to the introduction of a PVC tube into the home cage of males housed in groups of 4 was videorecorded and later scored by an observer blind to treatment status. As young adults, the sexual behaviour and performance of each male in the presence of a receptive adult female rat was similarly recorded and scored. Plasma 17β-oestradiol and testosterone level were measured in 5-8 males/group on pnd 37 and 105. It is not clear from the report if littermates were present in each treatment and age group and, if so, whether the statistical analysis took account of this.

There was no treatment-related effect on bodyweight. Three types of juvenile behaviour were identified by principal component analysis, and statistically significant differences between the control and the BPA group were reported for one. Biting/sniffing/climbing behaviours directed at the PVC tube were significantly lower in the BPA group. In the sexual behaviour assessment, 10/12 controls and 9/12 BPA treated males were active, and the data analysis was restricted to these animals. Only one element of sexual performance was significantly affected; latency to first intromission was reduced. Plasma testosterone levels were significantly lower than controls on pnd 37 (by 33%) and 105 (by 61%). Plasma 17β-oestradiol was not affected. In the ethinyl oestradiol group exploring, behaviours directed at the PVC tube and sexual performance were reduced, but there were no changes in hormone levels.

The effect of prenatal exposure to BPA on maternal nursing behaviour was investigated in CD-1 mice (Palanza *et al*. 2002). Groups of 10-12 mated females were exposed to BPA via the oral route, by feeding from a micropipette, to levels of 0 (corn oil vehicle control) or 0.01 mg/kg/day from gd 14-18. Pups were weaned on pnd 20. At 2-2.5 months of age female offspring (F_1 generation) were mated, and dosed with either the vehicle or 0.01 mg/kg BPA from gd 14-18, creating four treatment groups as follows: 20 control F_1 females receiving the vehicle only; 15 control F_1 females receiving BPA; 15 BPA F_1 females receiving the vehicle only; 15 BPA F_1 females receiving BPA. Maternal behaviour was monitored over a 2 hour period on each of pnd 2-15. The report did not state whether the order of testing was counterbalanced with respect to treatment group. Additionally, F_2 litter size, pup

bodyweights, cliff drop aversion and righting reflex were recorded. The statistical analysis provided an adjustment for litter effects.

F_1 females exposed to BPA either only prenatally or only as an adult spent significantly less time nursing and in the nest and more time nest building, resting alone, grooming and out of the nest. The only significant effect observed in F_1 females exposed to BPA both in utero and as an adult was increased time resting. There were no significant differences for the F_2 generation parameters. The lack of consistency between the effects seen the groups exposed either prenatally or as an adult and the group exposed during both periods suggests that these intergroup differences were unlikely to have been caused by BPA exposure.

The effect of prenatal exposure to BPA on *d*-amphetamine reinforcing effects was investigated in CD-1 mice (Laviola *et al*. 2005). Groups of 10-12 mated females were exposed to BPA via the oral route, by feeding from a syringe, to levels of 0 (corn oil vehicle control) or 0.01 mg/kg/day from gd 11-18. At pnd 60, 3 males and 3 females from each litter (1/sex/dose level of *d*-amphetamine) were subjected to conditioned place preference testing. The order of testing with respect to treatment group was counterbalanced. On the first day of the test procedure, animals were familiarised to the apparatus. On days 2 and 4 each animal received an intraperitoneal injection of 0, 1, or 2 mg/kg *d*-amphetamine and were confined to one compartment of the test apparatus for 20 minutes. On days 3 and 5 each animal was injected with saline and confined in another compartment for 20 minutes. On the final day of testing, each animal was given free access to the entire apparatus for 10 minutes without *d*-amphetamine or saline treatment and the time spent in each compartment and total locomotion activity was recorded.

Conditioned place preference occurred in control females following injection with either *d*-amphetamine dose, but was not observed in the BPA exposed females. In males, a preference displayed for the *d*-amphetamine-associated compartment was similar for the BPA exposed and control animals. There were no significant differences in locomotor activity between the BPA and control groups.

Ceccarelli *et al*. (2007) investigated the effect of juvenile BPA exposure on brain development of Sprague-Dawley rats. Groups of 14 juveniles/group (sex distribution not stated) were dosed by the oral route, using a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.04 mg/kg/day BPA from pnd 23 to 30. Another group of 14 juveniles were similarly exposed to 0.0004 mg/kg/day ethinyl oestradiol. Half the animals were killed on pnd 37 and half on pnd 90. Females killed on pnd 90 were killed in oestrus. Blood samples were taken and brains were processed for immunohistochemistry. ERα levels were analysed in three sexually dimorphic regions of the hypothalamus: arcuate nucleus, ventromedial nucleus and medial preoptic area. Serum testosterone and 17β-estradiol were determined.

The ERα analysis revealed just one statistically significant observation for comparisons between the control and same sex BPA groups; levels were higher (~2-fold) in the ventromedial nucleus among BPA females killed on pnd 37. On day 37, serum testosterone levels in the BPA males were significantly lower (by ~30%). 17β-estradiol levels were not affected by BPA treatment. In the ethinyl oestradiol group there were occasional differences in ERα levels, which were not consistent with the change in the BPA group. Also, testosterone levels were increased in males on pnd 37 and 17β-estradiol levels were increased in females on pnd 90 in the ethinyl oestradiol group. Overall, some evidence of differences in ERα in one of three sexually dimorphic regions of the hypothalamus was seen following BPA exposure to juvenile females.

Studies by researchers associated with Hoshi University, Japan

A series of five studies designed to investigate the effect of prenatal and postnatal exposure to BPA at maternal dose levels ranging from 0.006 - 250 mg/kg/day on the central dopaminergic system in mice were conducted by the Hoshi University investigators.

Suzuki *et al*. (2003) investigated the effect of prenatal and neonatal exposure to BPA on the dopamine D1 receptor-dependent rewarding effect, locomotion stimulation of methamphetamine and dopamine D_1 receptor activity in male ddY mice. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 2.5, 60, 250 mg/kg/day during gestation and lactation. A place-preference conditioning test was conduced on 6-10 male offspring per group (age not reported). In a six day conditioning period animals received either an injection of methamphetamine and immediately placed in a particular compartment for 50 min (days 1, 3, 5) or saline and placed in the other compartment (days 2, 4, 6) for the same time period. On day 7 each animal was, untreated, given free access to the entire apparatus for 15 min and the time spent in each compartment was recorded. Locomotor activity after either a saline or 2 mg/kg methamphetamine subcutaneous injection was automatically measured over a 3 hour period in 9-10 offspring/group. Further injections of methamphetamine were given at 7 day intervals to investigate if sensitisation to the locomotion-stimulating effects of methamphetamine was induced. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The effects on dopamine D1 receptor-mediated G-protein activation (indicating up-regulation of these receptors) by dopamine in limbic forebrain homogenates taken from three control and three 250 mg/kg/day animals (age when killed was not reported) were measured in a ^{35}S-guanosine-5´[γ-thio]-triphosphate binding assay. Protein levels of dopamine and vesicle monoamine transporters in the brain were determined by Western blot and mRNA levels of dopamine D1 receptor in the brain were determined by RT-PCR, also in three control and three 250 mg/kg/day animals. It is not clear if the statistical analysis used the litter or the pup as the experimental unit.

BPA treatment had no effect on maternal bodyweight or clinical condition. In place-preference conditioning testing, there was a dose-dependent and statistically significant increase in all BPA exposed groups in preference for the compartment associated with methamphetamine. Preference for this compartment was eliminated by injecting the animals with SCH23390, a dopamine D1 receptor antagonist. The stimulation of locomotion by methamphetamine was significantly enhanced (by ~80% at peak) in the BPA 250 mg/kg/day group, and this stimulation was more pronounced when methamphetamine injection was repeated. Dopamine-induced binding of ^{35}S-guanosine-5´[γ-thio]-triphosphate to membranes was increased in the BPA groups, indicating enhanced G-protein activation, which was eliminated following injection with SCH23390 or sulpiride, a dopamine D2 receptor antagonist. No changes were observed for expression of dopamine and vesicle monoamine transporter proteins. Expression of dopamine D1 receptor mRNA was significantly up-regulated to 130% of control levels in the high-dose BPA group.

Mizuo *et al*. (2004a) investigated the effect of prenatal and neonatal exposure to BPA on the rewarding effects, locomotion activity and receptor activity induced by morphine in male ddY mice. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 2.5, 60, 250 mg/kg/day during gestation and lactation. Place conditioning testing was conducted in 6-10 male offspring/group (litter distribution was not reported). Each animal was placed in one compartment of a testing apparatus following saline injection and in

a second compartment of the apparatus following morphine injection. On the next day each animal was given free access to both compartments and the time spent in each compartment was measured. Locomotor activity was automatically measured over a 3 hour period in 9-10 offspring/group after either a saline or 10 mg/kg morphine injection. The order of testing with respect to treatment group was counterbalanced. Morphine stimulated ^{35}S-guanosine-5´[γ-thio]-triphosphate binding to membranes (an indication of μ-opioid receptor mediated G protein activation) and expression of μ-opioid receptor mRNA were measured in midbrain homogenates taken from three control and three BPA 250 mg/kg/day animals.

BPA treatment had no effect on maternal bodyweight or clinical condition. In place-preference conditioning testing, there was a dose-dependent and statistically significant increase at 60 and 250 mg/kg/day in preference for the compartment associated with morphine. Locomotion at 250 mg/kg/day only was significantly increased (130 activity counts vs. 10 in controls) following morphine injection. Although place preference differences were observed, there were no affects of BPA on ^{35}S-guanosine-5´[γ-thio]-triphosphate binding or expression of μ-opioid receptor mRNA.

The effect of prenatal and neonatal exposure to BPA on functional changes in dopamine D3 receptors was investigated in male ddY mice (Mizuo *et al*. 2004b) Females (group size not reported) received BPA via the diet at estimated dose levels of 0 or 250 mg/kg/day during gestation and lactation. Offspring were killed (numbers and age not reported) and limbic forebrain homogenates were prepared. The effects on dopamine D3 receptor-mediated G-protein activation by 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT, a D3 receptor agonist) were measured in a ^{35}S-guanosine-5´[γ-thio]-triphosphate binding assay, and a dopamine D_3 receptor binding assay using [3H] PD128907 was conducted. The expression of dopamine D3 receptor mRNA was measured in a RT-PCR assay. Results for 3 animals/group (litter distribution not described) were reported.

BPA treatment had no effect on maternal bodyweight or clinical condition. It was found that G-protein activation by the D3 receptor agonist was markedly reduced in the BPA group, in comparison with controls. In the D_3 receptor binding assay, a decrease of the receptor density in the brain was found in the BPA exposed group.

Narita *et al*. (2006) investigated the effect of prenatal and neonatal exposure to BPA on the dopaminergic system of ddY strain mice, in a very poorly written report. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 0.006, 0.06, 0.6, 100, or 400 mg/kg/day during gestation and lactation. A series of investigations were conducted. Firstly, a place conditioning test was conducted in 6-14 mice/group (gender not specified). In the preconditioning phase mice were placed in compartment of the apparatus immediately after a saline injection and in another compartment following sc injection of 1 mg/kg morphine. On the day of testing, the amount of time spent in each compartment was recorded. Secondly, locomotor activity following a subcutaneous injection of 10 mg/kg morphine was measured for 3 hours in 5-15 mice from the 0, 0.006, 0.6 or 400 mg/kg groups. Also, dopamine induced binding of ^{35}S-guanosine-5´[γ-thio]-triphosphate was measured in limbic forebrain homogenates from 3 offspring of the 0, 0.006, 0.6 or 400 mg/kg groups. The order of testing with respect to treatment group was counterbalanced. It is not clear from the report if the statistical analysis was conducted using the litter or the individual pup as the experimental unit.

There were no effects on maternal bodyweight gain. In the place conditioning test, mice from the 0.006, 100 and 400 mg/kg/day groups spent more time (about 8-fold) in the compartment cage associated with morphine injection, compared with controls; no place preference was

shown by the BPA 0.06 and 0.6 mg/kg/day groups. In the locomotion test, activity after the morphine injection was increased (about 10-fold) at 0.006 and 400 mg/kg/day. In the binding assay, dopamine-induced binding was increased at 0.006, 0.6 or 400 mg/kg/day (by about 32, 18, and 56%, respectively), compared with controls. According to the authors, these findings suggest that prenatal and neonatal exposures to low BPA doses may potentiate central dopamine receptor dependent neurotransmission in the mouse. However, in the absence of a conventional dose-response relationship it is plausible that the observed inter-group differences were due to background variation.

Narita *et al*. (2007) provided a further investigation into the effect of prenatal and neonatal exposure to BPA on the dopaminergic system of male ddY strain mice. Mated females (group size not reported) received BPA via the diet at estimated dose levels of 0 or 400 mg/kg/day from either gd 0-7, gd 7-14, gd 14-20 or pnd 0-20. A similar series of investigations to those described by Narita *et al*. (2006) were conducted in 6-16 male offspring/group, at 7-9 weeks of age. In the place preference test, a clear preference for the compartment associated with morphine was seen in the males exposed for the periods gd 7-14 and pnd 0-20, but not for gd 0-7 or gd 14-20. In the locomotion test, activity after the morphine injection was markedly increased in the 7-14 and pnd 0-20 groups, but again the gd 0-7 or gd 14-20 groups were not affected. Similarly, dopamine-induced binding was potentiated in the gd 7-14 and pnd 0-20 groups, but not in the gd 0-7 or gd 14-20 groups.

Studies from Chemical Industry Institute for Toxicology (CIIT), USA

Three studies, focussing on brain structure, have been conducted at CIIT.

Kwon *et al*. (2000) examined the effect of prenatal and neonatal exposure to BPA on SDN-POA (Sexually Dimorphic Nucleus of the PreOptic Area) volume in female Sprague-Dawley rats (other reproductive parameters were also assessed). Groups of 8 mated females were dosed orally by gavage with 0 (vehicle control) 3.2, 32 or 320 mg/kg/day BPA from gd 13 to pnd 21. A positive control group received diethylstilbestrol at 1.5 mg/kg/day. On pnd 10, 1-3 female pups per litter were killed for measurement of SDN-POA volume. Other parameters measured in offspring included reproductive organ weights and histopathology in males and oestrous cyclicity, vaginal opening and lordosis behaviour in females. The statistical analysis was conducted using the litter as the experimental unit.

There was no evidence of maternal toxicity, based on bodyweight and organ weight analysis. BPA treatment had no effect on SDN-POA volume, or any of the other parameters investigated. In the positive control group there was an increase in maternal liver weight, increased SDN-POA volume and disrupted oestrous cycling. Thus, this study provided no evidence of an effect on SDN-POA volume of prenatal and neonatal BPA exposure maternal dose levels of up to 320 mg/kg/day.

The effect of short-term neonatal exposure to BPA on the development of the anterioventral periventricular nucleus of the hypothalamus (AVPV) was investigated in the Sprague Dawley rat (Patisaul *et al*. 2006). Group size was 5-8 pups/sex. BPA was administered by subcutaneous injection on pnd 1 and 2 at a dose level of approximately 100 mg/kg/day. A control group received the sesame oil vehicle. On pnd 19 the pups were killed and the brains were removed for immunohistochemical processing. The numbers of cells in the AVPV immunoreactive for ERα and/or tyrosine hydroxylase (TH) were counted.

As expected, based on earlier investigations, TH expression was sexually dimorphic; higher numbers were present in females. In the BPA group, the numbers of TH positive cells for females were similar to the female controls, but for males the numbers were increased (by ~75%) in comparison with the control males. For ERα expression, which is not sexually dimorphic, there were no differences between the control and BPA treated groups. The number of cells positive for both TH and ERα was substantially reduced in the medial AVPV for BPA females, reducing the marked sexual dimorphism normally present in this area. There were no differences in the numbers of double-positive cells among the males.

In a further study, the effect of short term neonatal exposure on the development of the AVPV and SDN-POA was investigated in male Sprague Dawley rats (Patisaul *et al*. 2007). Group size was 5-8 male pups. BPA was administered by subcutaneous (sc) injection on pnd 1 and 2 at a dose level of approximately 100 mg/kg/day. A vehicle control group received the sesame oil vehicle on these days. On PND 85, males were gonadectomised. Six age-matched female rats were ovariectomised and assigned to the study for comparative purposes. After a recovery period, the rats were given sc injections of 10 µg estradiol benzoate, and 48 hours later, a sc injection of 500 µg progesterone. The authors note that this protocol has consistently induced fos gene expression in GnRH neurons, leading to LH release in females. About 8 hours later the animals were killed and the brains removed for processing. The volumes of the AVPV, SDN-POA and calbindin-immunoreactive regions of the SDN-POA and the numbers of immunoreactive calbindin, GnRH and fos cells were counted.

BPA had no effect on the volumes of the SDN-POA, AVPV and immunoreactive regions of the SDN-POA. Also, there was no effect on the numbers of GnRH reactive cells. There was no fos gene expression in the males, indicating the absence of GnRH activation, as is normal for males. However, there was a significant increase in SDN-POA calbindin-positive nuclei (by ~40%), in comparison with controls.

Studies by researchers mainly from University of Tokyo, Japan

The Tokyo group has published two studies investigating the effects of prenatal and postnatal BPA exposure on behaviour, either at dose levels of 4 - 400 mg/kg/day, or 0.1 mg/kg/day.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in F344 rat (Negishi *et al.* 2003). Groups of 8 or 9 mated females were dosed orally by gavage with 0 (olive oil vehicle control), 4, 40 or 400 mg/kg/day BPA from gd 10 to pnd 20. Maternal bodyweights, litter size and pup bodyweights were recorded. Eight pups/sex/group were randomly selected for necropsy at 8 weeks of age and selected organ weights were recorded. Three behavioural evaluations were conducted in all offspring: spontaneous motor activity at about 4 weeks of age, an active avoidance test at about 4 or 8 weeks of age and open field behaviour at about 8 weeks of age. Spontaneous activity was measured using a heat sensor mounted above the examination cage, over 12 hours during a dark phase of the daily light cycle. The active avoidance test was conducted in a two compartment shuttle box. The animals were each subjected to 50 daily trials, with 50 seconds between each trial, on 3 consecutive days. For each trial a 5 second buzzer and light stimulus (the conditioned stimulus) was provided, followed by a 5 second electric shock (the unconditioned stimulus) delivered through the cage floor. A correct response was recorded when the animal moved to the other compartment during the 5 second buzzer/light stimulus. Open field behaviour was monitored over 5 minutes, during a dark phase of the daily cycle. Grooming, locomotion, stretching, rearing, or other activity was analysed using a computer assisted system. The

report did not state whether the order of testing was counterbalanced with respect to treatment group. It is not clear from the report if the statistical analysis was conducted using the litter or the individual pup as the experimental unit.

Maternal weight gain at 40 and 400 mg/kg/day was significantly reduced throughout most of the treatment period. The bodyweights of male offspring at 400 mg/kg/day were slightly, though significantly, lower than controls throughout much of the lactation (by ~10% on pnd 21) and post-lactation periods. For male offspring at 40 mg/kg/day and females at 40 and 400 mg/kg/day there were occasional statistically significantly lower bodyweights. Organ weights were not affected by maternal BPA treatment.

Spontaneous motor activity, in terms of activity 'counts', was not influenced by maternal BPA treatment; the authors drew attention to longer periods of inactivity in the BPA groups, but these differences did not follow a dose-related pattern. In the shuttle box active avoidance test, at 4 weeks of age the males at 40 and 400 mg/kg/day recorded a significantly greater proportion of correct responses, but a consistent dose-related response was not apparent across the 1st, 2nd and 3rd days of the trial. At 8 weeks of age the proportion of correct responses at 40 and 400 mg/kg/day was similar to the controls, although at 4 mg/kg/day the proportion of correct responses was lower than controls on the 1st day of the trial. Active avoidance behaviour for the females was similar for all four experimental groups. In the open field test there were no inter-group differences in locomotion, stretching, rearing or other behaviour. However, the proportion of time spent grooming for males at 4 mg/kg/day was significantly greater than the controls, but in the absence of a dose-related response this difference was considered unlikely to be due to BPA exposure.

The effect of prenatal exposure to BPA on behaviour of males was investigated in the F344 rat (Negishi *et al.* 2004). Groups of 10 or 11 mated females were dosed orally by gavage with 0 (corn oil vehicle control) or 0.1 mg/kg/day BPA from gd 3 to pnd 20. One male pup/litter was selected for a series of behavioural tests. These were: open field at 8 weeks of age, spontaneous activity at 12 weeks, passive avoidance at 13 weeks, elevated plus-maze at 14 weeks, active avoidance at 15 weeks and a monoamine-disruption test (a comparison of open field behaviour following intraperitoneal injections of 0.9% saline and the monoamineoxidase inhibitor trans-2-phenylcyclopropyl-amine hydrochloride (Tcy)). The report did not state whether the order of testing was counterbalanced with respect to treatment group.

There were no effects on maternal or pup bodyweights. Behaviour in the open field and elevated-plus maze, and spontaneous activity were not affected by BPA exposure. In the passive avoidance test there were no statistically significant differences between the control and BPA group for any of the parameters recorded, although the BPA exposed rats tended to delay entry into a dark compartment in the retention trial. In the active avoidance test BPA-treated offspring showed significantly fewer correct avoidance responses during the 1st, 2nd and 3rd of the four training sessions (each comprising of 25 trials), indicating slower learning. Also, among BPA-treated offspring there was an increase in the number of failures to respond to both the conditioned stimulus (buzzer and light) and unconditioned stimulus (electric shock) compared with the controls (2.5% failures, vs. ~0.3 in controls). In the monoamine disruption test, BPA exposed males failed to show the significant increase in the proportion time in locomotion behaviour in response to Tcy that was seen in controls (14 vs. 24%). However, a Tcy-induced significant reduction in rearing frequency among controls was also seen in the BPA group.

Studies by researchers associated with Kushi academic institutes, Fukuoka, Japan

The Kushi group has conducted five studies, investigating the effects of prenatal and postnatal BPA exposure at maternal exposure levels of 0.002 - 1.5 mg/kg/day on behaviour or brain structure.

The effect of prenatal and neonatal exposure to BPA on behaviour and brain development was investigated in the Wistar rat (Kubo *et al*. 2001). Groups of 5 mated females were administered BPA via the drinking water at 0 (vehicle control) or approximately 1.5 mg/kg/day during gestation and the lactation period. Open field behaviour was investigated in offspring (11-14/group, sex and litter distribution not reported) at 6 weeks of age. The report did not state whether the order of testing was counterbalanced with respect to treatment group. A passive avoidance test was conducted at 7 weeks (11-14/group). At 20 weeks of age the volumes of the sexually dimorphic nucleus of the preoptic area (SDN-POA) and locus coeruleus were measured (6-7/sex/group). It was not clear if the statistical analysis was conducted using the litter or individual pup as the experimental unit.

In the open field, the distance moved, rearing frequency and time spent in centre of the field were all significantly greater for control females than control males. In the passive avoidance test the latency to enter the dark chamber following a shock was significantly longer in control males compared with control females of the same group. For the BPA group, these gender-related differences were not present. This was due to differences in the behaviour of both sexes; the three open field parameters were higher and the passive avoidance test latency period shorter for BPA males in comparison with control males, and vise versa for the BPA females. However, there were no statistically significant differences when the results for BPA males and females were compared with their gender controls. The volume of the SDN-POA in the control group was significantly greater in males than females, and a similar difference was also present in the BPA group. In contrast, a gender difference observed in the control group for the volume of the locus coeruleus (~14% greater for females) was reversed in the BPA group (volume was greater for males). However, it should be noted that the difference between control males and BPA males was not statistically significant, and neither was the difference between control females and BPA females.

Kubo *et al*. (2003) investigated the effect of prenatal and neonatal exposure to BPA on behaviour and brain development in a second study. Groups of 5-6 mated female Wistar rats were administered BPA via the drinking water at 0 (vehicle control), 0.03 or 0.3 mg/kg/day (estimated intakes). As positive controls, groups of 5 mated females received either diethylstilbestrol (~0.0065 mg/kg) or resveratrol (~1.5 mg/kg/day) via the drinking water. The animals were probably exposed throughout gestation and the lactation period, although the report is not clear on this. Open field testing was conducted at 6 weeks of age on 24 animals/group; the sex and litter distribution of the tested pups was not reported. Sexual behaviour of males (in the presence of a receptive untreated female) and females (in the presence of a sexually vigorous untreated male) was assessed at 11-12 weeks of age in 7-13 animals/sex/group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. At 14 weeks of age the volume of the SDN-POA and locus coeruleus was measured (7-8/sex/group). Also, the number of neurones in the locus coeruleus was estimated. The study was conducted as 3 replicated blocks. The data were analysed using the individual pup as the experimental unit.

As with the previous Kubo *et al*. (2001) study, in the open field the distance moved, rearing frequency and time spent in centre of the field for control females were all significantly

greater than control males. With the exception of the distance moved in the BPA 0.03 mg/kg/day group where the normal significant sex difference was present, these sex differences were less marked and did not achieve statistical significance in the BPA group. The masking of the sex difference was due primarily to a greater distance moved and rearing frequency among BPA males and a reduced amount of time spent in the centre for the BPA females, in comparison with their gender controls. With regard to the assessment of sexual behaviour there was no evidence of a treatment-related effect in either males or females. In the diethylstilbestrol group, the open field distance moved, rearing frequency and time spent in centre of the field were significantly increased for both sexes. In the resveratrol group there were no effects on open field behaviour. Consistent with the Kubo *et al*. (2001) study, the volume of the SDN-POA was similar in both the control and BPA exposed groups and the gender difference observed in the control group for the volume of the locus coeruleus (greater for females) was reversed in the BPA group. However, a 'conventional' dose response relationship was not apparent for the locus coeruleus differences as the extent of the reversal was greater in the 0.03 mg/kg/day group. Differences in the locus coeruleus neurone count matched those observed for volume of this brain. In both the diethylstilbestrol and resveratrol groups the normal locus coeruleus sex difference was reversed but SDN-POA was not affected.

Fujimoto *et al*. (2006) conducted a range of behavioural testing in the offspring of mothers exposed to BPA during the last week of gestation. Groups of 6 Wistar rats received BPA via the drinking water from gd 13 to pnd 0 at 0 (vehicle control) or approximately 0.015 mg/kg/day. The following behavioural tests were conducted in the offspring (20-24/sex/group in each test): open field at 6 weeks of age, elevated plus maze at 7 weeks, passive avoidance at 8 weeks and forced swimming test at 9 weeks. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably conducted using the individual pup as the experimental unit.

In the open field test, treatment related differences were observed for rearing behaviour. The duration of rearing was significantly greater for BPA males (by ~50%) in comparison with the control males. Among the control offspring, the rearing frequency and duration was significantly greater for the females as compared with males, but this gender difference was not present in BPA group, due to both a comparative increase in rearing activity in males and a slight decrease in females of the BPA group. BPA treatment had no affect on the other parameters measured in the open field test. Behaviour in the elevated plus maze and passive avoidance test was similar for controls and the BPA group. In the forced swimming test, the duration of immobility was significantly greater (by ~75%) and the duration of limb movements was significantly less (by ~8%) for BPA males compared with control males. For females, the duration of diving was significantly greater (by ~28%) in the BPA group. Also, the significant gender difference in duration of struggling seen in the controls (longer for females) was not seen in the BPA group, due to both a comparative increase in duration for males and a slight decrease for females of the BPA group.

Kawai *et al*. (2003) investigated the effect of prenatal exposure on 'aggressive' behaviour in male CD-1 mice. Groups of 7-9 females were dosed by the oral route, using a micropipette, with BPA at 0 (corn oil vehicle control), 0.002 and 0.02 mg/kg/day from gd 11-18. 'Aggression' testing was conducted 8, 12 and 16 weeks of age in groups initially comprising of 26-32 male offspring randomly selected from each treatment group. Each male was placed in a cage with an 'opponent' mouse (a male specially selected from the control group, used for testing once/day) and their behaviour was observed for 7 mins. The report did not state whether the order of testing was counterbalanced with respect to treatment group. About 10

of the test mice from each group were killed at 9, 13 and 17 weeks of age for testes weight and serum testosterone level measurement. It was not clear from the report whether the statistical analysis had been conducted using the litter or the individual pup as the experimental unit.

'Aggression' scores, as determined by contact time with the 'opponent', were significantly increased (about 2-fold) at 8 weeks at both 0.002 and 0.02 mg/kg/day, compared to the control group at 8 weeks. No effect on aggression score was seen at 12 and 16 weeks. The lack of a conventional dose response relationship and absence of an effect at older ages suggests that the increased aggression score at 8 weeks was not related to BPA treatment.

Kawai *et al*. (2007) investigated the effect of prenatal exposure to BPA on the expression of ERα and ERβ on male ICR mice. Groups of 18 pregnant females were dosed by the oral route, using a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.002 mg/kg/day from gd 11-17. The pups were weaned pnd 21. At 4-5, 8-9 or 12-13 weeks of age, 8-12 randomly selected male offspring for each group were killed. Serum testosterone levels were measured. Brains were removed and processed for immunostaining with antibody to ERα, ERβ, serotonin and serotonin transporter. Immumoreactivity levels in sections of the dorsal raphe nucleus were assessed. It is not clear from the report if the analysis took account of litter effects.

The number of neurons expressing ERα and ERβ was significantly greater (by ~40-140%) in the BPA group at 5 and 13 weeks but not at 9 weeks, in comparison with controls. There were no differences in the numbers of serotonin immunoreactive neurones or in the immunoreactivity of serotonin transporter in the BPA group. Overall, this study suggested that prenatal BPA exposure might influence the numbers of ERα and ERβ in the brain of males, but in the absence of differences at 9 weeks of age this evidence is questionable.

Studies from Kyoto Prefectural University of Medicine

Two studies, investing brain structure using immunohistochemical techniques, were conducted by the Kyoto group.

Nakamura *et al*. (2006) investigated the effect of prenatal exposure to BPA on brain (neocortex) development in ICR/Jcl mice. Females (group size not reported) were dosed by subcutaneous injection with BPA at 0 (sesame oil vehicle control) 0.02 mg/kg/day from gd 0 to 16. Pregnant mice received an intraperitoneal dose of 0.5 mg 5-bromo-2'-deoxyuridine on either gd 10, 12, 14, or 16; the embryos were removed either 1 hour or 2-3 days later (10 embryos/group). The embryonic forebrains were dissected out and processed for immunohistochemistry using a range of primary antibodies. Morphometric analysis of the neocortex was conducted on 10 embryos from two or more dams/group/day of kill. Also, the expression of various genes within the forebrain was determined by RT-PCR.

BrdU-labelled cells examined 1 hour after BrdU injection showed no differences between the BPA and control groups, which indicated that the proliferation of precursor cells was not affected. The BrdU-labeled cells, analysed 2 days after BrdU injection, were decreased in the ventricular zone of BPA group killed on gd 14 and 16, whereas they were increased in the cortical plate at gd 14, compared with controls. Furthermore, the expression of Math3, Ngn2, Hes1, LICAM, and THRalpha was significantly upregulated at E14.5 in the BPA-treated group. According to the authors, these results suggest that BPA might disrupt normal neocortical development by accelerating neuronal differentiation/migration.

Tando *et al*. (2007) investigated the effect of prenatal and neonatal BPA exposure on brain development in ddY mice. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 4.5 or 1200 mg/kg/day during gestation and lactation until pnd 21. At 8-11 weeks of age offspring were killed and the brains were removed and processed for immunohistochemical detection of the following proteins: tyrosine hydroxylase (assessed in the substantia nigra), calbindin D-28 K, calretinin, and parvalbumin (assessed in the somatosensory cortex). Also, cell death was assessed using terminal transferase dUTP nick end labelling (TUNEL) staining.

The distribution and density of immunopositive staining for calbindin D-28K, calretinin, and parvalbumin (regarded as markers for GABAergic neurons) in the BPA groups was similar to controls. The volume and density of tyrosine hydroxlyase-positive (regarded as a marker for dopaminergic neurons) nuclei and fibres in the substantia nigra were reduced (by about 18%) in female offspring at 4.5 mg/kg/day BPA (but not at 1200 mg/kg/day), compared with controls. TUNEL staining did not reveal the presence of degeneration or cell death.

Studies from other Institutes

Five independent studies from other institutes, investigating the effects of BPA on various aspects of neurodevelopment, are available.

The effect of prenatal and postnatal exposure to BPA on behaviour was investigated in a conventional (similar to OECD test guideline 416) 2-generation study (Ema *et al*. 2001). Groups of 25 male and female Sprague Dawley rats (F_0 generation) were administered BPA by oral gavage at 0 (water vehicle control). 0.0002, 0.002, 0.02 or 0.2 mg/kg/day for 10 (males) or 2 (females) weeks prior to mating. Dosing of the females continued throughout gestation and lactation. At weaning, 1-2 F_1 pups/sex/litter were retained for continued dosing, as for their respective parents. At 5-6 weeks of age, 1 pup/sex/litter was observed in an open field for 3 min. At 6-7 weeks of age, 6 pups/sex/group was tested in a water-filled T-maze; time from start of run to touching the escape ramp and number of errors was recorded. The results were not presented in the report, but the authors stated that there were no BPA-related differences in open field behaviour or T-maze performance.

The effects of neonatal exposure to bisphenol A on Morris water maze performance were investigated in F344 rats (Carr *et al.* 2003). Groups of 10 neonatal rats of each sex were dosed orally, by gavage, with 0 (safflower oil vehicle control), 0.1 or 0.25 mg/kg/day BPA dissolved in safflower oil from pnd 1 to pnd14. An additional group of 10 neonates of each sex was similarly dosed with 17β-estradiol (E_2) at 0.072 mg/kg/day. Litter-mates were assigned to treatment groups such that there was one member of each treatment group for each sex in each litter.

On pnd 33 the rats were tested in a straight swimming channel, 150 cm in length. A trial consisted of placing each rat facing the wall at one end of the channel and the time taken to swim to a wire escape ramp at the other end of the channel was recorded. Each rat was subjected to 4 trials on one day, with a one minute interval between each trial. On pnd 34, Morris water maze testing commenced. The report did not state whether the order of testing was counterbalanced with respect to treatment group.

There were no treatment-related effects on bodyweight or on performance in the straight channel swimming test. In the Morris maze there were no statistically significant differences

between the control and BPA treated groups for the mean time taken to find the escape platform during the acquisition phase. However, in the memory retention test the time spent in the escape quadrant was less (23 vs. 29 seconds for males, 14 vs. 22 seconds for females) for animals from the BPA 0.25 mg/kg/day group when compared with controls, with the difference achieving statistical significance for the females. This was interpreted by the authors as indicating an impairment of the retention of spatial memory in the BPA group. For the E_2 group the only significant difference relative to controls was a prolonged time for males to find the platform on the 3rd day of the acquisition phase.

The effect of prenatal and neonatal exposure to BPA on numbers of corticotrophin releasing hormone (CRH) neurones in the brain was investigated using immunohistological techniques in the Wistar rat (Funabashi *et al*. 2004). Groups of 8-11 mated females received BPA via the drinking water during gestation and until weaning on gd 21 at 0 (vehicle control) or approximately 2.5 mg/kg/day. The study was conducted as two series of experiments, the results of which were combined. Offspring (8-11/sex/group were killed at between 4 and 7 months of ages for brain examination; females were killed in the pro-oestrus phase of the oestrous cycle. It was not clear from the report if 1/sex/litter was selected, or if control and exposed animals of each gender were killed at similar ages. The numbers of CRH-immunoreactive neurones in the anterior and posterior areas of the bed nucleus of the stria terminalis (BST) and in the preoptic area were counted.

In both the anterior and posterior BST the numbers of CRH-immunoreactive neurones for each sex were not significantly different in comparison with their respective controls. However, the statistically significant gender difference in numbers of neurones in the control group (numbers were about 2-fold higher in females) was not seen in the BPA exposed group. This was due to the presence of relatively higher numbers of neurones in males and lower numbers females. In the preoptic area there were no differences between the control and BPA exposed group in the numbers CRH-immunoreactive neurones.

Honma *et al*. (2006) investigated the effect of prenatal and neonatal exposure to BPA on brain development in female Sprague-Dawley rats. Groups of 6 mated female rats were dosed orally, by gavage, with BPA at dose levels of 0 (corn oil vehicle control), 4 or 40 mg/kg/day from gd 6 to pnd 20, when the offspring were weaned. Female offspring were killed, usually 4-6/sex/group, at 1, 3, 6 or 9 weeks of age and the brains were processed for analysis. The levels of the following neurotransmitters in a number of areas of the brain were analysed by HPLC: noradrenalin, dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid, serotonin and its metabolite 5-hydroxyindoleacetic acid, acetyl choline and its precursor/metabolite choline. No differences were reported at 1 week of age. At 3 weeks, levels of the dopamine metabolites, serotonin and its metabolites were increased in some brain areas of BPA exposed offspring. At 6 and 9 weeks of age there were few statistically significant differences in the BPA groups when compared with the controls. Overall, there were no discernable patterns to the variations of neurotransmitter levels and no conclusions could be drawn from this study.

Ryan and Vandenbergh (2006) investigated the effect of prenatal and neonatal exposure to BPA on aspects of the non-reproductive sexually dimorphic behaviour of ovariectomised female C57/B1/6 mice. Ovariectomised animals were used because, according to the authors, the potential confounding effects of oestrous cycling in sexually dimorphic behaviours are eliminated. Groups of mated females (group size probably 14-16) were dosed orally, by gavage, with 0 (corn oil vehicle control), 0.002 or 0.2 mg/kg/day BPA from gd 3 to pnd 21, when the offspring were weaned. Another group was similarly dosed with 0.005 mg/kg/day

ethinyl oestradiol. Litter size, anogenital distance, and pup bodyweight were recorded at weaning. At pnd 28 one randomly chosen female pup from each litter was surgically ovariectomised and retained for behavioural testing, which commenced at pnd 44. The following behavioural tests were conduced on 14-16 females/group: elevated plus maze, light-dark preference chamber (regarded as tests for anxiety), radial arm maze and modified Barnes maze (regarded as tests of spatial memory). The report did not state whether the order of testing was counterbalanced with respect to treatment group. The day on which cornified cells were first seen in a vaginal smear (regarded as onset of puberty) was recorded.

There were no effects of treatment on litter size, anogenital distance, and pup bodyweight at weaning. The onset of puberty was significantly earlier in the BPA 0.2 mg/kg/day group (by 4.5 days). There were no statistically significant differences in the parameters recorded in the elevated plus maze for the BPA groups, in comparison with the vehicle controls. In the light-dark preference chamber, the time spent in the light chamber was significantly less (by 52%) than vehicle controls at 0.2 mg/kg/day, indicating higher levels of anxiety. Performance in the spatial memory tests for the BPA groups was similar to that of the vehicle controls. In the ethinyl oestradiol group, time spent in the open arms of the elevated plus maze and in the light chamber was decreased and the number of errors in the radial arm and Barnes mazes was increased, regarded by the authors an indicative of a masculinisation of behaviour.

Weight of evidence assessment of developmental neurotoxicity studies

This assessment focuses on the reliability and consistency of the evidence. To help the reader, a summary table of the available developmental neurotoxicity studies has been added below.

Table 4.19 Summary of developmental neurotoxicity studies, using oral route unless otherwise stated

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Studies by linked researchers mainly from Universities of Florence, Siena, Rome, Calabria and Parma, Italy					
Farabollini *et al.* 1999 Rat n = 9 - 11	0, 0.04 mg/kg (prior to conception - pnd 21, 0.4 mg/kg (gd14 - pnd 6), by micropipette	Hole board, elevated-plus maze	Anxiety-related behaviour increased in hole board test in males and females. Anxiety-related behaviour decreased in elevated-plus maze in males.	Possibly inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques
Farabollini *et al.* 2002 Rat n = 8 - 13	0, 0.04 mg/kg, gd 0 - pnd 21, by micropipette. Cross fostering to create prenatal and post natal exposure groups	'Sociosexual' behaviour	Slight intensification of sexual behaviour in females. Slightly reduced sexual performance in males	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Aloisi *et al.* 2002 Rat n = 7	0, 0.040 mg/kg, by pipette, gd 0 - pnd 21. Cross fostering to create prenatal and post natal exposure groups	Response to pain (caused by sc injection of formalin) at 22 weeks of age	Response very marginally diminished	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Dessi-Fulgheri *et al.* 2002 Rat n = 9-11	0, 0.04 (prior to conception-pnd 21), 0.4 mg/kg (gd 14 - pnd 6), by pipette	Play behaviour	Differences in 4 out of 8 types of behaviour, in both sexes.	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Facciolo *et al.* 2002 Rat n = ?	0, 0.04, 0.4 mg/kg, oral, exposure period not stated	Effects on somatostatin receptor subtype 2 (sst_2) in limbic region of brain	Expression of some sst_2 states increased	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	
Facciolo *et al.* 2005 Rat n = 12	0, 0.04, 0.4 mg/kg by pipette, 8 d pre-mating-pnd 21 lactation	Effects of expression of somatostatin receptor subtype 3 (sst_3) mRNA in brain of females	Expression of sst_3 reduced in some areas and increased in an other. Differences were increased in presence of $\alpha GABA_A$ agonist.	Possibly inappropriate statistical methods. A mechanistic study of limited value for hazard assessment.	

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Adriani *et al.* 2003 Rat n = 9	0, 0.04 mg/kg, gd 0 - pnd 21, by micropipette	Novelty seeking, impulsivity. Open field with and without amphetamine challenge	Reduced novelty seeking behaviour in females and decreased impulsive behaviour in males. Partial inhibition of amphetamine stimulated open field activity. No open field differences without challenge.	Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques. Analyis of results used appropriate statistical unit
Porrini *et al.* 2005 Rat n = 10	0, 0.04 mg/kg, gestation - pnd 21, by micropipette	Play behaviour in females	Some aspects of female behaviour differed	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Della Seta *et al.* 2006 Rat , pups, n = ~25 males	0, 0.04 mg/kg, pnd 23 - 30, micropipette	Males juvenile play (response to black PVC tube placed in cage), sexual behaviour	Some marginal differences in play and sexual behaviour	Possibly inappropriate statistical methods. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Palanza *et al.* 2002 Mouse, n = 9 (F_0)	0, 0.01 mg/kg, gd 14 -18 to F_0 and F_1 females, by micropipette	Maternal nursing behaviour of F_1	No convincing evidence of an effect on nursing behaviour.	Possibly inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques
Laviola *et al.* 2005 Mouse n = 10 - 12	0, 0.01 mg/kg, gd 11 - 18	*d*-amphetamine-reinforcing effects at pnd 60 using conditioned place preference testing	Conditioned place preference not present in females. Males not affected	Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques. Analyis of results used appropriate statistical unit
Ceccarelli *et al.* 2007 Rat, pups n = 14	0, 0.04 mg/kg, pnd 23 - 30, oral	ERα levels in three sexually dimorphic region of the brain on pnd 37 and 90	Increase in ERα levels in medial preoptic area of females at pnd 37, but not at pnd 90. No differences in ERα levels among males	Single BPA treatment group. A mechanistic study of limited value for hazard assessment.	Analyis of results used appropriate statistical unit
Studies by researchers associated with Hoshi University, Japan					

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Suzuki *et al.* 2003 Mouse n = ?	0, 0.4, 100, 250 mg/kg bwt, in diet, mating to pnd 21	Male offspring: methamphetamine-induced rewarding effects and hyperlocomotion. G-protein activation in limbic forebrain, expression of dopamine D_1 receptor mRNA.	Preference for methamphetamine-associated compartment. Increased locomotion. G-protein activation increased and dopamine D_1 receptor mRNA upregulated at high dose level.	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	Behavioural testing conducted according to acceptable techniques.
Mizuo *et al.* 2004a Mouse n = ?	0, 2, 500, 2000 ppm in diet (~ 2.5, 60, 250 mg/kg), gest & neonatal period	Male offspring: morphine-induced rewarding effects and hyperlocomotion. G-protein activation and expression of μ-opioid receptor mRNA.	Preference for morphine-associated compartment at 60 and 250 mg/kg. Increased locomotion at 250 mg/kg. No effect on G-protein activation and mRNA	Possibly inappropriate statistical methods. Group size not reported.	Behavioural testing conducted according to acceptable techniques.
Mizuo *et al.* 2004b Mouse n = ?	0, 2000 ppm in diet (250 mg/kg) gd 0 - weaning	Male offspring: functional changes in dopamine D3 receptors	Attenuation of dopamine D3 receptor-mediated G-protein activation by 7-OH-DPAT in the mouse limbic forebrain. Decrease in D_3 receptor density	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	
Narita *et al.* 2006 Mouse n = ?	0, 0.006, 0.06, 0.6, 100, 400 mg/kg/day, in diet, gest and lact timing not reported	Effects on dopaminergic system- place conditioning and locomotion test, [^{35}S]GTPγS binding assay in response to morphine	Potentiation of dopamine receptor-dependent neurotransmission	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	Behavioural testing conducted according to acceptable techniques.
Narita *et al.* 2007 Mouse n = ?	0, 400 mg/kg/day, gd 0 - 7, 7 - 14, 14 - 20 or pnd 0 - 20	Males: effects on dopaminergic system- place conditioning and locomotion test, [^{35}S]GTPγS binding assay in response to morphine	Potentiation of dopamine receptor-dependent neurotransmission in gd 7-14 and pnd 0-20 groups, but not for gd 0-7 or gd 14-21	Possibly inappropriate statistical methods. Group size not reported. Single BPA treatment group. A mechanistic study of limited value for hazard assessment. Report poorly written.	Behavioural testing conducted according to acceptable techniques.
Studies from Chemical Industry Institute for Toxicology, USA					

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Kwon *et al.* 2000 Rat n = 8	0, 3.2, 32, 320 mg/kg/day, gavage, gd 13- pnd 21	SDN-POA volume	No effects		Analyis of results used appropriate statistical unit
Patisaul *et al.* 2006 Rat, pups n = 5 - 8	100 mg/kg/day pnd 1 - 2, subcutaneous route	Brain immunohistochemistry at pnd 19	Interference with normal testosterone associated masculinisation of the anteroventral periventricular nucleus	Single BPA treatment group. Small group size. A mechanistic study of limited value for hazard assessment.	Analyis of results used appropriate statistical unit
Patisaul *et al.* 2007 Rat, pups n = ?	66 mg/kg/day, pnd 1 - 2, subcutaneous route	Brain immunohistochemistry as adults	No effects on SDN-POA volume. Increase in calbindin +ve nuclei.	Single BPA treatment group. Group size not reported. A mechanistic study of limited value for hazard assessment.	Analyis of results used appropriate statistical unit
Studies by researchers mainly from University of Tokyo, Japan					
Negishi *et al.* 2003 Rat n = 8-9	0, 4, 40, 400 mg/kg, gavage, gd 10 - pnd 20	Spontaneous motor activity, active avoidance, open field	No consistent effects on behaviour.	Possibly inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques.
Negishi *et al.* 2004 Rat n = 8 – 10	0. 0.1 mg/kg, gavage, gd 3 - pnd 20	Males: spontaneous motor activity, passive and active avoidance, open field, elevated-plus maze, monoamine disruption test	Differences in active avoidance behaviour.	Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques. Analyis of results used appropriate statistical unit
Studies by researchers associated with Kushi academic institutes, Fukuoka, Japan					
Kubo *et al.* 2001 Rat n = 5	0, 1.5 mg/kg (some uncertainty), drinking water gd 0 - pnd 21	Open field, passive avoidance testing, also volume of SDN-POA and locus ceruleus, serum hormone levels	Anxiety-related behaviour slightly reduced and avoidance memory increased in males, opposite effect in females. Normal locus ceruleus volume gender difference reversed	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques.
Kubo *et al.* 2003 Rat n = 5	0, 0.03, 0.3 mg/kg, drinking water, probably gd 0 - pnd 21	Open field, sexual behaviour, volume of SDN-POA and locus ceruleus.	Anxiety-related behaviour slightly reduced in males and slightly increased in females. Normal locus ceruleus volume gender difference reversed.	Inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques.

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Fujimoto *et al.* 2006 Rat n = 6	0, 0.015 mg/kg drinking water , gd 13 - pnd 0	Open field, elevated plus maze, passive avoidance, forced swimming test, 20-24/gp tested	Open field rearing activity increased in males, decreased in females. Some differences in forced swimming test. No effects in elevated plus maze or passive avoidance tests.	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques.
Kawai *et al.* 2003 Mouse n = 7 - 9	0, 0.002, 0.02 mg/kg, gd 11-17, by micropipette	Aggressive behaviour in males, at 8, 12, 16 weeks of age	No convincing dose-related effects on aggression.	Possibly inappropriate statistical methods. Small group size.	
Kawai *et al.* 2007 Mouse n = 19	0, 0.002 mg/kg, gd 11 - 17, by micropipette	Brain expression of ERα and ERβ, in males	Number of neurones expressing ERα and ERβ increased at 5 and 13 weeks of age, but not at 9.	Single BPA treatment group. Statistical methods not clear. A mechanistic study of limited value for hazard assessment.	Analyis of results used appropriate as statistical unit
Studies from Kyoto Prefectural University of Medicine					
Nakamura *et al.* 2006 Mouse n = ?	0, 0.02 mg/kg, gd 0 - 16, subcutaneous route	Brain immunohistochemical investigation	Evidence of accelerated neuronal differentiation	Single BPA treatment group. Group size not reported and statistical approach not clear. A mechanistic study of limited value for hazard assessment.	
Tando *et al.* 2007 Mouse n = ?	0, 4.5, 1200 mg/kg/day, gd 0 - pnd 21, dietary	Brain immunohistochemical investigation for tyrosine hydroxylase, calbindin D-28 K, calretinin, and parvalbumin proteins	Reduction in number of tyrosine hydroxylase-positive nuclei and fibres in the substantia nigra among females exposed to the low dose. Other parameters not affected.	Group size not reported and statistical approach not clear. A mechanistic study of limited value for hazard assessment.	
Studies from other institutes					
Ema *et al.* 2001 Rat n = 25	0, 0.0002, 0.002, 0.02, 0.2 mg/kg/day, gavage, prenatal, postnatal	Conventional 2-generation study, with added endpoints for open field and water-filled T maze	No effects on open field behaviour or T-maze performance declared by authors	Actual results of behavioural testing not included in report.	GLP study. Behavioural testing conducted according to acceptable techniques. Analyis of results used appropriate statistical unit.

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Carr *et al.* 2003 Rat, pups, n = 10/sex	0, 0.1, 0.25mg/kg/day, pnd 1 - 14, gavage	Morris water maze	Reduction in memory retention in males and females at 0.25 mg/kg		Behavioural testing conducted according to acceptable techniques. Analyis of results used appropriate statistical unit
Funabashi *et al.* 2004 Rat n = 8-11	0, 2.5 mg/kg drinking water, prenatally and to pnd 21	Immunohistochemistry investigations of brain	Reduction in normal gender difference in numbers of corticotrophin-releasing hormone neurones in bed nucleus of stria terminalis	Possibly inappropriate statistical methods. Single BPA treatment group. A mechanistic study of limited value for hazard assessment.	
Honma *et al.* 2006 Rat n = 6	0, 4, 40 mg/kg/day, gd 6-pnd 20, gavage	Neurotransmitter levels in various regions of brain at 1, 3, 6, 9 weeks in female offspring	Results had no discernable pattern	Possibly inappropriate statistical methods. Small group size. A mechanistic study of limited value for hazard assessment.	
Ryan and Vandenbergh 2006 Mouse n = 14-16	0, 0.002, 0.2 mg/kg/day, gd 3 - pnd 21, gavage	Ovariectomised female offspring investigated in elevated plus maze, light-dark preference chamber, radial arm and modified Barnes mazes	No differences in special memory tests. Increased anxiety in light-dark preference testing.		Behavioural testing conducted according to acceptable techniques. Analyis of results used appropriate statistical unit.

n = group size gd = gestational day pnd = postnatal day

Reliability

A number of weaknesses in the available BPA developmental neurotoxicity studies can be identified.

With the exceptions of a conventional (similar to OECD test guideline 416) 2-generation study by Ema *et al*. (2001) and the behavioural studies of Della Seta *et al*. (2006) and Ryan and Vandenbergh (2006), the studies generally used relatively small group sizes, typically 7-10 litters. For some studies (Suzuki *et al*. 2003, Mizou *et al*. 2004, Narita, 2006. 2007, Tando *et al*. 2007, Patisaul *et al.* 2007) the group size was not stated in the report. Although an important influence of small group size is to reduce the power of the study to detect an effect, and as such is less of issue for a study that claims a treatment-related effect, this does reduce confidence in the reliability of the study.

For many studies involving maternal dosing and the selection of more than one pup/sex/litter for testing the statistical analysis appeared to have been conducted using the individual pup as the experimental unit. This is inappropriate because it is the litter, not the individual pup, which has been randomly assigned to the treatment groups. The failure to use the litter as the experimental unit will inflate the number of independent observations considered in the statistical analysis and increase the risk of a false positive (Type 1 error) outcome. For some studies it was not stated whether the litter or individual pup was selected as the experimental unit. Also, it is not clear from many of the study reports whether the statistical analysis took account of the non-independence of repeated measures in the same individual, as recommended in draft OECD Guideline 426 (Developmental neurotoxicity study). Failure to take account of this non-independence will, again, increase the risk of Type 1 errors.

All the study reports were obtained from the published peer-reviewed scientific literature and reporting was therefore necessarily brief. Thus, comprehensive information on the experimental conditions, methods, results and natural background variation of the parameters measured are not available. While this does not invalidate the studies, the capacity of the reader to critically appraise each study and assess the validity of the authors' conclusions may be restricted, and in such cases have a negative influence on confidence in each study.

With exception of the 2-generation study by Ema *et al*. (2001), none of the studies declared a compliance with the principles of GLP. Again, this does not invalidate the studies, but confidence in their reliability might have been greater if they had been conducted according to GLP.

When differences between control and treated groups are observed in toxicology studies, the results will usually be examined for dose-response relationships. The presence of consistent dose-response relationships will increase confidence that the observed differences were caused by the experimental treatment and not due to natural variation. A number of studies, notably most of the behavioural studies from the Italian team (Farabollini *et al*. 2002, Aloisi *et al.* 2002, Adriani *et al*. 2003, Della Seta *et al*. 2006, Palanza *et al*. 2002, Laviola *et al*. 2005), used just one exposure level of BPA and so there is no opportunity to evaluate observed differences between control and treated groups in the light of a dose response assessment. Consequently, confidence in the validity of claims of a causal effect of BPA exposure is reduced.

Developmental neurotoxicity testing is a relatively new and not fully established area in regulatory toxicity (evidenced by the fact that an OECD Guideline for developmental neurotoxicity testing has not yet been finalised) and therefore experience in the conduct and interpretation of the studies is limited. However, many of the behavioural tests conducted in the BPA studies are established tests that have been used extensively for a number of years in pharmacological research and are the types of tests (with the exception of those involving pharmacological challenge) recommended by draft OECD Guideline 426. These tests are considered relevant to human health. Concerning the brain receptor expression and immunohistochemical investigations, these techniques do not have an established role in regulatory toxicology and should be regarded tools for mode of action and mechanistic investigation rather than hazard identification tests.

Overall, many limitations have been identified. The reliability of the development neurotoxicity database is considered to be low because of the collective impact of these limitations.

Consistency of evidence: behavioural endpoints

The effect of prenatal and postnatal exposure to BPA on various aspects of behaviour (locomotory and exploratory activity, grooming, cognitive, emotional, social, sexual, response to pain and response to pharmacological challenge) have been investigated in rats or mice in 22 studies. A large range of maternal exposure levels, 0.0002-400 mg/kg/day, were investigated. The consistency of evidence for a genuine treatment-related effect on each of these behavioural endpoints will be considered in turn.

Open field testing for general locomotory and exploratory activity, grooming and anxiety was conducted in the following studies: Ema *et al*. (2001), Kubo *et al*. (2001), Kubo *et al*. (2003), Adriani *et al*. (2003), Negishi *et al*. (2003), Negishi *et al*. (2004) and Fujimoto *et al*. 2006. No effects were reported by Ema *et al*. (2001), in which exposure was to 0.0002-0.2 mg/kg/day, throughout the prenatal and postnatal periods. This study used the largest group size (n = 25 litters) of all the BPA studies reviewed and as such is likely to be the most reliable, but the weight that can be given to this study is limited because of incomplete reporting. The two Kubo *et al*. (2001 and 2003) studies used higher exposure levels of 0.03-1.5 mg/kg/day, dosing throughout gestation and lactation, and reported a decrease in anxiety-related behaviour (time spent away from the centre) in males and an increase in females, although the differences were slight. Adriani *et al*. (2003) reported no effects in open field testing following a saline injection in animals exposed during the prenatal and neonatal period at 0.004 mg/kg/day. Negishi *et al*. (2003) found no effects on open field behaviour at the relatively high exposure levels 4-400 mg/kg/day during the prenatal and postnatal periods. Also, Negishi *et al*. (2004) found no effects on open field behaviour of males (females not tested) at the lower exposure level of 0.1 mg/kg/day during the same periods. Finally, Fujimoto *et al*. (2006) reported increased rearing activity in males and females exposed to 0.0015 mg/kg/day during late gestation. Overall, there appears to be no convincing evidence of a consistent BPA-related effect on open field behaviour

Anxiety and related behaviour was also tested by other methods in the following studies: Farabollini *et al*. (1999, hole board test, elevated plus maze), Negishi *et al*. (2004, elevated plus maze), Fujimoto *et al*. (2006, elevated plus maze, forced swimming test), and Ryan and Vandenbergh (2006, elevated plus maze, light-dark preference chamber). Farabollini *et al*. (1999) reported increased anxiety-related behaviour in males and female rats in a hole board

test, and decreased anxiety-related behaviour in the elevated plus maze in males following prenatal and neonatal exposure to 0.04 or 0.4 mg/kg/day. Negishi *et al*. (2004) reported no changes in anxiety related behaviour in male rats (females not tested) at 0.1 mg/kg/day during the prenatal and neonatal periods. Fujimoto *et al*. (2006) also found no changes in anxiety related behaviour in males and female rats exposed to the very low exposure level of 0.0015 mg/kg/day during late gestation. Ryan and Vandenbergh (2006) found that anxiety behaviour was increased in females (males not tested) at 0.2 mg/kg/day prenatally and neonatally, but not at 0.002 mg/kg/day. Thus, the studies by Farabollini *et al*. (1999) and Ryan and Vandenbergh (2006) provide evidence of increased anxiety in rats (males and females, hole board test) and mice (females), respectively, at doses levels of 0.04-0.4 mg/kg/day, but evidence of decreased anxiety in male rats (elevated plus maze) was also seen in the study of Farabollini *et al.* (1999) and no evidence of an effect on anxiety in males was reported by Negishi *et al*. (2004) at similar dose levels. Overall, there does not appear to be a consistent pattern across species and gender in the results of the tests for anxiety.

Cognitive (learning and memory) testing was conducted in the following studies: Ema *et al*. (2001, T water maze), Kubo *et al*. (2001, passive avoidance), Negishi *et al*. (2003, active avoidance), Negishi *et al*. (2004, active and passive avoidance), Fujimoto *et al*. (2006, passive avoidance) and Carr *et al*. (2003, Morris water maze). No effects were reported by Ema *et al*. (2001), in which exposure was to 0.0002-0.2 mg/kg/day throughout the prenatal and postnatal periods. Kubo *et al*. (2001) reported an enhancement of avoidance memory in males, with the opposite effect in females at 0.03 or 0.3 mg/kg during the prenatal and neonatal periods. Negishi *et al*. (2003) found no consistent effects in active avoidance testing at the relatively high exposure levels of 4-400 mg/kg/day. Negishi *et al*. (2004) tested only males, exposed to 0.1 mg/kg/day during the prenatal and neonatal periods, and found no differences in a passive avoidance test. However, avoidance memory appeared to be decreased in an active avoidance test. Fujimoto *et al*. (2006) reported no effects in a passive avoidance test in males and females exposed to the very low dose of 0.0015 mg/kg/day during late gestation. Carr *et al*. (2003) noted changes in the memory testing phase in males and females exposed to 0.25 mg/kg/day during the neonatal period, but not at 0.1 mg/kg/day, indicative of an effect on spatial memory retention. Overall, a consistent picture does not emerge from the cognitive testing results.

Other aspects of behaviour investigated were as follows: Farabollini *et al*. (2002) investigated social and sexual behaviour in rats exposed to 0.04 mg/kg/day and noted some marginal differences, interpreted as a slight intensification of sexual behaviour in females and a slightly reduced sexual performance in males. Aloisi *et al*. (2002) investigated the response to pain in rats exposed to 0.04 mg/kg/day during the prenatal and neonatal periods and reported only very slight differences. Adriani *et al*. (2003) also conducted behavioural tests in rats also exposed to 0.04 mg/kg/day during these periods and reported changes interpreted as reduced novelty seeking in females and increased impulsivity in males. Porrini *et al*. (2005) used an identical exposure regime and noted some difference in play behaviour in females (males not tested). Dessi-Fulgheri *et al*. (2002) reported some differences in the play behaviour of rats exposed to 0.04 mg/kg during the prenatal and neonatal periods or 0.4 mg/kg/day during the late prenatal and early neonatal periods. Della Seta *et al*. (2006) exposed 3-4 week old juvenile male rats to 0.04 mg/kg/day and reported only very marginal changes in play and, at a later age, sexual behaviour. In mice, Palanza *et al*. (2002) found no convincing evidence of an effect on maternal nursing behaviour in females exposed during the prenatal period and/or as adults at 0.01 mg/kg/day. Kawai *et al*. (2003) reported no convincing effect on aggressive behaviour in male mice exposed during the late prenatal period to 0.002 or 0.02 mg/kg/day

BPA. These studies looked at different aspects of behaviour so it is not possible to assess the study results for consistency. However, it is noted that the reduced novelty seeking seen in females (Adriani *et al*. 2003) was not confirmed by changes in open field behaviour in females in the same study or in other studies.

A number of other studies investigated the behavioural response to pharmacological challenge. The aim of these studies was to detect possible effects of BPA on the organisation of the brain at the receptor or neurotransmitter level. Adriani *et al*. (2003) reported a partial inhibition of an amphetamine-stimulated increase in male rats, but not females, exposed to 0.04 mg/kg/day during the prenatal and neonatal period. Laviola *et al*. (2005) found that conditioned place preference for an amphetamine-associated compartment was abolished in female mice, but not in males, exposed to 0.01 mg/kg/day during late gestation. Negishi *et al*. (2004) found that a monoamine oxidase inhibitor (Tcy)-stimulated increase in open field activity was reduced in male rats (females not tested) exposed to 0.1 mg/kg/day during the prenatal and neonatal periods. Suzuki *et al*. (2003) found that the stimulating effects on locomotion of methamphetamine (which has very similar actions to amphetamine) and place preference for a methamphetamine-associated compartment was enhanced in male mice (females not tested) exposed to higher levels of BPA, 0.4-250 mg/kg/day, during the prenatal and neonatal periods. As both amphetamine and Tcy cause an increase in the levels of dopamine and noradrenalin, the results of the Adriani *et al*. (2003) and Negishi *et al*. (2004) studies can be regarded as showing a consistent effect. However, there is an inconsistency with the study of Laviola *et al.* (2005) because females only, rather than males, were affected. Suzuki *et al*. (2003) reported an enhancement, rather than an inhibition, of methamphetamine response, which is another inconsistency.

Several studies have investigated the behavioural response to morphine challenge. Mizou *et al*. (2004a) found a place preference for a morphine-associated compartment in male mice (females not tested) exposed 60 or 250 mg/kg/day BPA during the prenatal and postnatal periods, although the μ-opioid receptor activity was not affected. Narita *et al*. (2006, 2007) also reported some evidence for place preference for a morphine-associated compartment in mice exposed to the 0.006-400 mg/kg/day during the prenatal and neonatal periods. However, because of the absence of a conventional dose response relationship and lack of consistency of response within the Narita *et al.* (2007) study, the interpretation of these results is uncertain. Overall, the consistency of results for effects on the behavioural response to morphine cannot be assessed because of limited investigation.

Consistency of evidence: receptor expression in brain

Effects of BPA exposure at the receptor level have been investigated in nine studies.

Facciolo *et al.* (2002, 2005) reported some changes in expression of somatostatin receptor subtypes 2 and 3 following maternal exposure to 0.04 or 0.4 mg/kg/day, but these endpoints have not been investigated in other independent studies.

ER expression has been investigated in two recent studies, but the extent of investigation is too limited to assess consistency. Ceccarelli *et al*. (2007) reported an increase in ERα levels in the medial preoptic area of brain in females exposed to BPA at 0.04 mg/kg/day from pnd 23-30. Kawai *et al*. (2007) reported an increase in brain expression of ERα and ERβ following prenatal exposure to a maternal BPA level of$_3$ 0.002 mg/kg/day at 5 and 13 weeks of age but, inconsistently, not at 9 weeks.

The possible influence of prenatal and postnatal BPA exposure on different aspects of the central dopaminergic system was investigated in five studies conducted by the Hoshi University group. Suzuki *et al.* (2003) reported an upregulation of dopamine D_1 receptors in the male limbic forebrain at maternal exposure levels of 400 mg/kg/day in a study that also reported a BPA-associated enhanced response to methamphetamine. Mizou *et al*. (2004a) found no evidence of an effect on μ-opioid receptor expression in males. Mizuo *et al*. (2004b) found that the response to a dopamine D_3 agonist and D_3 receptor density in the male brain were both decreased at a maternal BPA dose level of 250 mg/kg/day. Narita *et al*. (2006, 2007) reported evidence of an activation of dopamine receptor mediated G-protein, but the interpretation of these results is uncertain because of the absence of a conventional dose response relationship and lack of consistency of response within the Narita *et al.* (2007) study. Overall, the extent of investigation of possible effects on the central dopaminergic system is too limited to allow an assessment for consistency

Consistency of evidence: brain morphology and brain chemistry

SDN-POA volume was measured in five studies. Kwon *et al*. (2000) found no effects following perinatal and neonatal exposure at maternal dose levels of 3.2-320 mg/kg/day. Patisaul *et al.* (2006, 2007) reported no effects on SDN-POA volume following subcutaneous administration at 100 mg/kg on pnd 1 and 2. At a lower range of maternal exposure levels, 0.03-1.5 mg/kg/day, Kubo *et al*. (2001, 2003) also reported no effects on SDN-POA volume. Kubo *et al*. (2001, 2003) also measured the volume of the locus coeruleus and reported a reversal of the normal gender difference in both studies. However, a conventional dose response relationship was not present and control group comparisons did not achieve statistical significance, so it is possible that these differences were due to chance rather that BPA exposure.

The following studies examined aspects of brain development that were not looked at in other studies: Funabashi *et al*. (2004) found differences in the numbers of CRN neurones; Honma *et al.* (2006) investigated neurotransmitter levels in various regions of the brain; Tando *et al.* (2007) reported reduced numbers of dopaminergic neurons in the substantia nigra at maternal exposures of 4.5 mg/kg/day and no differences in the numbers of GABAergic neurones in the cerebral cortex in females at 4.5 or 1200 mg/kg/day. Nakamura *et al.* (2006) reported evidence of accelerated neuronal differentiation at a maternal exposure, using the subcutaneous route, of 0.02 mg/kg/day.

Overall, with the exception of SDN-POA volume, the extent of brain morphology and brain chemistry investigations are very limited and so consistency between studies cannot be assessed.

Conclusions to weight of evidence assessment of developmental neurotoxicity studies

Confidence in the reliability of the developmental neurotoxicity database is low because of limitations in the design and reporting in all of the available studies. These limitations include small group size, inappropriate statistical analysis, brief reporting of methods and results, lack of compliance with GLP and use of one BPA dose level. The receptor/neurotransmitter level studies are regarded as mode of action or mechanistic investigations and cannot be used as the primary support for conclusion regarding the hazardous properties of BPA.

The consistency assessment shows that there is no discernable and reproducible pattern to the behavioural testing results. Most of the studies investigating effects at the receptor/neurotransmitter level and brain morphology have not been replicated by independent laboratories; so consistency cannot be assessed.

Overall, taking together the low confidence in the reliability of the developmental neurotoxicity studies and the lack of consistency in the results of behavioural testing, no conclusions can be drawn from these studies[3]. This opinion is very similar to that of EFSA (2006), who reviewed nine of the developmental neurotoxicity studies.

Developmental effects on female reproductive tract expressed in old-age.

In a study designed to investigate the effects of BPA on the development of the reproductive tract, groups of 24 neonatal CD-1 mice received subcutaneous injections of BPA at dose levels of 0 (corn oil vehicle control), 0.01, 0.1 or 1 mg/kg/day from pnd 1 to 5 (Newbold *et al.* 2007) The neonatal mice were drawn from the pooled offspring of an unspecified number of dams. The neonates were randomly fostered to litters each comprising of 8 female pups per dam. It was not stated whether fostered litter-mates received the same experimental treatment. The female mice were weaned on pnd 21 and maintained without further experimental treatment until sacrifice at 18 months of age. The uterus and ovaries/oviducts were removed and processed for examination by light microscopy. At 18 months, 18, 23, 20 and 16 out of 24 females from the control, 0.01, 0.1 and 1 mg/kg/groups, respectively, survived. Group mean bodyweights at 18 months were similar. The incidence of histopathological changes in the ovaries and uterus is presented below:

Table 4.20 Incidence (%) of histopathological changes in reproductive tract (Newbold et al. 2007)

Histopathological finding		Dose level (BPA mg/kg/day)			
		Control	0.01	0.1	1
Ovary/ oviduct	Presence of corpora lutea	100	96	90	88
	Ovarian cysts	39	35	70*	38
	Parovarian cysts	0	4	10	6
	Progressive proliferative lesion of oviduct	0	13	15	6
Uterus	Cystic endometrial hyperplasia	6	22	45**	25
	Adenomyosis	6	9	20	19
	Wolffian duct remnants in uterine wall	0	13	10	19
	Leiomyoma	0	4	10	6
	Atypical hyperpasia	0	4	5	0
	Stromal polyp	6	4	25	6

[3] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007.

* significantly different from control, Fisher's exact test, $p \leq 0.05$ ** significantly different from control, Fisher's exact test, $p \leq 0.01$

As shown in the above table, the incidence of a number of findings was increased in the BPA-treated groups. In particular, the incidence of cystic endometrial hyperplasia and ovarian cysts was statistically significantly increased at 0.1 mg/kg/day, in comparison with controls. However, these differences did not follow a dose-related pattern and could not therefore be attributable to BPA treatment. It is also noted that these findings are inconsistent with the results of a study by Yoshida et al (2004) (summarised in the carcinogenicity section) in which no effects of transplacental and lactational exposure to BPA (up to 6 mg/kg/day) were seen on ovarian and uterine histopathology in 15 months-old female rats.

Human case-control study

Sugiura-Ogasawara *et al*. (2005) investigated the possible association between recurrent miscarriage and serum BPA levels in a case-control study. A group of 45 women (mean age 31.6 years) with a history of 3 or more consecutive first-trimester miscarriages who attended Nagoya City University Hospital during a 17 month period were selected for the study. The subject selection method was not reported, although it was stated that cases with uterine abnormalities or chromosome abnormalities in either partner were excluded from the study. Thirty-two healthy non-pregnant women (mean age 32.0 years), with no history of pregnancy or infertility served as controls. The occupation of most of the cases was either housewife (20 subjects) or office worker (17 subjects), whereas the control subjects were doctors, nurses and secretaries employed by Nagoya Hospital. Immunological tests for parameters such as antinuclear bodies (ANAs, a sensitive marker for systemic lupus erythematous), antiphospholipid antibodies (aPLs) and natural killer (NK) activity and blood tests for hypothyroidism, diabetes mellitus and hyperprolactinaemia were conducted for all cases, but not for controls. Serum BPA levels were measured using an ELISA method in single fasting blood samples taken from all subjects 5-9 days after ovulation (Sugiura-Ogasawara, 2006).

The mean (± SD) serum BPA levels for the cases were significantly higher than controls (2.59 ± 5.23 vs. 0.77 ± 0.38 ng/ml). However, median BPA levels in the two groups were similar (0.71 vs. 0.705), so whether there was a genuine difference between the groups (as claimed by the author) is questionable. The large SD for cases and similar median values for cases and controls indicates that a small number of women with very high BPA levels were responsible for the higher group mean levels. A comparison of BPA levels within the cases showed that the higher levels of BPA were associated with ANA-positive status (10 cases) but not with hyperthyroidism (8 cases), luteal phase defect (9 cases), hyperprolactinaemia (5 cases) or aPL-positive status (6 cases). Thirty-five cases achieved pregnancies subsequent to the study, of which 17 miscarried again. The mean serum BPA levels for those who miscarried again was higher, though not statistically significantly, than those who did not (4.39 ± 8.08 vs. 1.22 ± 1.07 ng/ml), although the median values for the two groups were similar (0.71 vs. 0.91 ng/ml).

There are a number of limitations to this study (as pointed out by Berkowitz, 2006). Group sizes were relatively small, so the subjects may not be representative of the populations from which they are drawn. The cases and controls were not comparable in terms of occupation and may not have been comparable in terms of the presence of potential confounding factors. The BPA levels for the cases were measured at a time-frame that was not relevant to the induction of their miscarriages. The reliability of the reported BPA measurements is

uncertain because the ELISA method is not optimal for BPA in serum due to cross-reactivity and other problems (Fukata *et al*. 2006, discussed in section 4.1.1.3.2). A parametric statistical test (Welch's test) was used to compare mean BPA levels for the cases and controls, but the distribution of results indicate that use of a non-parametric would have been appropriate. Overall, given the significant limitations in the design and conduct of this study and lack of a convincing difference in serum BPA levels between the cases and controls, no conclusions can be drawn regarding an association between BPA exposure and recurrent miscarriage.

4.1.2.9.3 Impact of new information and summary of reproductive toxicity

The new 2-generation study in mice (Tyl *et al*. 2007) provides a comprehensive, definitive, investigation of the effects of BPA on reproduction at exposure levels spanning the low (µg/kg bw/day) to high (mg/kg bw/day) ranges. This study has shown that BPA causes adverse effect on pregnancy and the offspring, observed as a slightly increased duration of gestation, reduced pup bodyweight during lactation, a slight increase in the incidence of undescended testes at weaning, seminiferous tubule hypoplasia in offspring at weaning, and delayed acquisition of preputial separation, at 600 mg/kg/day, an exposure level that also caused mild parental toxicity. Fertility was not affected by BPA exposure, which resolves the previous uncertainty regarding the the NOAEL for fertility in mice. A study NOAEL for reproductive toxicity of 50 mg/kg/day has been identified. As there was no evidence of an adverse effect on the development of the male reproductive tract at µg/kg bw/day doses of BPA, the study resolves the uncertainties surrounding the potential to produce adverse effects on development at low doses. Thus, a NOAEL of 50 mg/kg/day for reproductive toxicity, which was a provisional position in the original risk assessment report, should be used in the risk assessment.

No conclusions could be drawn from the new developmental toxicity studies[4] or from a human study investigating the possible association between recurrent miscarriage and BPA exposure. Therefore, these studies do not influence the conclusions of the original risk assessment report.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Toxicokinetics: The toxicokinetics of BPA have been well studied in rats both *in vivo* and *in vitro*, and have been investigated to a lesser extent in mice and cynomolgus monkeys. Two studies have investigated the toxicokinetics and fate of an oral dose of labelled BPA in human volunteers.

In the species studied (rats, mice, monkeys, humans), the available evidence suggests that following oral administration, BPA is rapidly and extensively absorbed from the gastrointestinal tract. Analysis of plasma AUC values suggests that the extent of absorption from the GI tract is up to 86% in rats and up to 85% in monkeys. The only relevant human

[4] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

studies suggest that, on the basis of the recovery of labelled BPA-glucuronide from the urine, a relatively low dose of BPA (54-88 μg/kg) was completely absorbed after oral dosing.

An *in vitro* dermal absorption study using human skin found limited absorption of BPA at millimolar concentrations; the extent of absorption was in the region of 10% of the applied dose.

There are no data on the toxicokinetics of BPA following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and the high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first-pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

The available data indicate that BPA is subject to extensive first-pass metabolism following absorption from the gastrointestinal tract.

In all species studied, the major metabolic pathway involves conjugation of BPA to BPA-glucuronide. Studies conducted in rats suggest that in neonates the glucuronidation pathway is more susceptible to saturation than in adults indicating an age-dependent increase in metabolic capacity. *In vitro* studies with microsomal preparations also suggest species differences, with the rank order for the metabolic clearance rate per unit weight of tissue being mice > rats > humans. When the total clearance rates for the whole liver were calculated, the rank order was reversed (humans > rats > mice).

In addition to the glucuronidation pathway, *in vivo* and *in vitro* studies suggest that in the rat, BPA may be subject to limited oxididation to bisphenol O-quinone by cytochrome P450, and also to conjugation to BPA-sulphate and 5-hydroxy-BPA.

A study in pregnant mice given subcutaneous doses of BPA also found that glucuronidation was the major pathway for the metabolism of BPA, although dehydrated, sulphated and methoxylated conjugates of BPA were also produced. Some minor metabolites were double conjugates, such as a double conjugate of BPA with glucuronide and N-acetyl galactosamine which was found in the intestine, placenta, amniotic fluid and foetal tissue. A study in cynomolgus monkeys showed that BPA-glucuronide was the major metabolite, although there was evidence for production of a minor metabolite, possibly BPA-sulphate or 5-hydroxy-BPA. Studies conducted in humans provide evidence for the glucuronidation of BPA in man; some studies also found evidence for the sulphation of BPA.

Most studies investigating the distribution of BPA measured tissue radioactivity levels after giving labelled BPA to experimental animals. An oral dosing study in rats found that the tissue concentrations of BPA-derived-radioactivity were highest in the liver, kidney and carcass, and lowest in the brain and testes, and there were no large differences between adult and neonatal animals. A number of studies in rats suggest that BPA metabolites and especially free BPA have a limited distribution to the embryo/foetal or placental compartments following oral administration. No selective affinity of either yolk sac/placenta or embryo/foetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in a recent study in rats after oral dosing. However, maternal and embryo/foetal exposure to free BPA did occur, but systemic levels were found to be low due to extensive first-pass metabolism.

Regarding the distribution of free, unconjugated BPA to tissues after oral dosing, since free BPA is removed rapidly from the blood after absorption by first pass metabolism, it has been suggested that in rodents the availability of free BPA to extrahepatic tissues is likely to be limited following oral exposure. In adult rats it has been estimated that no more than 5-10% of the administered dose of free BPA is available to the tissues, although this figure may be higher in neonates. In humans, the systemic availability of free BPA is very low as enterohepatic recirculation of BPA does not occur.

In summary, there are differences between humans and rodents in the distribution of BPA. After oral administration, BPA is rapidly metabolised in the gut wall and the liver to BPA-glucuronide. This metabolite is devoid of endocrine activity. In humans, the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion. Due to the rapid biotransformation and excretion ($t_{1/2}$ = 5 hours) and plasma protein binding, peak free BPA concentrations in humans after oral exposure that are available for estrogen receptor binding are very low. In contrast, BPA glucuronide is eliminated in bile in rodents and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic recirculation results in slow excretion ($t_{1/2}$ = 15-22 hours) and increased systemic availability of free BPA in rodents.

This conclusion is supported by the observation that in urine of rats dosed orally with BPA, a part of the dose was excreted as free BPA in urine (1 -4 % of applied dose, whereas BPA-glucuronide in urine accounted for 20-40 % of applied dose). In both of the human studies and the monkey study free BPA was below the limit of detection in all urine and blood samples (equivalent to a ratio of free BPA to BPA-glucuronide of < 0.5 %). Since free BPA found in urine is translocated from blood to urine in the kidney, these observations of higher free BPA levels in urine of rats compared with primates further support the existence of species differences in blood levels of free BPA between rodents and humans with higher AUCs for free BPA in rats.

The major route of elimination in the rat is via the faeces. The available data indicate that the percentage of the administered dose recovered in the faeces is in the range 50% to 83%. Urinary excretion is of secondary importance in the rat, with 13% to 42% of the administered dose being recovered in the urine. Over 7 days post-dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post-dosing. A sex difference was also observed for urinary elimination, with females excreting approximately twice as much radioactivity (24-28%) than males (14-16%). A study in female SD rats found that excretion was not affected by pregnancy at 3 different stages of gestation. Data from a number of studies suggest limited excretion of BPA in the milk. However, the data do not allow a reliable quantitative determination to be made.

Following oral administration to rats, a high proportion of the administered dose (45-66%) was rapidly excreted in the bile in the form of BPA-glucuronide, with the rate of biliary excretion tending to be higher in males than females. Most of the faecal radioactivity was found to be in the form of free BPA. Since BPA has a high oral bioavailability in the rat, the free BPA found in the faeces is more likely to be derived from BPA-glucuronide excreted in the bile and hydrolysed to free BPA in the gastrointestinal tract rather than representing unabsorbed BPA which might have passed along the gastrointestinal tract into the faeces unchanged. Most of the urinary radioactivity was found to be in the form of BPA-glucuronide (82%) with free BPA and BPA-sulphate making minor contributions (14% and 4% respectively).

In contrast to the findings in rodents, in cynomolgus monkeys given BPA orally most of the administered dose (82–85%) was recovered in the urine, with only 2-3% of the dose being recovered in the faeces. In two studies in human volunteers given a low dose of BPA orally, the administered dose was completely recovered in the urine as BPA-glucuronide. No free BPA was detected and no gender differences in the kinetics of BPA-glucuronide in plasma and urine were reported.

Acute toxicity: No useful information is available on the effects of single exposure to BPA in humans. Oral LD_{50} values beyond 2,000 mg/kg are indicated in the rat and mouse, and dermal LD_{50} values above 2,000 mg/kg are evident in the rabbit. Few details exist of the toxic signs observed or of target organs. For inhalation, a 6-hour exposure to 170 mg/m^3 (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed. These data indicate that BPA is of low acute toxicity by all routes of exposure relevant to human health.

Irritation: Limited human anecdotal information of uncertain reliability is available from written industry correspondence suggesting that workers handling BPA have in the past experienced skin, eye and respiratory tract irritation. It cannot be determined whether the reported skin reactions were related to skin sensitisation or irritation. However, a recent well conducted animal study clearly shows that BPA is not a skin irritant. A recent well conducted animal study shows that BPA is an eye irritant; effects persisted until the end of the study (day 28 postinstillation) in 1 of 3 rabbits. Overall, taking into account the animal and human evidence, BPA has the potential to cause serious damage to the eyes.

Slight and transient nasal tract epithelial damage was observed in rats exposed to BPA dust at 170 mg/m^3 for 6 hours. Slight local inflammatory effects in the upper respiratory tract were observed in rats exposed to 50 mg/m^3 and 150 mg/m^3 of BPA in 2 and 13 week repeat inhalation studies, but were not observed at 10 mg/m^3 in the same studies. Increased duration of exposure did not increase the severity of the response at 50 and 150 mg/m^3. Taken together with anecdotal human evidence, these data suggest BPA has a limited respiratory irritation potential.

Skin sensitisation: With respect to skin sensitisation, there are several reports of patients with dermatitis responding to BPA in patch tests. However, it is unclear whether BPA or related epoxy resins were the underlying cause of the hypersensitive state. Anecdotal information indicates skin inflammation in workers handling BPA, although given the uncertain reliability of this information no conclusions can be drawn from it. In animals, the available studies are negative, but the test reports lack detail and no reliable justifications were given for the choice of concentrations used. In the study using the highest challenge concentration, 50% in a guinea pig closed-patch test, a sensitisation rate of 12.5% was obtained. Based on the findings from the most robust study, it appears that BPA may possess a skin sensitisation potential, albeit a limited one.

A recent LLNA study has shown that BPA does not possess skin sensitisation potential. However, in this study the concentration of BPA was not maximised. Therefore, there remains some uncertainty as to whether high concentrations (> 30%) of BPA can still exert skin sensitising activity. Similarly, a recent photo-LLNA has shown that BPA does not possess skin photo-sensitisation potential. However, again, in this study the concentration of BPA employed was not maximised. Although there are sporadic reports showing that BPA in the presence of UV light can elicit skin responses in humans, comprehensive medical surveillance data obtained from BPA manufacture plants has shown that no cases of skin

sensitisation have been identified among approximately 875 employees examined for several years. Due to the nature of these data, although it can be concluded that the risk of skin sensitisation is low under the exposure conditions experienced by these workers, a potential skin sensitisation hazard cannot be completely excluded.

Overall the new information shows that BPA does not possess a skin sensitisation potential under the exposure conditions tested. However, it cannot be excluded that high concentrations (> 30%) of BPA may exert skin sensitising activity and, in the presence of UV light, also photo-reactivity.

Respiratory sensitisation: There are no data from which to evaluate the potential of BPA to be a respiratory sensitiser. However, based on the lack of reports of cases of respiratory sensitisation, there are no grounds for concern for this endpoint.

Repeated dose toxicity: No useful information on the effects of repeated exposure to BPA in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in rats following exposure to 50 mg/m^3 (6 hours/day, 5 days/week for 13 weeks). There was no significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m^3. A NOAEL of 10 mg/m^3 was identified in rats in this 13-week study.

Oral studies in rats and mice have shown that the repeated dose toxicity of BPA involve effects on bodyweight gain, liver and kidney. A NOAEL of 50 mg/kg/day has been identified in multigeneration study in rats and a recent 2-generation study in mice for these effects. This NOAEL rather than the original LOAEL of 120 mg/kg/day for liver effects from the published report is taken forward to the risk characterisation.

There are no animal data available for repeated dermal exposure.

Overall, therefore, the inhalation NOAEL of 10 mg/m^3 for local effects on the respiratory tract and the oral NOAEL of 50 mg/kg/day for systemic effects are taken forward to the risk characterisation of repeated dose toxicity.

Mutagenicity: No human data regarding mutagenicity are available. However, BPA appears to have demonstrated aneugenic potential *in vitro,* positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems there is information that shows BPA disrupts microtubule formation. BPA has been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. However, some deficiencies in the conduct of these studies have been noted and the negative results cannot be taken as entirely conclusive. BPA does not appear to be aneugenic *in vivo*, since a recently conducted, standard mouse bone marrow micronucleus test has given a negative result. BPA was negative in a briefly reported dominant lethal study in rats but, given the limited details provided, this is not regarded as an adequate negative result. The only other data in somatic cells *in vivo* are from a ^{32}P-postlabelling assay, which showed that BPA is capable of producing DNA adduct spots in rat liver following oral administration. These adduct spots were not characterised fully.

Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies, it does not appear that BPA has significant mutagenic potential *in vivo*. Any aneugenic potential of BPA seems to be limited to *in vitro* test systems and is not of concern. The relevance of the finding that BPA can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health.

New information on the mutagenicity of BPA has shown that BPA produces an increase in congression failure, a misalignment of chromosomes during the metaphase stages of meiosis II in oocytes of female mice. However, in view of several methodological weaknesses and flaws identified in these new data along with the reporting inadequacies, and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis. Furthermore, these findings have not been confirmed in more recent publications. Therefore, the original conclusion from the published assessment that BPA has no significant mutagenic potential *in vivo*, is still valid.

Carcinogenicity: There are no human data contributing to the assessment of whether or not BPA is carcinogenic. In animals, BPA has not shown any significant carcinogenic activity in two standard oral cancer bioassays in rats and mice. No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, BPA did not exhibit properties that raise concern for potential carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m^3 in a 13 week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m^3.

New information on the potential carcinogenic and/or promoting effects of BPA in prenatal and neonatal rat models indicates that BPA does not possess significant carcinogenic potential and does not exert promoting activity on the carcinogenesis induced by established carcinogens/initiators in specific organs. Taking into account all of the animal data available the evidence suggests that BPA does not have carcinogenic potential.

Reproductive toxicity: BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with BPA glucuronide *in vitro.* It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories. Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of BPA on fertility and reproductive performance have been investigated in four good quality, comprehensive, studies: a 2-generation study in the rat, a multigeneration study in the rat, a continuous breeding study in the mouse and a 2-generation study in the mouse. Although no effect on fertility was seen in the rat 2-generation study, low-dose levels were employed (0.2-200 μg/kg/day). In the multigeneration study, an effect on fertility (reduction

in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse continuous breeding study, it is prudent to assume that BPA may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg/day and below in the multigeneration study. In the mouse 2-generation study, using dose levels of 0.003-600 mg/kg/day, no effects on fertility, reproductive organ weights and histopathology or sperm production were observed. However, the continuous breeding study in the mouse provided some evidence of an adverse effect on fertility. In the F_0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F_1 generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F_1 generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F_0 generation) there was no effect on epididymal weight. Furthermore, there were no effects on epididymal weight in the mouse two generation study. Overall, a NOAEL of 50 mg/kg/day can be identified for effects on fertility based on the rat multi-generation study.

No evidence that BPA is a developmental toxicant was observed in standard developmental toxicity studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day, respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1,000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F_1-F_3). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg. Similarly, effects on F_1 (but not F_2) pup bodyweight gain were observed in the mouse two generation study at 600 mg/kg/day, a dose level that also caused mild parental toxicity. Additionally, there was an increase in the incidence of undescended testes and seminiferous tubule hypoplasia in F_1 and F_2 offspring at weaning at 600 mg/kg/day, although similar effects were not seen in adult F_1 males. No adverse effects were seen at 50 mg/kg/day and below in the mouse study.

Several additional studies have focused on the potential of BPA to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at low dose levels, in the range 2–50 µg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. Also, no effects on male reproductive tract development were observed in a recent mouse two generation study, which was conduced specifically to help resolve the uncertainties surrounding the potential for BPA to affect development at low doses. Giving most weight to the negative ‘gold standard’ mouse two generation study, and taking account of the fact that there were no functional changes in reproductive parameters or reproductive organ

development at low dose levels in the rat multigeneration and two generation studies, it is concluded that BPA does not have an adverse effect on male reproductive tract development at low dose levels.

The effect of prenatal and perinatal exposure to BPA on neurological development has been investigated in a large number of recent studies. Many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. Although a number of these studies claimed to have detected a BPA-related effect on development, no firm conclusions could be drawn about developmental neurotoxicity because of a low level of confidence in the reliability of the studies and a lack of consistency in the results.[5]

Also, no conclusions could be drawn from a human study investigating the possible association between recurrent miscarriage and BPA exposure.

Thus, a NOAEL of 50 mg/kg/day can be identified for developmental toxicity, taken from the rat multigeneration study and the mouse two-generation study.

Summary: Overall, the hazardous properties of BPA have been evaluated in animals to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure and reproductive toxicity have been identified. No dose response information is available for eye irritation. A NOAEL of 10 mg/m^3 has been identified for repeated dose toxicity to the respiratory tract. A NOAEL of 50 mg/kg has been identified for systemic effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg has been established in multi- and two-generation studies for effects on fertility and development.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

To conduct the risk characterisation for workers and consumers, it is necessary to compare human exposure for the inhalation/dermal route with oral N(L)OAELs from repeated dose animal studies, because of the absence of significant inhalation/dermal toxicity data. A direct comparison between exposure and systemic effects (with the exception of effects on the liver) must take account of the first pass effect, which has been shown to limit systemic bioavailability by the oral route (see toxicokinetic section). To compensate for this limited oral bioavailability (shown to be around 5-10% of the administered dose in rodents – see toxicokinetic section), the oral animal N(L)OAELs have been reduced by a factor of 10 for the comparison of inhalation or dermal exposure and systemic effects. For effects on the

[5] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007.

liver, adjustment for the first-pass effect is inappropriate as these effects are not related to the systemic availability of free unconjugated BPA, but to the dose of BPA delivered to the liver, which is approximately the same for the oral route (only 10% higher) and the inhalation and dermal routes.

The toxicological starting points for the risk characterisation of BPA have been derived from animal studies. Therefore, to conduct the risk characterisation, extrapolation from animals to humans needs to be performed. New kinetic information in humans and primates has shown that at comparable exposure levels the blood concentrations of free BPA in humans are much lower than those in rodents, indicating that there are important quantitative differences in the fate of BPA between humans and rodents. Following absorption, BPA is metabolised in the liver to BPA-glucuronide, which is devoid of endocrine activity. In humans, the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion. Due to the rapid biotransformation and excretion ($t_{1/2}$ = 5 hours) and plasma protein binding, peak free BPA concentrations in humans that are available for estrogen receptor binding are very low. In contrast, in rats, BPA glucuronide is eliminated in bile and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic recirculation results in slow excretion ($t_{1/2}$ = 15-22 hours) and increased systemic availability of free BPA in rats. Free BPA has been shown both in vitro and in vivo to have biological (endocrine) activity. Its glucuronide is devoid of endocrine activity. Therefore, it appears reasonable to assume that free BPA is the toxic entity responsible for the different systemic effects (repeat dose effects and reproductive effects) of BPA.

It is proposed that in view of this evidence, for systemic effects (except effects on the liver), the default factor of 2.5 for remaining (kinetic and dynamic) interspecies differences (draft TGD, 2005) is reduced to 1. A reduction of the allometric scaling factor does not seem appropriate, as the observed species differences in the elimination and fate of BPA are not related to the basal metabolic rate. Although a precise quantification of this factor is not possible because no information is available on potential dynamic interspecies differences, a comparison of the elimination half-life in humans and rats indicates that rats would be 3-4 times more sensitive than humans to the effects of BPA, and hence humans would be 0.25-0.3 times more sensitive than rats, on the basis of kinetic differences. For effects on the liver, the rat-human differences in the systemic availability of free unconjugated BPA are unimportant, and hence, for these effects a reduced interspecies factor for remaining differences is not appropriate.

It should also be noted that, although some of the toxicological starting points derive from the mouse for which no oral kinetic data are available, the kinetics of BPA in mice are such that the mouse-human interspecies differences are likely to be even more pronounced than those between rats and humans. In vitro data and in vivo mouse data by the subcutaneous route of administration indicate that, while glucuronidation of BPA seems to be the major pathway of BPA biotransformation in rats, in mice, oxidation products of BPA have been identified after low-dose administration, suggesting possible formation of metabolites with higher oestrogenic potency. It should also be noted that there are major species differences between the mouse and the human, both in the physiology of gestation and in their toxicodynamic sensitivity to oestrogens, the mouse being particularly sensitive to oestrogens, which could predispose that species to sensitivity to weak oestrogens such as BPA. Therefore, the likely high sensitivity of the mouse to oestrogens has to be considered when using that particular species as a model for the risk assessment of BPA in humans.

4.1.3.2 Workers

The health effects of concern for BPA relevant to workers are eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure, and reproductive toxicity. There are no concerns for acute toxicity, skin irritation, respiratory sensitisation, mutagenicity and carcinogenicity and hence conclusion (ii) is drawn for these endpoints.

In order to carry out the risk characterisation for workers, the following assumptions have been made; the body weight of the average worker is 70 kg and the worker inhales 10 m^3 air per working day.

Eye irritation

No clinical signs of eye irritation were reported in an acute inhalation study in rats following exposure to 170 mg/m^3 BPA for 6 hours. However, anecdotal human evidence, albeit limited, suggests that eye irritation can occur following occupational exposure to BPA. A recent well conducted animal study showed that irritation was observed following instillation of BPA into rabbit eyes. However, provided good occupational hygiene practices are in operation, eye irritation is unlikely to be expressed. Conclusion (ii) is proposed for all scenarios.

Respiratory tract irritation

Anecdotal human evidence suggests that respiratory irritation has been reported in workers, though there are no quantitative details. In an acute inhalation study, slight and transient nasal tract epithelium damage was observed in rats following exposure to 170 mg/m^3 (the only concentration tested and the highest attainable concentration) for 6 hours. A NOAEL for these effects was not identified in this study. However, a NOAEL of 10 mg/m^3 was identified in rats from a 13-week repeated exposure study. Overall, it is considered that BPA may have limited respiratory tract irritation potential that should be considered for risk characterisation in particular in relation to short-term peak exposures. The Margins of Safety (MOS) between the NOAEL of 10 mg/m^3 and the worst case inhalation short-term exposure for each occupational scenario are shown in Table 4.21. The MOS values are evaluated by comparison with the minimal MOS (12.5). In Table 4.21a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.21 Margins of Safety for respiratory tract irritation

Scenario	Worst-case inhalation short-term exposure (mg/m^3)	MOS for respiratory tract irritation based on NOAEL of 10 mg/m^3	Conclusion
Manufacture of BPA	6	1.7	(ii)
Manufacture of PC	0.5	20	(ii)
Manufacture of articles from PC	negligible	Very high	(ii)
Manufacture of epoxy resin	4	2.5	(ii)
Powder coating manufacture	0.3	33	(ii)
Powder coating use	negligible	Very high	(ii)
Thermal paper manufacture	< 4	> 2.5	(ii)

Tin plating manufacture	negligible	Very high	(ii)

Table 4.21a Assessment factors applied for the calculation of the minimal MOS for respiratory tract irritation in workers from rat data

Uncertainty	Assessment factor
Interspecies differences[a]	2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	12.5

[a] for local effects the allometric scaling factor is not applicable

For all scenarios except manufacture of BPA and manufacture of epoxy resins, the MOS values are greater than the minimal MOS of 12.5. Therefore, these MOS are sufficient to conclude that respiratory tract irritation from exposure to short-term peaks will not occur, and conclusion (ii) is drawn. For manufacture of BPA and manufacture of epoxy resins, the MOS values of 1.7 and 2.5 respectively are lower than the minimal MOS of 12.5. This would normally lead to concern. However, considering that the MOSs were calculated comparing a NOAEL derived from a 13–week study and exposures of 6 h/day with 15 minutes peaks, and that the effects at the LOAEL were minor, conclusion (ii) is reached. These conlusions are consistent with the recent IOELV (Indicative Occupational Expousre Limit Value) of 10 mg/m^3 (8h-TWA) established by SCOEL (Scientific Committee on Occupational Exposure Limits) for BPA and the lack of a STEL value (SCOEL SUM 113, May 2004).

Skin sensitisation

Overall, while the data do not exclude a skin sensitising activity of BPA at high concentrations (> 30%), there is no evidence that this is a concern for workers in current BPA manufacturing plants (such workers are believed to represent the group most likely to be exposed to BPA dust). Consequently, repeated skin contact with high concentrations of BPA may result in dermatitic responses. In order to avoid this, skin exposure with high concentrations of BPA should be controlled for all exposure scenarios. Therefore, conclusion (iii) is drawn.

Repeated dose toxicity

Local effects on the respiratory tract

The principal effect following repeated exposure to BPA was slight local inflammatory effects in the upper respiratory tract. A NOAEL of 10 mg/m^3 was identified in rats for respiratory irritation from a 13-week study. It is particularly noted that the effects seen at 50 mg/m^3 were slight and that an increase in the exposure concentration to 150 mg/m^3 produced only a slightly greater response, indicating a shallow dose-response curve. Furthermore, extending the duration of exposure from 2 weeks to 13 weeks at these exposure concentrations had only marginal effects. The MOSs between this NOAEL of 10 mg/m^3 and the worst case inhalation 8h-TWA exposures for each occupational scenario are shown in

Table 4.22. The MOS values are evaluated by comparison with the minimal MOS (12.5). In Table 4.22a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.22 Margins of Safety for repeated dose local effects on the respiratory tract

Scenario	Worst-case inhalation 8h-TWA exposure (mg/m^3)	MOS for repeated dose local effects on the respiratory tract based on NOAEL of 10 mg/m^3	Conclusion
Manufacture of BPA	3	3.3	(ii)
Manufacture of PC	0.001	10,000	(ii)
Manufacture of articles from PC	0.001	10,000	(ii)
Manufacture of epoxy resin	0.7	15	(ii)
Powder coating manufacture	0.01	1,000	(ii)
Powder coating use	0.5	20	(ii)
Thermal paper manufacture	0.1	100	(ii)
Tin plating manufacture	0.05	200	(ii)

Table 4.22a Assessment factors applied for the calculation of the minimal MOS for repeated dose local effects on the respiratory tract in workers from rat data

Uncertainty	Assessment factor
Interspecies differences[a]	2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	12.5

[a] for local effects the allometric scaling factor is not applicable

For all scenarios except manufacture of BPA, the MOS values are greater than the minimal MOS of 12.5. Therefore, for these scenarios there is no concern and conclusion (ii) is drawn. For the manufacture of BPA the MOS value of 3.3 is lower than the minimal MOS of 12.5. This would normally lead to concern. However, it is considered sufficient (and thus, conclusion (ii) applies) for the following reasons:

- only minor effects were observed at the LOAEL and there is a shallow dose response curve;
- rats are obligate nasal breathers, therefore the amount deposited in nasal turbinates in humans would be less than in rats for comparable exposures;
- The lack of a lifetime inhalation study is not considered a concern as extending the duration of exposure from 2 weeks to 13 weeks had only marginal effects.

These conlusions are consistent with the recent IOELV (Indicative Occupational Expousre Limit Value) of 10 mg/m^3 (8h-TWA) established by SCOEL (Scientific Committee on Occupational Exposure Limits) for BPA (SCOEL SUM 113, May 2004).

Systemic effects

Oral studies in rats and mice have shown that the repeated dose toxicity of BPA involves effects on bodyweight gain, liver and kidney. An oral NOAEL of 50 mg/kg/day has been identified in a recent 2-generation study in mice for these effects (occurring at the next dose level of 600 mg/kg/day).

Effects on bodyweight gain and kidney

The MOSs between the NOAEL of 50 mg/kg (equivalent to an internal extrapolated NAEL of 5 mg/kg to account for first pass effect following oral exposure) for effects on bodyweight and kidney and the worst case body burdens arising from inhalation and dermal exposure for each scenario are shown in Table 4.23. The MOS values are evaluated by comparison with the minimal MOS (<35). In Table 4.23a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.23 Margins of safety for repeated dose toxicity (systemic effects on bodyweight and kidney)

Scenario	RWC inhalation 8h-TWA exposure (mg.m^{-3})	RWC inhalation body burden* (mg.kg^{-1}.day^{-1})	RWC dermal exposure# (mg.day^{-1})	RWC dermal body burden+ (mg.kg^{-1}.day^{-1})	RWC combined body burden (mg.day^{-1})	MOS for repeated dose systemic effects on bodyweight and kidney based on NAEL of 5 mg.kg^{-1}.day^{-1}	Conclusion
Manufacture of BPA	3	0.43	42	0.06	0.49	**10**	**(iii)**
Manufacture of PC	1x10^{-3}	1x10^{-4}	0.04	6x10^{-5}	1.6x10^{-4}	3x10^{4}	(ii)
Manufacture of articles from PC	1x10^{-3}	1x10^{-4}	0.04	6x10^{-5}	1.6x10^{-4}	3x10^{4}	(ii)
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3	**4**	**(iii)**
Powder coating manufacture	0.01	1x10^{-3}	2	3x10^{-3}	4x10^{-3}	1,250	(ii)
Powder coating use	0.5	0.07	2.3	3x10^{-3}	0.07	71	(ii)
Thermal paper manufacture	0.1	0.01	42	0.06	0.07	71	(ii)
Manufacture of tin plating	0.05	7x10^{-3}	42	0.06	0.07	71	(ii)

additive							

* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m^3 air inhaled per working day.

Taking into account area exposed

+ Assuming 10% absorption by the dermal route

Table 4.23a Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects on bodyweight and kidney in workers from mouse data

Uncertainty	Assessment factor
Interspecies differences[a]	7 x 1
Intraspecies differences	5
Differences between experimental conditions and exposure[b]	1
Dose response / Type of critical effect [c]	<1
Confidence of the database	1
Overall	<35

[a] Allometric scaling factor for the mouse x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1)

[b] Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. In a mouse 2-year study a LOAEL of 120 mg/kg/day was identified for minor effects on body weight gain.

[c] As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day and the effects at the LOAEL are rather minor, an AF lower than 1 is considered appropriate.

For all scenarios except manufacture of BPA and manufacture of epoxy resins the MOS values are greater than the minimal MOS (<35). These MOSs are considered sufficient to conclude that adverse repeated dose systemic effects will not occur in these scenarios. Therefore conclusion (ii) is drawn for these scenarios. For manufacture of BPA and manufacture of epoxy resins, MOS values of 10 and 4 have been calculated. These MOSs are significantly lower than the minimal MOS (<35). There is concern that adverse repeated dose systemic effects on bodyweight and kidney might occur in these scenarios, and thus, conclusion (iii) is drawn.

Effects on the liver

The MOSs between the NOAEL of 50 mg/kg for effects on the liver (for these effects, adjustment for the first-pass effect is inappropriate as liver effects are not related to the systemic availability of free unconjugated BPA, but to the dose of BPA delivered to the liver, which is approximately the same for the oral route and the inhalation and dermal routes) and the worst case body burdens arising from inhalation and dermal exposure for each scenario are shown in Table 4.24. The MOS values are evaluated by comparison with the minimal MOS (<87.5). In Table 4.24a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.24 Margins of safety for repeated dose toxicity (systemic effects on the liver)

Scenario	RWC inhalation	RWC inhalation	RWC dermal	RWC dermal	RWC combined	MOS for repeated	Conclusion

	8h-TWA exposure ($mg.m^{-3}$)	body burden* ($mg.kg^{-1}.day^{-1}$)	exposure# ($mg.day^{-1}$)	body burden+ ($mg.kg^{-1}.day^{-1}$)	body burden ($mg.day^{-1}$)	dose systemic effects on the liver based on NOAEL of 50 $mg.kg^{-1}.day^{-1}$	
Manufacture of BPA	3	0.43	42	0.06	0.49	102	(ii)
Manufacture of PC	$1x10^{-3}$	$1x10^{-4}$	0.04	$6x10^{-5}$	$1.6x10^{-4}$	$3x10^{5}$	(ii)
Manufacture of articles from PC	$1x10^{-3}$	$1x10^{-4}$	0.04	$6x10^{-5}$	$1.6x10^{-4}$	$3x10^{5}$	(ii)
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3	**40**	**(iii)**
Powder coating manufacture	0.01	$1x10^{-3}$	2	$3x10^{-3}$	$4x10^{-3}$	12,500	(ii)
Powder coating use	0.5	0.07	2.3	$3x10^{-3}$	0.07	710	(ii)
Thermal paper manufacture	0.1	0.01	42	0.06	0.07	710	(ii)
Manufacture of tin plating additive	0.05	$7x10^{-3}$	42	0.06	0.07	710	(ii)

* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m^3 air inhaled per working day.

Taking into account area exposed

+ Assuming 10% absorption by the dermal route

Table 4.24a Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects on the liver in workers from mouse data

Uncertainty	Assessment factor
Interspecies differences[a]	7 x 2.5
Intraspecies differences	5
Differences between experimental conditions and exposure[b]	1
Dose response / Type of critical effect [c]	<1
Confidence of the database	1
Overall	<87.5

[a] Allometric scaling factor for the mouse x default factor for remaining uncertainties (for effects on the liver, the rodent-human differences in the systemic availability of free unconjugated BPA are unimportant, and hence, for these effects a reduced interspecies factor for remaining differences is not appropriate.)

[b] Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. A NOAEL of 50 mg/kg bw/d was identified in parental generations in subchronic reproductive toxicity studies in the mouse. The LOAEL was 600 mg/kg bw/d. In a chronic study with

mice some liver effects were observed at a dose level of 120 mg/kg bw/d, but without an increase in severity at 650 mg/kg bw/d. Therefore it is judged that an additional factor to extrapolate the subchronic NOAEL to chronic exposure is not necessary.

[c] As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day, an AF lower than 1 is considered appropriate.

For all scenarios except manufacture of epoxy resins the MOS values are greater than the minimal MOS (<87.5). These MOSs are considered sufficient to conclude that adverse repeated dose systemic effects on the liver will not occur in these scenarios. Therefore conclusion (ii) is drawn for these scenarios. For manufacture of epoxy resins, an MOS of 40 has been calculated. This MOS is lower than the minimal MOS (<87.5). There is concern that adverse repeated dose systemic effects on the liver might occur in these scenarios, and thus, conclusion (iii) is drawn.

Toxicity to reproduction

An overall NOAEL of 50 mg/kg has been established in a multigeneration study in rats and a two generation study in mice for effects on fertility and development occurring at the next doses level of 500-600 mg/kg[6]. The MOSs between this NOAEL of 50 mg/kg (equivalent to an internal extrapolated NAEL of 5 mg/kg to account for first pass effect following oral exposure) and the worst case body burdens arising from inhalation and dermal exposure for each scenario are shown in Table 4.25. The MOS values are evaluated by comparison with the minimal MOS (20). In Table 4.25a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.25 Margins of safety for reproductive toxicity (effects on fertility and development)

Scenario	RWC inhalation 8h-TWA exposure ($mg.m^{-3}$)	RWC inhalation body burden* ($mg.kg^{-1}.day^{-1}$)	RWC dermal exposure# ($mg.day^{-1}$)	RWC dermal body burden+ ($mg.kg^{-1}.day^{-1}$)	RWC combined body burden ($mg.day^{-1}$)	MOS for reproductive toxicity based on NAEL of 5 $mg.kg^{-1}.day^{-1}$	Conclusion
Manufacture of BPA	3	0.43	42	0.06	0.49	**10**	**(iii)**
Manufacture of PC	$1x10^{-3}$	$1x10^{-4}$	0.04	$6x10^{-5}$	$1.6x10^{-4}$	$3x10^{4}$	(ii)
Manufacture of articles from PC	$1x10^{-3}$	$1x10^{-4}$	0.04	$6x10^{-5}$	$1.6x10^{-4}$	$3x10^{4}$	(ii)
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3	**4**	**(iii)**
Powder coating manufacture	0.01	$1x10^{-3}$	2	$3x10^{-3}$	$4x10^{-3}$	1,250	(ii)
Powder	0.5	0.07	2.3	$3x10^{-3}$	0.07	71	(ii)

[6] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

coating use							
Thermal paper manufacture	0.1	0.01	42	0.06	0.07	71	(ii)
Manufacture of tin plating additive	0.05	$7x10^{-3}$	42	0.06	0.07	71	(ii)

* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m^3 air inhaled per working day.

Taking into account area exposed

+ Assuming 10% absorption by the dermal route

Table 4.25a Assessment factors applied for the calculation of the minimal MOS for reproductive effects in workers from mouse and rat data

Uncertainty	Assessment factor
Interspecies differences[a]	4 x 1
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	20

[a] Allometric scaling factor for the rat x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1). The same NOAEL of 50 mg/kg/day has been identified from mouse and rat data; however, since the available information (see section 4.1.3.1) shows that the rat is a better model for humans than the mouse, the allometric scaling of the rat is considered more appropriate than that for the mouse.

For all scenarios except manufacture of BPA and manufacture of epoxy resins the MOS values are greater than the minimal MOS (20). These MOSs are considered sufficient to conclude that adverse reproductive effects (effects on fertility and development) will not occur in these scenarios. Therefore conclusion (ii) is drawn for these scenarios. For manufacture of BPA and manufacture of epoxy resins, MOS values of 10 and 4 have been calculated. These MOSs are significantly lower than the minimal MOS (20). There is concern that adverse reproductive effects might occur in these scenarios, and thus, conclusion (iii) is drawn.

4.1.3.2.1 Summary of risk characterisation for workers

The risk characterisation for workers leads to conclusion (iii) for repeated dose systemic effects and for reproductive toxicity during the manufacture of BPA and the manufacture of epoxy resins. In addition, conclusion (iii) is reached in relation to skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact with high concentrations of BPA.

4.1.3.3 Consumers

The health effects of concern for BPA relevant to the consumer are eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure, and reproductive toxicity (effects on fertility and development, including developmental neurotoxicity). There are no concerns for acute toxicity, skin irritation, respiratory sensitisation, mutagenicity and carcinogenicity and hence conclusion (ii) is drawn for these endpoints.

The relevant routes of exposure for the consumer are inhalation, oral and dermal.

Eye irritation, respiratory tract irritation and repeated dose local effects on the respiratory tract

The potential for eye and respiratory tract irritation and for repeated dose local effects on the respiratory tract could arise as a result of consumer use of paints and varnishes containing BPA. In these applications, the concentration of BPA in the product is $\leq$ 0.0004%. At these concentrations, there is no concern that the irritating properties of BPA will be expressed and therefore there are no concerns for these endpoints and conclusion (ii) is reached.

Skin sensitisation

Dermal exposure to BPA, leading to potential concerns for skin sensitisation, can result from the consumer use of paints, varnishes, wood fillers and adhesives which contain BPA. In these applications, the concentration of BPA in the product is $\leq$ 0.0004%. At these concentrations, there is no concern that the sensitising properties of BPA will be expressed and therefore there are no concerns for this endpoint and conclusion (ii) is reached.

Repeated dose systemic effects and reproductive toxicity

Oral exposure

Potential concerns for repeat dose toxicity (systemic effects on body weight, liver and kidney) and for reproductive effects arise from those consumer exposure scenarios which involve repeated exposure to BPA. Scenarios for which exposures are single, relatively rare events (application of paints and varnish, use of wood fillers, exposures immediately following dental treatment) are not relevant in relation to concerns for these endpoints, and thus will not be considered further.

The sources of consumer exposure which could result in repeated exposure to BPA are food contact applications (infant feeding bottles; polycarbonate tableware; wine from epoxy-resin lined vats; canned food and canned beverages). In addition, the use of adhesives will be considered in relation to these endpoints, since although it is generally unlikely to be a daily event, some consumers may have relatively frequent use. With the exception of adhesives use, each of these scenarios results in oral exposure only; use of adhesives results in dermal exposure only.

Some of these sources will result in exposure to adult and/or infant or child consumers. Table 4.26 gives calculations of body burdens for adult and/or infant and child consumers from sources involving oral exposure and MOSs for repeat dose toxicity (effects on bodyweight, kidney and liver) and reproductive effects (effects on fertility and development, including

developmental neurotoxicity)[7]. Body burdens have been calculated using the following assumptions: oral absorption is 100%; an adult consumer weighs 60 kg; a young child (1.5-4.5 years) weighs 11 kg; a 1-2 month baby weighs 4.5 kg; a 4-6 month baby weighs 7 kg, an infant (6-12 months) weighs 8.7 kg. Bodyweight values for adults and young children are based on UK data (HMSO, 1990, 1992, 1995).

The MOS values for repeated dose effects on bodyweighnt and kidney are evaluated by comparison with the minimal MOS of <70, and the MOS values for repeated dose effects on the liver are evaluated by comparison with the minimal MOS of <175. The MOS values for reproductive toxicity (effects on fertility and development) are evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.23c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

[7] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

Table 4.26 Calculated body burdens from oral exposure and MOSs for repeat dose toxicity (effects on bodyweight, kidney and liver) and reproductive effects (effects on fertility and development)

Source of exposure	Daily ingestion of BPA (mg/day)	Estimated body burden (mg/kg/day)	MOS		Conclusion	
			Repeated dose toxicity (effects on bodyweight, kidney and liver)[1]	Reproductive toxicity (effects on fertility and on development)[2]	Repeated dose toxicity (effects on bodyweight, kidney, and liver)	Reproductive toxicity (effects on fertility and development)
Infant feeding bottles (1-2 month baby)	0.035	0.008	6250	6250	(ii)	(ii)
Infant feeding bottles (4-6 month baby)	0.050	0.007	7 100	7 100	(ii)	(ii)
Canned food and beverages (infant 6-12 months)	0.0375	0.0043	11 628	11 628	(ii)	(ii)
Canned food and beverages (young child 1.5-4.5 years)	0.100	0.009	5 555	5 555	(ii)	(ii)
Canned food (adult)	0.050	8×10^{-4}	62 500	62 500	(ii)	(ii)
Canned beverages (adult)	0.020	3×10^{-4}	170 000	170 000	(ii)	(ii)
Wine (adult)	0.010	1.7×10^{-4}	300 000	300 000		
Canned food and beverages including wine (adult)	0.070	0.00125	42 000	42 000	(ii)	(ii)
Polycarbonate tableware (young child, 1.5-4.5 years)	0.010	9×10^{-4}	55 000	55 000	(ii)	(ii)
Polycarbonate tableware (adult)	0.015	2.5×10^{-4}	20 000	20 000	(ii)	(ii)
Canned food and beverages + polycarbonate tableware (young child, 1.5-4.5 years)	0.110	0.01	5 000	5 000	(ii)	(ii)
Canned food and beverages + polycarbonate tableware (adult)	0.085	0.00150	33 333	33 333	(ii)	(ii)

[1] Based on NOAEL of 50 mg/kg for effects on body weight, kidney and liver from 2-gen study in the mouse

[2] Based on NOAEL of 50 mg/kg from 2-gen study in the mouse

Table 4.26a Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects (effects on bodyweight and kidney) in consumers and humans exposed indirectly via the environmenrt from mouse data

Uncertainty	Assessment factor
Interspecies differences[a]	7 x 1
Intraspecies differences	10
Differences between experimental conditions and exposure[b]	1
Dose response / Type of critical effect [c]	<1
Confidence of the database	1
Overall	<70

[a] Allometric scaling factor for the mouse x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1)

[b] Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. In a mouse 2-year study a LOAEL of 120 mg/kg/day was identified for minor effects on body weight gain.

[c] As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day and the effects at the LOAEL are rather minor, an AF lower than 1 is considered appropriate.

Table 4.26b Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects (effects on the liver) in consumers and humans exposed indirectly via the environmenrt from mouse data

Uncertainty	Assessment factor
Interspecies differences[a]	7 x 2.5
Intraspecies differences	10
Differences between experimental conditions and exposure[b]	1
Dose response / Type of critical effect [c]	<1
Confidence of the database	1
Overall	<175

[a] Allometric scaling factor for the mouse x default factor for remaining uncertainties (for effects on the liver, the rodent-human differences in the systemic availability of free unconjugated BPA are unimportant, and hence, for these effects a reduced interspecies factor for remaining differences is not appropriate.)

[b] Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. A NOAEL of 50 mg/kg bw/d was identified in parental generations in subchronic reproductive toxicity studies in the mouse. The LOAEL was 600 mg/kg bw/d. In a chronic study with mice some liver effects were observed at a dose level of 120 mg/kg bw/d, but without an increase in severity at 650 mg/kg bw/d. Therefore it is judged that an additional factor to extrapolate the subchronic NOAEL to chronic exposure is not necessary.

[c] As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day, an AF lower than 1 is considered appropriate.

Table 4.26c Assessment factors applied for the calculation of the minimal MOS for reproductive effects (effects on fertility and development, including developmental neurotoxicity) in consumers and humans exposed indirectly via the environment from mouse and rat data

Uncertainty	Assessment factor
Interspecies differences[a]	4 x 1
Intraspecies differences	10
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1

Uncertainty	Assessment factor
Confidence of the database	1
Overall	40

[a] Allometric scaling factor for the rat x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1). The same NOAEL of 50 mg/kg/day has been identified from mouse and rat data; however, since the kinetic information shows that the rat is a better model for humans than the mouse, the allometric scaling of the rat is considered more appropriate than that for the mouse.

The margins between exposures and the NOAELs are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects (effects on fertility and development) respectively, for all the exposure scenarios in Table 4.23. These margins are considered not to give rise to concern and therefore conclusion (ii) is drawn. These conclusions are in agreement with those of the EFSA (2006) evaluation of BPA. EFSA (2006) sated that the conservative estimates of exposure in all population groups considered were well below the Tolerable Daily Intake (TDI) established by EFSA at 0.05 mg/kg bw/day.

Dermal exposure

In relation to potential exposure arising from the use of adhesives, exposure occurs only as a result of dermal contact. Based on the available information for dermal absorption, the contribution to total body burden arising from dermal exposure is calculated on the basis of 10% uptake. In order to compensate for first pass metabolism, the oral NOAEL of 50 mg/kg/day for repeated dose effects on bodyweight and kidney and reproductive toxicity (effects on fertility and development)[8] has been adjusted by a factor of 10 for comparison with the dermal exposure estimates. For the repeated dose effects on the liver, adjustment of the oral NOAEL of 50 mg/kg/day for the first-pass effect is inappropriate as liver effects are not related to the systemic availability of free unconjugated BPA, but to the dose of BPA delivered to the liver, which is approximately the same for the oral and dermal routes of exposure. The estimated exposures arising from the use of adhesives for an adult consumer and the resultant MOSs for repeated dose effects on bodyweight and kidney and for reproductive toxicity are shown in Table 4.27. The estimated exposures arising from the use of adhesives for an adult consumer and the resultant MOS for repeated dose effects on the liver are shown in Table 4.28. The MOS value for repeated dose effects on bodyweight and kidney is evaluated by comparison with the minimal MOS of <70 and the MOS value for repeated dose effects on the liver is evaluated by comparison with the minimal MOS of <175. The MOS value for reproductive toxicity is evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.27 Calculated body burdens and MOSs for repeat dose toxicity (effects on bodyweight and kidney) and reproductive effects (effects on fertility and development) as a result of use of adhesives (dermal exposure)

[8] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

Exposure to BPA per event (mg)	Estimated body burden (mg/kg/day)	MOS		Conclusion	
		Repeated dose toxicity(effects on bodyweight and kidney)[1]	Reproductive toxicity (effects on fertility and on development)[2]	Repeated dose toxicity(effects on bodyweight and kidney)	Reproductive toxicity(effects on fertility and on development)
0.014	2.3×10^{-4}	22 000	22 000	(ii)	(ii)

[1] NAEL = 5 mg/kg/day (allowing for first pass metabolism)

[2] NAEL = 5 mg/kg/day (allowing for first pass metabolism)

Table 4.28 Calculated body burdens and MOSs for repeat dose toxicity (effects on the liver) as a result of use of adhesives (dermal exposure)

Exposure to BPA per event (mg)	Estimated body burden (mg/kg/day)	MOS for repeated dose toxicity(effects on the liver)[1]	Conclusion
0.014	2.3×10^{-4}	220,000	(ii)

[1] NOAEL = 50 mg/kg/day

The margins between exposure and the NOAELs are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, for repeated dose effects on the liver and for reproductive effects (effects on fertility and on development), respectively. These margins are considered not to give rise to concern and therefore conclusion (ii) is drawn for this scenario.

4.1.3.3.1 Summary of risk characterisation for consumers

The risk characterisation for consumers leads to conclusion (ii) for all the endpoints as consumer exposure is very low.

4.1.3.4 Humans exposed via the environment

The key health effects relevant to humans exposed via the environment are reproductive toxicity (effects on fertility and on development)[9] and systemic effects following repeated exposure. Irritation, sensitisation and local effects on the respiratory tract are of low concern where exposure is dissipated throughout the environment and hence conclusion (ii) is reached for these endpoints. Furthermore, there are no concerns for acute toxicity, skin irritation,

[9] Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

respiratory sensitisation, mutagenicity and carcinogenicity and hence conclusion (ii) is drawn for these endpoints.

4.1.3.4.1 Regional exposure

In Table 4.14, the total daily human exposure to BPA via the environment is estimated to be 9.1×10^{-6} mg/kg/day for regional sources. Inhalation makes no contribution to this exposure estimate as it all comes from oral sources. Comparisons of this intake estimate with the oral NOAEL of 50 mg/kg/day for repeated dose toxicity (effects on bodyweight, kidney and liver) and for reproductive toxicity (effects on fertility and development) respectively to derive MOSs are shown in the table below (table 4.29). The MOS value for repeated dose effects on bodyweight and kidney is evaluated by comparison with the minimal MOS of <70 and the MOS value for repeated dose effects on the liver is evaluated by comparison with the minimal MOS of <175. The MOS value for reproductive toxicity is evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.29 Risk characterisation for repeated dose toxicity (effects on bodyweight, kidney and liver) and reproductive toxicity (effects on fertility and development, including developmental neurotoxicity) following exposure via the environment (regional sources)

Exposure		Effects (systemic)			
		Reproductive effects NOAEL 50 mg/kg/day		Repeated dose toxicity NOAEL 50 mg/kg/day	
Source	Value (mg/kg/day)	MOS	Conclusion	MOS	Conclusion
Regional	9.1×10^{-6}	5×10^{6}	(ii)	5×10^{6}	(ii)

Given the low levels of exposure for the regional scenario and the very large margins of safety, which are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects, respectively, these exposures are considered not to be of concern and hence conclusion (ii) is reached for regional sources.

4.1.3.4.2 Local exposure

For all local scenarios except BPA manufacture, inhalation makes no contribution to the exposure estimates as it all comes from oral sources. For BPA manufacture, inhalation makes a more significant contribution (0.3%), but still the oral route contributes the most (99.7%). The human health systemic effects of concern include reproductive toxicity (effects on fertility and development) and repeated dose toxicity (effects on bodyweight, kidney and liver). The highest local exposure is in the locality of plants producing BPA. Exposure is estimated to be 0.041 mg/kg/day. Comparisons of this intake estimate with the oral NOAEL of 50 mg/kg/day for reproductive toxicity and repeated dose effects respectively to derive MOSs are shown in the table below (Table 4.30). The MOS value for repeated dose effects on bodyweight and kidney is evaluated by comparison with the minimal MOS of <70 and the MOS value for repeated dose effects on the liver is evaluated by comparison with the

minimal MOS of <175. The MOS value for reproductive toxicity is evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.30 Risk characterisation for repeated dose toxicity (effects on bodyweight, kidney and liver) and reproductive toxicity (effects on fertility and development) following exposure via the environment (local sources)

Exposure		Effects (systemic)			
		Reproductive effects NOAEL 50 mg/kg/day		Repeated dose toxicity NOAEL 50 mg/kg/day	
Source	Value (mg/kg/day)	MOS	Conclusion	MOS	Conclusion
Local (BPA production)	0.041	1220	(ii)	1220	(ii)

Given the very large margins of safety, which are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects, respectively, these exposures are considered not to be of concern and hence conclusion (ii) is reached for local sources.

4.1.3.5 Combined exposure

The worst case combined exposure would be for someone exposed via the environment near to a BPA production plant, and who is also exposed via food contact materials (oral exposure from canned food and canned beverages and from polycarbonate tableware and storage containers) as described in section 4.1.1.2.

The maximum combined exposure from these sources is 1.45×10^{-3} and 0.043 mg/kg/day for the regional and local scenarios respectively (see section 4.1.1.4.2).

Comparisons of these intake estimates with the oral NOAEL of 50 mg/kg/day for reproductive toxicity and repeated dose effects respectively to derive MOSs are shown in the table below (Table 4.31). The MOS values for repeated dose effects on bodyweight and kidney are evaluated by comparison with the minimal MOS of <70 and the MOS values for repeated dose effects on the liver are evaluated by comparison with the minimal MOS of <175. The MOS values for reproductive toxicity are evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.31 Risk characterisation for repeated dose toxicity (effects on bodyweight, kidney and liver) and reproductive toxicity (effects on fertility and development) for combined exposure scenarios

Exposure		Effects (systemic)			
		Reproductive effects NOAEL 50 mg/kg/day		Repeated dose toxicity NOAEL 50 mg/kg/day	
Source	Value (mg/kg/day)	MOS	Conclusion	MOS	Conclusion

Regional	1.45 x10^{-3}	35 000	(ii)	35 000	(ii)
Local (BPA production)	0.043	1163	(ii)	1163	(ii)

Given the very large margins of safety, which are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects, respectively, these exposures are considered not to be of concern and hence conclusion (ii) is reached for combined exposure scenarios.

4.2 HUMAN HEALTH (PHYSICOCHEMICAL PROPERTIES)

The physicochemical properties of BPA are available from the literature although the exact values for end points such as vapour pressure can be difficult to verify. There will be slight variation in values quoted by manufacturers according to the nature of the material they produce. Given the low vapour pressure at normal temperatures, lack of flammability and the general stability, the risks arising from the physicochemical properties are small. In common with many organic materials, the finely powdered material is a significant dust explosion hazard (Grossel, 1988). However, this appears to be well known within the manufacturing industry and it is considered that there are adequate controls in place for this risk. Given the controls used during manufacture and use, the risk from this is small. Overall, the risk from physicochemical properties is low and conclusion (ii) is drawn.

5 RESULTS

5.1 RISKS TO HUMAN HEALTH

5.1.1 Human health (toxicity)

The key health effects of exposure to BPA are eye irritation, respiratory tract irritation, skin sensitisation, repeated dose local effects to the respiratory tract, systemic effects following repeated exposure and reproductive toxicity (effects on fertility and on development). No dose response information is available for eye irritation. A NOAEL of 10 mg/m^3 has been identified for repeated dose toxicity to the respiratory tract. A NOAEL of 50 mg/kg has been identified for systemic effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg has been established in a multigeneration study in rats and a two generation study in mice for effects on fertility and development.

5.1.1.1 Workers

The risk characterisation for workers leads to conclusion (iii) for repeated dose systemic effects and for reproductive toxicity during the manufacture of BPA and the manufacture of epoxy resins. In addition, conclusion (iii) is reached in relation to skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact.

Results

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied should be taken into account.

This applies to the manufacture of BPA and the manufacture of epoxy resins, in relation to concerns for repeated dose systemic effects and reproductive toxicity. In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact with high concentrations of BPA.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This conclusion applies in relation to repeated dose systemic effects and reproductive toxicity for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, thermal paper manufacture and manufacture of tin plating additive. This conclusion also applies in relation to eye and respiratory tract irritation and repeated dose local effects in the respiratory tract for all scenarios.

5.1.1.2 Consumers

For eye and respiratory tract irritation, for repeated dose local effects on the respiratory tract and for skin sensitisation, exposure is very low and it is concluded that there is no concern for these endpoints. For systemic effects following repeated exposure and for reproductive toxicity, conclusion (ii) is reached for all exposure scenarios.

Results

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This applies to all consumer exposure scenarios in relation to eye and respiratory tract irritation, skin sensitisation, repeated dose local effects on the respiratory tract, systemic effects following repeated exposure and reproductive toxicity.

5.1.1.3 Humans exposed via the environment

The key health effects relevant to humans exposed via the environment are reproductive toxicity (effects on fertility and on development) and systemic effects following repeated exposure. Irritation, sensitisation and local effects on the respiratory tract are of low concern where exposure is dissipated throughout the environment.

Given the low levels of exposure and the large margins of safety for both the regional and local exposure scenarios, there are no concerns for repeated dose toxicity and reproductive toxicity and hence conclusion (ii) is reached.

Results

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This applies to both regional and local exposure scenarios in relation to repeated dose systemic effects and reproductive toxicity.

5.1.1.4 Combined exposure

The worst case combined exposure would be for someone exposed via the environment near to a BPA production plant, and who is also exposed via food contact materials (oral exposure from canned food and canned beverages and from polycarbonate tableware and storage containers).

Given the very large margins of safety, there are no concerns for repeated dose toxicity and reproductive toxicity and hence conclusion (ii) is reached.

Results

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This applies in relation to repeated dose systemic effects and reproductive toxicity.

5.1.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

There are no significant risks from physico-chemical properties.

6 REFERENCES

Adriani, W., Seta, D. D., Dessi-Fulgheri, F., Farabollini, F., and Laviola, G. (2003). Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. *Environ Health Perspect* 111, 395-401.

Aikawa, H., Koyama, S., Matsuda, M., Nakahashi, K., Akazome, Y., and Mori, T. (2004). Relief effect of vitamin A on the decreased motility of sperm and the increased incidence of malformed sperm in mice exposed neonatally to bisphenol A. *Cell Tissue Res* 315, 119-24.

Akingbemi, B. T., Sottas, C. M., Koulova, A. I., Klinefelter, G. R., and Hardy, M. P. (2004). Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology* 145, 592-603.

Al-Hiyasat, A. S., Darmani, H., and Elbetieha, A. M. (2004). Leached components from dental composites and their effects on fertility of female mice. *Eur J Oral Sci* 112, 267-72.

Aloisi, A.M., Seta, D.D., Rendo, C., Ceccarelli, I., Scaramuzzino, A., and Farabollini, F. (2002) Exposure to the estrogenic pollutant bisphenol A affects pain behavior induced by subcutaneous formalin injection in male and female rats. *Brain Res*. 937:1-7

Alonso-Magdalena, P., Morimoto, S., E., Ripoll, C., Fuentes, E.., and Nadal, A. (2006). The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect* 114, 106-112.

Arakawa, C, Fujimaki, K., Yoshinaga, J., Imai, H., Serizawa, S., and Shiraishi, H. (2004). Daily urinary excretion of bisphenol A. *Environ. Health Prev. Med.* 9, 22-26.

Arenholt-Bindslev D., V. Breinholt, G. Schmalz, and A. Preiss, 1998, "Time-related bisphenol A content and estrogenic activity in saliva samples collected in relation to placement of dental fissures", *Journal of Dental Research*, 77(B): 692 (abstract 481). Cited by www.bisphenol-A.org/human/dental.html

Ashby, J. (2003). Endocrine disruption occurring at doses lower than those predicted by classical chemical toxicity evaluations: The case bisphenol A. *Pure Appl. Chem.* 75, 2167-2179.

Ashby, J., and Odum, J. (2004). Gene expression changes in the immature rat uterus: effects of uterotrophic and sub-uterotrophic doses of bisphenol A. *Toxicol Sci* 82, 458-67.

Ashby, J., Tinwell, H., Lefevre, P. A., Joiner, R., and Haseman, J. (2003). The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisphenol A between postnatal days 91-97. *Toxicol Sci* 74, 129-38.

Ashby, J., Tinwell, H., Odum, J., and Lefevre, P. (2004). Natural variability and the influence of concurrent control values on the detection and interpretation of low-dose or weak endocrine toxicities. *Environ Health Perspect* 112, 847-53.

Association of Plastics Manufacturers in Europe (2006). Answer to EFSA request for further information on uses of bisphenol A. D. Thomas, 23 January 2006.

Attia, M.S., Adler, I.D., Eichenlaub-Ritter, U, Ranaldi, R., and Pacchierotti, F. (2004). Aneuploidy studies in mouse germ cells with bisphenol A (abstract PW6022). In: 34th Annual Meeting of the European Environmental Mutagen Society, Maastrich, Netherlands.

Bae, B., Jeong, J. H., and Lee, S. J. (2002). The quantification and characterization of endocrine disruptor bisphenol-A leaching from epoxy resin. *Water Sci Technol* 46, 381-7.

Berkowitz G. (2006). Letters to the Editor; Limitations of a case–control study on bisphenol A (BPA) serum levels and recurrent miscarriage. *Hum Reprod* 21, 565-566.

Braunrath, R., Podlipna, D., Padlesak, S., and Cichna-Markl, M. (2005). Determination of bisphenol a in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection. *J Agric Food Chem* 53, 8911-7.

Brede, C., Fjeldal, P., Skjevrak, I., and Herikstad, H. (2003). Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. *Food Addit Contam* 20, 684-9.

Brenn-Struckhofova Z. & Cichna-Markl M. (2006). Determination of bisphenol A in wine by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection. *Food Addit Contam*, 23, 1227–35.

Calafat, A. M., Kuklenyik, Z., Reidy, J. A., Caudill, S. P., Ekong, J., and Needham, L. L. (2005). Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ Health Perspect* 113, 391-5.

Carr, R.L., Bertasi, F.R., Betancourt, A.M., Bowers, S.D., Gandy, B.S., Ryan, P.L., and Willard, S.T. (2003) Effect of neonatal rat bisphenol A exposure on performance in the Morris water maze. *J Tox Environ Health* Part A. 66, 2077-2088.

Cassidy, A., Bingham, S., and Setchell, K. (1995). Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *Br J Nutr* 74, 587-601.

Cassidy, A., Bingham, S., and Setchell, K. D. (1994). Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr* 60, 333-40.

CEC (1993). Commission of the European Communities. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food. Thirty first series. Luxembourg: Office for official Publications of the European Communities.

Ceccarelli, I., Della Seta, D., Fiorenzani, P., Farabollini, F., and Aloisi, A.M. (2007). Estrogenic chemicals at puberty change ERalpha in the hypothalamus of male and female rats. *Neurotoxicol Teratol*; 29, 108-15

CERHR (2007). NTP-CERHR report on the reproductive and developmental toxicity of bisphenol A. Interim draft. April 2007. Report available from http://cerhr.niehs.nih.gov.

Cohen, S. M., Meek, M. E., Klaunig, J. E., Patton, D. E., and Fenner-Crisp, P. A. (2003). The human relevance of information on carcinogenic modes of action: overview. *Crit Rev Toxicol* 33, 581-9.

Conolly, R. B., and Lutz, W. K. (2004). Nonmonotonic dose-response relationships: mechanistic basis, kinetic modeling, and implications for risk assessment. *Toxicol Sci* 77, 151-7.

Csanady, G. A., Oberste-Frielinghaus, H. R., Semder, B., Baur, C., Schneider, K. T., and Filser, J. G. (2002). Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein. *Arch Toxicol* 76, 299-305.

CSL (2004). A study of the migration of bisphenol A from polycarbonate feeding bottles into food simulants. Central Science Laboratory Test Report L6BB-1008 for the Boots Group; http://www.boots-plc.com/environment/library/250.pdf.

Daston, G. P., Cook, J. C., and Kavlock, R. J. (2003). Uncertainties for endocrine disrupters: our view on progress. *Toxicol Sci* 74, 245-52.

Degen, G. H., Janning, P., Diel, P., and Bolt, H. M. (2002). Estrogenic isoflavones in rodent diets. *Toxicol Lett* 128, 145-57.

Delclos, K. B., Bucci, T. J., Lomax, L. G., Latendresse, J. R., Warbritton, A., Weis, C. C., and Newbold, R. R. (2001). Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. *Reprod Toxicol* 15, 647 -663.

Della Seta, D., Minder, I., Belloni, V., Aloisi, A.M., Dessi-Fulgheri, F., and Farabollini, F. (2006). Pubertal exposure to estrogenic chemicals affects behaviour in juvenile and adult male rats. *Horm Behav* 50, 301-307

Dessi-Fulgheri, F., Porrini, S., and Farabollini, F. (2002). Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. *Environ Health Perspect* 110 Suppl 3, 403-7.

Diel, P., Schmidt, S., Vollmer, G., Janning, P., Upmeier, A., Michna, H., Bolt, H. M., and Degen, G. H. (2004). Comparative responses of three rat strains (DA/Han, Sprague-Dawley and Wistar) to treatment with environmental estrogens. *Arch Toxicol* 78, 183-93.

Dionisi, G., and Oldring, P. K. (2002). Estimates of per capita exposure to substances migrating from canned foods and beverages. *Food Addit Contam* 19, 891-903.

Domoradzki, J. Y., Pottenger, L. H., Thornton, C. M., Hansen, S. C., Card, T. L., Markham, D. A., Dryzga, M. D., Shiotsuka, R. N., and Waechter, J. M., Jr. (2003). Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages. *Toxicol Sci* 76, 21-34.

Domoradzki, J. Y., Thornton, C. M., Pottenger, L. H., Hansen, S. C., Card, T. L., Markham, D. A., Dryzga, M. D., Shiotsuka, R. N., and Waechter, J. M., Jr. (2004). Age and dose dependency of the pharmacokinetics and metabolism of bisphenol A in neonatal sprague-dawley rats following oral administration. *Toxicol Sci* 77, 230-42.

Durando, M., Kass, L., Piva, J., Sonnenschein, C., Soto, A. M., Luque, E. H., and Muñoz-de-Toro, M. (2007). Prenatal Bisphenol A Exposure Induces Preneoplastic Lesions in the Mammary Gland in Wistar Rats *Environ Health Perspect* 115(1), 80-86.

Earls AO, Clay CA and Braybrook JH (2000). Preliminary Investigation into the Migration of bisphenol-A from Commercially-Available Polycarbonate Baby Feeding Bottles. LGC Technical Report LGC/DTI/2000/005.

EC SCF (2002). European Commission (2002) Final opinion of the Scientific Committee on Food on Bisphenol A (Expressed on 17 April 2002) SCF/CS/PM/3936. http://ec.europa.eu/food/fs/sc/scf/out128_en.pdf

EFSA (2006) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A). Question number EFSA-Q-2005-100. Adopted on 29 November 2006. The EFSA Journal (2006) 428, 1 - 75.

Elswick, B. A., Miller, F. J., and Welsch, F. (2001). Comments to the editor concerning the paper entitled "Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals" by C. Gupta. *Exp Biol Med (Maywood)* 226, 74-5; discussion 76-7.

Elswick, B. A., Welsch, F., and Janszen, D. B. (2000). Effect of different sampling designs on outcome of endocrine disruptor studies. *Reprod Toxicol* 14, 359-67.

Ema, M., Fujii, S., Furukawa, M., Kiguchi, M., Ikka, T., and Harazono, A. (2001) Rat two-generation reproductive toxicity study of bisphenol A. *Reprod Toxicol* 15, 505-23

EU (2003). *European Union Risk Assessment Report. Bisphenol A, CAS No: 80-05-7*. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 3rd Priority List, Luxembourg: Office for Official Publications of the European Communities.

Everitt, J. I., and Foster, P. M. (2004). Laboratory Animal Science Issues in the Design and Conduct of Studies with Endocrine-active Compounds. *Ilar J* 45, 417-24.

Facciolo, R.M., Alo, R., Madeo, M., Canonaco, M., and Dessi-Fulgheri, F. (2002) Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst-2. *Environ Health Perspect*. 110 (Suppl 3), 397-402.

Facciolo, R.M., Madeo, M., Alo, R., Canonaco, M., and Dessi-Fulgheri, F. (2005). Neurobiological effects of bisphenol A may be mediated by somatostatin subtype-3 receptors in some regions of the developing rat brain. *Toxicol Sci*. 88, 477-484.

Farabollini, F., Porrini, S., and Dessi-Fulgheri, F. (1999) Perinatal exposure to the estrogenic pollutant bisphenol A affects behaviour in male and female rats. *Pharmacol Biochem Behav* 64, 687-994

Farabollini, F., Porrini, S., Della Seta, D., Bianchi, F., and Dessi-Fulgheri, F. (2002). Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. *Environ Health Perspect* 110 Suppl 3, 409-14.

Filser, J. G., Csanady, G. A., and Faller, T. (2003). Tissue burden and resulting effectiveness of bisphenol A and daidzein in humans in depencene of age. *Umweltforschungsplan des Bundesministers für Umwelt, Naturschutz und Reaktorsicherheit, UBA-FB Report No. 21602001/07*.

Festing, M.F.W. (2006) Design and statistical methods in studies using animal models of development. *ILAR J* 42, 5-14

Foster, W. G., Hughes, C. L., Chan, S., and Platt, L. (2002). Human developmental exposure to endocrine active compounds. *Environmental toxicology and Pharmacology* 12, 75-81.

FSA (2001). Food Standards Agency. Survey of Bisphenols in Canned Foods. Food Surveillance Information Sheet. Number 13/01. April 2001. Food Standards Agency, UK. Available on http://www.foodstandards.gov.uk/food_surv.htm.

Fujimoto, T., Kubo, K., and Aou, S. (2006) Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. *Brain Res* 1068, 49-55.

Fukata, H., Miyagawa, H., Yamazaki, N., and Mori, C. (2006). Comparison of Elisa- and LCMS-based methodologies for the exposure assessment of bisphenol A. *Toxicology Mechanisms and Methods* 16, 427-430.

Funabashi, T., Kawaguchi, M., Furuta, M., Fukushima, A., and Kimura, F. (2004). Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. *Psychoneuroendocrinology* 29, 475-85.

Funabashi, T., Sano, A., Mitsushima, D., and Kimura, F. (2003). Bisphenol A increases progesterone receptor immunoreactivity in the hypothalamus in a dose-dependent manner and affects sexual behaviour in adult ovariectomized rats. *J Neuroendocrinol* 15, 134-40.

Fung, E. Y., Ewoldsen, N. O., St Germain, H. A., Jr., Marx, D. B., Miaw, C. L., Siew, C., Chou, H. N., Gruninger, S. E., and Meyer, D. M. (2000). Pharmacokinetics of bisphenol A released from a dental sealant. *J Am Dent Assoc* 131, 51-8.
Gallard H, Leclercq A., Croué J.P. (2004) Chlorination of bisphenol A: kinetics and byproducts formation. Chemosphere 56, 465–473.

Goodman, J.E., McConnell, E.E., Sipes, I.G., Witorsch, R.J., et al (2006). An updated weight of the evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. *Critical Reviews in Toxicology* 35, 387-457.

Goodson, A., Robin, H., Summerfield, W., and Cooper, I. (2004). Migration of bisphenol A from can coatings--effects of damage, storage conditions and heating. *Food Addit Contam* 21, 1015-26.

Goodson, A., Summerfield, W., and Cooper, I. (2002). Survey of bisphenol A and bisphenol F in canned foods. *Food Addit Contam* 19, 796-802.

Gray, G.M., Cohen, J.T., Cunha, G, et al (2004). Weight of evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. *Human and Ecological Risk Assessment* 10, 875-921.

Guo, T. L., Germolec, D. R., Musgrove, D. L., Delclos, K. B., Newbold, R. R., Weis, C., and White, K. L., Jr. (2005). Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F1 generations of Sprague Dawley rats following developmental and adult exposures. *Toxicology* 211, 207-19.

Haighton, L. A., Hlywka, J. J., Doull, J., Kroes, R., Lynch, B. S., and Munro, I. C. (2002). An evaluation of the possible carcinogenicity of bisphenol A to humans. *Regul Toxicol Pharmacol* 35, 238-54.

Hanai, Y. (1997). Bisphenol-A Eluted from Nursing Bottles. Unpublished Data. Environmental Science Research Center, Yokohama National University.

Hanaoka, T., Kawamura, N., Hara, K., and Tsugane, S. (2002). Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidylether and mixed organic solvents. *Occup Environ Med* 59, 625-8.

Harvey, P. W., and Johnson, I. (2002). Approaches to the assessment of toxicity data with endpoints related to endocrine disruption. *J Appl Toxicol* 22, 241-7.

HMSO (1995). The Toddlers Survey. Gregory, J.R., Collins, D.L., Davies, P.S.W., Hughes, J.M., and Clarke. P,C. National Diet and Nutrition Survey; Children Aged 1.5- 4.5 years. 1: Report of the Diet and Nutrition Survey.

Ho, S-M., Tamg, W-Y., de Frausto, J. B., and Prins, G. S. (2006). Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res* 66(11), 5624-5632.

Homey, B., von Schilling C., Blumel J., Schuppe H-C., Ruzicka T., Ahr H-J., Lehmann P., and Vohr, H-W. (1998). An integrated model for the differentiation of chemical-induced allegic and irritant skin reactions (IMDS). *Toxicol and Appl Pharmacol* 153, 83-94.

Honma, S., Suzuki, A., Buchanan, D. L., Katsu, Y., Watanabe, H., and Iguchi, T. (2002). Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod Toxicol* 16, 117-22.

Honma, T., Miyagawa, M., Suda, M., Wang, R. S., Kobayashi, K., Sekiguchi, S. (2003) Effects of perinatal exposure to bisphenol A on brain neurotransmitters in female rat offspring.. 44(3):510-524

Horie, M., Yoshida, T., Ishii, R., Kobayashi, S., and Nakazawa, H. (1999). Determination of bisphenol A in canned drinks by LC/MS. *Bunseki Kagaku***,** 48, 579-588.

Hunt, P. A., Koehler, K. E., Susiarjo, M., Hodges, C. A., Ilagan, A., Voigt, R. C., Thomas, S., Thomas, B. F., and Hassold, T. J. (2003). Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 13, 546-53.

Ichihara, T., Yoshino, H., Imai, N., Tsutsumi, T., Kawabe, M., Tamano, S., Inaguma, S., Suzuki, S., and Shirai, T. (2003). Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. *J Toxicol Sci* 28, 165-71.

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., and Taketani, Y. (2002). Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum Reprod* 17, 2839-41.

Imanaka, M., Sasaki, K., Nemoto, S., Ueda, E., Murakami, E., Miyata, D., and Tonogai, Y. (2001). [Determination of bisphenol A in foods using GC/MS]. *Shokuhin Eiseigaku Zasshi* 42, 71-8.

Imanishi, S., Manabe, N., Nishizawa, H., Morita, M., Sugimoto, M., Iwahori, M., and Miyamoto, H. (2003). Effects of oral exposure of bisphenol A on mRNA expression of nuclear receptors in murine placentae assessed by DNA microarray. *J Reprod Dev* 49, 329-36.

Inoue, H., Tsuruta, A., Kudo, S., Ishii, T., Fukushima, Y., Iwano, H., Yokota, H., and Kato, S. (2005). Bisphenol a glucuronidation and excretion in liver of pregnant and nonpregnant female rats. *Drug Metab Dispos* 33, 55-9.

Inoue, K., Murayama, S., Takeba, K., Yoshimura, Y., and Nakazawa, H. (2003). Contamination of xenoestrogens bisphenol A and F in honey: safety assessment and analytical method of these compounds in honey. *Journal of Food Composition and analysis* 16, 497-506.

Inoue, K., Wada, M., Higuchi, T., Oshio, S., Umeda, T., Yoshimura, Y., and Nakazawa, H. (2002). Application of liquid chromatography-mass spectrometry to the quantification of bisphenol A in human semen. *J Chromatogr B Analyt Technol Biomed Life Sci*. 773, 97-102.

Inoue, K., Yamaguchi, A., Wada, M., Yoshimura, Y., Makino, T., and Nakazaw, H. (2001). Quantitative detection of bisphenol A and bisphenol A diglycidyl ether metabolites in human plasma by liquid chromatography-electrospray mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765, 121-6.

Jaeg, J. P., Perdu, E., Dolo, L., Debrauwer, L., Cravedi, J. P., and Zalko, D. (2004). Characterization of new bisphenol A metabolites produced by CD1 mice liver microsomes and S9 fractions. *J Agric Food Chem* 52, 4935-42.

Jefferson, W. N., Couse, J. F., Padilla-Banks, E., Korach, K. S., and Newbold, R. R. (2002). Neonatal exposure to genistein induces estrogen receptor (ER)alpha expression and multioocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions. *Biol Reprod* 67, 1285-96.

Jordakova, I., Dobias, J., Voldrich, M., and Poustka, J. (2003). Determination of bisphenol A, bisphenol F, bisphenol A diglycidyl ether and bisphenol F diglycidyl ether migrated from food cans using gas chromatography-mass spectrometry. *Czech Journal of Food Sciences* 21, 85-90.

Kang, J. H., Kito, K., and Kondo, F. (2003). Factors influencing the migration of bisphenol A from cans. *J Food Prot* 66, 1444-7.

Kang, J. H., and Kondo, F. (2002). Bisphenol A migration from cans containing coffee and caffeine. *Food Addit Contam* 19, 886-90.

Kang, J. H., and Kondo, F. (2003). Determination of bisphenol A in milk and dairy products by high-performance liquid chromatography with fluorescence detection. *J Food Prot* 66, 1439-43.

Kato, H., Ota, T., Furuhashi, T., Ohta, Y., and Iguchi, T. (2003). Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. *Reprod Toxicol* 17, 283-8.

Kato, H., Furuhashi, T., Tanaka, M., Katsu, Y., Watanabe, H., Ohta, Y., and Iguchi, T. (2006). Effects of bisphenol A given neonatally on reproductive functions of male rats. *Reproductive Toxicology* 22, 20-29.

Kawaguchi, M., Sakui, N., Okanouchi, N., Ito, R., Saito, K., Izumi, S., Makino, T., and Nakazawa, H. (2005). Stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography--mass spectrometry for measurement of phenolic xenoestrogens in human urine samples. *J Chromatogr B Analyt Technol Biomed Life Sci* 820, 49-57.

Kawai, K., Nozaki, T., Nishikata, H., Aou, S., Takii, M., and Kubo, C. (2003). Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. *Environ Health Perspect* 111, 175-8.

Kawai, K., Murakami, S., Senba, E., Yamanaka, T., Fujiwara, Y., Arimura, C., Nozaki, T., Takii, M., and Kubo, C. (2007) Changes in estrogen receptors alpha and beta expression in the brain of mice exposed prenatally to bisphenol A. *Regul Toxicol Pharmacol* 47, 166-70.

Kersting, M., Alexy, U., Sichert_Hellert, W., Manz, F. and Schoch, G. (1998). Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. *J Pediatr Gastroeneterol Nutr* 27, 547-552.

Kim, H. S., Han, S. Y., Kim, T. S., Kwack, S. J., Lee, R. D., Kim, I. Y., Seok, J. H., Lee, B.M., Yoo, S. D., and Park, K. L. (2002). No androgenic/anti-androgenic effects of bisphenol-A in Hershberger assay using immature castrated rats. *Toxicol Lett* 135, 111-123.

Kim, Y. H., Kim, C. S., Park, S., Han, S. Y., Pyo, M. Y., and Yang, M. (2003). Gender differences in the levels of bisphenol A metabolites in urine. *Biochem Biophys Res Commun* 312, 441-8.

Klein, K. O. (1998). Isoflavones, soy-based infant formulas, and relevance to endocrine function. *Nutr Rev* 56, 193-204.

Kubo, K., Arai, O., Ogata, R., Omura, M., Hori, T., and Aou, S. (2001) Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. *Neurosci Lett* 304, 73-6

Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., and Aou, S. (2003). Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci Res* 45, 345-56.

Kuklenyik, Z., Ekong, J., Cutchins, C. D., Needham, L. L., and Calafat, A. M. (2003). Simultaneous measurement of urinary bisphenol A and alkylphenols by automated solid-phase extractive derivatization gas chromatography/mass spectrometry. *Anal Chem* 75, 6820-5.

Kuo, H. W., and Ding, W. H. (2004). Trace determination of bisphenol A and phytoestrogens in infant formula powders by gas chromatography-mass spectrometry. *J Chromatogr A*1027, 67-74.

Kurebayashi, H., Betsui, H., and Ohno, Y. (2003). Disposition of a low dose of ^{14}Cbisphenol-A in male rats and its main biliary excretion as BPA glucuronide. *Toxicol Sci* 73, 17-25.

Kurebayashi, H., Harada, R., Stewart, R. K., Numata, H., and Ohno, Y. (2002). Disposition of a low dose of bisphenol a in male and female cynomolgus monkeys. *Toxicol Sci* 68, 32-42.

Kurebayashi, H., Nagatsuka, S., Nemoto, H., Noguchi, H., and Ohno, Y. (2005). Disposition of low doses of 14C-bisphenol A in male, female, pregnant, fetal, and neonatal rats. *Arch Toxicol* 79, 243-52.

Kwon, S., Stedman, D.B., Elswick, B.A., Cattley, R.C., and Welsch, F. (2000) Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. *Toxicol. Sci.* 55, 399-406.

Laviola, G., Gioiosa, L., Adriani, W., Palanza, P. (2005) d-Amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors. *Brain Res Bull* 65, 235-240.

Lee, H. J., Chattopadhyay, S., Gong, E. Y., Ahn, R. S., and Lee, K. (2003). Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicol Sci* 75, 40-6.

Lobenhofer, E.K., Cui, X., Bennett, L., Cable, P.L., Merrick, B.A., Churchill, G.A., and Afshari, C.A. (2004). Exploration of low-dose estrogen effects: identification of No Observed Transcriptional Effect Level (NOTEL). *Toxicol Pathol* 32, 482-92.

Lopez-Cervantes, J., and Paseiro-Losada, P. (2003). Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. *Food Addit Contam* 20, 596-606.

Lopez-Cervantes, J., Sanchez-Machado, D. I., Pastorelli, S., Rijk, R., and Paseiro-Losada, P. (2003). Evaluating the migration of ingredients from active packaging and development of dedicated methods: a study of two iron-based oxygen absorbers. *Food Addit Contam* 20, 291-9.

MAFF (1998). Joint Food Safety and Standards Group. Food Surveillance Information Sheet - Number 167, November 1998.

Mao, L., Sun, C., Zhang, H., Li, Y., and Wu, D. (2004). Determination of environmental estrogens in human urine by high performance liquid chromatography after fluorescent derivatization with p-nitrobenzoyl chloride. *Anal. Chim. Acta* 522, 241-246.

Markaverich, B., Mani, S., Alejandro, M. A., Mitchell, A., Markaverich, D., Brown, T., Velez-Trippe, C., Murchison, C., O'Malley, B., and Faith, R. (2002). A novel endocrine-disrupting agent in corn with mitogenic activity in human breast and prostatic cancer cells. *Environ Health Perspect* 110, 169-77.

Markey, C. M., Luque, E. H., Munoz De Toro, M., Sonnenschein, C., and Soto, A. M. (2001). In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 65, 1215-23.

Markey, C. M., Wadia, P. R., Rubin, B. S., Sonnenschein, C., and Soto, A. M. (2005). Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. *Biol Reprod* 72, 1344-51.

Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., and Hirose, M. (2004a). Dietary influence on the impact of ethinylestradiol-induced alterations in the endocrine/reproductive system with perinatal maternal exposure. *Reprod Toxicol* 18, 23-33.

Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Lee, K. Y., and Hirose, M. (2004b). Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals. *Arch Toxicol* 78, 232-40.

Matsumoto, A., Kunugita, N., Kitagawa, K., Isse, T., Oyama, T., Foureman, G. L., Morita, M., and Kawamoto, T. (2003). Bisphenol A levels in human urine. *Environ Health Perspect* 111, 101-4.

Matthews, J. B., Twomey, K., and Zacharewski, T. R. (2001). In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chem Res Toxicol* 14, 149-57.

Melnick, R., Lucier, G., Wolfe, M., Hall, R., Stancel, G., Prins, G., Gallo, M., Reuhl, K., Ho, S. M., Brown, T., Moore, J., Leakey, J., Haseman, J., and Kohn, M. (2002). Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environ Health Perspect* 110, 427-31.

Miyamoto, K. and Kotake, M. (2006, in press) Estimation of Daily Bisphenol A Intake of Japanese Individuals with Emphasis on Uncertainty and Variability. accepted for publication in Environmental Sciences (Journal of the Japan Society of Endocrine Disruptors Research)

Milman, H. A., Bosland, M. C., Walden, P. D., and Heinze, J. E. (2002). Evaluation of the adequacy of published studies of low-dose effects of bisphenol A on the rodent prostate for use in human risk assessment. *Regul Toxicol Pharmacol* 35, 338-46.

Miyamoto, K., and Kotake, M. (2006). Estimation of daily bisphenol a intake of Japanese individuals with emphasis on uncertainty and variability. *Environ Sci* **13**, 15-29.

Mizuo, K., Narita, M., Miyagawa, K., Narita, M., Okuno, E., and Suzuki, T. (2004a). Prenatal and neonatal exposure to bisphenol-A affects the morphine-induced rewarding effect and hyperlocomotion in mice. *Neurosci Lett*. 356(2), 95-8.

Mizuo, K., Narita, M., Yoshida, T., and Suzuki, T. (2004b). Functional changes in dopamine D3 receptors by prenatal and neonatal exposure to an endocrine disruptor bisphenol-A in mice. *Addict Biol* 9, 19-25.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., and Nakao, K. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J Clin Endocrinol Metab* 87, 5185-90.

Mountfort, K. A., Kelly, J., Jickells, S. M., and Castle, L. (1997). Investigations into the potential degradation of polycarbonate baby bottles during sterilisation with consequent release of bisphenol A. *Food Additives & Contaminants*, 14, 737-740.

Munguia-Lopez, E. M., Peralta, E., Gonzalez-Leon, A., Vargas-Requena, C., and Soto-Valdez, H. (2002). Migration of bisphenol A (BPA) from epoxy can coatings to jalapeno peppers and an acid food simulant. *J Agric Food Chem* 50, 7299-302.

Munguia-Lopez, E. M., and Soto-Valdez, H. (2001). Effect of heat processing and storage time on migration of bisphenol A (BPA) and bisphenol A-diglycidyl ether (BADGE) to aqueous food simulant from Mexican can coatings. *J Agric Food Chem* 49, 3666-71.

Munoz-de-Toro, M., Markey, C., Wadia, P. R., Luque, E. H., Rubin, B. S., Sonnenschein, C., and Soto, A. M. (2005). Perinatal exposure to Bisphenol A alters peripubertal mammary gland development in mice. *Endocrinology*.

Murray, T. J., Maffini, M. V., Ucci, A. A., Sonnenschein, C., and SotoA. M. (2007). Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reproductive Toxicology* 23(3), 383-390.

Naciff, J. M., Hess, K. A., Overmann, G. J., Torontali, S. M., Carr, G. J., Tiesman, J. P., Foertsch, L. M., Richardson, B. D., Martinez, J. E., and Daston, G. P. (2005). Gene Expression Changes Induced in the Testis by Transplacental Exposure to High and Low Doses of 17{alpha}-Ethynyl Estradiol, Genistein or Bisphenol A. *Toxicol Sci*.

Naciff, J. M., Jump, M. L., Torontali, S. M., Carr, G. J., Tiesman, J. P., Overmann, G. J., and Daston, G. P. (2002). Gene expression profile induced by 17alpha-ethynyl estradiol, bisphenol A, and genistein in the developing female reproductive system of the rat. *Toxicol Sci* 68, 184-99.

Naciff, J. M., Overmann, G. J., Torontali, S. M., Carr, G. J., Tiesman, J. P., and Daston, G. P. (2004). Impact of the phytoestrogen content of laboratory animal feed on the gene expression profile of the reproductive system in the immature female rat. *Environ Health Perspect* 112, 1519-26.

Nagao, T., Saito, Y., Usumi, K., Yoshimura, S., and Ono, H. (2002). Low-dose bisphenol A does not affect reproductive organs in estrogen-sensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage. *Reprod Toxicol* 16, 123-130.

Nagao, T., Wada, K., Kuwagata, M., Nakagomi, M., Watanabe, C., Yoshimura, S., Saito, Y., Usumi, K., and Kanno, J. (2004). Intrauterine position and postnatal growth in Sprague-Dawley rats and ICR mice. *Reprod Toxicol* 18, 109-20.

Nagel, S. C., vom Saal, F. S., Thayer, K. A., Dhar, M. G., Boechler, M., and Welshons, W. V. (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ Health Perspect* 105, 70-6.

Nagel, S. C., vom Saal, F. S., and Welshons, W. V. (1999). Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. *J Steroid Biochem Mol Biol* 69, 343-57.

Nakamura, K., Itoh, K., Yaoi, T., Fujiwara, Y., Sugimoto, T., and Fushiki, S. 2006) Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of bisphenol A. *J Neurosci Res* 84, 1197-1205.

Narita, M., Miyagawa, K., Mizuo, K., Yoshida, T., and Suzuki, T. (2006). Prenatal and neonatal exposure to low-dose of bisphenol-A enhance the morphine-induced hyperlocomotion and rewarding effect. *Neurosci Lett* 402, 249-252.

Narita, M., Miyagawa, K., Mizuo, K., Yoshida, T., and Suzuki, T. (2007) Changes in central dopaminergic systems and morphine reward by prenatal and neonatal exposure to bisphenol-A in mice: evidence for the importance of exposure period. *Addict Biol* 12, 167-72

Negishi, T., Kawasaki, K., Suzaki, S., Maeda, H., Ishii, Y., Kyuwa, S., Kuroda, Y., and Yoshikawa, Y. (2004). Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. *Environ Health Perspect* 112, 1159-64.

Negishi, T., Kawasaki, K., Takatori, a., Ishii, y., Kyuwa, S., Kuroda, Y., and Yoshikawa, Y. (2003). Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats. *Environmental Toxicology and Pharmacology* 14, 99-108.

Nerin, C., Fernandez, C., Domeno, C., and Salafranca, J. (2003). Determination of potential migrants in polycarbonate containers used for microwave ovens by high-performance liquid chromatography with ultraviolet and fluorescence detection. *J Agric Food Chem* 51, 5647-53.

Newbold, R. R., Jefferson, W. N., Padilla-Banks, E., (2007) Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reprod Toxicol* 24, 253-258

NIEHS (2001). National Toxicology Program's report of the Endocrine Disruptors Low-Dose Peer Review. Page A-58. August 2001. NTP, NIEHS, Research Triangle Park, NC, USA. Available at http://ntp.niehs.nih.gov/ntp/htdocs/liason/LowDosePeerFinalRpt.pdf.

Nikaido, Y., Yoshizawa, K., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., and Tsubura, A. (2004). Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol* 18, 803-11.

Nikaido, Y., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., and Tsubura, A. (2005). Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. *In Vivo* 19, 487-494.

Nishizawa, H., Manabe, N., Morita, M., Sugimoto, M., Imanishi, S., and Miyamoto, H. (2003). Effects of in utero exposure to bisphenol A on expression of RARalpha and RXRalpha mRNAs in murine embryos. *J Reprod Dev* 49, 539-45.

Odum, J., Tinwell, H., Tobin, G., and Ashby, J. (2004). Cumulative dietary energy intake determines the onset of puberty in female rats. *Environ Health Perspect* 112, 1472-80.

Ohkuma, H., Abe, K., Ito, M., Kokado, A., Kambegawa, A., and Maeda, M. (2002). Development of a highly sensitive enzyme-linked immunosorbent assay for bisphenol A in serum. *Analyst* 127, 93-7.

Olea, N., Pulgar, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A. M., and Sonnenschein, C. (1996). Estrogenicity of resin-based composites and sealants used in dentistry. *Environ Health Perspect* 104, 298-305.

Ouchi, K., and Watanabe, S. (2002). Measurement of bisphenol A in human urine using liquid chromatography with multi-channel coulometric electrochemical detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 780, 365-70.

Owens, J. W., and Chaney, J. G. (2005). Weighing the results of differing 'low dose' studies of the mouse prostate by Nagel, Cagen, and Ashby: Quantification of experimental power and statistical results. *Regul Toxicol Pharmacol* 43, 194-202.

Ozaki, A., and Baba, T. (2003). Alkylphenol and bisphenol A levels in rubber products. *Food Addit Contam* 20, 92-8.

Ozaki, A., Yamaguchi, Y., Fujita, T., Kuroda, K., and Endo, G. (2004). Chemical analysis and genotoxicological safety assessment of paper and paperboard used for food packaging. *Food Chem Toxicol* 42, 1323-37.

Pacchierotti, F., Ranaldi, R., Eichenlaub-Ritter, U., Attia, S., and Adler I.-D. (2007). Evaluation of aneugenic effects of bisphenol A in somatic and germ cells of the mouse. Submitted for publication.

Palanza, P. L., Howdeshell, K. L., Parmigiani, S., and vom Saal, F. S. (2002). Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environ Health Perspect* 110 Suppl 3, 415-22.

Patisaul, H.B., Fortino, A.E., and Polston, E.K. (2006) Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. *Neurotox Teratol*. 28, 111-118

Patisaul, H.B., Fortino, A.E., and Polston, E.K. (2007) Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A. *NeuroToxicol* 28, 1-12

PlasticsEurope (2007). Answer to HSE request for further information on medical surveillance data in BPA manufacture. M. Burcher, 26 October 2007.

Porrini, S., Belloni, V., Della Seta, D., Farabollini, F., Giannelli, G., and Dessi-Fulgheri, F. (2005). Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats. *Brain Res Bull* 65, 261-6.

Pottenger, L. H., Domoradzki, J. Y., Markham, D. A., Hansen, S. C., Cagen, S. Z., and Waechter, J. M., Jr. (2000). The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicol Sci* 54, 3-18.

Pritchett, J. J., Kuester, R. K., and Sipes, I. G. (2002). Metabolism of bisphenol a in primary cultured hepatocytes from mice, rats, and humans. *Drug Metab Dispos* 30, 1180-5.

Pulgar, R., Olea-Serrano, M. F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., Navajas, J. M., and Olea, N. (2000). Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. *Environ Health Perspect* 108, 21-7.

Putz, O., Schwartz, C. B., Kim, S., LeBlanc, G. A., Cooper, R. L., and Prins, G. S. (2001a). Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: I. Effects on the prostate gland. *Biol Reprod* 65, 1496-505.

Putz, O., Schwartz, C. B., LeBlanc, G. A., Cooper, R. L., and Prins, G. S. (2001b). Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: II. Effects on male puberty and the reproductive tract. *Biol Reprod* 65, 1506-17.

Quesada, I., Fuentes, E., Viso-Leon, M. C., Soria, B., Ripoll, C., and Nadal, A. (2002). Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *Faseb J* 16, 1671-3.

Ramos, J. G., Varayoud, J., Kass, L., Rodriguez, H., Costabel, L., Munoz-De-Toro, M., and Luque, E. H. (2003). Bisphenol a induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. *Endocrinology* 144, 3206-15.

Rivas, A., Lacroix, M., Olea-Serrano, F., Laios, I., Leclercq, G., and Olea, N. (2002). Estrogenic effect of a series of bisphenol analogues on gene and protein expression in MCF-7 breast cancer cells. *J Steroid Biochem Mol Biol* 82, 45-53.

Rodriguez-Mozaz, S., López de Alda, M.J. and Barceló, D. (2004). Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry. J. Chromatogr. A, 1045, 85-92.

Romero J., Ventura F., Gomez M. (2002) Characterisation of point samples used in drinking water reservoirs: Identification of endocrine disruptor compounds. Journal of Chromatographic Science 40, 191-197.

Rubin, B. S., Murray, M. K., Damassa, D. A., King, J. C., and Soto, A. M. (2001). Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ Health Perspect* 109, 675-80.

Rubin, B. S., Lenkowski, J. R., Schaeberle, C. M., Vandenberg, L. N., Ronsheim, P. M., and Soto, A. M. (2006). Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology* 147, 3681-3691.

Runyon, J., Noti, A., Grob, K., Biedermann, M., and Dudler, V. (2002). Isolation of the < 1000 Dalton Migrants from Food Packaging Materials by Size Exclusion Chromatography (SEC). *Mitt. Lebensm. Hyg. 93* 93, 57-72.

Ryan, B.C., and Vandenbergh, J.G. (2006). Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. *Hormones and Behav*. 50, 85-93

Safe, S. H. (2000). Endocrine disruptors and human health--is there a problem? An update. *Environ Health Perspect* 108, 487-93.

Sajiki, J., Takahashi, K., and Yonekubo, J. (1999). Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl*. 736, 255-261.

Sajiki, J. (2001). Determination of bisphenol A in blood using high-performance liquid chromatography-electrochemical detection with solid-phase extraction. *J Chromatogr B Biomed Sci Appl*. 755, 9-15.

Sakamoto, H., Yokota, H., Kibe, R., Sayama, Y., and Yuasa, A. (2002). Excretion of bisphenol A-glucuronide into the small intestine and deconjugation in the cecum of the rat. *Biochim Biophys Acta* 1573, 171-6.

Schonfelder, G., Flick, B., Mayr, E., Talsness, C., Paul, M., and Chahoud, I. (2002a). In utero exposure to low doses of bisphenol A lead to long-term deleterious effects in the vagina. *Neoplasia* 4, 98-102.

Schonfelder, G., Friedrich, K., Paul, M., and Chahoud, I. (2004). Developmental effects of prenatal exposure to bisphenol a on the uterus of rat offspring. *Neoplasia* 6, 584-94.

Schonfelder, G., Wittfoht, W., Hopp, H., Talsness, C. E., Paul, M., and Chahoud, I. (2002b). Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environ Health Perspect* 110, A703-7.

Schuler, M., P. Muehlbauer, P. Guzzie and D.A. Eastmond (1999) Noscapine hydrochloride disrupts the mitotic spindle in mammalian cells and induces aneuploidy as well as polyploidy in cultured human lymphocytes. *Mutagenesis* 14, 51-6.

Seidlova-Wuttke, D., Jarry, H., Christoffel, J., Rimoldi, G., and Wuttke, W. (2005). Effects of bisphenol-A (BPA), dibutylphtalate (DBP), benzophenone-2 (BP2), procymidone (Proc), and linurone (Lin) on fat tissue, a variety of hormones and metabolic parameters: A 3 months comparison with effects of estradiol (E2) in ovariectomized (ovx) rats. *Toxicology*, in press.

Seidlova-Wuttke, D., Jarry, H., and Wuttke, W. (2004). Pure estrogenic effect of benzophenone-2 (BP2) but not of bisphenol A (BPA) and dibutylphtalate (DBP) in uterus, vagina and bone. *Toxicology* 205, 103-12.

Shao, B., Han, H., Hu, J., Zhao, J., Wu, G., Xue, Y., Ma, Y., and Zhang, S. (2005). Determination of alkylphenol and bisphenol A in beverages using liquid chromatography/electrospray ionization tandem mass spectrometry. *Analytica Chimica Acta* 530, 245.

Sharpe, R. M., Rivas, A., Walker, M., McKinnell, C., and Fisher, J. S. (2003). Effect of neonatal treatment of rats with potent or weak (environmental) oestrogens, or with a GnRH antagonist, on Leydig cell development and function through puberty into adulthood. *Int J Androl* 26, 26-36.

Shimizu, M., Ohta, K., Matsumoto, Y., Fukuoka, M., Ohno, Y., and Ozawa, S. (2002). Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. *Toxicol In Vitro* 16, 549-56.

Shin, B. S., Yoo, S. D., Cho, C. Y., Jung, J. H., Lee, B. M., Kim, J. H., Lee, K. C., Han, S. Y., Kim, H. S., and Park, K. L. (2002). Maternal-fetal disposition of bisphenol A in pregnant Sprague-Dawley rats. *J Toxicol Environ Health A* 65, 395-406.

Simoneau C, Roeder G and Anklam E (2000). Migration of bisphenol-A from baby bottles: effect of experimental conditions and European survey. 2nd International Symposium on Food Packaging: Ensuring the Safety and Quality of Foods (ILSI conference), Vienna, Austria, 8-10 November 2000.

Snyder, R. W., Maness, S. C., Gaido, K. W., Welsch, F., Sumner, S. C., and Fennell, T. R. (2000). Metabolism and disposition of bisphenol A in female rats. *Toxicol Appl Pharmacol* 168, 225-34.

Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., and Nakashima, K. (2004). Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. *Biomed Chromatogr* 18, 501-7.

Stowell, C. L., Barvian, K. K., Young, P. C., Bigsby, R. M., Verdugo, D. E., Bertozzi, C. R., and Widlanski, T. S. (2006). A role for sulfation-desulfation in the uptake of bisphenol a into breast tumor cells. *Chem Biol* 13, 891-897.

Sugiura-Ogasawara M., Ozaki Y., Sonta S. I., Makino T., and Suzumori K. (2005) Exposure to bisphenol A is associated with recurrent miscarriage. *Hum Reprod* 20, 2325–2329.

Sugiura-Ogasawara M (2006) Letters to the Editor; Limitations of a case–control study on bisphenol A (BPA) serum levels and recurrent miscarriage *Hum Reprod* 21, 566-567.

Susiarjo, M., Hassold, T.J., Freeman, E., and Hunt, P. (2007). Bisphenol A exposure in utero disrupts early oogenesis in the mouse. *PLOS Genetics* 3(1), 1-8.

Suzuki, A., Sugihara, A., Uchida, K., Sato, T., Ohta, Y., Katsu, Y., Watanabe, H., and Iguchi, T. (2002). Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. *Reprod Toxicol* 16, 107-16.

Suzuki, M., Aoyama, T., Ohno, H., Nakashima, S., Iwama, M., and Mitani, K. (2004). Migration of bisphenol A from polyvinyl chloride products. *Kankyo Kagaku* 14, 375-379 (only abstract available in English).

Suzuki, T., Mizuo, K., Nakazawa, H., Funae, Y., Fushiki, S., Fukushima, S., Shirai, T., and Narita, M. (2003). Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: enhancement of the methamphetamine induced abuse state. *Neuroscience* 117, 639-44.

Takagi, H., Mitsumori, K., Onodera, H., Nasu, M., Tamura, T., Yasuhara, K., Takegawa, K., and Hirose, M. (2002). Improvement of a two-stage carcinogenesis model to detect modifying effects of endocrine disrupting chemicals on thyroid carcinogenesis in rats. *Cancer Lett* 178, 1-9.

Takagi, H., Shibutani, M., Masutomi, N., Uneyama, C., Takahashi, N., Mitsumori, K., and Hirose, M. (2004). Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life. *Arch Toxicol* 78, 97-105.

Takahashi, O., and Oishi, S. (2003). Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice. *Food Chem Toxicol* 41, 1035-44.

Takao, T., Nanamiya, W., Nazarloo, H. P., Matsumoto, R., Asaba, K., and Hashimoto, K. (2003). Exposure to the environmental estrogen bisphenol A differentially modulated estrogen receptor-alpha and -beta immunoreactivity and mRNA in male mouse testis. *Life Sci* 72, 1159-69.

Takao, Y., Lee, H. C., Kohra, S., and Arizono, K. (2002). Release of bisphenol A from food can lining upon heating. *Journal of Health Science* 48, 331-334.

Takashima, Y., Tsutsumi, M., Sasaki, Y., et al. (2001) Lack of effects of bisphenol A in maternal rats or treatment on response to their offspring to N-nitrosobis(2-hydroxypropyl)amine. *Journal of Toxicologic Pathology* 14, 87-98.

Takeuchi, T., and Tsutsumi, O. (2002). Serum bisphenol a concentrations showed gender differences, possibly linked to androgen levels. *Biochem Biophys Res Commun* 291, 76-78.

Takeuchi, T., Tsutsumi, O., Ikezuki, Y., Takai, Y., and Taketani, Y. (2004). Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. *Endocr J* 51, 165-9.

Tan, B. L., Kassim, N. M., and Mohd, M. A. (2003). Assessment of pubertal development in juvenile male rats after sub-acute exposure to bisphenol A and nonylphenol. *Toxicol Lett* 143, 261-70.

Tan, B. L., and Mustafa, A. M. (2003). Leaching of bisphenol A from new and old babies' bottles, and new babies' feeding teats. *Asia Pac J Public Health* 15, 118-23.

Tanaka, M., Nakaya, S., Katayama, M., Leffers, H., Nozawa, S., Nakazawa, R., Iwamoto, T., and Kobayashi, S. (2006). Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth. *Hum Exp Toxicol* 25, 369-373.

Tando, S., Itoh, K., Yaoi, T., Ikeda, J., Fujiwara, Y., and Fushiki, S. (2007). Effects of pre- and neonatal exposure to bisphenol A on murine brain development. *Brain and Develop* 29, 352-356

Teeguarden, J. G., and Barton, H. A. (2004). Computational modeling of serum binding proteins and clearance in extrapolations across life stages and species for endocrine active compounds. *Risk Anal* 24, 751-70.

Teeguarden, J. G., Waechter, J. M., Jr., Clewell, H. J., 3rd, Covington, T. R., and Barton, H. A. (2005). Evaluation of Oral and Intravenous Route Pharmacokinetics, Plasma Protein Binding, and Uterine Tissue Dose Metrics of Bisphenol A: A Physiologically Based Pharmacokinetic Approach. *Toxicol Sci* 85, 823-838.

Thayer, K. A., Ruhlen, R. L., Howdeshell, K. L., Buchanan, D. L., Cooke, P. S., Preziosi, D., Welshons, W. V., Haseman, J., and vom Saal, F. S. (2001). Altered prostate growth and daily sperm production in male mice exposed prenatally to subclinical doses of 17alphaethinyl oestradiol. *Hum Reprod* 16, 988-96.

Thigpen, J. E., Haseman, J. K., Saunders, H. E., Setchell, K. D., Grant, M. G., and Forsythe, D. B. (2003). Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. *Comp Med* 53, 607-15.

Thomson, B. M., Cressey, P. J., and Shaw, I. C. (2003). Dietary exposure to xenoestrogens in New Zealand. *J Environ Monit* 5, 229-35.

Thomson, B. M., and Grounds, P. R. (2005). Bisphenol A in canned foods in New Zealand: an exposure assessment. *Food Addit Contam* 22, 65-72.

Thuillier, R., Wang, Y., and Culty, M. (2003). Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors alpha and beta in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. *Biol Reprod* 68, 867-80.

Tinwell, H., Haseman, J., Lefevre, P. A., Wallis, N., and Ashby, J. (2002). Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. *Toxicol Sci* 68, 339-48.

Toyama, Y., Suzuki-Toyota, F., Maekawa, M., Ito, C., and Toshimori, K. (2004). Adverse effects of bisphenol A to spermiogenesis in mice and rats. *Arch Histol Cytol* 67, 373-81.

Tominaga, T., Negeshi, T., Hirroka, H., Miyachi, A., Inoue, A., Hayasaka I, and Yoshikawa, Y. (2006). Toxicokinetics of bisphenol A in rats, moeys and chimpanzees by the LCMS/ MS method. *Toxcicology* 226, 208-17.

Toyama, Y., and Yuasa, S. (2004). Effects of neonatal administration of 17beta-estradiol, beta-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis. *Reprod Toxicol* 19, 181-8.

Tsukioka, T., Brock, J., Graiser, S., Nguyen, J., Nakazawa, H., and Makino, T. (2003). Determination of trace amounts of bisphenol A in urine by negative-ion chemicalionization-gas-chromatography/mass spectrometry. *Anal. Sci*. 19, 151-3.

Tyl, R. W., Myers, C. B., Marr, M. C., Thomas, B. F., Keimowitz, A. R., Brine, D. R., Veselica, M. M., Fail, P. A., Chang, T. Y., Seely, J. C., Joiner, R. L., Butala, J. H., Dimond, S. S., Cagen, S. Z., Shiotsuka, R. N., Stropp, G. D., and Waechter, J. M. (2002). Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci* 68, 121-46.

Tyl, R.W., Myers, C.B., Marr, M.C. (2004a) Rangefinding study for the two-generation reproductive toxicity evaluation of 17β-estradiol (E2, CAS no. 50-28-2) administered in the feen to CD-1® Swiss mice (modified OECD 416). *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

Tyl, R.W., Myers, C.B., Marr, M.C. (2004b) Two-generation reproductive toxicity evaluation of 17β-estradiol (E2, CAS no. 50-28-2) administered in the feen to CD-1® Swiss mice (modified OECD 416). *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

Tyl, R.W., Myers, C.B., Marr, M.C. (2005) Thirteen-week rangefinding study for the two-generation reproductive toxicity evaluation of bisphenol A (BPA; CAS 80-05-7) administered in the feed to CD-1® (Swiss) mice. *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

Tyl, R.W., Myers, C.B., Marr, M.C., Sloan, C.S., Castillo, N.P., Veselica, M.M,. Seely, J.C., Dimond, S.S., Van Millar, J.P., Stropp, G.D., and Waechter, J.M. (2006). Two-generation study of dietary 17β-estradiol (E2) in CD-1® Swiss mice. *The Toxicologist* 90 (1-suppl.), 253 (Abstract No. 1242)

Tyl, R. W., Myers, C. B., and Marr, M. C. (2007). Draft Final Report: Two-generation reproductive toxicity evaluation of Bisphenol A (BPA; CAS No. 80-05-7) administered in the feed to CD-1® Swiss mice (modified OECD 416). *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

UK Committee on Toxicity (2001). TOX/MIN/2002/02. Minutes of the meeting held on 13 March 2001. Available at: http://www.food.gov.uk/multimedia/pdfs/cot08.pdf .

Vidaeff, A. C., and Sever, L. E. (2005). In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence. *Reprod Toxicol* 20, 5-20.

Vohr H.W. (2002). Bisphenol A: local lymph node assay in mice (LLNA/IMDS). Unpublished report. Bayer AG, Department of Toxicology, Friedrich-Ebert-Str., D-42096, Wuppertal. Report No AT00155.

Vohr H.W. (2003). Bisphenol A: study of photoreactive potential in mice (LLNA/IMDS). Unpublished report. Bayer AG, Department of Toxicology, Friedrich-Ebert-Str., D-42096, Wuppertal. Report No AT00413.

Volkel, W., Bittner, N., and Dekant, W. (2005). Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by HLPC-MS/MS. *Drug Metab Dispos*.

Volkel, W., Colnot, T., Csanady, G. A., Filser, J. G., and Dekant, W. (2002). Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol* 15, 1281-7.

vom Saal, F.S., Cooke, P., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani, S., and Welshons, W.V. (1998). A physiologically based approach to the study of bisphenol-A and other oestrogenic chemicals on the size of reproductive organs, daily sperm production, and behaviour. *Toxicol. Indust. Health* 14, 239-260.

Walsh, D. E., Dockery, P., and Doolan, C. M. (2005). Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [Ca2+]i in human breast cancer cells. *Mol Cell Endocrinol* 230, 23-30.

Watanabe, S., Wang, R. S., Miyagawa, M., Kobayashi, K., Suda, M., Sekiguchi, S., and Honma, T. (2003). Imbalance of testosterone level in male offspring of rats perinatally exposed to bisphenol A. *Ind Health* 41, 338-41.

Wenzel, A., Muller, J. and Ternes, T. (2003). Study on endocrine disruptors in drinking water. Fraunhofer Institute, Schmallenberg, Germany. Report ENV.D.1/ETU/2000/0083.

Wistuba, J., Brinkworth, M. H., Schlatt, S., Chahoud, I., and Nieschlag, E. (2003). Intrauterine bisphenol A exposure leads to stimulatory effects on Sertoli cell number in rats. *Environ Res* 91, 95-103.

Witorsch, R. J. (2002a). Endocrine disruptors: can biological effects and environmental risks be predicted? *Regul Toxicol Pharmacol* 36, 118-30.

Witorsch, R. J. (2002b). Low-dose in utero effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. *Food Chem Toxicol* 40, 905-912.

Wong, K. O., Leo, L. W., and Seah, H. L. (2005). Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles. *Food Additives and Contaminants*, 3.

Wozniak, A. L., Bulayeva, N. N., and Watson, C. S. (2005). Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca2+ fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ Health Perspect* 113, 431-439.

Yamada, H., Furuta, I., Kato, E. H., Kataoka, S., Usuki, Y., Kobashi, G., Sata, F., Kishi, R., and Fujimoto, S. (2002). Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. *Reprod Toxicol* 16, 735-9.

Yang, M., Kim, S. Y., Lee, S. M., Chang, S. S., Kawamoto, T., Jang, J. Y., and Ahn, Y. O. (2003). Biological monitoring of bisphenol a in a Korean population. *Arch Environ Contam Toxicol* 44, 546-51.

Ye, X., Kuklenyik, Z., Needham, L. L., and Calafat, A. M. (2005). Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal Chem* 77, 5407-13.

Yellayi, S., Naaz, A., Szewczykowski, M. A., Sato, T., Woods, J. A., Chang, J., Segre, M., Allred, C. D., Helferich, W. G., and Cooke, P. S. (2002). The phytoestrogen genistein induces thymic and immune changes: a human health concern? *Proc Natl Acad Sci U S A* 99, 7616-21.

Yoshida, M., Shimomoto, T., Katashima, S., Watanabe, G., Taya, K., and Maekawa, A. (2004). Maternal exposure to low doses of bisphenol A has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. *J Reprod Dev* 50, 349-360.

Yoshida, T., Horie, M., Hoshino, Y., and Nakazawa, H. (2001). Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography. *Food Addit Contam* 18, 69-75.

Yoshihara, S., Mizutare, T., Makishima, M., Suzuki, N., Fujimoto, N., Igarashi, K., and Ohta, S. (2004). Potent estrogenic metabolites of bisphenol A and bisphenol B formed by rat liver S9 fraction: their structures and estrogenic potency. *Toxicol Sci* 78, 50-59.

Yoshino, H., Ichihara, T., Kawabe, M., Imai, N., Hagiwara, A., Asamoto, M., and Shirai, T. (2002). Lack of significant alteration in the prostate or testis of F344 rat offspring after transplacental and lactational exposure to bisphenol A. *J Toxicol Sci* 27, 433-439.

Zalko, D., Soto, A. M., Dolo, L., Dorio, C., Rathahao, E., Debrauwer, L., Faure, R., and Cravedi, J. P. (2003). Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environ Health Perspect* 111, 309-19.

Zsarnovszky, A., Le, H. H., Wang, H. S., and Belcher, S. M. (2005). Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex:

potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. *Endocrinology* 146, 5388-96.

European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

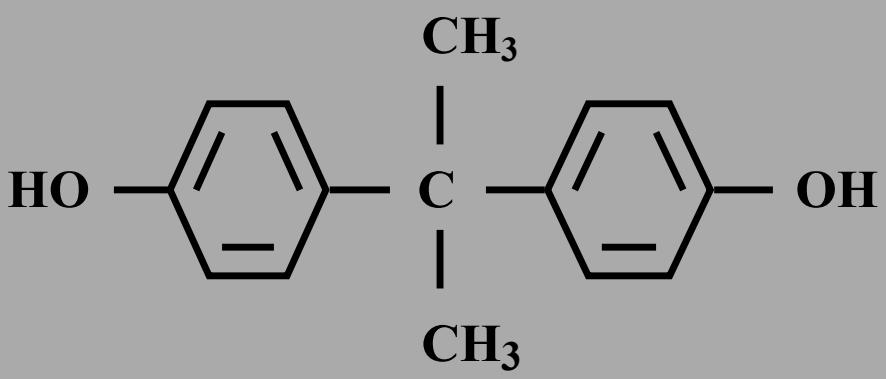
Existing Substances

European Union Risk Assessment Report

CAS No: 80-05-7

EINECS No: 201-245-8

4,4'-isopropylidenediphenol (bisphenol-A)



3rd Priority List

Volume: 37

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

EUR 20843 EN

European Union Risk Assessment Report
bisphenol-A
CAS: 80-05-7
EC: 201-245-8
PL-3
37

European Union Risk Assessment Report

4,4'-ISOPROPYLIDENEDIPHENOL

(BISPHENOL-A)

CAS No: 80-05-7

EINECS No: 201-245-8

RISK ASSESSMENT

Cataloguing data can be found at the end of this publication
Luxembourg: Office for Official Publications of the European Communities, 2003

Printed in Italy

4,4'-ISOPROPYLIDENEDIPHENOL

(BISPHENOL-A)

CAS No: 80-05-7

EINECS No: 201-245-8

RISK ASSESSMENT

Final Report, 2003

United Kingdom

This document has been prepared by the UK rapporteur on behalf of the European Union. The scientific work on the environmental part was prepared by the Building Research Establishment Ltd (BRE), under contract to the rapporteur.

Contact (human health): Health & Safety Executive
Industrial Chemicals Unit
Magdalen House, Stanley Precinct
Bootle, Merseyside L20 3QZ
e-mail: ukesrhh@hse.gsi.gov.uk
Fax: + 44 151 951 3308

Contact (environment) Environment Agency
Chemicals Assessment Section
Ecotoxicology and Hazardous Substances National Centre
Isis House, Howbery Park
Wallingford, Oxfordshire, OX10 8BD
e-mail: ukesrenv@environment-agency.gov.uk
Fax: +44 (0)1491 828 559

Date of Last Literature Search:	**1998**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2003**

(The last full literature survey was carried out in 1998 - targeted searches were carried out subsequently).

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

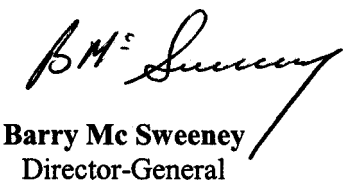
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.



Barry Mc Sweeney
Director-General
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

---

[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No: 80-05-7
EINECS No: 201-245-8
IUPAC name: 2,2-bis(4-hydroxyphenyl)propane (also known as bisphenol-A)

Environment

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the following scenarios for the water and sediment compartments:

Thermal paper recycling
Use as an inhibitor in PVC production
Preparation of additive packages for PVC processing
Use as an anti-oxidant in the production of plasticisers for use in PVC processing.

For these uses further refining the PNEC for water will not change the outcome of the assessment. Although these scenarios are referred to as generic in the exposure section, the PEC estimates are based on data from the industry and use areas and are considered representative. It appears unlikely that the provision of further information would alter the conclusions. The use of bisphenol-A in the manufacture of PVC resin is due to be phased out in Europe by the end of 2001 under a voluntary agreement by industry.

Conclusion (i) There is need for further information and/or testing.

This conclusion applies to the following scenarios for the water and sediment compartments:

Bisphenol-A production [4]
Epoxy resin production
Thermal paper production
Phenoplast cast resin processing
Use as an anti-oxidant in PVC processing
Use as a plasticiser in PVC processing
Regional concentration

These scenarios do not give rise to a risk when the PNEC based on the standard endpoint of egg hatchability is used. However, if a "conservative" PNEC based on research studies indicating effects on snails and sperm development in fish is used, all scenarios and the regional concentration give rise to a risk. There is considerable uncertainty over the validity of the lower PNEC. Recent research studies on snails have raised the possibility of effects at still lower concentrations. If these studies were to be used as the basis for a PNEC derivation, the much lower value would have implications for possible risk reduction measures. It is therefore considered that further studies on the toxicity of bisphenol-A to snails are needed, to provide a

[4] Four uses only take place on sites where bisphenol-A is produced. Emissions from these processes are included in the site-specific emissions for bisphenol-A production and so are not separately identified. These are:
Polyol/polyurethane production
Brake fluid manufacture
Polyamide production
Polycarbonate production

more robust basis for the derivation of a PNEC. The re-investigation of the effects on sperm development in fish is also required. The apparently elevated levels measured in sediment will also be considered when the aquatic assessment is refined.

Conclusion (i) also applies to the following uses of bisphenol-A for the terrestrial compartment:

Epoxy resin production
Phenoplast cast resin processing
Thermal paper recycling
Use as an inhibitor in PVC production
Preparation of additive packages for PVC processing
Use as an anti-oxidant in the production of plasticisers for use in PVC processing
Use as an anti-oxidant in PVC processing
Use as a plasticiser in PVC processing
Regional concentration

The equilibrium partitioning method has been used, so testing on terrestrial organisms could revise the PNEC. It is currently not clear what testing would be appropriate, as the most sensitive effects in aquatic organisms appear to be related to endocrine disruption. It is proposed to await the outcome of the further work on aquatic organisms before deciding on testing for the terrestrial compartment. In addition, the UK Department of Environment, Food & Rural Affairs is conducting research into endocrine disruption in the earthworm *Eisenia andrei* and bisphenol-A is one of the test compounds. The project aim is to develop molecular markers of exposure to, and population level effects of, endocrine disruption for use in field and laboratory studies. It is expected that this work will provide relevant information, to a timescale compatible with that of the aquatic tests.

A revision of the PNECoral value will also be considered if additional information on the interpretation of mammalian developmental data becomes available as a result of further studies being conducted for the human health assessment.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion applies to microorganisms in wastewater treatment plants and to the air compartment for all scenarios. It also applies to the terrestrial compartment for the following:

Bisphenol-A production
Thermal paper manufacture

This conclusion also applies to the water, sediment and terrestrial compartments for the following uses:

Unsaturated polyester production
Can coating production
Tyre manufacture
Alkoxylated bisphenol-A production
Tetrabromobisphenol-A production and use
Phenoplast cast resin production

For these six scenarios, emissions are negligible and PECs have not been calculated in this assessment (these processes are either completely dry, or any aqueous effluent produced is disposed of through incineration).

Human health

Human health (toxicity)

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached in relation to eye and respiratory tract irritation, effects on liver and for toxicity for reproduction (effects on fertility and on development) during the manufacture of bisphenol-A and the manufacture of epoxy resins. In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact.

Conclusion (i) There is need for further information and/or testing.

This conclusion is reached in relation to developmental toxicity. Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached in relation to eye and respiratory tract irritation, effects on liver following repeated exposure and effects on fertility for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, manufacture of PVC, thermal paper manufacture, manufacture of tin plating additive and manufacture of TBBA. This conclusion is also reached in relation to repeated dose toxicity to the respiratory tract for all scenarios.

Consumers

Conclusion (i) There is need for further information and/or testing.

This conclusion is reached in relation to developmental toxicity. Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

For all other endpoints, given that consumer exposure is very low, there are no concerns for human health effects as a result of consumer exposure.

Humans exposed via the environment

Conclusion (i) There is need for further information and/or testing.

This conclusion is reached in relation to developmental toxicity. Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on

development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

For all other endpoints, conclusion (ii) applies to exposures arising from both local and regional exposure scenarios.

Combined exposure

Conclusion (i) There is need for further information and/or testing.

This conclusion is reached in relation to developmental toxicity. Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This conclusion is reached in relation to liver effects following repeated exposure and effects on fertility.

Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached because there are no risks from physico-chemical properties arising from the use of bisphenol-A.

CONTENTS

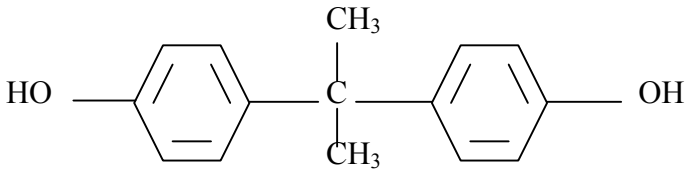
Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it.

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS-No: 80-05-7
EINECS-No: 201-245-8
IUPAC name: 2,2-bis(4-hydroxyphenyl)propane
Molecular weight: 228.29
Molecular formula: $C_{15}H_{16}O_2$
Structural formula:



Smiles notation Oc(ccc(c1)C(c(ccc(O)c2)c2)(C)C)c1

2,2-Bis(4-hydroxyphenyl)propane is more commonly known as bisphenol-A. The common name will be used throughout this report. Bisphenol-A may also be known by the following synonyms:

BPA (Common abbreviation)
2,2-Bis(4-hydroxyphenyl)propane
2,2-Bis(p-hydroxyphenyl)propane
p,p'-Isopropylidene-bisphenol
p,p'-Isopropylidene-di-phenol
Phenol, 4,4'-Isopropylidene-di
Diphenylol Propane
Parabis (Trademark)
Bis (4-hydroxyphenyl) dimethyl methane
Bis (4-hydroxyphenyl)propane
Dian (Trademark)
Dimethylmethylene-p,p'-di-phenol
Dimethyl Bis(p-hydroxyphenyl)methane
4,4'-Dihydroxy-2,2'-diphenyl propane
4,4'-Dihydroxydiphenyldimethyl methane
4,4'-Dihydroxydiphenyl propane
ß-Di-p-Hydroxyphenyl propane
p,p'-Dihydroxydiphenyldimethyl methane
p,p'-Dihydroxydiphenyl propane
2,2'-(4,4'-Dihdroxydiphenyl) propane
4,4'-Dihydroxydiphenyl-2,2'-propane
2,2'-Di(4-hydroxyphenyl) propane
2,2'-Di(4-phenylol) propane
4,4'-Isopropylidene bisphenol
4,4'-(1-methylethylidene)bisphenol

1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Purity

The purity of bisphenol-A is stated as being 99-99.8% depending upon the manufacturer. Impurities typically include phenol (<0.06%), ortho and para isomers of bisphenol-A (<0.2%) and water (<0.2%).

1.2.2 Additives

There are no stated additives used with bisphenol-A.

1.3 PHYSICO-CHEMICAL PROPERTIES

Bisphenol-A is a white solid at room temperature and usually occurs as flakes or a powder. It has a mild phenolic odour.

1.3.1 Melting point

The melting point of the commercial material is quoted at between 150-157°C (Sax and Lewis, 1996; Pohanish and Greene, 1996; IPCS, 1993; Merck Index, 1989; Hubbard, 1948; Bayer Leverkusen, 1989; Ullmann's Encyclopaedia of Industrial Chemistry, 1991). Early references to recrystallised material give a value of 153°C (Zinke and Greuters, 1905) although the exact purity is not known. The melting point of the commercial material will reflect the nature of the manufacturing process. As melting point is depressed by impurities the true melting point of the pure material will be reflected by the higher values i.e. circa 155-157°C. A value of 155°C will be used for the environmental modelling.

1.3.2 Boiling point

The "normal" boiling point is quoted as 360.5°C (DIPPR, 1994 and IUCLID). No original test data are cited.

A boiling point of 250-252°C at 13 mmHg or 1.7 kPa (von Braun, 1925) has been established. This value has been quoted more recently (Eyre and Spottiswood, 1965; Sax and Lewis, 1996) though without mention of the original reference. A value of 220°C at 4 mmHg or 0.5 kPa is quoted in several handbooks (CRC, 1995; Merck Index, 1989). The original reference to this value has not been established. A value of 220°C at 1 atmosphere (101.3 kPa) (Pohanish and Greene, 1996) is clearly wrong. The value relating to a pressure of 4 mm Hg (0.5 kPa) as quoted in the Merck Index is quoted in the reference to bisphenol-A in the International Chemical Safety Cards (IPCS, 1993).

Reports (Merck Index, 1989) suggest that at pressures higher than 8 mmHg (~ 1 kPa) decomposition occurs above 220°C. Decomposition at elevated temperatures is accompanied by emission of acrid and irritating fumes (Sax and Lewis, 1996).

A boiling point of 250-252°C at 13 mmHg (1.7 kPa) will be accepted as the original data have been reviewed. It is possible that some decomposition may occur.

The boiling point at atmospheric pressure will be accepted as ~ 360°C with decomposition.

1.3.3 Relative density

Relative densities of 1.195 kg/m^3 at 25°C (Sax and Lewis, 1996) and 1.13 kg/m^3 (Hubbard et al., 1948) are reported for bisphenol-A. Ullmann's Encyclopaedia of Industrial Chemistry (1991) reports values of 1.04 g/cm^3 at 20°C and 1.065 g/cm^3 at 160°C. The density will vary according to the manufacturer and the exact temperature of measurement. A density of 1.1-1.2 kg/m^3 at 25°C is judged acceptable for use in the risk assessment.

1.3.4 Vapour pressure

Vapour pressures of $4.1 \cdot 10^{-10}$ kPa and $5.3 \cdot 10^{-9}$ kPa at 25°C (Bayer AG, 1988) and 0.65 mm Hg (0.009 kPa) at 190°C (Kluwer, 1991) are reported for bisphenol-A. For environmental modelling purposes a vapour pressure of $5.3 \cdot 10^{-9}$ kPa at 25°C will be used.

1.3.5 Water solubility

Ullmann's Encyclopaedia of Industrial Chemistry (1991) reports the solubility of bisphenol-A in water as 0.322 wt% at 83°C. Water solubilities of 120 mg/l at 25°C (Howard et al., 1990) and 301 mg/l at room temperature (Bayer AG, 1988) are reported. A value of 300 mg/l will be used for environmental modelling purposes.

1.3.6 n-Octanol-water partition coefficient (Kow)

The Dow material safety data sheet for bisphenol-A gives a value for the octanol-water partition coefficient (as log Kow) of 3.32. This is the same as the value given in Hansch and Leo (1979), Korenman and Gorokhov (1973) and Howard et al. (1990). Eadsforth (1983) determined an n-octanol/water partition coefficient of 160 (log Kow 2.2) for bisphenol-A using a reverse phase HPLC method. Bayer AG (1993) reports a calculated value of 3.5 and a measured value of 3.4. The log Kow is estimated as 3.64 using the SRC KOWWIN program. A log Kow of 3.4 will be used for environmental modelling purposes.

1.3.7 Flash point

Sax and Lewis (1996) report a flash point of 79.4°C for bisphenol-A. Ullmann's Encyclopaedia of Industrial Chemistry (1991) reports a value of 227°C. The Dow material safety data sheet for bisphenol-A gives a value of 213°C conducted to ASTM D92-66. DIPPR (1994) report a value of 212.78°C. Kluwer (1991) reports a value of 207°C. A value of circa 207°C will be used in this assessment.

1.3.8 Autoflammability

Ullmann's Encyclopaedia of Industrial Chemistry (1991) reports an ignition temperature of 510°C. Grossel (1988) reports a value of 570°C and Kluwer (1991) reports a value of 600°C.

A value of 532°C is quoted by The Society of the Plastics Industry (1997). It should be noted that decomposition would start to occur before these temperatures are reached.

1.3.9 Explosivity

Bisphenol-A is not explosive in the conventional sense or when considering structure or chemical groupings. The finely powdered material is however, a significant dust explosion hazard and dust control is necessary for safe handling. The Dow material safety data sheet for bisphenol-A states that bisphenol-A is dust explosion class 3. Grossel (1988) quotes a minimum exposable concentration of 0.012 g/l with a maximum oxygen concentration of 5% to prevent ignition.

1.3.10 Oxidising properties

Bisphenol-A is not an oxidising agent on the basis of structural considerations.

1.3.11 Summary of physico-chemical properties for bisphenol-A

The physico-chemical properties of bisphenol-A are summarised in **Table 1.1**. The table also notes which values have been used in the environmental exposure calculations.

Table 1.1 Physico-chemical properties for bisphenol-A

Parameter	Value	Comments
Physical state at normal temperature and pressure	White solid flakes or powder	Depends upon manufacturing process
Melting point	155-157°C 155ºC used in environmental models	Depends upon manufacturing process
Boiling point	360°C at 101.3 kPa	Decomposition is also likely
Relative density	circa 1.1-1.2 kg/m^3 at 25°C	
Vapour pressure	$5.3 \cdot 10^{-9}$ kPa used in environmental models	See text for more details
Solubility in water	300 mg/l used in environmental models	
Partition coefficient	Log Kow circa 3.3-3.5 3.4 used in environmental models	
Flash point	circa 207°C	
Autoflammability	circa 532°C	
Explosive limits (in air)	Minimum explosive concentration 0.012 g/l with oxygen > 5%	
Oxidising properties	Not an oxidising agent	

1.4 CLASSIFICATION

The classification and labelling of bisphenol-A has recently been discussed (January 2002) and provisional agreement has been reached, as follows:

Classification:	Repr. Cat. 3; R62 Xi; R37-41, R43
Labelling:	Xn R37-41-43-62 S2-26-36/37-39-46

R62 states: Possible risk of impaired fertility

Toxicity to reproduction category 3 is for substances which cause concern for human fertility, generally on the basis of:

1) results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2;

2) other relevant information.

Xi indicates	"irritant"
Xn indicates	"harmful"
R37 states:	Irritating to respiratory system
R41 states:	Risk of serious damage to eyes
R43 states:	May cause sensitisation by skin contact
S(2) states:	Keep out of the reach of children
S26 states:	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37 states:	Wear suitable protective clothing and gloves
S39 states:	Wear eye/face protection
S46 states:	If swallowed, seek medical advice immediately and show this container or label

For the environment, bisphenol-A has acute L/EC_{50}s in the range 1-15.5 mg/l [5], is biodegradable and is not bioaccumulative. It therefore does not fit the current criteria for classification. The rapporteur considers that the observed effects at low concentrations in longer-term studies justify the application of suitable risk and safety phrases to this substance, but that further discussion is needed on what these should be, and more generally on how to include such effects in the classification system.

[5] Salt-water organisms appear to be of similar sensitivity to freshwater organisms. The lowest reported acute toxicity value is a 96-hour EC_{50} of 1 mg/l (based on cell number) for *Skeletonema costatum*, a marine alga, which could indicate that classification with R50 is appropriate. However the study was longer than the 72 hours indicated in the classification requirements and the EC_{50} value was derived using a non-linear extrapolation method. The rapporteur has re-analysed the original data using probit analysis in accordance with the OECD guideline, and this gives an EC_{50} of 1.1 mg/l. A second measure of toxicity from the same study (chlorophyll content) gave a 96-hour EC_{50} of 1.8 mg/l, and all of the other available acute toxicity results are greater than 1 mg/l. On the balance of the available information the acute toxicity of bisphenol-A to aquatic organisms is considered to lie above 1 mg/l.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Four companies within the EU manufacture bisphenol-A. There are a total of six production sites based in Germany, The Netherlands, Belgium and Spain. The total amount of bisphenol-A manufactured within the EU, based upon submissions to CEFIC by the manufacturers, is estimated at approximately 700,000 tonnes/year (taken from 1996, 1997, 1998 and 1999 data). According to EU statistics in 1997 the total imports of bisphenol-A into the EU were 8,010 tonnes/year and exports from the EU were 1,887 tonnes/year. Figures from the American Society of the Plastics Industry indicate that in 1997 American manufacturers exported 5,855 tonnes to Europe, while imports from Europe were 8,509 tonnes. From the data submitted by the EU manufacturers net exports are in the region of 25,000 tonnes/year for 1998 and net imports in the region of 3,000 tonnes/year. There is some discrepancy between the EU and American import and export values, which are based upon 1997 and 1998 industry data, respectively but this is not considered significant in relation to the total tonnage involved. For the purposes of this assessment a representative EU consumption of bisphenol-A is estimated to be approximately 690,000 tonnes/year from producer and end user data.

Bisphenol-A is manufactured from phenol and acetone by an acid or alkaline catalysed condensation reaction. Industrially it is produced by an acid catalysed reaction. This is because in the alkali catalysed reaction the formation of by-products is increased. Acidic ion exchangers with bivalent sulphur compound promoters attached are more commonly used than mineral acids. Phenol is often used in excess as the solvent to avoid formation of higher condensation products.

In the production process phenol and acetone are injected into a reactor filled with a cation exchanger. Conversion to bisphenol-A occurs at about 75°C. The mixture passes into a concentrator where it is freed of water and acetone under reduced pressure. Bisphenol-A crystallises out when cooled and is then washed with phenol and distilled out under reduced pressure. The bisphenol-A produced is usually of a very high purity.

2.2 USES

2.2.1 Uses within the EU

Table 2.1 summarises the amount of bisphenol-A used within different applications. This is based upon submissions made by the bisphenol-A manufacturers and end users to CEFIC.

Table 2.1 Bisphenol-A use pattern data

Use pattern data	Tonnes/year	Percentage of EU consumption
Polycarbonate production	486,880	71.1
Epoxy resin production	171,095	25.0
Phenoplast resins	8,800	1.3
Unsaturated polyester resin production	3,000	0.4
Can coating manufacture	2,460	0.4
Use PVC production and processing	2,250	0.3
Alkyloxylated bisphenol-A manufacture	2,020	0.3
Thermal paper manufacture	1,400	0.2
Polyols/Polyurethane manufacture	950	0.1
Modified polyamide production	150	<0.1
Tyre manufacture	110	<0.1
Brake fluid	45	<0.1
Minor uses	5,990	0.9
EU Consumption	**685,000**	

Notes: Figures in the table are approximate and based upon industry submissions for the years 1996-1999.
Minor uses include sales to chemical merchants and minor sales. The uses of these minor sales are not expected to be different from those mentioned above

The information in **Table 2.2** has been obtained from the Danish Product Register (personal communication, 6 July 1998). In the Danish Product Register 915 products are reported as containing bisphenol-A. The total quantity of bisphenol-A used in products is reported as 151 tonnes/year. More detailed information is given in **Table 2.2**.

Table 2.2 Information from the Danish Product Register (June 1998)

Product type	Bisphenol-A concentration in product	Number of products	Quantity of bisphenol-A (Tonnes/year)
Insulation materials	0-1%	3 Total: 5	<1 Total: 55
Process regulators (Hardeners)	0-1% 1-5% 5-10% 10-20% 20-50%	60 39 15 22 23 Total: 160	<1 2 1 <1 17 Total: 20
Fillers	0-1% 1-5%	42 3 Total: 50	<1 16 Total: 16

Table 2.2 continued overleaf

Table 2.2 continued Information from Danish Product Register

Product type	Bisphenol-A concentration in product	Number of products	Quantity of bisphenol-A (Tonnes/year)
Softeners	Information classified as confidential on register		
Adhesives, binding agents	0-1% 5-10% 20-50%	180 3 3 Total: 201	<1 <1 <1 Total: 4
Construction materials	0-1% 10-20% 20-50%	29 3 9 Total: 45	<1 <1 2 Total: 2

Notes: If the number of products within a category is sufficiently low the information is considered to be confidential and is marked as such. If the number of products within a given concentration interval is too small the line is deleted. This can result in totals larger than the sum of the above mentioned numbers.

2.2.2 Description of the uses of bisphenol-A

2.2.2.1 Polycarbonate production

One of the main uses of bisphenol-A is in the production of polycarbonate. Approximately 487,000 tonnes/year bisphenol-A is used in the production of polycarbonates, at five sites within the EU. World-wide production of polycarbonate is estimated at 1 million tonnes/annum (Polycarbonate Resin Manufacturers Group of Japan, 1999). The polycarbonate is then sold on to processors who form it into finished products for consumer use. The following is general information on the different methods that may be employed in the production of polycarbonate. It does not specifically reflect the methods currently used within the EU - these processes are confidential to the manufacturing companies - but is considered to be representative.

Polycarbonates are prepared commercially by two processes: Schotten-Baumann reaction of phosgene and an aromatic diol in an amine-catalysed interfacial condensation reaction; or via base-catalysed transesterification of a bisphenol with a monomeric carbonate (Kirk-Othmer, Vol. 19, 1996).

Most bisphenol-A polycarbonate is produced by an interfacial polymerisation process utilising phosgene. This method involves stirring a slurry or solution of bisphenol-A and 1-3% of a chain stopper, such as phenol, p-*t*-butylphenol, or *p*-cumylphenol, in a mixture of methylene chloride and water, while adding phosgene in the presence of a tertiary amine catalyst. Sodium hydroxide solution is added to maintain the correct reaction pH. The by-product of the reaction is sodium chloride, which concentrates in the aqueous phase. The polymer dissolves into the methylene chloride phase. Phosgene addition continues until all the phenolic groups are converted to carbonate functionalities. Some hydrolysis of phosgene to sodium carbonate may also occur. When the reaction is complete, the methylene chloride solution of polymer is washed first with acid to remove residual base and amine, then with water. The aqueous sodium chloride stream can be reclaimed, ultimately regenerating phosgene. There are many variations to this process, including the use of many different types of catalysts, continuous or semi-continuous processes and methods which rely on formation of bischloroformate oligomers followed by polycondensation (Kirk-Othmer, Vol. 19, 1996).

Methods for the isolation of the polymer product include antisolvent precipitation, removal of solvent in boiling water, spray drying, and melt devolatisation using a film evaporator. The polymer must be isolated dry, to avoid hydrolysis, and essentially be devoid of methylene chloride (Kirk-Othmer, Vol. 19, 1996).

An alternative method of production of polycarbonates is via a transesterification route. The transesterification process utilises no solvent during polymerisation and thus eliminates the use of chlorinated solvents. In the process diphenyl carbonate and bisphenol-A are combined with small amounts of basic catalysts in a melt reactor, in which phenol begins to be liberated. As the raw materials pass from reactor to reactor the temperature of the reaction is increased and higher vacuum is applied, so that phenol can be removed, driving the process towards the polymer. This method requires high purity starting material (Kirk-Othmer, Vol. 19, 1996).

Polycarbonates produced from bisphenol-A generally have good optical clarity, impact resistance and ductility at room temperature and below. This makes them ideally suited to a wide range of end applications. Polycarbonates may be fabricated by conventional thermoplastic processing operations. The maximum residual content of bisphenol-A in polycarbonate is reported as 50 mg/kg. Typically the residual content is < 10 mg/kg. At present there are no legal restrictions on the amount of bisphenol-A that can be present in the finished product.

Among the uses reported for polycarbonate are the following:

- compact disc manufacture;
- solid and multi wall sheet in glazing applications and film;
- food contact containers, e.g. returnable milk and water bottles (e.g. used in water cooler machines) and baby bottles;
- medical devices;
- as polycarbonate blends for diverse injection moulded, functional parts used mainly in the electric/electronics industry and the automotive industry. Examples from the electric/electronics industry include alarm devices, car telephone and mobile phone housings, coil cores, displays, computer parts, household electrical equipment, lamp fittings and power plugs. Examples from the automotive industry include car head and rear light reflectors and coverings, bumpers, radiator and ventilation grilles, safety glazing, inside lights, motor cycle wind shields and protective helmets;
- as modified high heat resistant copolycarbonates of bisphenol-A used mainly in the automotive and electric/electronics industry.

2.2.2.2 Epoxy resin production

Epoxy resin production is the second largest user of bisphenol-A in the EU. Approximately 171,000 tonnes/year of bisphenol-A is used in the production of epoxy resins per year, with around 90% being used at 8 known sites.

There are a number of different epoxy resins, which vary depending upon the starting constituents. However, diglycidyl ethers of bisphenol-A derived from bisphenol-A and epichlorohydrin are still among the most widely used epoxy resins (Kirk-Othmer, Vol. 9, 1994).

Liquid epoxy resins may be synthesised by a two-step reaction of an excess of epichlorohydrin to bisphenol-A in the presence of an alkaline catalyst. Initially the dichlorohydrin of bisphenol-A is

produced. The intermediate product then undergoes dehydrohalogenation with an alkali. In the preparation of commercial pure diglycidyl ether of bisphenol-A (DGEBPA) an excess of epichlorohydrin is used in order to minimise polymerisation of the reactants to higher molecular weight species (Kirk-Othmer, Vol. 9, 1994).

Advanced epoxy resins can be manufactured according to the taffy- (one step) or fusion (two step) process. In the taffy process, bisphenol-A reacts directly with epichlorohydrin in the presence of caustic soda. At the completion of the reaction, the mixture consists of an alkaline brine solution and water-resin emulsion and recovery of the product is accomplished by the separation of phases, washing the resin with water and removal of water under vacuum (Kirk-Othmer, Vol. 9, 1994).

In the advancement process, sometimes referred to as the fusion method, liquid epoxy resin is chain extended with bisphenol-A in the presence of a catalyst to yield higher polymerised products. The reaction is carried out at elevated temperatures. The finished product is isolated by cooling the molten resin and crushing or flaking or by allowing it to solidify in containers (Kirk-Othmer, Vol. 9, 1994).

The bisphenol-A derived epoxy resins are most frequently cured with anhydrides, aliphatic amines, or polyamides, depending on the desired end properties. Some of the desired properties are superior electrical properties, chemical resistance, heat resistance, and adhesion. Conventional epoxy resins range from low viscosity liquids to solid resins (Kirk-Othmer, Vol. 9, 1994). The uses of epoxy resins include as protective coatings, structural composites, electrical laminates, electrical applications and adhesives.

2.2.2.3 Other applications

Bisphenol-A is used in a range of other applications. In some cases use may be restricted to one or two sites in the EU. All of the uses reported appear to involve bisphenol-A in the manufacture of a product or as an intermediate in chemical production.

As well as epoxy resins bisphenol-A may be used in the production of a number of other resins including phenoplast resins, phenolic resins, and unsaturated polyester resins. Often resin manufacturers group all the resins they produce as epoxy resins, so it is difficult to determine the total amount of these other resins produced. Industry estimates are that 8,800 tonnes/year bisphenol-A is used in the production of phenoplast resins, and that 3,000 tonnes/year bisphenol-A is used in the production of unsaturated polyester resins. No values are available as to the amount of bisphenol-A used in the production of phenolic resins.

Phenoplast and phenolic resins are based upon the reaction products of phenols (bisphenol-A in this case) with formaldehyde. The phenolic resins formed using bisphenol-A are used in low colour moulding compounds and coatings (Kirk-Othmer, Vol. 18, 1996). There are two unsaturated polyester resin groups based upon bisphenol-A: bisphenol fumarates which are used in applications involving highly corrosive environments; and bisphenol-A epoxy dimethacrylates which have high flexural properties and high tensile elongation (Kirk-Othmer, Vol. 19, 1996).

Epoxy can coatings are based on high molecular weight epoxy resins made by advancing liquid epoxy resin with bisphenol-A. CEPE (the trade organisation representing the can coatings business) estimates that there are 5 sites within the EU carrying out this operation and that the total tonnage of bisphenol-A used is 2,460 tonnes/year.

The total amount of bisphenol-A used for thermal paper production within the EU is estimated at 1,400 tonnes/year. It is used as an additive in the coating that is applied to the paper, and its main function is as a developing agent when the paper is heated. The bisphenol-A in the paper reacts when it is heated; however if the paper is not completely developed residual bisphenol-A may remain. One tonne of bisphenol-A produces approximately 75 tonnes/year of thermal paper. Based upon a total usage of 1,400 tonnes/year bisphenol-A the total amount of thermal paper manufactured that contains bisphenol-A is 105,000 tonnes/year.

2,250 tonnes/year of bisphenol-A are sold per year for use in PVC manufacture and processing. There are four reported uses of bisphenol-A within the industry: as an inhibitor or reaction "killing" agent during the polymerisation stage of PVC production; as an anti-oxidant in PVC processing; as a constituent of an additive package used in PVC processing; and as an anti-oxidant in the production of plasticisers used in PVC processing. The use of bisphenol-A in the manufacture of PVC resin is due to be phased out in Europe by the end of 2001 under a voluntary agreement by industry.

2,020 tonnes/year of bisphenol-A are used in the production of ethoxylated bisphenol-A. This is reportedly used as an intermediate in the manufacture of some forms of epoxy resins. In the process bisphenol-A is charged to the first vessel and melted out at 140°C. A catalyst is added under vacuum and the bisphenol-A is then ethoxylated. Production is done on a batch wise basis.

950 tonnes/year of bisphenol-A are sold per year for use in the production of polyols that are used in the production of polyurethane. This use is only thought to occur at one site within the EU. In the process bisphenol-A is a reactant in the production of rigid polyols. The hydroxyl group of the bisphenol-A molecule reacts with propylene oxide to form a polyether binding. The polyol is then reacted with isocyanate to form a rigid polyurethane foam. Any residual bisphenol-A in the polyol reacts with the isocyanate. The production process is dry.

A small amount (45 tonnes/year) of bisphenol-A is sold for use in the production of brake fluid. Bisphenol-A is added to the brake fluid as an anti-oxidant.

110 tonnes/year of bisphenol-A are sold for use in tyre manufacture. This use is only thought to occur at one site within the EU. Bisphenol-A is used as a compounding ingredient for the manufacture of car tyres. The highly automated compounding step usually involves the blending of styrene butadiene rubber with highly aromatic extender oils, carbon black and various amine accelerators for the curing process. The compounding process is a dry operation with no aqueous effluents. The role of bisphenol-A, which is used in small quantities, as an anti-oxidant, is not fully understood in terms of imparting technological advantage to the cured elastomers. In the presence of the other compounding ingredients and during the curing process, the bisphenol-A becomes incorporated into the polymer matrix. Although it is used as an anti-oxidant this appears to be specifically for the compounding phases and it is presumably intended to protect the materials at this stage. There is no indication that it is intended to be the major anti-oxidant in the actual tyres, and so it is not expected to be present at significant levels in the finished product. As an anti-oxidant it will also react to give complex products so a proportion will be used up in this way.

150 tonnes/year of bisphenol-A are used in the production of modified polyamide. This use is only thought to occur at one site within the EU, which is also a bisphenol-A production plant. The modified polyamide grades produced have reduced moisture absorption conferring improved dimensional stability to the finished parts. The modified polyamide is produced via a dry process in closed systems. Bisphenol-A is introduced into polyamide at an average concentration of less than 8% by means of a compounding extruder. Bisphenol-A functions as an

additive, being tightly bound within the polar polyamide matrix. The modified polyamide is used for finished parts with improved dimensional stability mainly in electrotechnical applications.

Bisphenol-A may also be used in the production of tetrabromobisphenol-A (TBBPA), which is used as a flame retardant. Production of TBBPA in the EU stopped in early 1998 and then recommenced at the end of 1999 for a six-month period. After 2000 the company does not plan on restarting production. TBBPA is used as reactive monomer in the production of flame retardant polymers such as brominated epoxy resins for printed circuit boards. In this application TBBPA is fully reacted in the polymer backbone and residual TBBPA levels in the brominated epoxy resins are typically below 100 ppm. As TBBPA only contains trace amounts of bisphenol-A (typically less than 3 ppm) then the amount of bisphenol-A present in the polymeric material will be negligible. Any residual bisphenol-A in TBBPA would also be able to react in the polymerisation process. Hence the possibility of subsequent release of bisphenol-A from TBBPA used in this way is considered to be negligible.

[There are some indications that TBBPA may be used as an additive flame retardant in some cases, and that further derivatives of TBBPA are also used in this way. This type of use results in the inclusion of TBBPA in the polymer matrix rather than incorporation into the polymer chain. The same will be true of any residual bisphenol-A in the TBBPA. The amounts of bisphenol-A involved are likely to be small (especially in further derivatives) but this area requires further investigation and should be considered in the risk assessment of TBBPA, which is on the fourth priority list for risk assessment.]

According to the Merck Index bisphenol-A may be used in fungicide formulations (Merck Index, 1989). Following consultation with industry this use does not appear to occur in the EU and so is not considered in the assessment.

2.3 TRENDS

From the data presented by industry to CEFIC there would appear to be an increase in demand for bisphenol-A. Demand for bisphenol-A by the polycarbonate industry is thought to be increasing, though this can be affected by the economic climate due to the high use in automotive manufacture (demand for new cars usually follows economic conditions closely).

2.4 LEGISLATIVE CONTROLS

No environmental legislative controls specific to bisphenol-A are known.

In recognition of the ability of bisphenol-A to migrate from food contact materials into food, Specific Migration Limits (SMLs) have been set for the protection of the consumer. The EU legislation relating to the SML for bisphenol-A into food is 3 mg bisphenol-A per kg food (3 ppm). This migration limit is slightly lower in Japan with a maximum of 2.5 ppm. There is no SML in the US as instead calculations are conducted on a daily intake basis (personal communication).

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 Environmental releases

Emissions and predicted environmental concentrations (PECs) have been estimated in this section in accordance with the Technical Guidance Document (TGD) (EC, 1996). Where information specific to the production and use of bisphenol-A is available this has been used in the assessment, so that for some areas the assessment is based on site-specific information. For other areas the default emission factors have been used, where possible in combination with information on the likely amounts used on sites. Where only an estimated number of sites are available, the approach has been to calculate the average usage and then apply a factor of five to account for variability. The particular approach taken is described in each case. For the regional emissions, where specific information about the quantities produced or used on sites is available, the largest site tonnage is used if it is more than 10% of the total EU tonnage. Where the local use quantity is estimated as the 'average times five' (i.e. not an 'actual' site) then the regional emissions have been taken as 10% of the total for the EU.

3.1.1.1 Production of bisphenol-A

Bisphenol-A is produced at six sites within the EU. The total tonnage of bisphenol-A produced is approximately 700,000 tonnes/year (based upon 1997, 1998 and 1999 data). Bisphenol-A producers within the EU have supplied information on releases from their production sites via CEFIC. These data are confidential and are summarised in **Table 3.1**. All of the production plants undertake some form of bisphenol-A processing in addition to production. The releases presented in **Table 3.1** are combined releases for both production and processing.

Table 3.1 Summary of environmental releases data from bisphenol-A production sites

Site	Air		Effluent (After wastewater treatment)		Receiving waters
	Measured levels	Release	Measured levels	Release	
BPA1	<0.2 mg/Nm^3 (outlet) <0.5 μg/Nm^3 (50 m from site)	<0.012 kg/day <4.4 kg/year	<70 μg/l	0.76 kg/day 277 kg/year	Flow rate $8.64 \cdot 10^6$ m^3/day
BPA2	2.9 mg/Nm^3 (outlet discontinuous) 0.1 μg/Nm^3 (outlet)	0.00017 kg/day 0.0605 kg/year	0.69 μg/l	0.017 kg/day 6.1 kg/year	Flow rate $2.068 \cdot 10^8$ m^3/day
BPA3	<1 mg/Nm^3 (dust)	<1 kg/day (dust) <365 kg/year (dust)	~0.005 mg/l	0.31 kg/day 113 kg/year	Flow rate $8.08 \cdot 10^7$ m^3/day
BPA4		0.03 kg/day 10 kg/year		0.19 kg/day 70 kg/year	River estuary $2.49 \cdot 10^7$ m^3/day

Table 3.1 continued overleaf

Table 3.1 continued Summary of environmental releases data from bisphenol-A production sites

Site	Air		Effluent (After wastewater treatment)		Receiving waters
	Measured levels	Release	Measured levels	Release	
BPA5		1.58 kg/day (dust) 575 kg/year (dust)	Up to 192 μg/l	$2.14 \cdot 10^{-4}$ kg/day 0.08 kg/year	River estuary $2.59 \cdot 10^{6}$ m³/day
BPA 6	10 mg/Nm³ (dust)	0.08 kg/day (dust) 31.2 kg/year (dust)	Up to 30 μg/l	0.072 kg/day 25.8 kg/year	Sea Dilution factor 100

Nm³: volume in m³ at standard temperature and pressure

In the risk assessment all the site-specific scenarios will be taken forward as conditions at each site such as flow rate of receiving waters vary considerably.

For the regional and continental scenarios the largest emissions to air (575 kg/year) and to receiving water (277 kg/year) will be taken as regional emissions. The sum of the remaining emissions of 410 kg/year to air and 215 kg/year to receiving waters will be taken as continental emissions.

3.1.1.2 Polycarbonates

3.1.1.2.1 Releases during production

Bisphenol-A is used in the production of polycarbonate at 5 sites within the EU. At each of these sites bisphenol-A production also occurs. In line with the TGD the emissions from these plants are combined with the emissions from the production of bisphenol-A, and considered in the section detailing releases from bisphenol-A production plants. Releases from a polycarbonate-only production site are detailed below (site PC1). This plant ceased operation at the end of 2000. These emissions are retained for illustration but are not taken forward in the assessment. The total amount of bisphenol-A used in the production of polycarbonate is estimated from company submissions to be 486,880 tonnes/year.

Site PC1

Site PC1 produced polycarbonate from bisphenol-A in a continuous wet process in a closed system. The plant operated for 289 days/year. No other use of bisphenol-A was reported at the site.

The release of bisphenol-A to air was estimated as 0.5 kg/24 hours (based upon measured data). This gave a yearly release of 144.5 kg/year.

The release of bisphenol-A to water was estimated as 0.7 kg/24 hours (based upon measured data). This gave a yearly release of 202.3 kg/year. The effluent from the production plant was released directly to receiving waters and the dilution in the receiving waters was 200.

3.1.1.2.2 Releases during processing

Volatile loss of bisphenol-A to air during processing

Ligon et al. (1997) studied the evolution of volatile organic compounds from polymers during extrusion operations. The study looked at three polycarbonate polymers containing between 94-99.5% polycarbonate. For all the polycarbonate blends studied bisphenol-A was not detected in the vent gases from the extrusion apparatus.

In further work Ligon et al. (1998) studied the evolution of volatile organic compounds from polymers during moulding operations. The study, which looked at several polymer blends, included three polycarbonate polymers (94-99.5% polycarbonate). For all the polycarbonate blends studied bisphenol-A was not detected in the vent gases from the moulding apparatus.

Polycarbonate losses during processing

Processing of polycarbonate may increase residual bisphenol-A levels if the incorrect operating conditions are employed. The major causes of polycarbonate degradation during processing are: the presence of water in the polycarbonate before processing; the use of too high a processing temperature; and use of additives that promote degradation. To overcome these problems polycarbonate manufacturers provide information on proper processing conditions and handling information. As long as these guidelines are followed the formation of bisphenol-A due to degradation during processing, should be negligible under normal conditions of processing and use.

3.1.1.2.3 Releases during use

A polycarbonate producer has supplied some information and additional information has been taken from the "Q&A Concerning polycarbonate and bisphenol-A" booklet produced by the Polycarbonate Resin Manufacturers Group of Japan (1999).

Migration

Residual bisphenol-A present in polycarbonate is retained very effectively in the polymer matrix. This results in extremely low extractability by aqueous, alcohol or fat-containing media. Several studies have been reported concerning migration of bisphenol-A into foodstuffs. All the tests show very low levels of migration into food. For further details on the migration rates selected for the human health risk assessment see Section 4.1.1.2.1.

Information on the washing of polycarbonate bottles has been provided by industry. Rates of migration into foodstuffs are not relevant to assess possible releases from this process. Results from standard migration studies with water (Howe and Borodinsky, 1998) showed no detectable migration of bisphenol-A following six hours extraction at 100°C. The detection limit corresponded to a migration rate of 50 ng/in^2 (7.8 ng/cm^2). This will be used as a limit value in estimating releases from bottle washing.

The assumptions for the scenario on bottle washing are as follows:

- bottle size 19 l; weight 0.8 kg; total surface area (internal plus external) 8,194 cm^2 (1,270 in^2),
- average bottle lifetime 2 years; washing frequency 25 times per year,
- washing time 4 · 15 (i.e. 60) seconds at 60°C; water use 8 l per bottle per wash,
- 8,000 bottles cleaned per day at representative site,
- total use of bisphenol-A in bottles 7,500 tonnes per year.

Migration is assumed to be proportional to the square root of the duration; so to convert from 6 hours (= 21,600 seconds) exposure to 60 seconds a factor of square root (60/21600) is used. Hence the migration rate for the bottle washing scenario is <0.41 ng/cm^2. For a surface area of 8,194 cm^2, this is <3.4 μg per bottle per wash, and for 8,000 washings per day the daily release to WWTP is <27 mg. The volume of water used is 64 m^3/day.

In the EU, 7,500 tonnes of polycarbonate is used for bottles each year; at a weight of 0.8 kg per bottle this corresponds to 9,375,000 bottles. With an average lifetime of two years, this means there are 18,750,000 bottles in use at one time. The release from one washing of one bottle was <3.4 μg (from above), so for 25 washings per year the annual emission per bottle is 85 μg. Together with the total number of bottles this gives an estimated release from washing of 1.6 kg per year in the EU. This is split as 0.16 kg/year to the regional environment, and 1.44 kg/year to the continental.

Hydrolysis

Hydrolysis of a polymer may potentially give rise to the monomers it was formed from, in this case bisphenol-A. The most likely application of polycarbonate that can result in hydrolysis of the polymer is thought to be use in solid and multi-wall sheet in outdoor applications. Here weathering effects may lead to the breakdown of the polymer.

In laboratory studies thermal, thermo-oxidative, hydrolytic and UV-radiation induced ageing processes which lead to discoloration ("yellowing") of polycarbonate have been investigated for bisphenol-A formation. None of the mechanisms investigated lead to the formation of significant quantities of bisphenol-A.

Polycarbonate solid and multi-wall sheet in outdoor applications are produced as multiple layer structures by coextrusion or by coating with acrylate lacquers. Coating the polycarbonate sheet with acrylate lacquer, results in the core of the sheet being protected by a cap layer containing a UV stabiliser. In the case of coextruded sheets, all layers consist of polycarbonate as the polymer matrix.

A polycarbonate producer has performed an investigation into the weathering of polycarbonate sheet samples. In the experiment polycarbonate sample sheets were exposed to accelerated UV and rainwater weathering conditions.

In the experiment 204 samples of polycarbonate sheet with a total surface area of 0.98 m^2, a weight of 2.038 kg and a residual bisphenol-A content of 6 mg/kg were exposed to different amounts of light, heat and water to simulate different weather conditions. Samples of circulating water within the system were taken for analysis of bisphenol-A. Based upon the results the amount of bisphenol-A released after 2,000 hours weathering to the circulating water was 1.35 mg/m^2 exposed polycarbonate. This period of accelerated weathering is roughly equivalent to over 9 years in typical European climate conditions, so that the annual loss rate was ~0.15 mg/m^2.

The total amount of polycarbonate used for single and multi walled sheeting within Europe is not known. A different polycarbonate producer estimated that their production of 20,750 tonnes of polycarbonate used in sheeting produced a total surface area of 8.7 km^2. Taking the release factor of 0.15 mg/m^2 this would result in a release of 1.3 kg/year. Therefore even if the quantity of polycarbonate used in sheeting is significantly more than this, releases due to weathering are still likely to be small compared to environmental releases during processing. These releases will not be considered further in this risk assessment report.

3.1.1.3 Epoxy resins

3.1.1.3.1 Releases during production

Bisphenol-A is used in the production of epoxy resins within the EU, and information on releases has been received from eight sites. Of the eight sites for which information is available, two sites are also bisphenol-A production sites. For these sites the releases of bisphenol-A are combined and reported under bisphenol-A production. Releases from sites carrying out epoxy resin production only are detailed below (ER1-6). The total amount of bisphenol-A used in the production of epoxy resins is estimated at 171,095 tonnes/year from company submissions. Of this amount 158,007 tonnes/year (92% of total tonnage) are used at the sites for which site-specific information is available. Small-volume sales account for the remaining tonnage. These sales are to approximately 20 customers with the amount sold being in the range of 200-800-tonnes/year per site. As site-specific information is available covering 92% of the total tonnage of bisphenol-A used in the production of epoxy resins these data will be taken as representative of releases from all epoxy resin sites.

Site ER1

Site ER1 produces epoxy resins by a batch/solvent method from bisphenol-A. The effluent from the epoxy resin plant passes to a biotreat reactor. The concentration of bisphenol-A in the effluent from the biotreat reactor is reported as <5 μg/l. The daily releases of bisphenol-A in the effluent from the plant are calculated as <0.075 kg/day. Sludge from the biotreat plant is incinerated.

Site ER2

Site ER2 produces epoxy resins by a batch fusion method. There are no aqueous effluents from this process.

Site ER3

Site ER 3 produces epoxy resins by a batch fusion method. Bisphenol-A is handled in closed systems and there are no releases to air or water from the process.

Site ER4

This site operates two processes that use bisphenol-A. The first process is the reaction of bisphenol-A with epichlorohydrin to make liquid epoxy resins. The second process is the reaction of liquid epoxy resin with bisphenol-A to obtain solid resins with different molecular weights. Overall emissions to air are reported as 25 kg/year. Overall emissions to wastewater are reported as 0.24 kg/day. The wastewater undergoes primary treatment. On-site biological treatment of waste was due to start in July 1999 but no information has been supplied confirming this and therefore the original data will be used. The company gives the influent concentration of bisphenol-A as 2.8 μg/l. The effluent concentration is reported as 1.8 μg/l and was predicted to go down to 0.8 μg/l after the biological treatment plant came online. The effluent is discharged to an off-site wastewater treatment plant. The sludge produced by the off-site wastewater treatment plant is used for agricultural purposes. The daily release of bisphenol-A from the on-site wastewater treatment plant (after primary treatment only) is calculated as 0.16 kg/day.

Site ER 5

This site produces epoxy resins by the reaction of bisphenol-A and epichlorohydrin using sodium hydroxide as a catalyst. The company gives the influent concentration of bisphenol-A as 2 mg/l and the effluent concentration as 0.03 mg/l. The sludge produced by the plant is incinerated. Using the wastewater treatment plant characteristics the daily release of bisphenol-A from the wastewater treatment plant is calculated as 0.72 kg/day.

Site ER 6

At site ER 6 epoxy resins are produced by reacting bisphenol-A with epichlorohydrin with caustic addition. Bisphenol-A is handled in closed systems and there are no releases to air or water from the process.

Regional and continental releases

The regional and continental emissions are calculated using the sum of site-specific data and application of a release factor to the remaining tonnage. The sum of release to water after wastewater treatment from the site-specific data is 290.25 kg/year. From the site-specific data the highest release factor for bisphenol-A to water after wastewater treatment is $8.64 \cdot 10^{-3}$ kg/tonne bisphenol-A processed. (Note for sites ER4 and ER5 the number of days processing is taken as 300 days/year and at site ER1 as 350 days/year). Applying this factor to the tonnage for sites for which no release information is available (13,088) gives a release of 113 kg/year. The sum of site-specific and the calculated release data is 1.335 kg/day (403.25 kg/year), this is taken as the total release. For modelling in EUSES the largest release to receiving waters from site-specific data (216 kg/year Site ER5) will be used for the regional releases; the remaining releases from site-specific data and calculated release data (187.25 kg/year) will be used for the continental releases. These releases are after wastewater treatment.

3.1.1.3.2 Releases during use

An epoxy resin manufacturer has provided the following information on releases during the use of epoxy resins.

Epoxy resins are used in a range of applications including the electrical and electronic industry, building and construction industry, powder coatings, and can and coil coatings. The potential for release of bisphenol-A from epoxy resins is low. The residual monomer content of bisphenol-A in the epoxy resin as produced is a maximum of 1,000 ppm. The residual bisphenol-A will be further reacted when the product is used (i.e. when the epoxy resin is cured). For food contact uses a specific migration limit of 3 mg/kg food or food simulant has been established within the EU.

3.1.1.4 Phenoplast cast resins

8,800 tonnes/year of bisphenol-A are used in the production of phenoplast cast resins. There are three companies manufacturing phenoplast cast resins in the EU (personal communication Bayer, 2001). For one of these companies joint emissions from all resin production are reported under epoxy resins. For the other two companies (covering 93% of bisphenol-A used in resin production) the following information emission information has been reported.

Site PCR1

At this site the resin is prepared in a batch operated reflux reactor using a closed loop reflux system for cooling the boiling reactor mixture. The company reports that emissions during the production process are negligible. The bisphenol-A content of the final resin is approximately 0.2% on a molar basis.

The company subsequently processes the resin on-site in the manufacture of high-pressure laminate compact panels. These are prepared by impregnating paper or coating wood fibres with a resin diluted in water. The final stage of the process is the panel pressing. During the paper impregnation process gases are emitted. These gases are passed to a thermal incineration plant, and the company states that there are no emissions of bisphenol-A to air. During coating of the wood fibres with resin process gases are passed over a condenser. The wastewater from the condenser is treated as dangerous waste and sent to an off-site wastewater treatment plant for treatment. The concentration of bisphenol-A in the wastewater sent off-site is approximately 10 mg/l; and after treatment the total concentration of phenols is 0.1 μg/l. The wastewater from the off-site treatment plant is subsequently treated in a municipal wastewater treatment plant.

Based upon information supplied by the company an emission factor, for release of bisphenol-A to wastewaters during this process, of 0.000027 is calculated.

Site PCR2

At the second site the resin is prepared via a batch process in a closed system. The effluent from the process is disposed of via incineration and there are no releases of bisphenol-A to the environment.

From the information reported there would appear to be no releases of bisphenol-A to the environment during the production of phenoplast cast resins and these are not considered further in the risk assessment. The information reported on the use of these resins in the production of high-pressure laminate compact panels suggests that there is a potential for releases to the environment during processing (though not from this site). No information is available as to the use pattern of the resin produced at the other sites. Therefore as a worst-case scenario it will be assumed that the resin is used in a similar application at one processing site in the EU. Applying the release factor calculated for site PCR1 gives a local release of 60 kg/year or 0.24 kg/day if averaged over 250 days. For the continental and regional scenarios a 90% and 10% split will be used, with use averaged over 365 days; this gives releases of 54 kg/year (0.15 kg/day) for the continental scenario and 6 kg/year (0.016 kg/day) for the regional scenario. These emissions will be taken as going to wastewater.

3.1.1.5 Unsaturated polyester resins

3,000 tonnes/year of bisphenol-A are sold annually for use in unsaturated polyester resin production. There are thought to be between five and ten sites within the EU using bisphenol-A in this application.

No information on releases to air is available, but it is probably reasonable to assume that any losses to air are volatile losses during processing. The default release factor for use as a chemical intermediate for a low volatile substance is 0. Therefore releases to air will be assumed to be negligible.

Since the process is dry and does not produce any liquid effluent it will not be considered further in the risk assessment report.

3.1.1.6 Can coating production

Can coatings are produced by the reaction of an epoxy resin with bisphenol-A. The total amount of bisphenol-A used at the five known sites is 2,460 tonnes/year. The extent to which the following site-specific data cover all can coating industry is unknown. It is not certain if bisphenol-A is used in the same way in all can coating applications or just in certain specialised applications.

Site CC1

A worst-case emission to air during raw materials handling of 97.5 kg/year is reported. Any spillages of material and waste bags are sent to landfill for disposal. Aqueous distillate may collect from some polymers, and this may contain bisphenol-A. This distillate is sent for off-site disposal by incineration. There are no other aqueous emissions.

Site CC2

Any spillages of material and waste bags are sent to landfill for disposal. There is a small quantity of bisphenol-A in process water and in the separator. This is sent for off-site disposal by incineration. Cleaning solvent is sent off-site for incineration. There are no other aqueous emissions.

Site CC3

Any spillages of material and waste bags are sent to landfill for disposal. There is a small quantity of bisphenol-A in process water. This is sent for off-site disposal by incineration. Cleaning solvent is sent off-site for incineration. There are no other aqueous emissions.

Site CC4

Concentration of bisphenol-A in dust emissions is less than 20 mg/m^3. There is no outlet into sewage waste streams or natural waterways. There is no aqueous reactor cleaning. Any waste bags and spillages are sent off-site for incineration.

Site CC5

There are no aqueous emissions of bisphenol-A, and so they will not be considered further in this risk assessment.

No information on releases to air is available, but it is probably reasonable to assume that any losses to air are volatile losses during processing. The default release factor for use as a chemical intermediate for a low volatile substance is 0. Therefore releases to air will be assumed to be negligible.

3.1.1.7 Thermal paper production

Information has been received from six thermal paper manufacturers operating at seven sites within the EU on the use and release of bisphenol-A. The usage from these six manufacturers

accounts for approximately 1,400 tonnes bisphenol-A per year. It is not certain that all thermal paper manufacturers within the EU have been covered. However as the tonnage from the site-specific data agrees well with the total tonnage reported by the bisphenol-A producers, the data from these companies will be taken as representative for the use of bisphenol-A in thermal paper manufacture.

Site PAPER 1

This site uses 180 kg/day bisphenol-A (54 tonnes/year). Aqueous release is 0.6 kg/day to a wastewater treatment plant. The wastewater treatment plant has a capacity of 600 m^3/day and typical operating conditions are 300 m^3/day. The wastewater treatment plant treats water by settlement of solids and then biological treatment. The dilution factor for effluent from the plant is 1,000. The company has calculated the concentration of bisphenol-A in the influent to the wastewater treatment plant as 2.3 mg/l. The company estimates that 90% of the bisphenol-A released is degraded and the remaining 10% adsorbed to the sewage sludge. The company's assumption that bisphenol-A is completely removed in the wastewater treatment plant by adsorption to sludge appears to be unrealistic. Using the default emission to wastewater for bisphenol-A in the wastewater treatment plant (12%) gives an emission of 0.072 kg/day (21.6 kg/year) to receiving waters. The sludge produced at the plant is disposed of to a controlled landfill.

Site PAPER 2

This site uses 300 tonnes/year bisphenol-A (1,000 kg/day). The company estimates from measured data that 0.056 kg/day bisphenol-A goes to the wastewater treatment plant. The wastewater treatment plant has a capacity of 2,800 m^3/day. The wastewater is treated by pH adjustment, polymer dosing and sedimentation of solids. Using the default emission to wastewater for bisphenol-A in the wastewater treatment plant (12%) gives an emission of 0.0067 kg/day (2.016 kg/year) to receiving waters. The company has measured the influent and effluent concentration of bisphenol-A as 0.02 mg/l and 0.006 mg/l respectively. The sludge from the wastewater treatment plant is disposed of to a controlled landfill.

Site PAPER 3

This site uses 2.5-3 tonnes/year bisphenol-A (9-10 kg/day). The total amount of wastewater produced is 1,000 m^3/day. Wastewater from the site goes to a wastewater treatment plant with a capacity of 85,000 m^3/day. The sludge from the plant is treated as a special waste.

No details on the quantities released, fate in the wastewater treatment plant or dilution in receiving waters are available. In Appendix I of the TGD default releases are given for pulp, paper and board production (Table A3.12). In using these tables bisphenol-A will be taken as being used as an additive in paper production. The relevant Main Category is 2 (Inclusion into or onto a matrix) and the Use Category is 31 (Impregnation agents). This use category is chosen because it is felt to more accurately reflect the use of bisphenol-A than other use categories since they refer more to chemicals added to the water during paper manufacture. Processing is assumed to occur at large sites within the EU. From Table A3.12 the default emissions to air are 0 and to wastewater 0.05. Using this default release estimation the release from PAPER 3 to wastewater is 0.5 kg/day. Using the default emission to wastewater for bisphenol-A in the wastewater treatment plant (12%) gives an emission of 0.06 kg/day (18 kg/year) to receiving waters.

Site PAPER 4

This site uses 20 tonnes/year bisphenol-A (66.7 kg/day). Dust emissions from the plant are collected in dust cleaning equipment for disposal. Wastewater from the plant is cleaned in a mechanical and biological wastewater treatment plant. The wastewater treatment plant has a capacity of 5,000 m^3/day. The concentration of bisphenol-A has been measured in the effluent of the plant as <0.01 mg/l, and this gives a maximum emission of 0.05 kg/day or 15 kg/year to receiving waters. The dilution rate in the receiving waters is not known. Sludge from the wastewater treatment plant is incinerated.

Site PAPER 5

This site uses 483 tonnes/year bisphenol-A (1,610 kg/day). Wastewater from the plant is cleaned in a mechanical and biological wastewater treatment plant. The wastewater treatment plant has a capacity of 1,500 m^3/day. The concentration of bisphenol-A has been measured in the effluent of the plant as <0.01 mg/l, which gives a maximum emission of 0.015 kg/day or 4.5 kg/year to receiving waters. The dilution rate in the receiving waters is not known. Sludge from the wastewater treatment plant is incinerated.

Site PAPER 6

This site uses 242 tonnes/year bisphenol-A (807 kg/day). Wastewater from the plant is treated prior to discharge to receiving waters. The capacity of the wastewater treatment plant is 250 m^3/day. In the effluent the company reports a phenol index of 0.39 mg/l. This is a measurement of total phenol and gives a bisphenol-A concentration of 0.47 mg/l, assuming that bisphenol-A is the only phenol present. This gives an emission of bisphenol-A to receiving waters of 0.12 kg/day (36 kg/year). The dilution factor of effluent in receiving waters is 500. The sludge produced from the wastewater treatment plant is incinerated.

Site PAPER 7

This site uses 300 tonnes/year bisphenol-A (1,000 kg/day). The site produces 2,000 m^3 effluent per day. In this effluent the company reports a phenol index of <0.1 mg/l. This is a measurement of total phenol and gives a bisphenol-A concentration of <0.12 mg/l, assuming that bisphenol-A is the only phenol present. This gives a maximum daily emission of bisphenol-A from the site of <0.24 kg/day (<72 kg/year). Wastewater from the site is treated at a wastewater treatment plant with a capacity of 70,000 m^3/day. This gives a dilution factor for effluent from the site in the wastewater treatment plant of 35. Using the default emission to wastewater for bisphenol-A in the wastewater treatment plant (12%) gives an emission of 0.0288 kg/day (8.64 kg/year) to receiving waters. The dilution rate of effluent in receiving waters has a low of 10 and a high of 30. The sludge produced from the wastewater treatment plant is incinerated.

Regional and Continental Emissions

The sum of all the emissions to receiving waters after wastewater treatment is 106 kg/year. For input into the EUSES model to calculate the regional and continental concentrations the highest value from a site (36 kg/year for PAPER 6) will be used for the regional emission. The sum of emissions from the remaining sites (70 kg/year) will be used for the continental scenario.

3.1.1.8 Thermal paper recycling

Information on the releases of bisphenol-A during thermal paper recycling has been supplied by the European Thermal Paper Association (ETPA) and some of its member companies. Other information is taken from the emission scenario document for the pulp, paper and board industry (Chapter 7 of the TGD).

According to the emission scenario document possible releases to the environment from the recycling of thermal paper are as follows. Most of the colour former and co-reactant in waste thermal paper remain unreacted except where printing has occurred; this is assumed to represent a very small proportion of the waste and is ignored. On alkaline pulping, the colour former hydrolyses and this and the co-reactant are 100% released to water. A worst-case assumption for bisphenol-A is that it is all released to water during recycling (this is equivalent to assuming a de-inking rate of 100%). The emission scenario document also states that at least primary sedimentation is carried out at all paper mills and that this process will remove 50% of poorly water soluble substances. Thus the overall release of bisphenol-A to wastewater from the process could be around 50% of that used.

The emission scenario document suggests that as a worst-case approach, the total amount of paper containing a substance is recycled at 10 sites (i.e. a maximum of 10% of the substance at a site). The default recycling rate is given as 50% of the total paper use. Thus the amount of bisphenol-A in thermal paper at a paper mill using recycled paper is 70 tonnes/year. 50% of the release is adsorbed during primary treatment. The number of days recycling is given as 250 days/year.

Using the above data the emissions of bisphenol-A after on-site primary treatment from recycling of thermal paper are as follows:

Local	35,000 kg/year (140 kg/day) to wastewater
Regional	35,000 kg/year (95.8 kg/day) to wastewater
Continental	315,000 kg/year (863 kg/day) to wastewater

In line with the Emission Scenario Document (ESD) for paper recycling these emissions will be taken as going to a wastewater treatment plant only.

The European Thermal Paper Association (ETPA) commissioned a report on release of bisphenol-A during thermal paper recycling (TNO, 2000). The report is based upon the tonnage of bisphenol-A used in thermal paper in Germany (300 tonnes/year) as estimated by the ETPA. Four scenarios are considered in the report: TGD default, TGD default using industry variables, and two branch-specific scenarios using data on the industry in Germany. The TGD scenario is similar to the scenario used above with the following exceptions: a removal rate for adsorption of 80% is used instead of 50%; a de-inking rate of 90% is used instead of 100%; and 10 recycling sites within Germany are considered compared to 10 sites in the EU. The industry scenario assumes a lower de-inking percentage than the TGD assessment (80% compared to 90%), 90% removal by adsorption, 350 days working per year and 69 recycling sites. The branch-specific scenario uses the TGD defaults apart from the number of working days and number of recycling sites which are taken from the industry scenario and water flow rates and dilution rates which are based upon site-specific data.

Information for a thermal paper recycling company indicates that it processes 3,600 tonnes/annum thermal paper waste which has a bisphenol-A content of 0.7% (25.2 tonnes). The concentration of bisphenol-A in the influent to the on-site wastewater treatment plant was measured at 1.82 mg/l. Bisphenol-A was not detected in the effluent from the wastewater

treatment plant (detection limit of analytical method 10 μg/l). As a worst-case scenario a concentration in wastewater treatment plant effluent of 10 μg/l (equivalent to the detection limit) will be taken forward in the risk assessment.

From the data in the TNO report it would appear that the default number of recycling sites used in the ESD document may be too low (10 for the EU compared to 69 in Germany) and the number of working days should be closer to 350 than 250 days per year. The site-specific information received would appear to support the TNO risk assessment with the total amount of bisphenol-A in thermal paper processed at the site being 25.2 tonnes. This compares to 70 tonnes for the ESD default and 30 tonnes in the TNO assessment. It should be noted that the site-specific data are from a company in Germany and presumably it is covered by the scenarios in the TNO report. However, it is not proposed to change the tonnage or number of days processing used in the generic scenario calculated above as this is taken to represent a realistic worst-case scenario. More information on recycling sites across Europe and not just Germany would be required to make a better judgement as to an alternative worst case. However, the data in the TNO report do represent a realistic scenario for one state in the EU and are probably more realistic than the generic scenario. To take this into account the PECs calculated in the TNO document will be considered alongside the PEC derived from the generic scenario presented here.

3.1.1.9 PVC production and processing

The total amount of bisphenol-A used in the PVC industry is approximately 2,250 tonnes/year. There are four possible uses of bisphenol-A associated with PVC production and processing. These are as follows:

- Use as an inhibitor or reaction "killing" agent during the polymerisation stage of PVC production. The total tonnage of bisphenol-A used is 200-250 tonnes/year. Use occurs at approximately 10 PVC production sites within the EU (20% of PVC producers). The European Council of Vinyl Manufacturers (ECVM) has announced that the use of bisphenol-A in the manufacture of PVC resin will be phased out in Europe by the end of 2001. As this is a voluntary phase out the use will be considered in the risk characterisation. However the effect of removing its contribution to the regional environment will be considered in Appendix 2.
- Use as an anti-oxidant during the processing of PVC. The total tonnage of bisphenol-A used is 500 tonnes/year. There are a large number of sites using bisphenol-A for this purpose, industry estimates vary from 200-500 sites within the EU. The amount of bisphenol-A used per site is approximately 1-3 tonnes/year.
- Incorporation into an additive package which is subsequently sold onto PVC processors for use. The total tonnage of bisphenol-A used is 500 tonnes/year. There are approximately 10-20 sites within the EU making additive packages that incorporate bisphenol-A. No information on the end use of these additive packages is known, though usage is thought to be similar to direct use of bisphenol-A as an anti-oxidant.
- Use as an anti-oxidant in the production of plasticisers used in PVC processing. The total tonnage of bisphenol-A used is approximately 1,000 tonnes/year. There are approximately seven sites within the EU that undertake this process.

Site-specific data

Site-specific data are available for two PVC production sites accounting for 112 tonnes/year bisphenol-A use per year. Both of these sites use bisphenol-A as an inhibitor during the production of PVC. One of these sites is also a bisphenol-A production site and so emissions are included in the production section. The concentration of bisphenol-A in the effluent from the PVC production plant on the site is measured at approximately 22 mg/l, before wastewater treatment. For the other site PVC 1 the bisphenol-A concentration in the effluent is measured at 17-18 mg/l. This effluent is then diluted between 10 to 24 times upon entry to the wastewater treatment plant. The concentration of bisphenol-A in effluent from the wastewater treatment plant is measured as less than 5 μg/l. This is discharged to a lake with an outlet to sea. The sludge from the wastewater treatment plant is incinerated (oily waste) or landfilled (solid waste).

Site-specific data are available for a typical plant using bisphenol-A as an anti-oxidant in the production of plasticisers. Bisphenol-A is supplied to the site in closed containers, which are unloaded directly into the mixing vessel to prepare a 10% solution. Dust emissions are calculated as 81 kg/year, these are then washed to drain and end up in the wastewater stream. The wastewater treatment plant has a flow of 0.3 m^3/sec and the receiving water flow varies between 700 and 3,000 m^3/sec with a mean flow of 1,500 m^3/sec, this gives a mean dilution factor of 5,000. The emission factor for release of bisphenol-A from this process to wastewater is calculated as 0.001.

Generic emission scenario

In calculating generic emissions for the use of bisphenol-A in the PVC industry, use is made of the Use Category Document on Plastic Additives (UCD, 1998). The Use Category Document gives information on the likely releases of plastic additives such as anti-oxidants and plasticisers during plastics processing, use and disposal. Bisphenol-A is used as an anti-oxidant either on its own or as part of an additive package during PVC processing and during production of plasticisers for use in PVC. According to the Use Category Document anti-oxidants are used in rigid PVC formulations and in particular PVC meant for use in building applications. The typical concentration of anti-oxidant in the PVC is 0.2%.

The Use Category Document gives the following release factors for anti-oxidants during use. For bisphenol-A, losses for powders with a particle size > 40 μm and low volatile compounds are taken as representative.

Losses during raw material handling: initially, some emissions will be to air, but ultimately all particulates will be removed or settle and losses will be to solid waste or to wastewater as a result of wash down. Material remaining in packaging will be assumed to go to solid waste from the plastics processing site and will not be considered further in this assessment. For powders of particle size > 40 μm losses of 0.2% to solid waste/water are estimated, which will be taken as going to wastewater. A loss of 0.01% of solid waste as residue in bags will also occur.

Losses during compounding: initial losses will be to atmosphere, but ultimately particulates will be removed or will settle, and vapours will condense, resulting in losses to both solid waste and aqueous washings. For powders of particle size > 40 μm losses to solid waste/water of 0.01% are estimated. For low volatility compounds losses of 0.002% are estimated. Assuming that both of these losses ultimately result in losses to wastewater gives a total loss of 0.012% to wastewater during compounding.

Losses during conversion: For use of anti-oxidants in PVC processing, closed processes are the most common and will be taken as the default. Initial losses will be to atmosphere. Subsequent condensation could result in losses to liquid waste. For a low volatile compound in a closed process the losses are estimated as 0.002% to air. These will be taken as condensing and going to wastewater. For smaller sites (processing < 750 tonnes plastics per year) the release factors should be increased by a factor of 10.

For bisphenol-A all volatile losses to air during raw materials handling, compounding and conversions are taken as condensing out and passing to wastewater. This is because bisphenol-A is a low-volatility compound and is reasonably soluble. Taking these volatile losses as going to wastewater is thought to be a realistic worst-case scenario. It is noted that in other assessments a split between air and water of 50:50 has been assumed. This seems more appropriate in these other specific cases as the substances concerned have very low solubilities.

Use as an inhibitor in PVC production

For the use of bisphenol-A in the production of PVC as an inhibitor during or at the end of the polymerisation process it is reported that approximately 2/3 bisphenol-A is incorporated into the polymer, the remaining 1/3 is lost to wastewater. The amount of bisphenol-A used per site or per tonne of PVC produced is not known. The total amount of bisphenol-A used as an inhibitor in the production of PVC is reported as 200-250 tonnes/year. The site-specific data gives the amount used per site as 34 and 78 tonnes bisphenol-A per year. Based upon these data a default tonnage of 50 tonnes bisphenol-A used per year at a site will be used, and production will be assumed to be on a continuous basis (300 days a year). This gives a daily release rate to wastewater of 55.5 kg/day. For the continental and regional scenarios the release figure will be applied to the total tonnage (250 tonnes), this gives a release to wastewater of 83 tonnes/year (227 kg/day based upon 365 days production per year). For the continental scenario 90% of this amount will be used (204 kg/day / 74.7 tonnes/year) and for the regional scenario 10% (23 kg/day / 8.3 tonnes/year).

Use as an anti-oxidant in PVC processing

Approximately 500 tonnes/year bisphenol-A is used as a stabiliser in the processing of PVC. This occurs at a large number of sites within the EU (Industry estimate 200-400 sites) with the amount used per site being 1-3 tonnes/year. No information on the releases expected is available therefore the Use Category Document on Plastics Additives (UCD, 1998) will be used to generate the default releases. Assuming 3 tonnes of bisphenol-A is used per year on-site at a concentration of 0.2% in the PVC the total amount of PVC produced would be 1,500 tonnes/year. Production of this amount will be taken as being fairly continuous over a year and the number of days processing will be taken as 250 days/year. Using the Use Category Document the following releases are calculated.

Local (3 tonnes/year per site, 250 days use per year):

Losses during raw materials handling:	6 kg/year (0.024 kg/day) to wastewater 0.3 kg/year as residue in bags (not considered further in RAR)
Losses during compounding:	0.36 kg/year (0.0014 kg/day) to wastewater
Losses during conversion:	0.06 kg/year (0.0002 kg/day) to wastewater
Total:	6.42 kg/year (0.0256 kg/day) to wastewater

For continental and regional scenarios these loss factors are applied to the total tonnage used (500 tonnes/year). The regional tonnage is taken as 10% of the continental tonnage and is subtracted from the continental tonnage to avoid double counting of emissions. Losses during service may also be considered for the regional and continental scenarios. The following releases are calculated for the regional and continental scenarios.

Continental (450 tonnes/year, 365 days use per year):

Losses during raw materials handling:	900 kg/year to wastewater 45 kg/year as residue in bags (not considered further in RAR)
Losses during compounding:	54 kg/year to wastewater
Losses during conversion:	9 kg/year to wastewater
Total:	963 kg/year to wastewater

Regional (50 tonnes/year, 365 days use per year):

Losses during raw materials handling:	100 kg/year to wastewater 5 kg/year as residue in bags (not considered further in RAR)
Losses during compounding:	6 kg/year to wastewater
Losses during conversion:	1 kg/year to wastewater
Total:	107 kg/year to wastewater

Preparation of additive packages for PVC production

There are between 10-20 sites in the EU which prepare additive packages for use with PVC. Approximately 500 tonnes/year of bisphenol-A is incorporated into these packages at these sites. Releases of bisphenol-A may occur during the preparation of additive packages and the subsequent use of the additive package. Releases during use of the additive package will be taken as the same as use of bisphenol-A as an anti-oxidant in PVC processing. This is because the function of bisphenol-A is the same in the additive package as if it was used directly and the number of sites and amount used is thought to be similar for direct use and additive package use.

In the absence of any information on releases during the production of the additive packages, it will be treated as a compounding stage i.e. mixing of several different materials together to form a master batch. The releases therefore occur during raw materials handling and in compounding. No information is available as to the amount of bisphenol-A used per site. If the amount used was divided equally between 10 sites then 50 tonnes/year would be used per site. As a worst case it will be assumed that one site uses five times the average amount, i.e. 250 tonnes/year bisphenol-A a year is used at one site in the preparation of additive packages. Using the Use Category Document on Plastic Additives (UCD, 1998) gives the following releases:

Local (250 tonnes/year per site, 250 days use per year):

Losses during raw materials handling:	500 kg/year (2 kg/day) to wastewater 25 kg/year as residue in bags (not considered further in RAR)
Losses during compounding:	30 kg/year (0.12 kg/day) to wastewater
Total:	530 kg/year (2.12 kg/day) to wastewater
Losses during use:	As for bisphenol-A use as an anti-oxidant in PVC processing

Continental (450 tonnes/year, 365 days use per year):

Losses during raw materials handling:	900 kg/year to wastewater 45 kg/year as residue in bags (not considered further in RAR)
Losses during compounding:	54 kg/year to wastewater
Total additive production:	954 kg/year to wastewater
Losses during use:	963 kg/year to wastewater

Regional (50 tonnes/year, 365 days use per year):

Losses during raw materials handling:	100 kg/year to wastewater 5 kg/year as residue in bags (not considered further in RAR)
Losses during compounding:	6 kg/year to wastewater
Total additive production:	106 kg/year to wastewater
Losses during use:	107 kg/year to wastewater

Use as an anti-oxidant in the production of plasticisers used for PVC processing

Information on the use of bisphenol-A as an anti-oxidant for plasticisers used in the processing of PVC has been supplied by the European Council for Plasticisers and Intermediates (ECPI).

Site-specific releases during production of the plasticiser are detailed above and are calculated as 81 kg/year for a local site. Assuming that the emission factor from the typical site applies at all sites using bisphenol-A as an anti-oxidant gives a total release of bisphenol-A of 112 kg/year to wastewater. Based upon these release estimates 81 kg/year will be used for the local and regional scenarios and 31 kg/year for the continental scenario. These estimates will be used in preference to default emissions contained in the UCD as they are based upon measured data. (The emissions calculated using information from the UCD are 1,908 kg/year for the continental scenario and 212 kg/year for the regional scenario.)

Use as a plasticiser in PVC processing

As well as losses during the production of the plasticiser there may be additional losses during the use of the plasticiser. No information is available as to the amount of plasticiser used per site. As a worst case it is assumed that all the flexible PVC used for electrical applications (53,900 tonnes/year based upon UK data in the UCD on plastics additives) is processed at one site. The bisphenol-A is present in the plasticiser at a concentration of 0.2% and the plasticiser is present in the PVC at 30%. Therefore the total amount of plasticiser used on the site is 16,170 tonnes/year, containing 32 tonnes/year bisphenol-A. Flexible PVC for electrical applications is processed in partially open systems. It is noted that in the Risk Assessment Reports for di-isodecyl phthalate and di-isononyl phthalate the amounts of plasticisers used on sites were up to 4600 tonnes per year, somewhat lower than assumed here. The releases estimated here may therefore be over-estimates.

The Use Category Document on Plastic Additives (UCD, 1998) gives the following releases for plasticisers during use. As with the releases associated with anti-oxidant use, solid wastes and volatile losses to atmosphere are taken as ultimately being lost to wastewater.

Losses during raw materials handling:	0.01% to wastewater
Losses during compounding:	0.002% to wastewater
Losses during conversion:	0.002% to wastewater

These release factors will be applied to the bisphenol-A content of the plasticiser.

Local (32 tonnes/year, 250 days processing per year):

Losses during raw materials handling:	3.2 kg/year (0.0128 kg/day) to wastewater
Losses during compounding:	0.64 kg/year (0.00256 kg/day) to wastewater
Losses during conversion:	0.64 kg/year (0.00256 kg/day) to wastewater
Total:	4.48 kg/year (0.0179 kg/day) to wastewater

Continental (900 tonnes/year, 365 days processing per year):

Losses during raw materials handling:	90 kg/year to wastewater
Losses during compounding:	18 kg/year to wastewater
Losses during conversion:	18 kg/year to wastewater
Total:	126 kg/year to wastewater

Regional (100 tonnes/year, 365 days processing per year):

Losses during raw materials handling:	10 kg/year to wastewater
Losses during compounding:	2 kg/year to wastewater
Losses during conversion:	2 kg/year to wastewater
Total:	14 kg/year to wastewater

There may also be losses from the products during use, and these are detailed in the next section.

Losses during service

The estimates of losses during the service life of PVC articles are based on the factors used in other assessments dealing with additives to PVC (phthalate esters, MCCPs). Further details are given in those reports. For products such as roofing and cabling, specific emission factors related to surface area have been developed in these assessments. Information from industry indicates that virtually all the plasticisers which contain bisphenol-A as an anti-oxidant are used in these two areas, with the majority going into the insulation and sheathing of electrical cables. These two applications are taken to involve PVC of similar thickness and are both single sided in terms of emission surfaces, and so the same emission factors are used for both. The emission factors derived in the assessments for the phthalates are 1.05 g/m^2/year for outdoor losses, and 9.5 mg/m^2/year for indoor losses. The outdoor loss factor is considered to apply to use in the open air, not to buried cables where releases are not expected to be significant. Indoor losses are considered to arise only though evaporation, as cables are not subjected to washing or polishing. The breakdown of use between indoor and outdoor use is estimated to be 50:50. Of the amount used outdoors, 80% (i.e. 40% of the total) is used underground.

The surface area of cables and roofing sheet in relation to the amount of plasticiser used is taken as 532 m^2/tonne of plasticiser, and the bisphenol-A content of the plasticiser is taken as 0.5%. A service life of 30 years is used for cables. The estimated emissions are as follows:

Indoor:

Plasticiser usage	135,000 tonnes/year
Surface area	$2.15 \cdot 10^9$ m^2
Loss factor	9.5 mg/m^2/year
Plasticiser loss	20.5 tonnes/year
Bisphenol-A loss	0.10 tonnes/year

These losses are all to air.

Outdoor - open air:

Plasticiser usage	18,200 tonnes/year
Surface area	$0.29 \cdot 10^9$ m^2
Loss factor	1,050 mg/m^2/year
Plasticiser loss	305 tonnes/year
Bisphenol-A loss	1.5 tonnes/year

It is assumed that the loss of bisphenol-A from outdoor cables will be distributed equally between air, surface water and soil.

For the other areas of use for bisphenol-A in PVC no specific information is available on the types of product involved. It seems likely that these will be similar to the products considered above, but in the absence of specific information the more general factors above will be used, recognising that this probably over-estimates the potential for release. (Yashimoto and Yasuhara (1999) measured bisphenol-A in synthetic leather as well as in electrical cords and plugs, which might be expected to be internal uses.) The annual losses are taken to be 0.05% to air and 0.15% through leaching - the latter is assumed to go to soil and surface water in the ratio 50:50. The factors for leaching emissions apply to external use. A lifetime of 30 years for electrical cables and wires has been proposed in other assessments. This may be an over-estimate for uses such as electrical plugs and electrical cords (and also synthetic leather), but has been used to maintain consistency with the calculations above and with other assessments. (It might also be noted that bisphenol-A is present as an anti-oxidant and functions by reacting with reactive species to protect the material, producing complex products as a result. Therefore over the course of the product's lifetime the concentration of bisphenol-A is expected to decrease.)

Applying a lifetime of 30 years to the emission factors from above gives the following annual emission rates: $0.05\% \cdot 30 = 1.5\%$ per year to air: $0.15\% \cdot 30 = 4.5\%$ leached per year, 2.25% to water and 2.25% to soil. These give the emissions in **Table 3.2** from PVC articles in use. The emissions relating to the use of plasticisers as calculated above are also included in the table, together with the estimated total emissions from products in use.

Table 3.2 Emissions from use of PVC products containing bisphenol-A

	Regional (tonnes/year)			Continental (tonnes/year)		
Use	**Air**	**Surface water**	**Soil**	**Air**	**Surface water**	**Soil**
Anti-oxidant in PVC processing	0.75	1.1	1.1	6.75	10	10
Preparation of additive packages for PVC processing	0.75	1.1	1.1	6.75	10	10
Anti-oxidant in the production of plasticisers	0.06	0.05	0.05	0.54	0.45	0.45
Total	**1.56**	**2.25**	**2.25**	**14.04**	**20.45**	**20.45**

3.1.1.10 Polyols/polyurethane

950 tonnes/year of bisphenol-A are used at one site in the production of polyols that are used in the production of polyurethane. The site is also a bisphenol-A production site. Hence, emissions are included in the production section. No other sites using bisphenol-A in this application are known. The polyol production process is a dry process.

3.1.1.11 Brake fluid manufacture

Bisphenol-A is used in the production of brake fluids at one site that is also a bisphenol-A production site. Hence, the emissions from this site are included in the production section. The concentration of bisphenol-A in the effluent from the brake fluid operations at the production site is measured as <0.1 mg/l, which is before any wastewater treatment.

In use the brake fluid is likely to be subjected to heat and pressure. As it is added to the brake fluid as an anti-oxidant, bisphenol-A may also be expected to react during product use, effectively being destroyed. Spent brake fluid is usually disposed of by professional personnel as chemical waste. It is therefore probably reasonable to assume that the amount of bisphenol-A reaching the environment during product use and disposal is very low. Therefore, the potential for release during brake fluid use will not be considered further.

3.1.1.12 Tyre manufacture

110 tonnes/year of bisphenol-A are used as a compounding ingredient in tyre manufacture. This is assumed to be all used by one manufacturer within the EU at one site. The compounding process is a dry operation with no aqueous effluents.

In the presence of the other compounding ingredients and during the curing process, the bisphenol-A becomes incorporated into the polymer matrix. Although it is used as an anti-oxidant this appears to be specifically for the compounding phases and it is presumably intended to protect the materials at this stage. There is no indication that it is intended to be the major anti-oxidant in the actual tyres, and so it is not expected to be present at significant levels in the finished product. As an anti-oxidant it will also react to give complex products so a proportion will be used up in this way. As a consequence, during the lifetime use of car tyres, there should be no significant environmental release of bisphenol-A.

Releases of bisphenol-A from tyre manufacture and use will not be considered further during this assessment.

3.1.1.13 Polyamide production

150 tonnes/year of bisphenol-A is used at one site for the production of modified polyamide grades. As this site is also a bisphenol-A production plant, combined emissions from production and use are reported in Section 3.1.1.1. No other sites using bisphenol-A in this application are known. The production of the modified polyamide is a dry process in a closed system.

3.1.1.14 Alkoxylated bisphenol-A

One company has reported using 2,020 tonnes/year bisphenol-A in the production of alkoxylated bisphenol-A, which is subsequently sold as an intermediate in the production of epoxy resins. The company operates two sites within the EU, and information from these two sites is detailed below.

Site AO1

Any bisphenol-A effluent arising from the production process comes from washing the handling conveyor. The washings go to the site drainage system which discharges direct to receiving waters.

Site AO2

Spillages during delivery are swept up and sent for incineration. The insides of production vessels tend to become coated in bisphenol-A powder. At the end of a production campaign the vessels are washed out with solvent to remove the bisphenol-A. The used solvent is sent for incineration. The vessels are then rinsed with water that goes to drain. The site drainage system terminates at the site lagoon. Sludge from the lagoon may be used for agricultural purposes in accordance with local regulations.

From the site-specific data, releases to the environment during production of the alkoxylated bisphenol-A appear to be negligible and this use will not be considered further in the risk assessment report. The formation of bisphenol-A during the use of alkoxylated bisphenol-A is not expected to occur to any significant extent. The alkoxylated bisphenol-A is chemically bound into the resin produced and so environmental releases of bisphenol-A are expected to be negligible.

3.1.1.15 Production of tetrabromobisphenol-A

Production of tetrabromobisphenol-A in the EU ceased in early 1998, and recommenced at the end of 1999 for a further six-month period. After 2000 the company concerned had no plans to restart production. Releases from the production plant based upon site-specific data are reported here. These releases and subsequent PECs will be calculated despite plans to cease production during 2000 to allow for any possible future production of this substance.

In the manufacturing process a reactor is charged with bisphenol-A and solvents. Bisphenol-A is then brominated by the addition of bromine. The solvent used is distilled off and the remaining slurry is cooled, crystallised and centrifuged to separate the solid product. The mother liquor from the centrifuge is treated separately for re-use. The solid product is dried before storage. During the process vapours are separated from solvent via distillation, active carbon adsorption and a catalytic afterburner, and any recovered solvent is returned to the process. Wastewater is separated and treated in a wastewater treatment plant consisting of biological treatment and an active carbon filter system. The treated wastewater is then passed to a municipal wastewater treatment plant. The use of bisphenol-A occurs in closed vessels. Releases of bisphenol-A from the process are therefore taken as negligible and will not be considered further in this assessment.

3.1.1.16 Disposal of waste products

Polycarbonate is typically used in the production of functional parts in long life applications. Use periods are typically in the range of 5 to 20 years. At the end of their lifetime direct reuse of the product in another application is not usually possible as function and design of the product is likely to have changed. Waste material may be directly processed into articles, which have inferior properties or used as a secondary raw material added to virgin material for the production of recycled grades. Recycling of polycarbonate from some applications is not feasible without significant prior treatment (for example material which is dirty or significantly discoloured due to weathering). At present some used polycarbonate is collected for processing into recycled material where it is economically feasible to do so. The remaining polycarbonate is likely to be disposed of to landfill or via municipal waste incineration.

Articles containing epoxy resins are typically disposed of via landfill or municipal waste incineration. There is not thought to be any recycling or recovery of epoxy resins, though some products containing them may be recycled.

Incineration of products containing bisphenol-A, will effectively destroy any free bisphenol-A present in the product.

There may be potential for residual bisphenol-A to leach from materials disposed of to landfill. Yamamoto and Yasuhara (1999) measured the leaching of bisphenol-A from samples of plastics into water. The rates of leaching found varied considerably, with the highest amount leached from a sample of synthetic leather, presumed to consist of PVC. Around 11% of the amount of bisphenol-A in this material leached to water in two weeks. It is not clear from the report whether the samples tested were taken from materials which had been in use, or from waste material direct from the manufacturers. The same authors (Yasuhara et al., 1997) measured bisphenol-A in the leachate from hazardous waste landfills in Japan, at concentrations up to 12.3 μg/l. They were not able to identify the source material for the substance. Wenzel et al. (1998) (reference quoted in personal correspondence with German CA) measured bisphenol-A in leachate water from three landfill sites in Germany with an average concentration of 81 μg/l (see Section 3.1.4.2). It is not possible from this information to make an estimate of the potential release of bisphenol-A after disposal of articles to landfill. Therefore the significance of leachate from landfills containing bisphenol-A is unknown.

3.1.1.17 Regional and continental exposure

Table 3.3 Summary of regional and continental releases

Process	Air (kg/year)		Emission to wastewater treatment plants (kg/year)		Emission to receiving waters (kg/year)	
	Regional	Continental	Regional	Continental	Regional	Continental
Bisphenol-A production [a]	575	410			277	215
Polycarbonate bottle washing [b]			0.10	1.0	0.05	0.4
Epoxy resin production [a]					216	187
Phenoplast cast resin processing [b]			4.2	38	1.8	16
Thermal paper production [a]					36	70
Thermal paper recycling [c]			35,000	315,000		
PVC – Inhibitor during production process [b]			5,810	52,290	2,490	22,410
PVC – Anti-oxidant during processing [b]			75	674	32	289
PVC – Preparation of additive packages [b]			74	668	32	286
PVC –Use of additive package [b]			75	674	32	289
PVC – Anti-oxidant in plasticiser production			81	31		
PVC – Plasticiser use [b]			10	88	4	38
Losses from PVC articles in use [a]	1,560	14,040			2,250	20,450
Total	**2,135**	**14,450**	**41,129**	**369,464**	**5,371**	**44,250**
Total in kg/day (averaged over 365 days)	**5.8**	**39.6**	**112.7**	**1,012.2**	**14.7**	**121.2**

a) releases to receiving waters calculated in the text (taking into account any WWTP)
b) releases to wastewatercalculated in the text; these are split 70:30 between WWTP and receiving waters in the table
c) ESD indicates all emissions go to WWTP

In addition to the releases in the table, there are also releases to soil of 2,250 kg/year in the regional environment, and 20,450 kg/year in the continental environment.

3.1.2 Environmental fate

3.1.2.1 Degradation in the environment

3.1.2.1.1 Atmospheric degradation

The rate constant for the reaction of bisphenol-A with hydroxyl radicals in the atmosphere and pseudo first-order rate constant for degradation in air are estimated by the AOPWIN program as $80.6 \cdot 10^{-12}$ $cm^3 \cdot molec^{-1} \cdot sec^{-1}$ and by EUSES as 3.48 d^{-1}, respectively. From this rate constant the half-life for the reaction of hydroxyl radicals with bisphenol-A in the atmosphere is calculated by EUSES as 0.2 days. The fraction of chemical absorbed to aerosol particles is calculated by EUSES as 0.385. Bisphenol-A released to the atmosphere is therefore likely to be degraded by reaction with hydroxyl radicals.

3.1.2.1.2 Aquatic degradation

Abiotic degradation

No information on the hydrolysis or photolysis of bisphenol-A in water is reported. The physical and chemical properties of bisphenol-A suggest that hydrolysis and photolysis are likely to be negligible.

Biodegradation studies

A number of biodegradation studies are reported for bisphenol-A, and these include a number of standardised tests. The results from these studies are presented below and summarised at the end of this section.

West and Goodwin (1997a) evaluated the ready biodegradability of bisphenol-A using the OECD 301F manometric respirometry test. Bisphenol-A of 99.7% purity (confirmed by HPLC) was used in the test. The theoretical oxygen demand (ThOD) of bisphenol-A was calculated as 2.52 mg O_2/mg. The inoculum used in the experiment consisted of activated sludge mixed liquor collected from a municipal sewage treatment plant. The experimental details followed the procedures detailed in the OECD 301F test to Good Laboratory Practice (GLP) standards. However, the temperature used in the experiment was 27.1°C, which is 2.1°C above the range of temperatures quoted in the OECD guidelines. The initial concentrations of bisphenol-A used in the experiment were 7 mg/l and 25 mg/l. Oxygen consumption and CO_2 evolution were measured over 28 days and removal of dissolved organic carbon (DOC) from the biodegradation reactions was determined after 28 days. The time required for achieving 10% degradation for the bisphenol-A ranged from 5.6 days (7 mg/l bisphenol-A) to 6.1 days (25 mg/l bisphenol-A) with biodegradation exceeding the 60% degradation level after an additional 3.5 days (7 mg/l bisphenol-A) and 5.0 days (25 mg/l bisphenol-A). Ten days following the defined lag periods biodegradation averaged 77.6% and 73.7% for the 7 mg/l and 25 mg/l reactions. The maximum degradation levels averaged 84.6% and 81.7% of the ThOD for the 7 mg/l and 25 mg/l reactions respectively after 28 days. The rate and extent of bisphenol-A mineralisation observed indicate that bisphenol-A can be classified as "readily biodegradable". Evolution of CO_2 resulting from mineralisation of bisphenol-A closely followed biodegradation of the compound as measured

from oxygen consumption. Maximum yields of CO_2 ranged from 73.0% to 80.1% of $ThCO_2$ indicating nearly complete conversion of the added organic carbon to CO_2.

West and Goodwin (1997b) repeated the above experiment at a lower temperature of 22.5°C to meet OECD guidelines for this test. Apart from the temperature the experimental conditions were the same as those from West and Goodwin (1997a). The results of the experiments confirmed the earlier test result that bisphenol-A could be classed as "readily biodegradable" according to the OECD 301F manometric respirometry test. The time required to achieve 10% degradation for the bisphenol-A ranged from 4.7 days (7 mg/l bisphenol-A) to 5.2 days (25 mg/l bisphenol-A). Ten days following the defined lag periods biodegradation averaged 92.3% and 77.1% for the 7 mg/l and 25 mg/l reactions. The maximum degradation levels averaged 93.1% and 81.0% of the ThOD for the 7 mg/l and 25 mg/l reactions respectively after 28 days.

Stone and Watkinson (1983) studied the biodegradation of bisphenol-A in the OECD 301D closed bottle test and the OECD 301B modified Sturm test. They also conducted an inhibition test on the growth of *Pseudomonas fluorescens*. The theoretical oxygen demand (ThOD) was calculated as 2.53 mg O_2/mg and the theoretical carbon dioxide demand ($ThCO_2$) as 2.90 mg CO_2/mg.

In the closed bottle test the initial test concentration used was 3 mg/l. The oxygen concentration in the bottles was measured at 5, 15 and 28 days. At the end of the test no degradation was observed. Inhibition of microbial activity was negligible under the test conditions.

In the modified Sturm test the initial concentration of bisphenol-A used was 20 mg/l. The test medium was dispensed into the Sturm vessels, inoculated and aerated with CO_2 free air. The extent of biodegradation was measured at 3, 7, 11, 18, 25, 27 and 28 days by titrating the total carbon dioxide released from the incubation. On day 27 the medium was acidified to release the total carbon dioxide by day 28. At the end of the test no degradation was observed.

In the microbial inhibition test the IC_{50} for the inhibition of growth of *Pseudomonas fluorescens* by bisphenol-A was 54.5 mg/l.

Turner and Watkinson (1986) studied the biodegradation of bisphenol-A using a modified SCAS procedure. The microorganisms used in the test were obtained from a municipal wastewater treatment plant. The initial test concentration of bisphenol-A was 20 mg/l. Removal of bisphenol-A was measured by % dissolved organic carbon (DOC) and UV adsorption spectroscopy. After 24 to 30 days the %DOC removal of bisphenol-A was 87-95%, the drop in DOC coincided with the disappearance in the UV absorption peak for bisphenol-A. The lag phase before degradation of bisphenol-A was observed to be 13 to 17 days. Based upon these results, the authors classified bisphenol-A as inherently biodegradable. This test is designed to measure inherent biodegradability and it is not possible to draw any conclusions about ready biodegradability. It is probably more correct to say that this test shows that bisphenol-A is at least inherently biodegradable, but it is not possible to classify the biodegradation of bisphenol-A using this test.

Matsui et al. (1988) studied the biodegradability of organic substances in an activated sludge test using inocula from an industrial wastewater treatment plant. The conditions in the aeration container were 2-3 g/l MLSS (mixed liquor suspended solids); air flow 150 ml/min and water temperature 25-30°C. The initial concentration of bisphenol-A in the sample was adjusted to 58 mg/l. In the experiment, 2 litres of wastewater was added to 0.5 litres of activated sludge. The sample was then aerated for 23 hours. After one-hour sedimentation 2.0 litres of the supernatant solution were replaced by the sample water. The sludge was acclimated for a total of 24 hours before the first samples were taken. The chemical oxygen demand (COD) was determined with

$KMnO_4$ and total organic carbon (TOC) was measured. The biodegradability of bisphenol-A was determined from the initial concentration and the concentration after 24 hours in terms of both COD and TOC. For bisphenol-A the removal was 72% COD and 57% TOC. It is not possible to say from these results whether bisphenol-A is readily biodegradable, but the results indicate that bisphenol-A is at least inherently biodegradable.

Dorn et al. (1987) studied the degradation of bisphenol-A in natural waters. The waters used in the experiments were taken from the Houston Ship Channel in the vicinity of a bisphenol-A manufacturing plant. Four sample waters were used in the experiments; fresh water control; chemical plant treated process effluent, water taken 180 meters downstream of the effluent discharge and from the ship channel (receiving waters). Each water sample was spiked with 3.0 mg/l bisphenol-A, and aerated for 8 days. Samples were taken for analysis by HPLC each day. In the effluent water sample bisphenol-A was found to decrease after 24 hours with 37% removal of bisphenol-A after 48 hours; after 5 days the concentration of bisphenol-A was below the detection limit (<0.1 mg/l). In the waters sample taken downstream of the effluent outflow bisphenol-A concentrations started to decrease after 48 hours and by 72 hours the concentration was below the detection limit. In the ship channel water the concentration of bisphenol-A began to decrease after 4 days and was below the detection limit by 8 days. In the control experiment no removal of bisphenol-A was observed. Bisphenol-A removal appeared to be rapid once the system had become acclimated. The waters which already received bisphenol-A or were near the bisphenol-A effluent outflow became acclimated first.

A river die-away study looking at the degradation of bisphenol-A in shake flask microcosms and respirometer tests is reported by Klečka et al. (2000). The objectives of the study were to determine the range of half-lives for bisphenol-A biodegradation that might be expected in surface waters and evaluate the effect of pre-exposure and adaptation of microorganisms to bisphenol-A. Water and sediment samples used in the tests were collected upstream and downstream from wastewater treatment plants known to treat wastewater containing bisphenol-A. Samples were collected from following rivers in the United States and Europe; Ohio (USA), Ware (USA), Monte Sano Bayou (USA), Mississippi (USA), Rhine (Germany), Elbe (Germany) and Westerschelde (The Netherlands). Bisphenol-A was not detected in any of the river water samples prior to the addition of the test compound. River die-away studies for ^{14}C bisphenol-A were conducted in parallel with respirometer studies using water samples from the Rhine and Ohio Rivers. A slightly longer lag phase was observed in the shake flask tests compared to respirometer studies but this was found to be insignificant. The reason for this was found to be due to enrichment of the headspace gases in the microcosms in the shake flask studies with pure oxygen. Degradation in the remaining rivers was studied by the respirometer method only. The results indicated rapid biodegradation of bisphenol-A after an initial lag phase. Based upon all the results the average lag-phase was 3.4 days and the average half-life after acclimation was 1.2 days. There was no significant difference between the tests conducted with different river waters or river waters upstream or downstream from wastewater treatment plants. All the tests showed extensive mineralisation of bisphenol-A to CO_2 indicating complete degradation by the end of the incubation period (≤ 18 days). The authors concluded that bisphenol-A is rapidly degraded in natural waters following a period of adaptation. Prior exposure of microorganisms does not appear to significantly affect the rate of adaptation or half-life of bisphenol-A.

Lobos et al. (1992) studied the biodegradation of bisphenol-A by a gram-negative aerobic bacterium. Sludge taken from a wastewater treatment plant serving a plastic manufacturing plant was enriched on bisphenol-A. The enriched sludge produced a microbial consortium capable of degrading a solution of 0.2% bisphenol-A after 1-week incubation. Gram-negative bacteria -

Strain MV1 was isolated as capable of degrading bisphenol-A. The growth of strain MV1 on bisphenol-A was best achieved under aerobic conditions. Under the experimental conditions 60% of carbon in bisphenol-A was mineralised to carbon dioxide, 20% of the carbon became associated with the bacterial cells and 20% of the soluble organic carbon remained in the medium. The main metabolites were identified as 2,2-bis(4-hydroxylphenyl)-1-propanol, 4-hydroxyacetophenone and 2,3-bis(4-hydroxyphenyl)-1,2-propanediol with trace amounts of 4-hydroxybenzoic acid. Formation of metabolites was rapid in the first 8 hours then their concentrations slowly declined. The concentration of 2,2-bis(4-hydroxylphenyl)-1-propanol was found to be proportional to cell growth. 2,3-bis(4-hydroxyphenyl)-1,2-propanediol was formed after 20 hours incubation. In experiments with rapid aeration, levels of 4-hydroxyacetophenone were formed that were inhibitory to the bacteria. The proposed pathways for the degradation of bisphenol-A by strain MV1 by the authors are given in Figure 3.1.

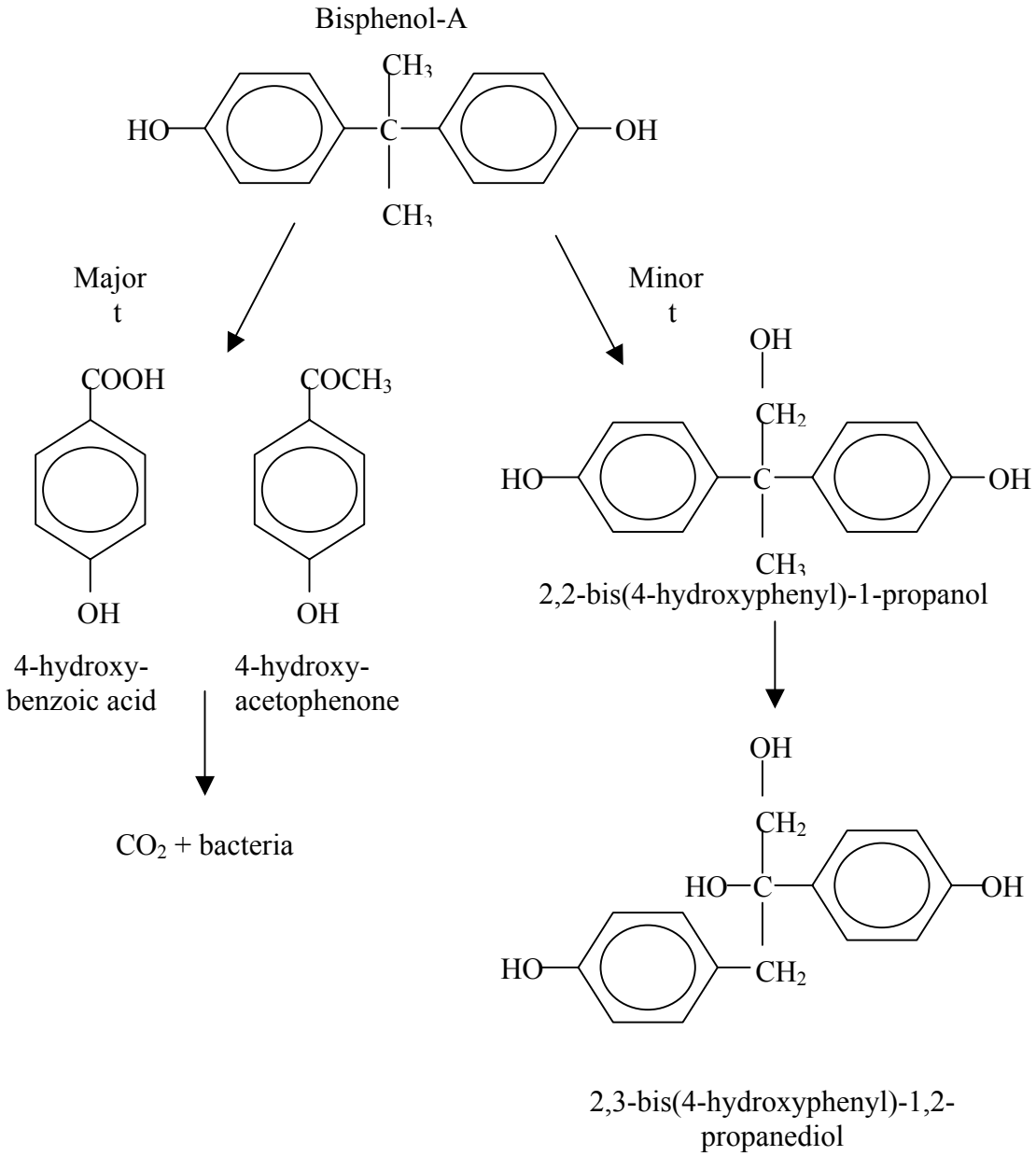


Figure 3.1 Possible degradation pathways of bisphenol-A

Furun et al. (1990) studied the treatment of wastewater containing bisphenol-A by biological processes, activated carbon adsorption and large pore resin adsorption. The results from the experiments looking at the removal of bisphenol-A from wastewaters using activated carbon adsorption and large pore resin adsorption are also reported in Section 3.1.2.2.1. The ability of biological wastewater treatment to degrade bisphenol-A was tested using the activated sludge treatment process. The synthetic wastewater feed comprised 200 mg/l bisphenol-A only. The activated sludge used in the test was taken from a plant treating petrochemical wastewater. The microorganisms were adapted to bisphenol-A over one week exposure. After two weeks exposure the removal rate of bisphenol-A was 99.7%. The biological treatment experiment was also conducted on the effluent from a polycarbonate production plant. The wastewater contained about 100 mg/l of bisphenol-A, and a certain amount of triethylamine. The acclimation stage of the activated sludge lasted about two months. After a period of adaptation the bisphenol-A removal rate from the effluent was 99.4%. The authors noted that actual production wastewater often contains high levels of sodium chloride which can cause the biological treatment to be ineffective.

Alexander and Batchelder (1975) reported the following parameters for bisphenol-A: theoretical oxygen demand 2.52 mg O_2/mg; chemical oxygen demand (COD) with dichromate 2.31 mg O_2/mg; COD with alkaline $KMnO_4$ 1.76 mg O_2/mg; biological oxygen demand 0.66 mg O_2/mg (after 5 days), 1.42 mg O_2/mg (after 9 days) and 1.78 mg O_2/mg (after 20 days). These results show a 5-day BOD/ThOD of 26% and a 20 day BOD/ThOD of 70.6%.

The removal of bisphenol-A in a biopond system is reported as greater than 95.5% (DOW, 1984). No indication is given about the main removal mechanism.

The results of an aerobic biodegradation study on an unnamed substance are reported by Mobil Oil Corporation (1993). The substance studied is not identified in the report though an accompanying document indicates that the substance is bisphenol-A. The study substance was assessed for biodegradability at an initial test concentration of 10 mg carbon/l using the EPA shake flask method with an unacclimated sewage/soil inoculum. In 28 days, 83.6% of the carbon in the study mixture was converted to CO_2. In the control 71.7% of the carbon was converted to CO_2. The study substance met the criteria for readily biodegradable meeting the 10 day test window. The supporting information on the identity of the test substance is such that this study can be considered valid for risk assessment purposes.

Shell (1999) reports the results from an anaerobic biodegradation study. However, problems with the controls in the experiment mean that the test gives little information of use about the anaerobic biodegradation of bisphenol-A.

Voordeckers et al. (2002) studied the fate of bisphenol-A in anaerobic media derived from estuarine sediments taken from between Staten Island and New Jersey in the USA. Sediment samples were mixed with an inorganic anaerobic medium, with specific additions to promote methanogenesis, sulphate-, iron (III)- or nitrate-reducing conditions. Bisphenol-A was added to each medium to a concentration of 200 μM. After 162 days of monitoring, no significant loss of bisphenol-A was seen in any of the live cultures or in autoclaved controls.

Summary of aquatic biodegradation studies

Results from a number of biodegradation studies are reported for bisphenol-A. In the OECD 301F manometric respirometry test bisphenol-A meets the criteria for ready biodegradability. However in the OECD 301D closed bottle test and OECD 301B modified Sturm test no biodegradation was observed. In a modified SCAS procedure bisphenol-A met the criteria for

inherently biodegradable substances, although this test can not give any indication of the potential for bisphenol-A to undergo ready biodegradation.

Measured levels of bisphenol-A before and after wastewater treatment at chemical plant and major users of bisphenol-A suggest a high level of removal. It is not possible to say if this is via adsorption to sludge or biodegradation, although based upon its chemical properties biodegradation is likely to be the major removal mechanism.

From the biodegradation studies reported bisphenol-A would appear to be readily biodegradable, possibly with a short period of adaptation. The default rate constant for biodegradation in wastewater treatment plant is k=1 h^{-1} for a readily biodegradable substance meeting the 10-day window. This value will be used in the assessment. The resulting fate in a wastewater treatment plant as estimated by EUSES is 12% to water and 6.2% to sludge, with 81.9% degraded and a negligible fraction to air.

A number of studies on the degradation of bisphenol-A in natural waters are reported. Removal appears to be rapid once the waters have become acclimatised to bisphenol-A. The reported lag-phases before degradation are between 3-8 days. After the lag phase removal was rapid with 50% removal in 1-2 days and 100% removal in 2 to 17 days. These data would appear to indicate that in natural waters bisphenol-A may be classed as readily biodegradable meeting the 10-day test window. The default rate constant for biodegradation of $4.7 \cdot 10^{-2}$ d^{-1} probably under-estimates the removal rate, as it corresponds to a half life of 15 days with 97% removal taking 75 days. However this value has been used in the risk assessment as a conservative approach.

3.1.2.1.3 Degradation in soil

No information is available as to the degradation rate of bisphenol-A in soil. Therefore, the degradation rate will be estimated from the degradation rate of bisphenol-A in surface water and soil-water partition coefficient. The half-life for biodegradation of bisphenol-A in soil and the first order rate constant for degradation in soil are calculated by EUSES as 30 days and 0.0231 d^{-1}, respectively. This is based upon bisphenol-A being readily biodegradable in surface waters.

3.1.2.2 Distribution

3.1.2.2.1 Adsorption

Furun et al. (1990) studied the treatment of wastewater containing bisphenol-A by biological processes, activated carbon adsorption and large pore resin adsorption. The results from the experiments looking at the removal of bisphenol-A from wastewater by biological processes are also reported in Section 3.1.2.1.2. Static and dynamic adsorption studies were carried out using activated carbon.

In the static adsorption study 500 mg activated carbon was added to 100 ml bisphenol-A solution (347.6 mg/l). After shaking for 2 hours the residual bisphenol-A concentration was determined and the average adsorption capacity of the activated carbon was calculated as 44.7 mg bisphenol-A/g carbon (by weight). The regeneration capacity of the activated carbon using sodium hydroxide was found to be very poor.

In the dynamic adsorption study activated carbon was packed into an adsorption column and a 100 mg/l bisphenol-A solution was pumped through it. The bisphenol-A concentration in the

effluent was measured every hour. The adsorption capacity of the activated carbon was determined as 50 g bisphenol-A/l activated carbon. As with the static experiment the regeneration capacity of the activated carbon using sodium hydroxide was found to be very poor.

Further adsorption studies were carried out using an adsorption resin. In static adsorption tests carried out on six different resins the adsorption capacities were found to be between 7.5 to 21.0 mg bisphenol-A /g wet resin. Of the resins tested, two were found to be just as efficient at adsorbing bisphenol-A after regeneration with sodium hydroxide.

These studies do not allow the adsorption coefficients for other environmental media to be estimated and the TGD methods as implemented in EUSES have to be used. The equation used to predict the Koc value is that for hydrophobic chemicals in general as described in the TGD, using a log Kow value of 3.40. The derived partition coefficients are as follows:

Koc	715 l/kg	Organic carbon-water partition coefficient
Kp_{soil}	14.3 l/kg	Solids-water partition coefficient in soil
Kp_{sed}	35.8 l/kg	Solids-water partition coefficient in sediment
Kp_{susp}	71.5 l/kg	Solids-water partition coefficient in suspended matter
$K_{susp\text{-}water}$	18.8 m^3/m^3	Suspended matter-water partition coefficient
$K_{soil\text{-}water}$	21.7 m^3/m^3	Soil-water partition coefficient
$K_{sed\text{-}water}$	18.7 m^3/m^3	Sediment-water partition coefficient

These data indicate that bisphenol-A is likely to be moderately adsorbed to solids upon release to the environment.

3.1.2.2.2 Precipitation

Bisphenol-A is not volatile and is relatively short lived in the atmosphere. Therefore, it is unlikely to enter the atmosphere in large amounts. Removal of bisphenol-A by precipitation is therefore likely to be negligible and the resulting rainwater concentration very low. As the lifetime of bisphenol-A in the atmosphere is relatively short it is unlikely to be transported a long distance from its point of emission. Any resultant concentrations in soil due to precipitation are therefore likely to be close to the point of emission.

3.1.2.2.3 Volatilisation

The volatilisation of bisphenol-A from surface water to air may be estimated by the Henry's Law constant. This is calculated using EUSES as $4.03 \cdot 10^{-6}$ $Pa \cdot m^3 \cdot mol^{-1}$ for bisphenol-A. The air-water partitioning coefficient ($K_{air\text{-}water}$) may be derived from the Henry's law constant and is calculated as $1.7 \cdot 10^{-9}$ m^3/m^3 for bisphenol-A. Both the Henry's law constant and air-water partitioning coefficient are very low suggesting that volatilisation is unlikely to be a significant removal mechanism for bisphenol-A from water systems.

3.1.2.2.4 Distribution from EQC model

The EQC model as distributed by the OECD was used to estimate the overall fate of bisphenol-A in the environment. The degradation rates as estimated in EUSES were used (as indicated in Sections 3.1.2.1.2 and 3.1.2.1.3), and the releases to air, soil and water were in the same ratio as those used in the EUSES modelling. The resulting distribution in the level III model was: 73.7%

in water; 22.3% in soil; 4% in sediment. If equal emission rates to the three compartments are used the distribution is: 83% soil; 16% water; 1% sediment.

3.1.2.3 Accumulation and metabolism

For bisphenol-A, measured data on bioconcentration in fish are reported in one test conducted by MITI (1977). Bioconcentration data on species from other trophic levels are not reported. No data on metabolism are reported. Bioconcentration factors have been calculated for fish and earthworms using QSARs as detailed in Chapter 4 of the TGD.

Bioconcentration factors for bisphenol-A have been measured by MITI (1977). Bioconcentration factors were determined for carp (*Cyprinus carpio*) exposed to bisphenol-A concentrations of 150 μg/l and 15 μg/l in a flow through system. The carp were exposed to bisphenol-A for six weeks. At the 150 μg/l exposure concentration bioconcentration factors of 5.1 to 13.3 were measured over the 6-week exposure period. At the 15 μg/l exposure concentration bioconcentration factors of <20 to 67.7 were measured over the 6-week exposure period. Bisphenol-A was judged to have a low bioaccumulation potential.

This study was conducted to MITI guidelines. The concentrations used in the study were determined by carrying out an acute toxicity test prior to the accumulation study. In the acute toxicity test a 48-hour LC_{50} of 15 mg/l was determined for killifish (*Oryzias latipes*). The fish used in the study were acclimatised to the test conditions prior to the start of the study. The length of the test appears to have been sufficient to allow a steady state between the concentration of bisphenol-A in fish and water to be achieved. A dispersant (HCO-40) was used to make up the exposure solutions. Although the current OECD guidance recommends that dispersants should not be used in bioconcentration tests, this material is included in the list of acceptable agents if they are used. It was used at concentrations well below the suggested maximum. Measured concentrations in water ranged from 145.9 to 155.7 μg/l for the higher concentration and from 15 to 15.5 μg/l at the lower concentration. Levels in the fish were more variable: 0.79-1.94 mg/kg at the higher concentration and 0.21-1.05 mg/kg at the lower. The test is considered valid for the determination of the bioconcentration factor of bisphenol-A in fish.

Lindholst et al. (2001) exposed rainbow trout (*Oncorhynchus mykiss*) to bisphenol-A in a flow-through system at a concentration of 100 μg/l. The levels of bisphenol-A were measured in the blood plasma, liver and muscle tissues of the fish. Levels in blood plasma reached a maximum concentration within the first twelve hours of exposure; maximum values for liver and muscle were reached after 24 and 48 hours, respectively. The bioconcentration factors for all three sample types were 3.5-5.5. The uptake of bisphenol-A in the blood plasma coincided with the appearance of the glucuronidated derivative; the steady state concentration of this metabolic product was 2-2.5 times that of bisphenol-A. The lower partition coefficient of the metabolite makes it easier to excrete.

The accumulation of bisphenol-A in freshwater clams (*Pisidium amnicum*) has been studied at ecologically relevant low temperatures (Heinonen et al., 2002). Uptake and depuration rates were measured using ^{14}C-labelled substance at temperatures between 2 and 12°C. Both uptake and depuration rates increased with temperature, although the uptake rate decreased slightly at the highest temperature. The bioconcentration factor was calculated from the concentration ratios at steady state and from the two rates. The maximum value was obtained at 8°C by both methods, as 144 based on concentrations and 134 based on rates.

Measured concentrations of bisphenol-A in surface water and fish have been reported by the Japanese Environment Agency. At two locations measured concentrations in both water and fish were recorded though no information on sampling time and species fish is reported. At a number of other sites bisphenol-A was only detected in water samples and not fish. If these data are used to derive BCFs a wide range of values is obtained; the majority of these are below the measured BCF, though some are higher. However, these data are not considered valid for use due to the lack of information correlating fish exposure to measured concentrations in surface and the inconsistencies observed between different sites.

A bioconcentration factor for fish can be calculated from the log Kow. For bisphenol-A a log Kow of 3.4 is taken as the most representative value. This gives a calculated BCF for fish of 155.

The measured bioconcentration factor in fish suggests that bisphenol-A has a low potential for bioaccumulation in fish, in contrast to the moderate potential indicated by the log Kow value. A slightly higher potential is indicated by the measured bioconcentration in freshwater clams (up to 144). Measured data are preferred over calculated values when the studies are valid. A BCF of 67 for fish will be used in the risk assessment, and the accumulation in clams will be considered in the risk characterisation.

A bioconcentration factor for earthworms of 7.9 kg/kg is estimated using QSARs (as implemented in EUSES).

3.1.3 Aquatic compartment (incl. sediment)

3.1.3.1 Predicted environmental concentrations in water

The predicted environmental concentrations (PECs) for water are calculated using the methods detailed in the TGD (Chapter 3 Sections 2.3.7. and 2.3.8.3.). In summary the relevant equations are:

$$Clocal_{inf} = \frac{Elocal_{water} \cdot 10^6}{EFFLUENT_{stp}} \quad \text{(17 TGD)}$$

$$Clocal_{eff} = \frac{Clocal_{inf} \cdot Fstp_{water}}{} \quad \text{(18 TGD)}$$

$$EFFLUENT_{stp} = CAPACITY_{stp} \cdot WASTEWinhab = 2{,}000{,}000 \text{ l/d} \quad \text{(19 TGD)}$$

$$Clocal_{water} = \frac{Clocal_{eff}}{(1 + Kp_{susp} \cdot SUSP_{water} \cdot 10^{-6}) \cdot DILUTION} \quad \text{(30 TGD)}$$

$$DILUTION = \frac{EFFLUENT_{stp} + FLOW}{EFFLUENT_{stp}} \quad \text{(31 TGD)}$$

$$= 10 \text{ [Default]}$$

$$Clocal_{water,ann} = Clocal_{water} \cdot \frac{Temission}{365} \quad \text{(32 TGD)}$$

$$PEClocal_{water} = Clocal_{water} + PECregional_{water} \quad \text{(33 TGD)}$$

$$PEClocal_{water,ann} = Clocal_{water,ann} + PECregional_{water} \quad \text{(34 TGD)}$$

Explanation of symbols:

$Clocal_{inf}$	Concentration in untreated wastewater [mg/l]
$Clocal_{eff}$	Concentration of the chemical in the STP-effluent [mg/l]
$Clocal_{water}$	Local concentration in surface water during emission episode [mg/l]
$Clocal_{water,ann}$	Annual average local concentration in surface water [mg/l]
$PEClocal_{water}$	Predicted environmental concentration during episode [mg/l]
$PEClocal_{water,ann}$	Annual average predicted environmental concentration [mg/l]
$PECregional_{water}$	Regional concentration in surface water [mg/l] (Section 3.1.3.2)
$Elocal_{water}$	Local emission rate to (waste) water during episode [kg/d]
Temission	No of days per year that emission takes place [d/year]
$Fstp_{water}$	Fraction of emission directed to water by STP [0.12 for bisphenol-A, as estimated by EUSES for ready biodegradability]
$EFFLUENT_{stp}$	Effluent discharge rate of stp [l/d]
$CAPACITY_{stp}$	Capacity of the STP [default 10,000]
$WASTEW_{inhab}$	Sewage flow per inhabitant [200 l/d]
DILUTION	Dilution factor [default 10]
FLOW	Flow rate of the river [l/d]
Kp_{susp}	Solids-water partitioning coefficient of suspended matter [default 71.5 l/kg]
$SUSP_{water}$	Concentration of suspended matter in water [default 15 mg/l]

Calculation of the local PEC requires the addition of the regional PEC to the local concentrations. The PECregional is calculated using EUSES as 0.12 µg/l (see Section 3.1.3.2). (For processes where there are no releases to water the PEClocal is the same as the PECregional.)

3.1.3.1.1 Bisphenol-A production sites

Releases from bisphenol-A production sites, based on confidential information provided by the companies to CEFIC, are given in Section 3.1.1.1. The data are releases to surface water, after wastewater treatment where this occurs. The data relate to both production and processing activities since some form of processing occurs at all production sites.

Site BPA1

The average concentration of bisphenol-A in the effluent from production site BPA1 is <70 µg/l. The dilution rate of the effluent in receiving waters is calculated as 794 based upon river flow rates and effluent flow rates. After dilution in receiving waters the concentration of bisphenol-A is calculated as 0.09 µg/l. The PEC_{water} is calculated as 0.21 µg/l.

Site BPA2

The concentration of bisphenol-A in the effluent from production site BPA2 is 0.69 µg/l. The dilution rate is calculated from the effluent flow rate and river flow rate as 8,620. After dilution in the receiving waters the concentration of bisphenol-A is calculated as 0.08 ng/l. The PEC_{water} is calculated as 0.12 µg/l.

Site BPA3

The company reports the concentration of bisphenol-A in different waste streams and the flow rates of these waste streams. Using this information the concentration of bisphenol-A in receiving waters is calculated as 5.3 ng/l. The PEC_{water} is calculated as 0.12 μg/l.

Site BPA4

The daily emission of bisphenol-A from site BPA4 is 0.19 kg/day to surface waters. This is based upon a measured concentration of bisphenol-A in the effluent of 1.8 ppb (1.8 μg/l). The $Clocal_{eff}$ for the plant is therefore taken as 1.8 μg/l. The dilution rate of the effluent from site is given by the company as 260, based upon effluent flow rates from the plant and the 10^{th} percentile flow rate for the receiving waters. This gives a $Clocal_{water}$ of 7 ng/l and a PEC_{water} of 0.12 μg/l.

Site BPA5

The daily emission of bisphenol-A from site BPA5 is $2.14 \cdot 10^{-4}$ kg/day in the effluent from the plant. The flow rate of the effluent from the plant is 5,000 m^3/day, which goes into the effluent from a publicly owned treatment works with a flow rate of 100,000 m^3/day. The receiving water is a tidal flow system, with a high flow rate of 120 m^3/sec and a low flow rate of 30 m^3/sec. The dilution in receiving waters of the effluent from the publicly owned treatment works is calculated as 26.9 based upon low flow conditions. The measured concentration of bisphenol-A in effluent from the plant ranges from not detected (detection limit 20 μg/l) up to 192 μg/l; this gives a concentration range of 0.95 to 9.14 μg/l after dilution with effluent from the publicly owned treatment plant and 0.035 to 0.32 μg/l in the receiving waters. As a worst case the highest measured concentration will be taken forward in the risk characterisation, although it should be noted that the majority of measured concentrations were below the detection limit. The PEC_{water} is calculated as 0.44 μg/l. The company has also conducted its own modelling studies using the Scaldis model which gives the calculated amount of bisphenol-A in the mixing zone of the river as 50 ng/l. The difference between the Scaldis model result and the TGD calculation is due to the different parameters used in the calculation; in particular the Scaldis model appears to take into account both high and low flow dilution rates. In line with the precautionary principle the calculated PEC_{water} of 0.44 μg/l will be used in the assessment.

Site BPA6

This site produces BPA for use on-site in further processing. None of the bisphenol-A produced at this site is sold on for use elsewhere. The plant has a biological wastewater treatment plant. From monitoring data of the plant the highest measured level of bisphenol-A in the effluent is 30 μg/l. This concentration will be taken as the worst-case scenario, however it should be noted that the majority of measurements are significantly below this concentration. The effluent from the wastewater treatment plant is discharged to sea, and the dilution of the effluent in the seawater 100 m from the shore is calculated as 100. This gives a $Clocal_{water}$ of 0.3 μg/l and PEC_{water} of 0.42 μg/l.

3.1.3.1.2 Polycarbonates

Production

Polycarbonate is produced at five sites within the EU, and at each of these sites bisphenol-A production also occurs. The releases from polycarbonate production at these sites are considered with the releases from bisphenol-A production in Section 3.1.3.1.1.

Another polycarbonate production site only ceased production at the end of 2000. The PEC from this site was calculated as 0.24 μg/l. This is not considered further in the risk assessment and is given for information only.

Washing of polycarbonate bottles

In Section 3.1.1.2.3 the release of bisphenol-A from the washing of polycarbonate bottles at a representative site was estimated. The following values have been calculated from this, assuming discharge to a standard WWTP:

Daily release to wastewater	<27 mg
Inflow concentration	<0.014 μg/l
Effluent concentration	<1.6 ng/l
Concentration in local receiving waters	<0.16 ng/l
PEC	0.12 μg/l

3.1.3.1.3 Epoxy resin production

Some bisphenol-A production plants also carry out epoxy resin production. For these plants the release of bisphenol-A from the site are combined and the combined PECs are reported under bisphenol-A production. PECs from sites carrying out epoxy resin production only are detailed below.

Site ER1

The highest concentration of bisphenol-A in the effluent from the site is reported as <5 μg/l. As a worst case 5 μg/l will be taken as a maximum concentration. The flow rate of the receiving waters is 2,000 m^3/sec ($172.8 \cdot 10^6$ m^3/day). The resultant dilution factor is calculated as 11,000. This gives the resultant concentration of bisphenol-A in receiving water as 0.5 ng/l. The PEC_{water} is calculated as 0.12 μg/l.

Site ER2

There are no aqueous effluents from this site. The PEC in the receiving waters is therefore taken as equivalent to the background concentration of bisphenol-A in the environment.

Site ER 3

There are no aqueous effluents from this site. The PEC in the receiving waters is therefore taken as equivalent to the background concentration of bisphenol-A in the environment.

Site ER4

Site ER4 reports an effluent concentration of 1.8 μg/l ($Clocal_{eff}$). This is expected to drop to 0.8 μg/l following installation of biological treatment in July 1999. The minimum flow rate of the receiving water is 0.5 m^3/sec and the average flow of the wastewater treatment plant is 1 m^3/sec. This gives a minimum dilution rate of effluent in the receiving waters of 1.5. This gives a $Clocal_{water}$ of 1.2 □g/l. Adding the background concentration on gives a $PEClocal_{water}$ of 1.32 □g/l.

Site ER5

Site ER5 reports an effluent concentration of 0.03 mg/l. The dilution rate of the effluent from the plant in the receiving waters is reported as 1,000. This gives a $Clocal_{water}$ of 0.03 μg/l. Adding the background concentration on gives a $PEClocal_{water}$ of 0.15 μg/l.

Site ER6

There are no aqueous effluents from this site. The PEC in the receiving waters is therefore taken as equivalent to the background concentration of bisphenol-A in the environment.

3.1.3.1.4 Phenoplast cast resins

Site-specific information is available for the sites producing phenoplast cast resins in the EU. From the available information there are no environmental releases of bisphenol-A during the production process therefore no PECs are calculated for this use.

For processing of phenoplast cast resins a generic worst-case scenario based upon releases from an existing processing plant is used.

Daily release rate to wastewater	0.24 kg/day
Influent concentration	0.12 mg/l
Effluent concentration	14.4 μg/l
Concentration in local receiving waters	1.44 μg/l
PEC	1.56 μg/l

3.1.3.1.5 Thermal paper production

Six companies operating at seven sites within the EU have provided site-specific data. The site-specific data accounts for the total tonnage of bisphenol-A, used by thermal paper manufacturers. Therefore, these data are taken as been representative of bisphenol-A use by the thermal paper industry.

Site PAPER 1

The company has estimated the influent concentration of bisphenol-A to be 2.3 mg/l. The company estimated that bisphenol-A would be completely removed during wastewater treatment by adsorption to sludge. The wastewater treatment plant has a capacity of 600 m^3/day, though typically treats 300 m^3/day waste. The receiving waters have a flow rate of 300,000 m^3/day, which gives a dilution factor for effluent from the plant of 1,000.

The company assumption that bisphenol-A is completely removed in the wastewater treatment plant by adsorption to sludge appears to be unrealistic. In EUSES the fraction of input to the

wastewater treatment plant directed to surface water is estimated as 0.12. If this release factor is applied to the influent concentration of 2.3 mg/l the resultant effluent concentration is 276 μg/l. Applying the dilution factor of 1,000 to this gives a local concentration 0.28 μg/l and a $PEClocal_{water}$ of 0.40 μg/l.

Site PAPER 2

The company has measured the influent and effluent concentrations of bisphenol-A to be 0.02 mg/l and 0.006 mg/l, respectively. The removal rate of bisphenol-A in the wastewater treatment plant is estimated as 70%, by adsorption to sludge. The wastewater treatment plant has a capacity of 2,800 m^3/day. The receiving waters have a flow rate of 146,880 m^3/day, so this gives a dilution factor for effluent from the plant of 53. Applying this dilution factor to the effluent concentration gives a local concentration in surface water of 0.11 μg/l and a $PEClocal_{water}$ of 0.23 μg/l.

Site PAPER 3

The company reports a release of 0.5 kg/day to wastewater and the total volume of effluent produced as 1,000 m^3/day. This is treated at a wastewater treatment plant with a capacity of 85,000 m^3/day. The calculated $Clocal_{eff}$ is 0.7 μg/l. Using the standard dilution of 10, the $Clocal_{water}$ is 0.07 μg/l and the $PEClocal_{water}$ is 0.19 μg/l.

Site PAPER 4

The company reports the concentration of bisphenol-A in the effluent from the wastewater treatment plant as <0.01 mg/l. No information on the dilution rate in the receiving waters is reported, therefore the default dilution factor of 10 is used. This gives a $Clocal_{water}$ of <1 μg/l and a $PEClocal_{water}$ of <1.12 μg/l.

Site PAPER 5

The company reports the concentration of bisphenol-A in the effluent from the wastewater treatment plant as <0.01 mg/l. No information on the dilution rate in the receiving waters is reported so the default dilution factor of 10 is used. This gives a $Clocal_{water}$ of <1 μg/l and a $PEClocal_{water}$ of <1.12 μg/l.

Site PAPER 6

The company reports the concentration of total phenols in the effluent from the plant as 0.39 mg/l. Assuming that this is all bisphenol-A gives a bisphenol-A concentration of 0.47 mg/l. The dilution factor for effluent from the plant is 500 which gives a $Clocal_{water}$ of 0.94 μg/l and a $PEClocal_{water}$ of 1.06 μg/l.

Site PAPER 7

The company reports the concentration of total phenols in the effluent from the plant as <0.1 mg/l. Assuming that this is all bisphenol-A gives a bisphenol-A concentration of <0.12 mg/l. The plant effluent is diluted by a factor of 35 at the local wastewater treatment plant, and this gives a bisphenol-A concentration of <3.43 μg/l in the plant influent. Using the default fraction to water (12%) gives a bisphenol-A concentration in the plant effluent of <0.411 μg/l.

The dilution factor for effluent from the plant is 10 at low flow, and this gives a $Clocal_{water}$ of <0.04 μg/l and a $PEClocal_{water}$ of <0.16 μg/l.

3.1.3.1.6 Thermal paper recycling

Releases of bisphenol-A to water have been estimated from the recycling of thermal paper containing bisphenol-A.

In order to calculate the PEC for recycling of thermal paper, knowledge of the water use in the process is needed. The emission scenario document for the pulp, paper and board industry gives water usage figures of 5-15 m^3/tonne paper recycled from the flotation processes, whereas washing can use between 5-100 m^3/tonne paper. The total water usage for the production of specific types of paper is 40-75 m^3/tonne for printing and writing paper, 57 m^3/tonne for tissue paper and 24-35 m^3/tonne for newsprint.

In order to estimate the worst-case concentration at a recycling site it will be assumed that the site only recycles thermal paper and uses around 5,250 tonnes/year of paper (i.e. the recycling rate for thermal paper is 50% of the total EU consumption (105,000 tonnes/year), with 10% of the thermal paper being recycled on one site as indicated in the ESD. Using a water consumption rate of 35 m^3/tonne gives a total water usage rate of 183,750 m^3/year for this volume of paper. According to the ESD, some paper recycling plants may carry out only primary treatment before discharge of effluent to surface water via a municipal wastewater treatment plant (the release estimate is based on the amount released after this primary treatment process). Further, the ESD also indicates that if the effluent is emitted to a wastewater treatment plant off-site, the size of the treatment plant is likely to be larger than average due to the large volume of wastewater generated. PECs are therefore calculated assuming that no further wastewater treatment other than primary treatment occurs on site, and that the effluent from the plant is treated at an off-site wastewater treatment plant, which is in line with the approach recommended in the ESD.

A release to water after primary wastewater treatment on-site of 140 kg/day and a volume of wastewater of 735 m^3/day give a $Clocal_{effluent}$ of 190 mg/l. This is then diluted by a fraction of 10 in the influent to the off-site wastewater treatment plant (to account for the dilution in the influent to the off-site WWTP) which gives a $Clocal_{inffluent}$ of 19 mg/l, $Clocal_{effluent}$ of 2.28 mg/l and $Clocal_{water}$ of 0.23 mg/l. Addition of the $PEC_{regional}$ as background concentration gives a $PEClocal_{water}$ of 230 μg/l.

The above calculation is based upon the ESD for pulp and paper. TNO (2000) have conducted a risk assessment of bisphenol-A from the recycling of thermal paper for the European Thermal Paper Association (ETPA). As discussed in Section 3.1.1.8 there are a number of differences between the generic scenario used here and the scenarios in the TNO risk assessment. To allow for the use of site-specific data in the assessment the worst-case branch specific PEC of 18 μg/l calculated in the TNO report will be taken forward in this risk assessment.

Site-specific data from a thermal paper recycler suggests that the concentration of bisphenol-A in the effluent from the plant is less than the detection limit of 10 μg/l. The dilution rate of wastewater from the plant is 19.2. Taking a worst-case scenario of a bisphenol-A concentration of 10 μg/l gives a $Clocal_{water}$ of 0.52 μg/l and a $PEClocal_{water}$ of 0.64 μg/l.

3.1.3.1.7 PVC Production and Use

Site-specific data

Site-specific information is available for two sites. Both of these sites use bisphenol-A as an inhibitor during the production of PVC. One of these sites is also a bisphenol-A producer and emissions from this plant are reported earlier. It is worth noting though that the mean effluent concentration of bisphenol-A from the PVC operation on the site is measured at 22 mg/l. For the second site the influent concentration of bisphenol-A to its wastewater treatment plant is measured at 17-18 mg/l. After on-site treatment the concentration is <5 μg/l, and there is further dilution in the receiving waters. As the dilution rate is not known, the default dilution rate of 10 is applied to give a concentration in receiving waters of 0.5 μg/l and a $PEClocal_{water}$ of 0.62 μg/l.

Generic scenarios

Use as an inhibitor in the production of PVC

Daily release rate to wastewater	55.5 kg/day
Influent concentration	27.75 mg/l
Effluent concentration	3.33 mg/l
Concentration in local receiving waters	333 μg/l
PEC	333 μg/l

The calculated influent concentration of bisphenol-A is 27.75 mg/l which agrees well with the influent concentrations available from the site-specific data (22-35 mg/l for one site and 17-18 mg/l for the other site). However, the resultant PECs are very different. This is due to a number of factors, including the removal rate used for bisphenol-A in the wastewater treatment plant and the dilution rate of material in the wastewater treatment plant. In the absence of any other data default assumptions have to be used for the generic calculation, though the results differ widely from the site-specific data available.

Use as an anti-oxidant in PVC processing

Daily release rate to wastewater	0.0256 kg/day
Influent concentration	12.8 μg/l
Effluent concentration	1.54 μg/l
Concentration in local receiving waters	0.15 μg/l
PEC	0.27 μg/l

Preparation of additive packages for PVC processing

Daily release rate to wastewater	2.12 kg/day
Influent concentration	1.06 mg/l
Effluent concentration	127μg/l
Concentration in local receiving waters	12.7 μg/l
PEC	12.8 μg/l

Use as an anti-oxidant in the preparation of plasticisers for use in PVC processing

Daily release rate to wastewater	0.32 kg/day
Influent concentration	0.16 mg/l
Effluent concentration	19.2 μg/l

Concentration in local receiving waters	1.9 μg/l
PEC	2.0 μg/l

Use as a plasticiser in PVC processing

Daily release rate to wastewater	0.0179 kg/day
Influent concentration	8.95 μg/l
Effluent concentration	1.07 μg/l
Concentration in local receiving waters	0.107 μg/l
PEC	0.23 μg/l

3.1.3.2 Regional and continental PEC calculations

The regional and continental PECs have been calculated using EUSES. The inputs to the regional and continental models are as follows:

	Regional	**Continental**
Air	5.8 kg/day	39.6 kg/day
Wastewater	112.7 kg/day	1,012.2 kg/day
Receiving waters	14.7 kg/day	121.2 kg/day
Industrial soil	6.2 kg/day	56 kg/day

The calculated PECregional for surface water is 0.12 μg/l and PECcontinental for surface water is 0.015 μg/l.

3.1.3.3 PECs for sediment

The predicted environmental concentrations for the sediment compartment have been calculated from the surface water concentrations using the equilibrium partitioning method in accordance with the TGD. The local PEC values are in **Table 3.4**. The regional and continental sediment concentrations from EUSES are $1.6 \cdot 10^{-3}$ mg/kg wet wt and $2.4 \cdot 10^{-4}$ mg/kg wet wt, respectively.

3.1.3.4 PEC for wastewater treatment plants

The PEC for wastewater treatment plant is taken as equivalent to the $Clocal_{eff}$ (concentration of bisphenol-A in the effluent from a wastewater treatment plant). Estimates are given in **Table 3.4**.

3.1.3.5 Summary of PECs for the aquatic compartment

Table 3.4 summarises the PECs for bisphenol-A calculated from site-specific data and generic scenarios. Where site-specific data are available for more than one site, the site marked with an asterisk (*) indicates that it has the highest PEC_{water} and is considered further in the risk assessment report for the sediment, terrestrial and secondary poisoning scenarios.

Table 3.4 PECs for bisphenol-A in wastewater treatment plants, surface water and sediment

	PEC_{stp} (mg/l)	$Clocal_{water}$ (μg/l)	PEC_{water} (μg/l)	$PEC_{sediment}$ (mg/kg wet wt)
Site-specific				
BPA 1	0.07	0.09	0.21	
BPA 2	0.00069	0.00008	0.12	
BPA 3	0.005	0.0053	0.12	
BPA 4	0.0018	0.007	0.12	
BPA 5 (*)	0.19	0.32	0.44	0.007
BPA 6	0.03	0.3	0.42	
ER 1	0.005	0.0005	0.12	
ER 2, ER 3, ER 6	0	0	0.12	
ER 4 (*)	0.0018	1.2	1.32	0.02
ER 5	0.03	0.03	0.15	
PAPER 1	0.276	0.28	0.40	
PAPER 2	0.006	0.11	0.23	
PAPER 3	0.0007	0.07	0.19	
PAPER 4	<0.01	<1.0	<1.12	
PAPER 5	<0.01	<1.0	<1.12	
PAPER 6 (*)	0.47	0.94	1.06	0.02
PAPER 7	0.000411	<0.04	<0.16	
PVC Production	<0.005	0.5	0.62	
Generic scenarios				
Polycarbonate bottle washing	$1.6 \cdot 10^{-6}$	0.00016	0.12	0.002
Thermal paper recycling	2.28	230	230	3.71
Thermal paper recycling (TNO branch specific value)	0.073	18	18	
Phenoplast cast resin processing	0.0144	1.44	1.56	0.025
PVC – Inhibitor during production process	3.33	333	333	5.4
PVC – Anti-oxidant during processing	0.00154	0.15	0.27	0.004
PVC – Preparation of additive packages	0.127	12.7	12.8	0.2
PVC – Anti-oxidant in plasticiser production	0.019	1.9	2.0	0.033
PVC – Plasticiser use	0.00107	0.107	0.23	0.0036

* Site-specific data marked with a * are taken forward in EUSES for modelling sediment, atmospheric, terrestrial and indirect exposure of man via the environment as appropriate.

3.1.3.6 Measured levels in the aquatic compartment

3.1.3.6.1 Surface water

Hendriks et al. (1994) measured bisphenol-A in river water samples from several locations on the Rhine in The Netherlands. The organic matter of the water samples was concentrated and removed by sedimentation. The water was then passed over 2 column beds containing XAD-4 resin. In the first column adsorption occurred at pH 7 and in the second column at pH 2. The columns were subsequently eluted with ethanol and ethanol/cyclohexane. This was followed by azeotropic distillation with ethanol/cyclohexane/water and ethanol/cyclohexane. After evaporation the ethanol was concentrated. Fractionation of the XAD isolates was carried out in five successive extraction steps with cyclohexane, diethylether, ethylacetate, ethanol and ethanol/water. The isolates and the fractions were analysed by GC and HPLC. The cyclohexane fraction containing the majority of the organic compounds was also analysed by mass spectrometry. Bisphenol-A was found in the cyclohexane fraction. Bisphenol-A at a concentration of 0.119 μg/l was detected in a sample from Lobith near the German border. It was not detected at any of the other sampling sites or in a later sample taken from the same site.

The concentration of bisphenol-A in the River Elbe in Germany and the Czech Republic is reported by Gandrass (1999) (reference quoted in personal correspondence with German competent authority (CA)). In measurements made during September 1998 the concentration ranged from 1.5 to 1,290 ng/l. Other measurements for 1998 give a range of 3.7-16.2 ng/l with a mean of 8.4 ng/l. The analytical method used was GC/ECD with a detection limit of 0.3 ng/l.

Statchel (1999) (reference quoted in personal correspondence with German CA) reported bisphenol-A concentrations in the River Elbe and its tributaries of below the detection limit up to 125 ng/l. The analytical method used was GC/MS; the detection limit for the method is not quoted.

Fromme et al. (1988) (reference quoted in personal correspondence with German CA) reported bisphenol-A concentrations in river water and lake water samples taken from around Berlin. The total number of samples was 65. The arithmetic mean of bisphenol-A concentrations was 23 ng/l and the geometric mean was 8 ng/l. The highest concentration measured was 410 ng/l and the 90^{th} percentile was 45 ng/l. Samples were analysed by HPLC with fluorescence detection and a detection limit of 5 ng/l.

Wenzel et al. (1998) (reference quoted in personal correspondence with German CA) reported bisphenol-A concentrations from 52 surface water sites in Germany. Bisphenol-A was found to be above the detection limit of 0.1 ng/l at 39 of the 52 sites. The maximum concentration detected was 229 ng/l, the average was 46.7 ng/l and the 90^{th} percentile 98 ng/l. The analytical method used was GC.

Boutrup et al. (1998) (reference quoted in personal correspondence with German CA) failed to detect bisphenol-A in fresh water samples taken in the County of Aarhus in Denmark. The detection limit of the analytical method used was 100 ng/l.

Sattelberger et al. (1999) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in surface waters in Austria. Bisphenol-A was not detected in 23 out of 34 samples. The maximum concentration detected was 65 ng/l and the mean concentration was 32 ng/l. The detection limit for the method used was 10 ng/l.

del Olmo et al. (1997) (reference quoted in personal correspondence with German CA) analysed seawater from near Malaga in Spain and spring water from an agriculture area of Spain. They

used a GC/MS analytical method with a detection limit of 0.6 μg/l. They failed to detect bisphenol-A in any of the samples.

Belfroid et al. (1999) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in surface water in the Netherlands. The concentration of bisphenol-A ranged from 3.5-160 ng/l. The analytical method used was a GC/MS system.

Staples et al. (2000) measured the concentration of bisphenol-A in receiving waters upstream and downstream of US manufacturers and processors. The analytical method used was COCI/GC/EI/MS (Cool On Column Injection/Gas Chromatography/Electron Impact/Mass Spectrometry) with a detection limit of 1 μg/l. Bisphenol-A was not detected in any of the surface water samples in 1996 or at six of seven sites in 1997. At the seventh site in 1997 bisphenol-A concentrations ranged from 2 to 8 μg/l upstream and 7 to 8 μg/l downstream. The authors noted that receiving waters at the site had no measurable flow at the time of measurement and the concentrations measured corresponded to undiluted effluent.

Matsumoto et al. (1977) identified bisphenol-A at a concentration of 10-90 ng/l in a river water sample taken from the Tama River in Tokyo. They used a GC/MS method for the analyses and bisphenol-A was identified by comparison with known retention time and spectra. The authors speculated that the bisphenol-A was present due to industrial activity.

The Japanese Environment Agency (1996) measured the concentration of bisphenol-A in surface water in Japan. The concentration of bisphenol-A ranged from 0.01-0.268 μg/l; the detection limit for the method used was 0.01 μg/l.

The Japanese Ministry of Construction (1998) measured the concentration of a range of potentially endocrine disrupting chemicals in 109 rivers in Japan. Bisphenol-A was measured using a GC/MS system with a detection limit of 0.01 μg/l. Bisphenol-A was not detected in 109 out of 256 samples. In 86 samples the concentration was between 0.01-0.03 μg/l, in 47 samples between 0.03-0.1 μg/l, in 12 samples between 0.1-0.3 μg/l and in two samples it was >0.3 μg/l (1.4 μg/l and 0.31 μg/l).

Matsumoto (1982) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in river water in the Tokyo area of Japan. The average concentration of bisphenol-A was 0.12 μg/l and the range of levels measured was 0.06-1.9 μg/l. The authors failed to detect bisphenol-A in unpolluted inland river waters. They used a GC/MS system for analysis.

3.1.3.6.2 Sediment

Boutrup et al. (1998) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in freshwater and marine sediments in Denmark. The analytical method used had a 2 μg/kg detection limit. In freshwater lake sediment the concentration of bisphenol-A was <10 μg/kg dry weight in diffuse samples and 35 μg/kg dry weight in samples 100 m downstream from a wastewater treatment plant. In river sediment the concentration ranged from 3.5 to 150 μg/kg dry weight with the highest concentration observed in river water receiving wastewater from several towns. In marine sediment the concentration of bisphenol-A ranged from below the detection limit up to 13 μg/kg dry weight with the highest concentration being observed in sediments near a wastewater treatment plant outflow.

Wenzel et al. (1998) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in sediments in fresh water lakes in Germany. Bisphenol-A was

detected in 11 out of 12 samples (detection limit 2 μg/kg dry weight) and range from 17.8-190.4 μg/kg dry weight. The average concentration was 81.3 μg/kg dry weight and the median concentration was 49.2 μg/kg dry weight. The analytical method used was GC/MS.

Fromme et al. (1998) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in sediment samples taken from surface waters in Berlin, Germany. Bisphenol-A was measured at concentrations greater than the detection limit (5 μg/kg dry weight) in 19 out of 23 samples. The maximum concentration measured was 150 μg/kg dry weight, the arithmetic mean was 42 μg/kg dry weight, the geometric mean was 27 μg/kg dry weight and the 90% percentile was 75 μg/kg dry weight. The analytical method used was HPLC with a fluorescence detector.

Belfroid et al. (1999) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in sediment samples in the Netherlands. From the available information they appear to be from a mixture of freshwater and marine water sources. The results are quoted as <68, <213 and <50 (saline), all as ng/l dry weight. These are assumed to be the detection limits in the different samples. The analytical method used was GC/MS.

The Japanese Environment Agency (1996) measured the concentration of bisphenol-A in mud in Japan. The concentration of bisphenol-A ranged from 5.9-600 μg/kg dry weight, the detection limit for the method used was 5 μg/kg dry weight.

CEFAS (2002) analysed archived sediment samples from 50 locations around England. The locations were selected as part of a survey of chlorinated paraffin levels, and relate to plastics processing activities as well as including some background sites. Samples were extracted using an n-hexane:dichloromethane:acetone mixture, and analysed using liquid chromatography - mass spectrometry. The lowest quantification limit for bisphenol-A by this method was 2.7 μg/kg dry weight. Bisphenol-A was not detected at this limit in 48 of the 50 samples. The two positive detections, which were from samples in different locations, contained 57 and 154 μg/kg dry weight.

3.1.3.6.3 Wastewater treatment plants

Körner et al. (1998) measured the concentration of bisphenol-A in the influent and effluent of a municipal sewage plant in Germany. In the influent to the plant the concentration of bisphenol-A was 0.556 μg/l. The effluent sample was taken 8 hours after the influent concentration was measured and was 0.155 μg/l. This corresponds to a 72.1% reduction of bisphenol-A in the wastewater treatment plant. The analytical method used was GC/MS, the detection limit of the method used was between 10 and 20 ng/l.

Wenzel et al. (1998) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in effluents from a range of wastewater treatment plants in Germany. Analysis of samples was by GC with a detection limit of 0.1 ng/l. In effluents from municipal wastewater treatment plants the concentration of bisphenol-A ranged from 2 to 314 ng/l, the average concentration was 88.2 ng/l and the 90^{th} percentile concentration was 267.5 ng/l. In effluents from municipal wastewater treatment plants with medium industrial influents the concentration of bisphenol-A ranged from 55.7 to 74.8 ng/l and the average concentration was 67.8 ng/l. In effluents from municipal wastewater treatment plants with influents from medium industrial activity and hospitals the concentration of bisphenol-A ranged from 21.6 to 701.8 ng/l, the average concentration was 46.3 ng/l and the 90^{th} percentile concentration was 461.7 ng/l. In effluents from municipal wastewater treatment plants with

influents from large and medium industrial activity and hospitals the concentration of bisphenol-A ranged from 3 to 653.3 ng/l and the average concentration was 234.3 ng/l. In the effluent from an industrial wastewater treatment plant the concentration of bisphenol-A was 110.6 ng/l.

Fromme et al. (1998) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in effluents from municipal wastewater treatment plants in Berlin. The maximum concentration measured was 160 ng/l, the arithmetic mean was 80 ng/l, the geometric mean was 60 ng/l and the 90^{th} percentile was 150 ng/l. The analytical method used was HPLC with fluorescence detection. The detection limit for the method was 5 ng/l.

Scharf et al. (1998) (reference quoted in personal correspondence with German CA) measured the concentration of bisphenol-A in the influent, middle fraction and effluent from a wastewater treatment plant in Vienna. The analytical method was GC/MS and had a detection limit of 50 ng/l. The concentration of bisphenol-A in the influent ranged from 1.1-2.3 μg/l with a mean of 1.5 μg/l. The concentration of bisphenol-A in the middle fraction ranged from 0.94-1.6 μg/l with a mean of 1.3 μg/l. The concentration of bisphenol-A in the effluent ranged from 0.72-1.2 μg/l with a mean of 0.9 μg/l. The reduction of bisphenol-A in the wastewater treatment plant was approximately 40%.

Scharf (1999) (reference quoted in personal correspondence with German CA) measured the influent and effluent concentration of bisphenol-A in 17 wastewater treatment plants including three industrial wastewater treatment plants. The bisphenol-A concentration ranged from 0.196-8.425 μg/l with a mean of 2.005 μg/l in the influents and from 0.067-0.884 μg/l with a mean of 0.346 μg/l in the effluents. The detection limit for the analytical method used was 50 ng/l.

Belfroid et al. (1999) (reference quoted in personal correspondence with German CA) measured bisphenol-A in wastewater treatment plants of varying designs and receiving influents from varying sources. The concentration of bisphenol-A in influent ranged from 0.25 μg/l to 2 μg/l and in effluent from 0.022 μg/l to 0.37 μg/l. The analytical method used was GC/MS.

Clark et al. (1991a) measured the concentration of non-volatile organics in effluent from publicly owned treatment works in New Jersey using particle beam liquid chromatography/mass spectrometry. Bisphenol-A was detected in effluent from two of the three treatment plants sampled at concentrations of 25 μg/l and 8 μg/l. The two wastewater treatment plants containing bisphenol-A are identified in a paper by Clark et al. (1991b) as receiving 27% industrial waste and 18% industrial waste respectively. The plant in which bisphenol-A was not detected received no industrial wastes.

3.1.3.7 Discussion of aquatic compartment data

The site-specific PECs for bisphenol-A production and polycarbonate production are based upon measured data and the resultant environmental concentrations should reflect actual conditions quite accurately. However, the addition of the PECregional to these concentrations does increase the PECs in some cases by a significant amount. There are few measured levels which can be related to specific activities. The US measurements near to bisphenol-A processors indicate levels of <1 μg/l, which is in reasonable agreement with most of the site-specific data.

For the generic scenarios the calculated PECs are generally higher than the PECs calculated for site-specific scenarios.

The PECregional is based upon realistic worst-case assumptions. In particular the regional emission from the generic scenarios is very high when compared to the regional emissions from site-specific scenarios. However the calculated regional background concentration (0.12 μg/l) agrees well with measurements in surface waters which are not associated with specific emissions. The maximum values measured exceed the calculated level in some cases, but the mean values for European waters are below the calculated level, as are the 90th percentile values where these are presented. This agreement may indicate that most of the significant sources of bisphenol-A have been accounted for in the estimation of regional emissions.

There are only a few data on levels in sediments, and these cannot easily be related to specific activities. The site-specific calculated values are of the same order as the measured values, while the generic calculated values are 1-2 orders of magnitude higher in some cases. The regional sediment concentration is 1.6 μg/kg dry weight, lower than many of the measured levels. It cannot be ruled out that the measured sediment levels are from sites directly affected by emissions, but this is unlikely to be the case for all measurements. This may indicate a greater retention in sediment than predicted from other properties. A comparison of the measured levels in water and sediments from individual studies suggests that the sediment levels are higher than would be predicted from the water levels; this comparison should be treated with caution as the water and sediment samples are not necessarily from the same locations (in some studies they appear to be from different locations). Another possibility is a reduced degradation in sediment, although reducing the degradation rate in sediment for the EUSES calculations has little effect on the concentration predicted. The database of sediment levels is limited in coverage. This area may need to be re-considered when refining the risk assessment (see Section 3.3).

While they are based upon worst-case assumptions the local PECs are judged acceptable for use in the risk assessment. The site-specific data are also judged acceptable for use in the risk assessment.

3.1.4 Terrestrial compartment

3.1.4.1 Predicted environmental concentrations

There are not thought to be any direct exposures of bisphenol-A to the terrestrial environment, except through accidental spillage. The routes of exposure of bisphenol-A to the soil will therefore be through atmospheric deposition and application of sewage sludge. The majority of release scenarios considered involve the use of bisphenol-A at production or processing sites having on-site wastewater treatment facilities. The sludge from these industrial plants may not necessarily be applied to agricultural soil but instead be disposed of via some other route such as incineration or landfill. The data received from the bisphenol-A production companies suggests that this is the case for bisphenol-A production and processing at industrial sites such as chemical plants. In calculating soil concentrations of bisphenol-A, sludge from wastewater treatment plants will be taken as applied to agricultural soil for the site-specific data except where site-specific data specifies an alternative disposal route. This is a precautionary approach and takes account of a realistic worst-case scenario.

The scenario for washing of polycarbonate bottles has not been included in the calculations as the releases to the wastewater treatment plant are negligible.

The EUSES model has been used to calculate the PECs in soil. PECs have not been calculated for most of the site-specific scenarios since the information obtained indicates that disposal of the sludge produced is either by incineration or to a controlled landfill as special waste. The

exception to this is the epoxy resin producer ER 4 which applies sludge to agricultural land in accordance with local regulations. Therefore a PEC_{soil} is calculated for this site. **Table 3.5** gives details of the PECs calculated for soil.

Table 3.5 PECs for soil

	PEC Agricultural soil averaged over 30 days (mg/kg wet wt)	PEC Agricultural soil averaged over 180 days (mg/kg wet wt)	PEC Natural soil averaged over 180 days (mg/kg wet wt)
Site-specific			
ER 4	0.463	0.152	0.0604
Generic Scenarios			
Phenoplast case resin processing	0.0199	0.0065	0.0027
PVC – Inhibitor during production process	4.59	1.5	0.598
PVC – Anti-oxidant during processing	0.0022	0.0008	0.0004
PVC – Preparation of additive packages	0.175	0.0575	0.0229
PVC – Anti-oxidant in plasticiser production	0.027	0.0087	0.0035
PVC – Plasticiser use	0.0016	0.0006	0.0003
Thermal paper recycling	3.14	1.03	0.41

The regional and continental PECs for agricultural soil are calculated as $9.9 \cdot 10^{-5}$ mg/kg wet wt and $9.7 \cdot 10^{-6}$ mg/kg wet wt, respectively. The regional and continental PECs for natural soil are calculated as $7.1 \cdot 10^{-5}$ mg/kg wet wt and $5.5 \cdot 10^{-6}$ mg/kg wet wt, respectively.

As noted in Section 3.1.5 below, only bisphenol-A production sites have direct emissions to air, and indirect emissions of bisphenol-A to air from wastewater treatment plants are calculated to be very low. The resulting air concentrations are low and hence so are the potential for deposition to soil. The highest air concentration calculated in Section 3.1.5 (and hence the highest deposition rate) leads to a concentration in soil through deposition of 0.0014 mg/kg wet weight. This is lower than any of the values in **Table 3.5** for 30-day soil levels, and so the contribution of deposition to the soil levels is ignored.

3.1.4.2 Measured levels

Measured levels of bisphenol-A in sewage sludge, agricultural slurry, and groundwater are reported and summarised below. No measurements of bisphenol-A in soil have been reported.

Wenzel et al. (1998) (reference quoted in Personal Correspondence with German CA) measured the concentration of bisphenol-A in slurry from animal farming at sites in Germany. Based upon 10 samples the range of bisphenol-A in the slurry was 56.9 μg/kg dry weight to 1112.3 μg/kg dry weight, the average concentration was 210.8 μg/kg dry weight and the 90th percentile was 35.4 μg/kg dry weight. A GC/MS analytical method was used having a detection limit of 2 μg/kg dry weight.

Rudel et al. (1998) measured the concentration of bisphenol-A in groundwater in Massachusetts, USA. The concentration of bisphenol-A in a plume from a wastewater treatment plant was 3-29 ng/l with an average concentration of 16 ng/l. The concentration of bisphenol-A in a plume from a landfill/seepage lagoon was 4-1,410 ng/l with an average of 320 ng/l. The analytical method used was GC/MS with a detection limit of 3.6 ng/l.

Wenzel et al. (1998) (reference quoted in Personal Correspondence with German CA) measured the concentration of bisphenol-A in sewage sludge samples from a range of wastewater treatment plants in Germany. Analysis of samples was by GC/MS with a detection limit of 0.1 μg/kg dry weight. In sludge from municipal wastewater treatment plants the concentration of bisphenol-A ranged from 20.9 to 1,363.3 μg/kg dry weight, the average concentration was 293.0 μg/kg dry weight and the 90th percentile concentration was 555.2 μg/kg dry weight. The concentration of bisphenol-A in sludge from municipal wastewater treatment plants with medium industrial influents ranged from 3.9 to 291.7 μg/kg dry weight, the average concentration was 86.8 μg/kg dry weight and the 90th percentile was 228.8 μg/kg dry weight. Municipal wastewater treatment plants with influents from medium industrial activity and hospitals gave concentrations of bisphenol-A in sludge ranging from 8.8 to 777.4 μg/kg dry weight, the average concentration was 235.5 μg/kg dry weight and the 90th percentile concentration was 433.7 μg/kg dry weight. The concentration of bisphenol-A in sludge from municipal wastewater treatment plants with influents from large and medium industrial activity and hospitals ranged from 137.4 to 855 μg/kg dry weight, the average concentration was 391.6 μg/kg dry weight and the 90th percentile was 650 μg/kg dry weight. In the sludge from an industrial wastewater treatment plant the concentration of bisphenol-A was 370 μg/kg dry weight. In comparison the calculated concentrations in dry sewage sludge from EUSES for the generic scenarios are 2-4333 mg/kg, or around three orders of magnitude higher.

Wenzel et al. (1998) (reference quoted in Personal Correspondence with German CA) measured the concentration of bisphenol-A in leachate water from three landfill sites in Germany. The range of levels measured was 24.82 μg/l to 145.9 μg/l bisphenol-A with an average concentration of 81.08 μg/l. The analytical method used was GC with a detection limit of 0.1 ng/l.

Yasuhara et al. (1997) measured the concentration of bisphenol-A in leachate from eight landfills in Japan. Bisphenol-A was detected in the leachates from five of the sites (detection limit not given). The concentration ranged from 0.15 μg/l to 12.3 μg/l, with the median value being 0.35 μg/l. The analytical method used was GC/MS.

3.1.5 Atmospheric compartment

The PEC in air is calculated using EUSES. Direct emissions of bisphenol-A to air are reported for bisphenol-A production sites only. For the other uses of bisphenol-A no direct emissions to air are reported and the generic scenarios give zero emissions to air. Therefore $PEClocal_{air}$ is only calculated for the bisphenol-A production site with the highest emission. For the remaining sites the $PEClocal_{air}$ is taken as equivalent to the regional concentration of bisphenol-A in air, i.e. background levels. It should be noted that there may be an indirect emission of bisphenol-A from the wastewater treatment plant at these sites. However this amount is very small (0.00000163%) and will have a negligible contribution to the PEC_{air}. **Table 3.6** gives details of the calculated $PECs_{air}$.

Table 3.6 PECs of bisphenol-A in air

Site	PEC_{air} (mg/m^3)
Bisphenol-A production – worst case	$3.61 \cdot 10^{-4}$
Regional	$2.08 \cdot 10^{-10}$
Continental	$1.61 \cdot 10^{-11}$

Matsumoto and Hanya (1980) measured the concentration of bisphenol-A in atmospheric fallout samples taken from a residential area of Tokyo. Bisphenol-A was found at the following deposition rates: 0.2, 0.04, 0.06, 0.07 and 0.08 μg/m^2/day. The calculated deposition fluxes for the bisphenol-A production sites range from $5.7 \cdot 10^{-7}$ to 4.16 μg/m^2/day. This includes the range of values measured by Matsumoto and Hanya.

Yamamoto and Yasuhara (1999) quote work done by Kamiura et al. (1997); the concentration of bisphenol-A in air was found to be between 2.9-3.6 ng/m^3 in samples taken in Japan. No information on the sample locations or analytical method employed is given. These values are much higher than the calculated regional level, but much lower than the levels calculated for specific sites.

3.1.6 Secondary poisoning

EUSES has been used to calculate the concentration of bisphenol-A in fish and earthworms and the concentration in human intake media for the assessment of indirect exposure to bisphenol-A in the environment. For fish the measured bioconcentration factor is used. For earthworms, plants, cattle, etc., the uptake factors are estimated from the log Kow value in accordance with the TGD. The results should be treated as a reasonable worst-case scenario and the limitations of the model considered in interpreting the results. **Tables 3.7** and **Table 3.8** summarise the results for bisphenol-A.

Table 3.7 PECs for secondary poisoning

	Concentration in fish from surface water for predators (mg/kg)	Concentrations in earthworms from agricultural soil for predators (mg/kg)
Site-specific		
Bisphenol-A production	0.017	$3.37 \cdot 10^{-3}$
Epoxy Resin production	0.041	0.6
Thermal Paper production	0.034	$6.7 \cdot 10^{-4}$
Generic scenario		
Phenoplast cast resin processing	0.0476	0.0263
Thermal paper recycling	6.32	4.06
PVC – Inhibitor during production process	7.7	5.93
PVC – Anti-oxidant during processing	0.0113	$3.4 \cdot 10^{-3}$
PVC – Preparation of additive packages	0.301	0.227
PVC – Anti-oxidant in plasticiser production	0.052	0.035
PVC – Plasticiser use	0.0102	$2.58 \cdot 10^{-3}$

The Japanese Environment Agency (1996) measured the concentration of bisphenol-A in fish in Japan. The concentration of bisphenol-A ranged from 15-287 μg/kg wet weight; the detection limit for the method used was 13 μg/kg wet weight. The calculated concentrations in fish for specific sites and for some of the generic scenarios are in reasonable agreement with these measurements.

Table 3.8 Concentrations for humans exposed via the environment

	Concentration in drinking water (mg/l)	Concentration in wet fish (mg/kg)	Concentration in plant roots (mg/kg)	Concentration in plant leaves (mg/kg)	Concentration in milk (mg/kg wet weight)	Concentration in meat (mg/kg wet weight)	Concentration in air (mg/m^3)	Total daily intake (mg/kg day)
Site-specific								
Bisphenol-A production	$3.93 \cdot 10^{-4}$	0.027	$1.49 \cdot 10^{-3}$	1.96	$2.64 \cdot 10^{-3}$	$8.35 \cdot 10^{-3}$	$3.61 \cdot 10^{-4}$	0.0338
Epoxy resin production	0.012	0.074	0.3	0.065	$4.85 \cdot 10^{-5}$	$1.53 \cdot 10^{-4}$	$2.08 \cdot 10^{-10}$	$3.22 \cdot 10^{-3}$
Thermal paper production	$8.86 \cdot 10^{-4}$	0.06	$1.4 \cdot 10^{-4}$	$3.14 \cdot 10^{-5}$	$1.02 \cdot 10^{-6}$	$3.21 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$1.25 \cdot 10^{-4}$
Generic scenarios								
Phenoplast cast resin processing	$1.29 \cdot 10^{-3}$	0.0875	0.013	$2.81 \cdot 10^{-3}$	$2.98 \cdot 10^{-6}$	$9.42 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$3 \cdot 10^{-4}$
Thermal paper recycling	0.187	12.6	2.03	0.441	$4.45 \cdot 10^{-4}$	$1.41 \cdot 10^{-3}$	$2.08 \cdot 10^{-10}$	0.0448
PVC – Inhibitor during production process	0.227	15.4	2.97	0.643	$6.01 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$	$2.08 \cdot 10^{-10}$	0.0591
PVC – Anti-oxidant during processing	$2.19 \cdot 10^{-4}$	0.0148	$1.51 \cdot 10^{-3}$	$3.28 \cdot 10^{-4}$	$4.45 \cdot 10^{-7}$	$1.41 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$4.46 \cdot 10^{-5}$
PVC – Preparation of additive packages	$8.8 \cdot 10^{-3}$	0.595	0.114	0.0246	$2.3 \cdot 10^{-5}$	$7.3 \cdot 10^{-5}$	$2.08 \cdot 10^{-10}$	$2.27 \cdot 10^{-3}$
PVC – Anti-oxidant in plasticiser production	0.0014	0.0964	0.0173	0.00374	$3.63 \cdot 10^{-6}$	$1.15 \cdot 10^{-5}$	$2.08 \cdot 10^{-10}$	$3.58 \cdot 10^{-4}$
PVC – Plasticiser use	$1.88 \cdot 10^{-4}$	0.0127	$1.1 \cdot 10^{-3}$	$2.39 \cdot 10^{-4}$	$3.62 \cdot 10^{-7}$	$1.15 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$3.64 \cdot 10^{-5}$
Regional	**$1.14 \cdot 10^{-4}$**	**$7.74 \cdot 10^{-3}$**	**$1.96 \cdot 10^{-4}$**	**$4.37 \cdot 10^{-5}$**	**$1.85 \cdot 10^{-7}$**	**$5.86 \cdot 10^{-7}$**	**$2.08 \cdot 10^{-10}$**	**$1.78 \cdot 10^{-5}$**

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

3.2.1 Aquatic compartment (incl. sediment)

3.2.1.1 Toxicity test results

3.2.1.1.1 Fish

Table 3.9 summarises the available toxicity data for fish.

For freshwater species the lowest acute toxicity value is a 96-hour LC_{50} of 4.6 mg/l (nominal concentration) for the fathead minnow (*Pimephales promelas*). The test conditions and methods are fully described in the test report, and this test is considered valid for use in the PNEC derivation.

For saltwater species the lowest acute toxicity value is a 96-hour LC_{50} of 7.5 mg/l (measured concentration) for the sheepshead minnow (*Cyprinodon variegatus*). The test method used appears to be acceptable, although no information is given as to temperature, pH or dissolved oxygen during the test.

Bayer AG (1999b) report the results of juvenile growth test on *Oncorhynchus mykiss* using bisphenol-A. The test followed the proposed OECD guideline for "Fish, Juvenile growth test". A $NOEC_{Growth\ rate}$ of 3.64 mg/l (arithmetic mean of analytical values) and a $LOEC_{Growth\ rate}$ of 11.0 mg/l (arithmetic mean of analytical values) were reported.

A multi-generation study has been conducted on fathead minnows (*Pimephales promelas*) to examine possible endocrine effects (Sumpter et al., 2001). This study is described in Section 3.2.1.1.5. As part of this study, two early life stage studies were carried out on eggs produced by the F0 and F1 generations. Survival and growth were also monitored throughout the exposures of the F0 and F1 generations in the main study. The overall results from the study were NOECs for mortality of 640 μg/l, for growth of 160 μg/l and for reproduction of 16 μg/l.

3.2.1.1.2 Aquatic invertebrates

Table 3.10 summarises the available toxicity data for aquatic invertebrates.

For freshwater species two 48-hour EC_{50} values based upon immobilisation of *Daphnia magna* are reported. Stephenson (1983) reports a value of 3.9 mg/l based upon nominal concentrations. Alexander et al. (1985c) reports a value of 10.2 mg/l based upon measured concentrations. In both tests the methods used are fully documented. The test result based upon measured concentrations is preferred for use in the derivation of the PNEC.

Table 3.9 Summary of toxicity test results to fish

Species	Age/size	Stat/ flow	Temp (°C)	Dissolved oxygen (mg/l)	Hardness (mg $CaCO_3$/l)/ salinity (‰)	pH	Endpoint	Conc. (mg/l)	Test method	Reference
Freshwater species										
Oncorhynchus mykiss Rainbow trout	Fingerlings 2-2.5 g	Static	15	10.2	230	8.4	96-hour LC_{50}	3-5	Not given	Reiff (1979)
	2-year-old	Semi Static	16-21.5	8.4			24-hour LC_{100} 48-hour NOEC	7 5	Not given	Lysak and Marcinek (1972)
Brachydanio rerio Zebra fish		Semi-Static					14-day NOEC 14-day LOEC	3.2 (m) 10.15 (m)	OECD 204*	Bayer AG (1999a)
Oryzias latipes Killifish	0.15-0.5 g	Static		7			48-hour LC_{50}	15	Not given	MITI (1977)
	Adult	Semi-Static					72-hour LC_{50}	7.5	Not given	Tabata et al. (2001)
	Embryo	Semi-Static					72-hour LC_{50}	5.1	Not given	
Xiphophorus helleri Swordtail fish	Adult	Semi-Static					96-hour LC_{50}	17.93	Based upon OECD 204	Kwak et al. (2001)
Pimephales promelas Fathead minnow	1.96 cm 0.103 g	Static	17.0-17.5	>65% saturation		7.0-8.1	96-hour LC_{50}	4.7		Alexander et al. (1985a) Alexander et al. (1988)
	3.0 cm 0.41 g	Flow	16.1-17.9	>81% saturation		7.6-8.0	96-hour LC_{50}	4.6	ASTM * E-35.21	
Saltwater species										
Menidia menidi Atlantic silverside	0.37 g 43 mm	Flow	22-23	6.7-7.7	20-21	7.9-8.3	96-hour LC_{50}	9.4 (m)	Not given*	Springborn Bionomics (1985a) Alexander et al. (1988)
Cyprinodon variegates Sheepshead minnow		Flow					96-hour LC_{50}	7.5 mg/l (m)		Emmitte (1978)

m measured (others are assumed to be nominal)
* these studies are considered valid; the others are “use with care”

Table 3.10 Summary of toxicity test results to aquatic invertebrates

Species	Age/size	Stat/ flow	Temp (°C)	Dissolved oxygen (mg/l)	Hardness (mg $CaCO_3$/l) salinity (‰)	pH	Endpoint	Concentration (mg/l) m = measured n = nominal	Test method	Reference
Freshwater										
Daphnia magna Water flea	24 hours	Semi static	20±2	8.5-9.8	15.1-16.1	7.7-8.1	21-day $NOEC_{reproduction}$ 21-day $LOEC_{reproduction}$	> 3.146 (m) > 3.146 (m)	OECD 202 GLP*	Bayer AG (1996)
	First instar	Static	19.8-20.4	> 96% saturation		8.0-8.3	24-hour EC_{50} (Immobilisation) 48-hour EC_{50} (Immobilisation)	15.5 (m) (14.4-16.7) 10.2 (m) (9.2-11.4)	ASTM E-35.21*	Alexander et al. (1985c) Alexander et al. (1988)
	< 24 hours	Static	20±2	8.6-8.8	170	8.3-8.4	24-hour EC_{50} (Immobilisation) 48-hour EC_{50} (Immobilisation)	10 (n) (8.6-12) 3.9 (n) (3.1-5.0)		Stephenson (1983)
Saltwater										
Acartia tonsa copepod	10-12 day-old	Static	20		18		24-hour LC_{50} 48-hour LC_{50}	5.1-6.3 (n) 3.4-5.0 (n)		Kusk and Wollenberger (1999)
Mysidopsis bahia Mysid shrimp	6 day-old	Flow	24-25		20	7.5-8.1	24-hour LC_{50} 48-hour LC_{50} 72-hour LC_{50} 96-hour LC_{50} 96-hour NOEC	3.3 (m) (2.6-5.7) 1.6 (m) (1.3-1.9) 1.2 (m) (0.92-1.2) 1.1 (m) (0.92-1.2) 0.51	*	Springborn Bionomics (1985b) Alexander et al. (1988)

m measured (others are assumed to be nominal)
* these studies are considered valid; the others are “use with care”

A 21-day $NOEC_{reproduction}$ of >3.146 mg/l for *Daphnia magna* is reported by Bayer (1996). In the test report the method used is fully documented and the test concentration is measured. At the highest concentration tested (nominal concentration of 3.2 mg/l) no effect on reproduction was observed. The $NOEC_{reproduction}$ is therefore given as greater than >3.146 mg/l (measured concentration). While this test does not give a true NOEC value, it is considered to be valid for use in determining the $PNEC_{water}$ for bisphenol-A.

For saltwater species the most sensitive acute result is a 96-hour LC_{50} of 1.1 mg/l reported for the mysid *Mysidopsis bahia*. The test conditions and methods are fully described in the test report, and the test is considered to be valid.

Further aquatic invertebrate toxicity test results relating to endocrine disruption are described in Section 3.2.1.1.5.

3.2.1.1.3 Aquatic algae and plants

Toxicity test results are reported for two algae species, and these are summarised below.

Alexander et al. (1985b; 1988) report 96-hour EC_{50} values, based upon cell count and total cell volume, of 2.73 mg/l and 3.10 mg/l for the green alga *Selenastrum capricornutum,* respectively. Both of the test results are based upon changes in biomass. The test report describes the test methods and test concentrations, and this test may be considered valid for use in the PNEC derivation. In addition to the EC_{50} values reported, the percentage inhibition of cell count and cell volume is reported for the concentrations tested. From these data it is possible to derive an EC_{10} using probit analysis. The calculated 96 hour EC_{10} values are 1.36 mg/l based upon cell count and 1.68 mg/l based upon cell volume.

Stephenson (1983) reports a 96-hour EC_{50} of 2.5 mg/l, based upon cell count, for the green alga *Selenastrum capricornutum*. The test report describes the test method used, however it does not give details of the test conditions. The test concentration is based upon nominal concentrations. This result should be used to support the data presented by Alexander et al. (1985).

Springborn Bionomics Inc. (1985c) (also published in Alexander et al. (1988)) report 96-hour EC_{50} values, based upon cell count and chlorophyll content, of 1.0 mg/l and 1.8 mg/l, respectively for the marine alga *Skeletonema costatum*. The test report describes the test methods and test concentrations, and is considered valid for use in the PNEC derivation. However the method used to estimate the effect concentrations was non-linear interpolation. The percentage inhibition of cell count and chlorophyll content is reported for the concentrations tested. These original data have been analysed by the rapporteur using probit analysis in accordance with the OECD Guideline. The resulting EC_{50} for cell count is 1.1 mg/l, and that for chlorophyll content is 1.4 mg/l. It is also possible to derive EC_{10} values using the probit analysis. The calculated 96-hour EC_{10} values are 0.69 mg/l based on chlorophyll content and 0.40 mg/l based upon cell count.

The TGD indicates that if a long-term NOEC is not available then an EC_{10} for a chronic test which is obtained by extrapolation using appropriate statistics, such as probit analysis, can be considered a NOEC. For algae studies it is generally accepted that a 72-hour (or longer) NOEC value can be considered as a chronic result. Therefore the EC_{10} values derived for algae species are treated as equivalent to long-term NOEC values in the derivation of the PNEC value.

3.2.1.1.4 Microorganisms

Dow (1988) report the determination of an acute bacterial toxicity test carried out using bisphenol-A. The test was performed to good laboratory practice guidelines. Cultures of *Pseudomonas putida* from an agar solidified medium were added to culture vessels and incubated at 25°C for 18 hours with bisphenol-A. The growth rate of the bacteria was measured by turbidimetry. The highest concentration tested was 320 mg/l, and at this concentration no inhibition of cell growth was observed. (Note that this concentration is slightly above the water solubility of 300 mg/l.)

Stone and Watkinson (1983) conducted an inhibition test on the growth of *Pseudomonas fluorescens* as part of their studies on bisphenol-A biodegradation. They reported an IC_{50} of 54.5 mg/l for the inhibition of the growth of *Pseudomonas fluorescens* by bisphenol-A.

3.2.1.1.5 Endocrine disrupting effects

The investigation of endocrine disrupting effects in environmental species is a rapidly developing area. At present, there are no fully established test methods comparable to those for more traditional endpoints such as mortality or reproduction. The results included in the following sections tend to fall into two types. The first are those using the standard test protocols to look for the effect of endocrine-disrupting substances on these "traditional" endpoints, with in some cases the addition of further biochemical investigations such as for vitellogenin synthesis. The second type are those using novel endpoints or novel species to try to develop a useful system for identifying endocrine disrupting substances or to investigate the mechanism by which effects are produced. Results from the first type of test can generally be validated more easily, as the experimental details are usually better reported, and so are generally preferred for risk assessment. However, studies of the other type may demonstrate other effects, and although they may not allow a definite NOEC to be determined they need to be considered in the assessment.

The rapidly developing nature of this area means that new studies are being conducted and presented all the time, many of which may be relevant to this assessment. In order to present as broad a view as possible, some studies only available as extended abstracts have been included in this assessment - this is noted in the descriptions of the studies.

Fish studies

For fish, a multigenerational study on the fathead minnow (*Pimephales promelas*) is reported by Sumpter et al. (2001). This was a long-term study and considered effects of bisphenol-A on the F0, F1 and F2 generations. Exposure was to nominal concentrations of bisphenol-A (1 µg/l, 16 µg/l, 160 µg/l, 640 µg/l and 1,280 µg/l) in a flow-through system. Fish were also exposed to a dilution water control throughout the experiment. The study was started with adult fish at 120 days post hatch, with 60 fish per treatment level. At day 42 of the study, eight breeding pairs per treatment were randomly selected and used to assess the fecundity of the F0 generation. Spawnings of 50 embryos from single females were used in hatchability trials. Two cohorts of eggs from these breeding pairs were taken and used in two separate early life stage studies (commencing on days 56 and 155 of the study). Fish larvae from the hatchability trials were discarded at the end of the trials, but those from the early life stage studies were transferred to the progeny tanks to form the F1 generation. The F0 breeding pairs were sacrificed on day 164 of the study. Other adult fish in the F0 generation were sacrificed after 43 and 71 days of the exposure.

Fish of the F1 generation were continuously exposed through to sexual maturity. On day 275 of the study (when the F1 fish were an average of 150 days old) eight breeding pairs were randomly selected and a similar series of tests to those above conducted: fecundity measurements on the F1 generation; hatchability trials on the F2 generation; and an early life stage test on the F2 generation. Adults from the F1 generation not selected for breeding were sacrificed on day 295 of the study. The study was terminated at 431 days from the start with the sacrifice of the F1 breeding pairs.

Nominal test concentrations were confirmed by measurements of bisphenol-A in the test media. During the experiment information was recorded on fish survival, fecundity and hatchability of eggs. Upon sacrifice, intact fish, dissected gonads and blood plasma of the F0 and F1 fish were analysed for vitellogenin, gonad growth and histology of the gonads. For male fish, the gonad histology included a scoring of the various testicular cell types in order to assess the progression of spermatogenesis.

The study concluded that bisphenol-A acts as a weak estrogen *in vivo* to fathead minnow exposed to bisphenol-A via water. The overall NOEC for conventional endpoints of survival, growth and reproduction based on the hatchability of the F2 generation is 16 μg/l. For vitellogenin production a NOEC of 16 μg/l is determined. Some growth endpoints, including gonad size, show NOEC values of <16 μg/l at individual monitoring points, but not consistently over the course of the experiment.

The observations on the testes of the male fish showed that exposure to bisphenol-A had a significant effect on the development of sex cell types compared to the controls. Measurements were made on the relative proportions of each cell type in the tissue, not the absolute numbers of cells. The cells develop from spermatogonia through spermatocytes and spermatids to spermatozoa. For the F0 generation, regression analysis showed that there were dose-related effects of bisphenol-A on the proportion of different cell types. The lowest effective concentration for these responses was 640 μg/l (spermatogonia) and 16 μg/l (spermatozoa). The highest exposure concentration (1,280 μg/l) caused a five-fold decrease in the relative occurrence of mature spermatozoa while spermatocytes, spermatids and other cell types varied by up to 10%. The relative proportion of spermatogonia increased from ~12% in the controls to 83% at the highest concentration. The NOEC for a reduced proportion of spermatozoa is 1 μg/l. For the F1 generation, there was a positive dose-related effect of bisphenol-A on the proportion of spermatogonia, and an inhibitory effect on the proportion of the testes occupied by spermatozoa. The lowest effect concentration for these responses was 1 μg/l for both spermatogonia and spermatozoa. (This aspect of the study has since been questioned, see comments following the study conclusions.)

From the data it is not possible to say that inter-generational sensitivity increased or decreased because the F0 generation fish were introduced to the test system as sub-adults, whereas the F1 generation was exposed to bisphenol-A throughout their lives.

From the data the report derived the following conclusions:

- LOEC (survival, 164 days) 1,280 μg/l.
- NOEC (growth, 164 days) 160 μg/l.
- The size of the gonads of female F0 fish, were significantly greater than that of the controls at 1 μg/l on day 43. However, no significant effects were seen at 16 μg/l and subsequently the NOEC rose to 1280 μg/l (day 71) and 160 μg/l (day 164). In males the NOEC for effects on

gonad size was <1 μg/l on day 43 but subsequently rose to 1280 μg/l (day 71) and 160 μg/l (day 164). Therefore, the NOEC for consistent or dose-related effects is taken as 160 μg/l.

- NOEC (egg production) 160 μg/l for the F1 generation and 640 μg/l for the F0 generation. This is based upon the number of eggs produced per female per day.
- NOEC (hatchability of eggs) 160 μg/l for the F1 generation and 16 μg/l for the F2 generation.
- NOEC (vitellogenin production) 16 μg/l, for F0 males and F1 generation males and females.
- Effects on the different stages of male spermatozoa development were seen at lower concentrations, with a NOEC value for the proportion of spermatogonia and spermatozoa of 1 μg/l for the F0 generation and a LOEC of 1 μg/l for the F1 generation. The hatchability of eggs was affected only at 160 μg/l or greater.

Overall, effects based upon the survival, and reproductive fitness of fathead minnows exposed to bisphenol-A from F0 breeding adults to F2 offspring occurred at concentrations of 640 μg/l bisphenol-A and higher, with hatchability of F2 eggs slightly but significantly reduced at 160 μg/l.

Two independent experts in fish histopathology have subsequently reviewed the parts of this study relating to spermatogenesis (D Dietrich, personal communication). It is noted that the study was designed to look for effects on reproduction, hatching and growth. The sampling and examination of gonad tissues for sperm cell types was added after the study design had been implemented, and so the experimental design was not optimised to look at these effects. Some short-comings of this part of the study were identified in relation to the number of fish sampled from each exposure level, the taking of tissue samples from the testes and their preparation for counting, and the number of cells counted in each sample. While these short-comings and general test design are not considered to make the study invalid for population effects in terms of reproduction, hatchability and growth, the experts concluded that the weaknesses in the spermatogenesis data make them unsuitable as the basis for deriving a PNEC. This view is supported by one of the main authors of the study. Both the NOEC for egg hatchability and the LOEC for sperm cell distribution will be considered in the general discussion on endocrine effects later in this assessment.

Lindholst et al. (2000) studied the estrogenic response to bisphenol-A in rainbow trout (*Oncorhynchus mykiss*). Rainbow trout were exposed to bisphenol-A via a continuous flow-through system. Vitellogenin concentrations were measured during the exposure period (12 days). A significant induction of vitellogenin synthesis was observed in the 500 μg/l bisphenol-A exposure group over the study period. In lower exposure groups (40 and 70 μg/l) steadily increasing levels of vitellogenin were observed between 6 and 12 days only. Based upon the data the LOEC for vitellogenin production is taken as 40 μg/l.

In a further study, Lindholst et al. (2001) again exposed rainbow trout (*Oncorhynchus mykiss*) to bisphenol-A in a continuous flow-through system (at 100 μg/l), and also through intraperitoneal injection (at a tissue concentration of 35 mg/kg). Both male and female fish showed increased levels of vitellogenin in the injection exposures, with a lag period of 3-5 days for females and 5-7 days for males. Measured levels of bisphenol-A in the livers of the fish had decreased almost to the detection limit before the increase in vitellogenin was noted. Fish in the continuous exposures did not show significantly higher levels of vitellogenin up to the end of the experiment after seven days.

Pawlowski et al. (2000) studied the estrogenic response of bisphenol-A in cells from rainbow trout (*Oncorhynchus mykiss*) and the variation of the response with temperature. Estrogenic response was measured *in vitro* using cultured hepatocytes from male rainbow trout using a non-

radioactive dot blot/RNAse protection assay and by RT-PCR. They found that bisphenol-A was estrogenic with a relative potency of 10^{-4} to 10^{-5} of that of 17ß-estradiol. They also found that a higher response rate was measured at 18°C than 14°C with a LOEC of 10 µM (2.3 mg/l) after 48 hours exposure at 14°C and a LOEC of 1 µM (0.23 mg/l) after 48 hours exposure at 18°C. The lowest LOEC measured for vitellogenin induction was 0.1 µM (23 µg/l) after 96 hours exposure at 18°C.

Shioda and Wakabayashi (2000) exposed male medaka (*Oryzias latipes*) to a natural estrogen (17ß-estradiol) and three estrogenic substances including bisphenol-A. After 14 days exposure, one male medaka was kept with two female medaka for spawning. The results indicated that bisphenol-A at a concentration of 2.3 mg/l caused a decrease in the number of hatchlings. No effects were observed at lower concentrations tested (68 µg/l, 0.23 mg/l and 0.68 mg/l). This study was designed to look at the effects of endocrine disrupters on reproduction due to *in vivo* exposure of the medaka. Due to the different study protocols used it is not possible to compare the estimated potency of bisphenol-A with that of 17ß-estradiol.

Smeets et al. (1999) determined the *in vitro* estrogenic potential of bisphenol-A using cultured hepatocytes from the male carp (*Cyprinus carpio*). Estrogenicity was measured as induction of vitellogenin. Bisphenol-A was found to induce vitellogenin production with a relative potency of $1 \cdot 10^{-4}$ to 17ß-estradiol and a LOEC of 50 µM (11 mg/l). Bisphenol-A was also found to exhibit cytotoxic effects at 100 µM (22 mg/l) the highest concentration of bisphenol-A tested.

Bowmer and Gimeno (2001) have studied the effects of bisphenol-A on the development of the male carp reproductive tract when exposed during sexual differentiation (only an extended abstract of this study was available at the time of writing). Male carp (*Cyprinus carpio*) were exposed to nominal concentrations of 10, 32, 100, 320 and 1,000 µg/l bisphenol-A under flow through conditions, during the period of sexual differentiation (from 45 to 55 days post hatch onwards). Two experiments were performed, the first conforming to the OECD principles of GLP. In both experiments nominal concentrations were confirmed by analysis. In the first experiment 28- and 49-day NOECs for growth (wet weight) were >600 and 100 µg/l bisphenol-A; in the second experiment 28- and 56-day NOECs were both 226 µg/l. In the first experiment 28- and 49-day NOECs for oviduct formation were 100 and 16 µg/l bisphenol-A while in the second experiment they were 60 and 17 µg/l.

Schäfers et al. (2001) studied the estrogenic impact of bisphenol-A on zebrafish (*Brachydanio rerio*) in a full life cycle study (only an extended abstract on the work has been seen). They found that juvenile growth, time until first spawning, egg production and fertilisation rate were affected by bisphenol-A exposure. The EC_{50} and NOEC for fertilisation rate were 1.45 mg/l and 0.76 mg/l, respectively. Bisphenol-A showed a lower estrogenic potency than ethinylestradiol. Similar results were found by Fenske et al. (2001) who looked at alterations in vitellogenesis and reproduction in zebra fish exposed to ethinylestradiol and bisphenol-A.

Tabata et al. (2001) studied the effect of bisphenol-A on mature male Japanese Medaka (*Oryzias latipes*). No concentration monitoring was undertaken to confirm the exposure levels. They found that after two weeks exposure to 100 µg/l bisphenol-A, female specific proteins could be detected in the fish, but no effects were observed at 0.1 or 10 µg/l exposure. After five weeks exposure female specific proteins were found in the 10 µg/l exposure group but not in the 0.1 µg/l exposure group. Abnormalities in the gonad tissue were observed in the 100 µg/l bisphenol-A exposure group in one animal of the sixteen exposed. There was no observation of any sex bias towards females in any of the bisphenol-A exposure groups.

Kwak et al. (2001) exposed swordtail fish (*Xiphophorus helleri*) to bisphenol-A in short-term tests (72 hours) to determine the effect of bisphenol-A on vitellogenesis and damage to testes, and in long-term tests (60 days) to examine the effect on sword (tail) length (a secondary sexual characteristic in males). Semi-static exposure conditions were used, but no concentration monitoring was undertaken. Vitellogenin expression was noted in a dose dependant manner with no induction at 0.4 mg/l bisphenol-A, but induction was observed at 2 and 10 mg/l. Binding studies to detect cell damage (apoptosis or necrosis) showed a reduction in the proportion of healthy cells at all three exposure concentrations. However, histological examination of testis tissue taken from fish exposed to 0.4 or 2 mg/l bisphenol-A, failed to show any apoptotic cells. Apoptotic cell masses and other injured cells were observed at 10 mg/l bisphenol-A though no lesions were observed. In tests on swordtail length a significant reduction in length was observed at 0.002 and 0.02 mg/l bisphenol-A but not at 0.0002 mg/l bisphenol-A. The authors also determined the acute toxicity of bisphenol-A in a 96-hour test, the result of which is included in **Table 3.9**.

Discussion of fish results

The clearest indication that bisphenol-A is estrogenic to fish was provided by the concentration-related increase in the plasma vitellogenin concentration observed in the fathead minnow (Sumpter et al., 2001), rainbow trout (Lindholst et al., 2000) and male carp (Smeets et al., 1999). Vitellogenin synthesis in fish is widely considered to be a reliable and sensitive indicator of exposure to estrogenic chemicals (Sumpter and Jobling, 1995). Sumpter et al. (2001) report a LOEC of 160 μg/l and a NOEC of 16 μg/l for vitellogenin production, and these values fit with the LOECs of 23 μg/l and 40 μg/l for vitellogenin production reported by Pawlowski et al. (2000) and Lindholst et al. (2000). While both of these studies did not test concentrations low enough to derive NOEC values they did measure the relative potency of bisphenol-A to 17ß-estradiol. All of the studies showed a similar relative potency of bisphenol-A to that of 17ß-estradiol of around 10^{-4}. This and the similar concentration for LOEC values between the studies suggests that the NOEC values from these other studies should be in a similar concentration range to the NOEC of 16 μg/l. Based upon the available data a NOEC of 16 μg/l can be assumed for vitellogenin production in fish.

Bisphenol-A can also bind to the estrogen receptor of fish (Kloas et al., 2000) though with a lower affinity than estradiol has for the receptor.

Although vitellogenin is a biomarker for exposure to estrogenic substances, the ecological significance of its presence is not yet known - the relationship between biomarkers for endocrine disruption and ecological effects is currently being investigated by a number of workers. The most sensitive end point for population-relevant effects from the existing fish studies is the NOEC for egg hatchability for the F2 generation of 16 μg/l reported by Sumpter et al. (2001). This is the same as the NOEC for vitellogenin production from the same study.

Other parameters can be sensitive to both estrogens and xenoestrogens. These include inhibition of testis growth by natural and synthetic estrogens and xenoestrogens (Panter et al., 1998; Jobling et al., 1996). Estrogens are also known to inhibit spermatogenesis in male fish (Billard et al., 1981) and exposure of fish to estradiol and nonylphenol has been shown to affect testicular structure (Miles-Richardson et al., 1999; Flammarion et al., 2000; Jobling et al., 1996). High concentrations of both estrogens and xenoestrogens can induce the development of ovotestes in male fish or cause complete feminisation (Hartley et al., 1998; Gary and Metcalfe, 1997).

Many of these effects were observed in the study with the fathead minnow (Sumpter et al., 2001). The growth of gonads was reduced in the F0 generation by day 164 (NOEC 160 μg/l).

Spermatogenesis was partially inhibited, with increased proportions of spermatogonia and decreased proportions of mature spermatozoa in the F1 generation at the lowest concentration tested (1 µg/l). As noted above, there are significant concerns about the reliability of the sperm cell results from this work. It is also noted that based on the hatchability results these changes did not appear to have an effect on the ability of the male fish to fertilise eggs successfully. No intersex fish were observed in this study.

Bowmer and Gimeno (2001) observed a NOEC for oviduct formation in male carp of 16 µg/l. It is noted that this is the same as the NOEC for egg hatchability observed by Sumpter et al. (2001).

The study on swordtail fish (Kwak et al., 2001) largely showed responses at similar concentrations to other studies and support these data. The significance of the changes in sword length is not understood, but it is thought that the length of the sword has an influence on mating success, with female fish preferring males with longer swords. It is not clear what degree of change should be considered to be significant. The separation between exposure levels was an order of magnitude, and there was no measurement of concentration during the exposures. The study is therefore not considered suitable for use in defining the PNEC, but it is noted that the LOEC from this study is higher than the LOEC for sperm effects in the fathead minnow.

The results indicate that bisphenol-A acts as a weak estrogen to fish, though it is a lot less active than either oestradiol or ethinyloestradiol. Both the NOEC for egg hatchability in fathead minnows (16 µg/l) and the LOEC for apparent effects on sperm cells (1 µg/l) in the same species will be taken forward for further discussion in the PNEC derivation and risk characterisation sections.

Aquatic invertebrate studies

Caspers (1998) has studied the estrogenic effects of bisphenol-A in *Daphnia magna*. The study looked at the moulting behaviour of parthenogenetic females. Moulting behaviour has been claimed to be a toxicological endpoint, which is able to reflect effects of endocrine disruption. The author did not notice any change in moulting behaviour of *Daphnia magna* at exposure concentrations of 0.316 mg/l and 3.16 mg/l. The author did comment that using moulting behaviour as an ecotoxicological end-point might not be the most suitable endpoint to study endocrine disruption.

Andersen et al. (2001) studied the effects of a range of substances on the development of nauplii of the saltwater copepod *Acartia tonsa*. Semi-static exposures were used, with solution renewal after three days; no monitoring of concentrations was carried out. The exposures were carried out for five days or until at least 50% of the organisms had undergone metamorphosis from the nauplius to copepodit stage, whichever was the longer. The larval development rate was expressed as the ratio of copepodits to the sum of nauplii and copepodits. The EC_{50} value established for this effect was 0.55 mg/l, and the EC_{10} value was 0.10 mg/l. Although this is a relatively short test in terms of duration, it assesses what is considered to be a sensitive endpoint.

Oehlmann et al. (2000), Schulte-Oehlmann et al. (2000) and Oehlmann et al. (2001) looked at the effect of bisphenol-A on prosobranch snails. In the first study two species were used, the freshwater ramshorn snail (or Apple snail) (*Marisa cornuarietis*) and the marine dog whelk (*Nucella lapillus*). Adult *Marisa cornuarietis* were exposed to nominal concentrations of bisphenol-A (1, 5, 25, and 100 µg/l) under semi-static laboratory conditions (with renewal every 24 hours) for five months and in a complete life-cycle test for 12 months. Both experiments included a solvent control. No analysis of the exposure solutions was carried out in these

experiments. In both experiments with *Marisa* a complex number of alterations referred to as "superfeminisation" occurred. Effects included the enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production. These effects were statistically significant at each test concentration when compared to the control, and were concentration dependent with the exception of mortality, which was virtually the same in all four bisphenol-A exposure groups (13.3-15.7% compared to control mortality of 3.8%). The cumulative numbers of eggs and the cumulative number of egg masses increased with increasing bisphenol-A concentrations. The hatching success of eggs from the organisms in the five month experiment (used to start the life cycle test) was not affected by exposure to bisphenol-A.

Adult *Nucella lapillus* from the field were exposed for three months in the laboratory to concentrations of 1, 25 and 100 μg/l, again with renewal every 24 hours. Superfeminisation with enlarged pallial sex glands and an enhancement of oocyte production was observed. No oviduct malformations were found probably due to species differences in gross anatomical structure of the pallial oviduct. A lower percentage of exposed specimens had ripe sperm stored in their vesicula seminalis and male *Nucella* exhibited a reduced length of penis and prostate gland when compared to the control. Statistically significant effects were observed at all the test concentrations. The authors concluded that the results show that prosobranchs are sensitive to endocrine disruption at the lowest concentrations of bisphenol-A tested (1 μg/l nominal).

As a follow up to the above studies, Schulte-Oehlmann et al. (2000) and Oehlmann (2001) exposed *Marisa cornuarietis* to a series of lower bisphenol-A concentrations. The same semi-static exposure system was used, and the duration of exposure was 180 days. The nominal exposure concentrations were 0.05-1.0 μg/l. In this experiment the concentrations in the exposures were checked by analysis by sampling on three occasions. The initial concentrations in the exposures were close to the nominal values. Observations over the 24-hour period between the changes of solution showed that the concentration of bisphenol-A decreased with time. After two months of the experiment, the half-life of bisphenol-A in the exposure solutions was around six hours. After four months the half-life had decreased to two hours and a similar value was found after six months. The concentrations were measured at 2-hour intervals; these were used to calculate average exposure concentrations over a 24-hour period as a time weighted average. The detection limit was 30 ng/l.

The phenomenon of superfeminisation was again observed in all of the treated groups (with the exception of the 0.05 μg/l (nominal) group), at a lower level of incidence than in the high concentration experiment (although the level of incidence in the one concentration common to both studies, 1 μg/l, was the same). Mortality was not significantly enhanced in any of the bisphenol-A groups in comparison to the controls. Egg production was also stimulated as in the previous experiment, although the results over the whole 180-day exposure period showed a significant increase only at the two highest concentrations. The authors observe that the exposure period in this second experiment included the season of the year (October to February) when spawning activity in *Marisa* increases naturally. It was therefore considered that the effect of bisphenol-A might be masked to some degree by the natural increase. (The first experiment took place completely outside this active season.) The experimental results were therefore split into three periods of 60 days, with the middle period containing the season of greatest natural spawning activity. The initial 60-day period showed an increase in the cumulative numbers of eggs and spawning masses in the exposed organisms, with a significant increase over the control for all but the lowest exposure level. Over the middle period, the animals exposed to bisphenol-A showed a reduction in the cumulative number of spawning masses in all treated groups when compared to the control; in the final 60-day period the pattern was similar to that in the first

period. Based on the cumulative egg production over the first 60 days of exposure, the following effect concentrations were obtained: LOEC 48.3 ng/l; NOEC 7.9 ng/l; EC_{10} 13.9 ng/l (all based on the average exposure levels calculated from the measured concentrations).

Further studies by the same authors (J. Oehlmann, personal communication) show similar effects (e.g. stimulation of egg production) to varying degrees in a number of other species of snail.

Discussion of aquatic invertebrate results

The data presented on aquatic invertebrates suggest that at present the mode of effect of bisphenol-A on the endocrine system is poorly understood. The tests reported to date have all been part of wider work programmes aimed at trying to identify suitable methods to test for endocrine disruption in aquatic invertebrates. The tests need to be evaluated with this consideration in mind.

The work undertaken by Anderson et al. (1981) was intended to develop a method as a test for endocrine disrupting activity in crustaceans, as the process of moulting and metamorphosis is believed to be controlled by the hormone system. Based on the ratios of toxicity in conventional short-term tests and the metamorphosis assay, the authors did not identify bisphenol-A as exhibiting enhanced toxicity in this test system. However, given the work done by Caspers (1998) this result is not necessarily unexpected as he noted that using moulting behaviour as an ecotoxicological end-point might not be the most suitable endpoint to study endocrine disruption. There would therefore appear to be some debate as to the relevance of this end point in endocrine studies.

The studies on prosobranch snails by Oehlmann and co-workers (Oehlmann et al., 2000; Schulte-Oehlmann et al., 2000; Oehlmann et al., 2001) indicate effects at low concentrations. The main underlying effect appears to be a stimulation of egg and spawning mass production. In a proportion of the snails this can lead to a rupture of internal organs and the death of the animal (this appears to depend on the morphology of the pallial oviduct, and the observation is so far restricted to one species (*Marisa*)). Changes to other organs in the animals were also observed.

The experiments were carried out over two concentration ranges, high (1-100 μg/l) and low (0.05-1 μg/l). Concentrations were not measured in the first (high concentration) experiment. Measurements in the second (low concentration) experiment showed that the concentrations of bisphenol-A decreased rapidly over the 24-hour period between renewals of solution. The rate of disappearance was more rapid at later times in the test, despite the renewal of the solutions each day. Bisphenol-A is not susceptible to rapid abiotic degradation in solution, so it would appear that biodegradation or metabolism occurred in the solutions, with some indication of adaptation over the course of the experiment. In view of the rapid disappearance of the substance, the nature of the chemical species present in the exposures is unclear, particularly in the later parts of the experiment. For example, for a half-life of six hours only 6% of the substance would remain after 24 hours; for a half-life of two hours there would be effectively none of the substance left after twelve hours. The report of the second experiment also indicates that the control exposure solutions initially contained 30-40 ng/l of bisphenol-A at the first time of sampling for analysis. This was found to be due to leaching of the test compound from the plastic tubes used in the filter systems of the exposure tanks. Following replacement of these tubes with glassware, bisphenol-A could not be detected in the control group in the subsequent sampling.

As noted in the description of the studies above, the period of the second, low concentration, experiment included the natural spawning season of the snails. This makes it difficult to compare the two studies. Both experiments included a nominal exposure concentration of 1 μg/l. The cumulative egg production over the first 60 days of the second experiment (taken as a period less

affected by the natural spawning) was much higher than that seen over the 180 days of the high concentration experiment at the same exposure level, but the incidence of females with malformed oviducts was identical in both 1 μg/l exposure groups. The cumulative egg production in the control of the low concentration experiment was similarly higher than that for the high concentration experiment. These observations presumably relate to the difference in the natural spawning rate, and make it difficult to distinguish the effects due to the substance.

In view of the apparent instability of the substance under the exposure conditions used, and the possible overlap with natural changes, it is considered that the effect concentrations from these studies are not suitable for use in the derivation of the PNEC. Nevertheless, the apparent sensitivity of snails to bisphenol-A is of concern, and further work is needed to clarify these issues. This will be taken into account in the risk characterisation section.

Amphibian studies

Kloas et al. (1999) reported the development of a model for the investigation of endocrine-disrupting chemicals using the amphibian *Xenopus laevis* (African clawed frog). As part of this work they exposed tadpoles of *Xenopus* at 2-3 days post-hatch to nominal concentrations of bisphenol-A. Solutions were renewed three times per week, and exposure continued until metamorphosis occurred in approximately 90% of all animals - this took around 12 weeks. The two exposure concentrations used were 10^{-7} M (23 μg/l) and 10^{-8} M (2.3 μg/l). After exposure the animals were examined for differentiation into males and females. The higher exposure concentration produced a statistically significant increase in the number of female phenotypes in relation to the controls. The ratio of the sexes in the control exposures was 60:40 male:female and in the 23 μg/l exposure group was 36:64 male:female. A decreased male:female ratio was also observed in the 2.3 μg/l test group though the result was not significant comparable to the controls.

Pickford et al. (2000) reported the results of a study investigating the effects of bisphenol-A on larval growth, development and sexual differentiation on the African clawed frog (*Xenopus laevis*). This study was conducted in an attempt to repeat the original findings by Kloas et al. and establish a dose-response relationship. The test was initiated with 4-day-old larvae. Hatching of larvae occurred principally on day 2 post-fertilisation, exposure to the test substance therefore commenced approximately 2 days post-hatching. A dynamic flow-through test system was used with four replicate test vessels for each test concentration, dilution water and positive control. In the experiments 17ß-estradiol was used as a positive control. Larvae were exposed to 1, 2.3, 10, 23, 100 and 500 μg/l nominal concentrations of bisphenol-A. The larvae were observed daily for mortality, behaviour and appearance. Test conditions were monitored throughout the study. Growth and development assessments were performed on all larvae from one replicate per treatment group on exposure days 32 and 62. Larvae were sacrificed upon reaching the froglet stage of development for analysis. The test was terminated at day 90 which corresponds to 94 days after fertilisation. The NOEC for larval survival was calculated as 500 μg/l based upon pooled data results. At 32 days and 62 days post fertilisation there were no significant differences in growth or development between the test concentrations, the positive control or the dilution water control. The sex ratios were assessed pre- and post- fixation to allow comparison with the method used by Kloas et al. (1999), with statistical analysis being undertaken on the post-fixation results only. No significant difference from the expected 50:50 sex ratio were observed in any of the test concentrations or the dilution water control, while a significant feminisation was observed in the positive control group. The exposure of larvae to bisphenol-A did not result in an increase in gross gonadal abnormalities in stage 66 froglets. There was no significant difference in time to metamorphosis in any of the test concentrations of bisphenol-A compared to the dilution water control. There was no significant difference in total lengths in any

of the test concentrations compared to the dilution water control. There were no significant differences in weight between any of the test concentrations of bisphenol-A, and the dilution water control.

Discussion of amphibian results

It is not clear why the two experiments on the same species produced different results. Some of the possible reasons are the differences in experimental design between the two experiments (in particular flow-through versus static conditions), analytical monitoring, number of replicates and statistical analytical methods used. The original study by Kloas et al. was aimed at developing a method to investigate endocrine effects rather than to determine a no-effect level, while the second study by Pickford et al. was designed to establish a no-effect level for a range of effects. There are also still questions in relation to the use of a parameter such as the sex ratio in risk assessments: what other factors influence the ratio; what is the normal range of values for the ratio in healthy populations? In view of the lack of agreement between the studies and the outstanding questions, this endpoint will not be considered quantitatively in the risk characterisation.

General discussion on the use of endocrine data in the risk assessment report

The comments at the beginning of Section 3.2.1.1.5 should be kept in mind here as well.

The most sensitive effect that has a clear and indisputable ecological relevance is egg hatchability in the fathead minnow, with an NOEC of 16 μg/l. The NOEC is furthermore obtained from a high quality study which is regarded as reliable in all regards and of significance in relation to the endpoint for fish populations by fish experts involved in the evaluation of the study. This is the same as the NOEC for vitellogenin production in males in the same species (seen as an indicator of endocrine effects) and oviduct formation in male carp. These results will be taken forward to the PNEC derivation as the "conventional" endpoint.

Endocrine disrupting effects other than vitellogenin production may be occurring at levels lower than 16 μg/l. These effects include disruption to male spermatozoa development in fathead minnow at 1 μg/l and the superfeminisation observed in prosobranch snails at <1 μg/l. Sumpter in his work on fathead minnow notes that despite the effect on spermatozoa development at 1 μg/l there is no effect on egg hatchability or the ability of the fish to reproduce. This raises the question of the ecological significance of this effect as the viability of the population and its ability to reproduce appears to be unaffected at this concentration. A group of independent experts in the UK was consulted over the relevance of these results and whether they should be used in the risk assessment. This consultation took place before the re-evaluation of the sperm results by Dietrich et al. Some experts thought that hatchability was the most relevant parameter for use in the risk assessment as it was seen as related to populations, and considered that the sperm results should not form the basis of the assessment. The measurement of the relative amounts of each cell type, rather than the actual numbers of cells, was questioned. Other experts cautioned that the design of the study, with paired male and female fish, meant that the males did not have to compete for reproductive success as they would in the wild, which suggests that a more conservative approach is appropriate. A further view was that any effect on the sperm cells should be considered as a relevant effect for the assessment. In view of this mixture of views, the LOEC of 1 μg/l for effects on sperm cells will be considered in the risk characterisation, bearing in mind the uncertainty over these results raised by the reinvestigation by Dietrich et al.

The work by Oehlmann and co-workers on prosobranch snails shows clear effects at 1 μg/l with possible effects being observed at concentrations as low as 0.014 μg/l (EC_{10} for enhanced egg production following 60 days exposure). There are some questions regarding this work, in particular the rapid degradation of bisphenol-A in the test solutions, the increasing rate of degradation in the course of the experiment, and the combination of natural changes in spawning rates with the effects of the substance. This does not mean that the study should be discounted; molluscs have been shown to be sensitive organisms in tests with other chemicals, in particular certain organotin compounds where responses occur at very low concentrations. The response of a variety of molluscs to bisphenol-A exposure appears to show a consistent pattern, whereby egg production is stimulated at times outside of the normal breeding periods. This could effectively be forcing the organism to use energy reserves at a time of sexual repose, with a consequent possible reduction in fecundity during the following normal breeding season. Therefore this is a potentially important adverse effect. Consultation with independent experts on this issue produced a reasonable consensus, with most agreeing that the results with snails were not currently suitable for use directly in the risk assessment (for PNEC derivation), but all agreeing that the effect was potentially significant and that further work on molluscs was needed.

(Information relating to endocrine disrupting effects in mammals is discussed in Section 4.1.2.9.1).

3.2.1.1.6 Sediment dwelling organisms

Whale et al. (1999) studied the acute toxicity of bisphenol-A to the benthic amphipod *Corophium volutator*. Artificial sediment was prepared following guidelines in the OECD (1984) earthworm acute toxicity test. Bisphenol-A with 98% purity was added to the sediment by a spiking procedure with and without the presence of acetone as a carrier solvent. *Corophium volutator* were added to the test system and exposed to bisphenol-A for 10 days. The condition of the organisms was assessed daily as active, immobilised or dead. The resultant LC_{50} (based on mortality) and EC_{50} (based on total adverse effects) values were calculated using probit analysis. The concentration of bisphenol-A in sediment was measured using solvent extraction and liquid chromatography. The pore-water concentration of bisphenol-A was estimated from the sediment concentration using the equilibrium partitioning model approach. The 10-day LC_{50} values calculated for acetone and direct spiked tests based on bulk sediment concentrations were 46 and 60 mg/kg dry weight, respectively. The corresponding 10-day EC_{50} values were 31 and 36 mg/kg dry weight for acetone and direct spiked tests, respectively. The endpoints of the toxicity tests based upon interstitial water concentrations were also determined; the 10-day LC_{50} values were 1.4 and 1.6 mg/l for acetone and direct spiked tests, respectively and the 10-day EC_{50} values were 1.1 and 1.3 mg/l for acetone and direct spiked tests, respectively.

Watts et al. (2001) studied the effect of bisphenol-A on development and reproduction in the freshwater invertebrate *Chironomus riparius*. Midge larvae were exposed to a range of sediment concentrations and raised until the adults emerged. The time to emergence, sex ratio, number of adults, egg production and egg viability were all measured. The sediments in the experiment were spiked with stock solutions of bisphenol-A, and the concentrations of bisphenol-A in the stock solution were confirmed by analysis. The sediment was artificial, containing 15% organic matter; the resultant bisphenol-A concentrations were not measured in the sediment or the exposure water. The authors found that emergence of male and female adults were significantly delayed in the second generation of adults at bisphenol-A concentrations of 78 ng/l to 0.75 mg/l (these are stock solution concentrations and not the actual exposure concentrations in sediment). There was no observable effect on the first generation adults, and no effect on sex ratio or total number of adults produced in either generation. The authors noted that although time of

emergence of adults was affected, the results in general do not suggest that the criteria examined, although validated as indicators of general sediment toxicity, could be used to detect oestrogenic effects. In this experiment it is not possible to estimate the actual level of exposure in the test system which may be substantially different from the stock solution concentrations due to adsorption and degradation of bisphenol-A. This study is not considered valid for use further in the risk assessment.

3.2.1.2 PNEC derivation for aquatic species

$PNEC_{water}$

In deriving the $PNEC_{water}$ consideration needs to be given to short-term and chronic toxicity studies for fish, amphibians, aquatic invertebrates and algae. The guidelines for deriving the PNEC given in the TGD are based upon population effects, e.g. effects on ability to reproduce and species mortality, and do not directly cover endocrine disruption as an endpoint. The available toxicity data on bisphenol-A suggest that endocrine disruption may be the most sensitive endpoint, although in deriving the PNEC the ecological significance of these effects needs to be taken into account.

For bisphenol-A the lowest NOEC value for a conventional endpoint is that for egg hatchability in fathead minnows, at 16 μg/l. This is also the NOEC for vitellogenin production in males of the same species and oviduct formation in male carp. As there are long-term NOEC values available for fish, invertebrates and algae a factor of 10 can be used on the NOEC in accordance with the usual TGD method to give a PNEC of 1.6 μg/l.

Other effects were seen in the fathead minnow study, notably on the development of spermatozoa. Partial inhibition of sperm development was noted in both the F0 and F1 generations, with a LOEC of 1 μg/l although there is some uncertainty associated with the conduct of the study in establishing this result. There are also indications of possible effects in snails at similar or lower levels. The TGD does not currently provide guidance on how to incorporate such results into the risk assessment process. In the absence of such guidance, a preliminary approach could be to apply an assessment factor of 10 to the LOEC to derive a "conservative" PNEC of 0.1 μg/l for use in the assessment. Further consultation with independent experts on this issue produced a divided response, with some considering the endpoint to be unsuitable for risk assessment (it should be noted that the authors of the fathead minnow study do not consider that the risk assessment should be based solely on this value). Others considered that the use of an assessment factor of 10 on such a LOEC was overly conservative, others that it was appropriate. There is also the evidence of possible effects at similar or even lower concentrations in snails. Although this effect is also open to some uncertainty in interpretation (and a PNEC cannot currently be derived directly from the snail data), it lends some support to the derivation of an alternative PNEC. The impact of this alternative "conservative" PNEC of 0.1 μg/l (and the potentially lower effect concentrations for snails) will therefore be considered in the risk characterisation section along with the traditionally derived PNEC of 1.6 μg/l. Additional studies of the potential effects of bisphenol-A on sperm development in fathead minnow and on snails are planned to resolve the uncertainties associated with the conservative PNEC.

$PNEC_{microorganisms}$

Two tests with microorganisms are reported for bisphenol-A, an IC_{50} test with *Pseudomonas fluorescens* and a NOEC on cell growth for *Pseudomonas putida*. The TGD indicates that tests with *Pseudomonas fluorescens* should not be used to determine the PNEC for microorganisms as *Pseudomonas fluorescens* uses glucose as a substrate. Results of a cell multiplication test with *Pseudomonas putida* may be used with care.

For *Pseudomonas putida* a NOEC based on cell growth of $\geq$320 mg/l is reported. This is not a true NOEC as it is the highest concentration used in the test and no effects were observed at this concentration. However, in the absence of any other data this value will be used as the NOEC for the derivation of a PNEC for microorganisms. For a NOEC from a specific population the $PNEC_{microorganisms}$ is set equal to the NOEC value. Therefore the $PNEC_{microorganisms}$ for bisphenol-A is taken as 320 mg/l.

$PNEC_{sediment}$

For bisphenol-A there are limited data on the toxic effects of bisphenol-A to benthic organisms. Based upon a 10-day EC_{50} for *Corophium volutator* of 36 mg/kg dry weight (lowest value for direct spiked tests) and using an assessment factor of 1,000 a $PNEC_{sediment}$ of 36 µg/kg dry weight is calculated. As the data set is very limited a $PNEC_{sediment}$ derived from the $PNEC_{water}$ using the equilibrium partitioning method has also been calculated for comparison. The calculated $PNEC_{sediment}$ values are 26 µg/kg wet weight (60 µg/kg dry weight) using the $PNEC_{water}$ of 1.6 µg/l and 1.6 µg/kg wet weight (3.7 µg/kg dry weight) using the more conservative $PNEC_{water}$ of 0.1 µg/l. The PNECs derived by the two methods are similar. The equilibrium partitioning approach should be suitable for a substance such as bisphenol-A. The database for aquatic organisms is much more extensive than that for sediment organisms, and so more confidence can be placed in the result. In addition, the sediment study also derived acute toxicity values based on the measured interstitial water concentrations in the test. The resulting $L(E)C_{50}$ values (1.1-1.4 mg/l) are the same as the lower end of the values for aquatic invertebrates. Taking all the evidence together, the assessment for aquatic organisms can be considered to be protective for the sediment compartment. The calculated $PNECs_{sediment}$ based on the partitioning approach will be taken forward in the assessment.

3.2.2 Terrestrial compartment

There are no toxicity tests results available for terrestrial species. Therefore the $PNEC_{soil}$ will be derived from the $PNEC_{water}$ by a partitioning equilibrium in line with the recommendations of the TGD. The $PNEC_{soil}$ is derived by the following relationship:

$$PNEC_{soil} = \frac{K_{soil-water}}{RHO_{soil}} \cdot PNEC_{water} \cdot 1000$$

RHO_{soil}	Bulk density of wet soil	1,700 kg/m^3
$K_{soil\text{-}water}$	Partition coefficient soil-water	21.7 m^3/m^3

For bisphenol-A this gives a $PNEC_{soil}$ of 23 µg/kg wet weight based on the conventional aquatic PNEC and 1.3 µg/kg wet weight based on the conservative aquatic PNEC.

3.2.3 Atmosphere

There are no known biotic or abiotic effects of bisphenol-A in the atmosphere, and in particular effects on plants due to atmospheric exposure are unknown. Based on structural considerations, it is unlikely to be an ozone depleter or greenhouse gas, nor is it thought to contribute to low-level ozone formation. It is therefore not possible to derive a PNEC.

3.2.4 Secondary poisoning

3.2.4.1 Avian studies

Berg et al. (2000) studied the effects of bisphenol-A on sex organ development in quail and chicken embryos. Bisphenol-A was injected (67 and 200 μg/g) into the yolk of quail (*Coturnix japonica*) and chicken (*Gallus domesticus*) eggs during incubation and the embryos examined two days before anticipated hatching. At 200 μg/g egg bisphenol-A induced Müllerian duct (embryonic oviduct) malformation in female quail embryos, and feminisation of the left testis (ovotestis) in male chicken embryos.

In further work Halldin et al. (2000) examined the embryonic uptake and distribution of bisphenol-A and the effect on variables related to reproduction in adult quail following injection into the yolk of embryonated eggs. Bisphenol-A at 200 μg/g egg did not cause any significant estrogen-like effects on either reproductive behaviour, testis morphology, egg laying ability or oviduct morphology. Measurements of radiolabelled bisphenol-A suggested that it was readily metabolised and excreted.

As both studies use an exposure route that is not relevant for the environment, they cannot be used in the risk assessment.

3.2.4.2 PNEC derivation

The toxicity data available on avian species are not suitable for use in risk assessment; thus a PNEC is derived from laboratory mammal data. Details of the mammalian studies are presented in Section 4. The study from which the NOAEL is taken was a three-generation multi-dose level feeding study on rats. The NOAEL of 50 mg/kg body weight is based on a reduction in litter size. Although other effects seen at the next concentration are thought to be indirect effects caused by maternal toxicity, this reduction may be due to a direct effect of bisphenol-A on fertility. Using the conversion factor of 20 from Appendix VII of the TGD and a further factor of 3 to allow for the fact that calorific content of a laboratory diet is higher than the diet of fish-eating mammals and birds, this NOAEL is equivalent to a daily dose of 330 mg/kg food. The TGD recommends the use of an assessment factor of 10 on chronic studies. Therefore the PNECoral is 33 mg/kg food.

3.3 RISK CHARACTERISATION

There are a number of use areas where emissions are expected to be negligible and for which PECs have not been calculated in this assessment (these processes are either completely dry, or any aqueous effluent produced is disposed of through incineration). These are:

- unsaturated polyester production,
- can coating production,
- tyre manufacture,
- alkoxylated bisphenol-A production,
- tetrabromobisphenol-A production and use,
- phenoplast cast resin production.

There are also a number of uses which currently only take place on sites where bisphenol-A is produced. Emissions from these processes are included in the site-specific emissions for bisphenol-A production and are not separately identified. These uses are:

- polycarbonate production,
- polyols/polyurethane production,
- brake fluid manufacture,
- polyamide production.

3.3.1 Aquatic compartment (incl. sediment)

3.3.1.1 Surface water

The main releases of bisphenol-A to the aquatic environment occur during its production and subsequent processing. The risk assessment report has considered the main stages during which bisphenol-A may be released into the environment. In considering these stages use has been made of site-specific data and generic scenarios.

In the aquatic compartment bisphenol-A is classed as readily biodegradable and it is not expected to be persistent in the aquatic environment.

A range of toxicity tests on aquatic species is reported and has been discussed in the preceding sections. From these tests two PNECs have been developed. The first of these is derived from the "conventional" endpoint of the hatchability of eggs in a multi-generation fish test, and is 1.6 μg/l. It is also supported by NOECs from other studies for vitellogenin production in male fathead minnows and for oviduct formation in male carp.

Another PNEC can be derived from effects seen on spermatogenesis in fathead minnows in the same study from which the egg hatchability NOEC was taken. The lowest concentration producing a difference in the distribution of the four sperm cell types in the testes was 1 μg/l; a factor of 10 applied to this LOEC gives a PNEC of 0.1 μg/l. As noted in the earlier discussions there are significant concerns over this aspect of the study and hence uncertainties over the actual level of effects for this endpoint. There are also indications of effects on snails which may occur at levels of 1 μg/l or possibly below, but the studies currently available do not allow a reliable PNEC to be established.

The risk characterisation will therefore use the "conventional" PNEC initially, but will also consider the effect of using the more "conservative" PNEC based on sperm effects in fish, and

the possible effects at lower concentrations in snails. The conservative PNEC is derived from studies about which there are concerns, and so it will not be used to make a definite decision on whether a risk exists. Instead it will be used to determine whether further work is needed to provide a sounder scientific basis for an assessment of these endpoints.

Table 3.11 compares the calculated PECs with the $PNEC_{aquatic}$ of 1.6 μg/l for bisphenol-A (polycarbonate bottle washing gives rise to a negligible concentration compared to the regional PEC and so it is not included). From the table it can been seen that the PEC/PNEC ratios generated using site-specific data are less than 1 for all sites; hence no environmental risks would be expected from the production or use of bisphenol-A at these sites based on this PNEC.

For the generic scenarios PEC/PNEC ratios greater than 1 are obtained for thermal paper recycling, use of bisphenol-A as an inhibitor in PVC production, preparation of additive packages for use with PVC and as an anti-oxidant in the production of plasticisers for use with PVC. For the remaining generic scenarios the PEC/PNEC ratios are less than 1 indicating that their use does not give rise to concern for the environment based on the current PNEC.

Table 3.11 PEC/PNEC ratios for bisphenol-A in wastewater treatment plant, surface water and sediment

	PEC_{stp} (mg/l)	PEC_{stp} / PNEC	PEC_{water} (μg/l)	PEC_{water} / PNEC
Site-specific [a]				
Production (BPA 5)	0.19	0.0006	0.44	0.28
Epoxy resin production (ER 4)	0.0018	0.000006	1.32	0.83
Thermal paper production (PAPER 6)	0.47	0.0015	1.06	0.67
PVC production (PVC 1)[c]	<0.005	<0.00002	0.62	0.39
Generic scenarios				
Phenoplast cast resin processing	0.0144	0.000045	1.56	0.98
Thermal paper recycling	2.28	0.007	230	143
Thermal paper (TNO branch specific value)	0.073	0.00023	18	11.25
PVC – Inhibitor during production process[b]	3.33	0.01	333	208
PVC – Anti-oxidant during processing	0.00154	0.000005	0.27	0.17
PVC – Preparation of additive packages	0.127	0.0004	12.8	8
PVC – Anti-oxidant in plasticiser production	0.019	0.00006	2.0	1.25
PVC – Plasticiser use	0.00107	0.000003	0.23	0.14
Regional				
Regional			0.12	0.075

a) For specific sites only the site giving the highest PEC in surface water is included in the table. The ratios for other sites will be lower than the value in the table.
b) Production of PVC resin using bisphenol-A is due to be voluntarily phased out by the end of 2001 in Europe.

The conservative PNEC of 0.1 μg/l is exceeded by the regional concentration of 0.12 μg/l, and hence using this PNEC all scenarios would give rise to concern (with the exception of the site-specific scenarios for three of the epoxy resin producers, as these have no emissions to water).

Appendix 2 contains a "what if" analysis to examine the effect of removing the emissions from those uses already identified as a risk using the higher PNEC value (this is not intended to pre-

judge any risk management decisions). This results in a regional concentration of 0.024 µg/l, which is below both of the PNEC values. Although risk reduction might still be required for all of the remaining scenarios (as their Clocal values are above the lower PNEC), this would have implications for any risk reduction strategy.

The effects on snails also need to be considered. The reported studies indicate effects at ~1 µg/l and possibly at levels down to ~10 ng/l. However, the studies currently available are not considered suitable for derivation of a PNEC. Clearly, if the no effect level were as low as indicated, then the "what if" regional concentration would also be of concern.

Results

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the following uses of bisphenol-A:

- thermal paper recycling, [6]
- use as an inhibitor in PVC production,
- preparation of additive packages for PVC processing,
- use as an anti-oxidant in the production of plasticisers for use in PVC processing.

Although these scenarios (with the exception of thermal paper production) are referred to as generic in the exposure section, the PEC estimates are based on data from the industry and use areas and are considered representative. It appears unlikely that the provision of further information would alter the conclusions. The use of bisphenol-A in the manufacture of PVC resin is due to be phased out in Europe by the end of 2001 under a voluntary agreement by industry.

Conclusion (i) There is need for further information and/or testing.

This conclusion applies to all of the areas of use which are not identified as a risk based on the "traditional" higher PNEC (1.6 µg/l):

- bisphenol-A production,
- epoxy resin production,
- thermal paper production,
- phenoplast cast resin processing,
- use as a anti-oxidant in PVC processing,
- use as a plasticiser in PVC processing,
- regional concentration.

Further work is required in relation to the toxicity of bisphenol-A to snails, to allow a NOEC for these effects to be determined. The rapporteur proposes that the studies should be carried out on the Apple Snail *Marisa cornuarietis* (with other species if possible). Some initial preparatory research will be required in order to be able to develop a test programme; an expert group,

[6] Of possible relevance to this scenario is the recent finding that chlorinated bisphenol-A has been detected in the effluent from paper recycling mills (Fukazawa et al., 2001). The data are insufficient to make any estimates of the amount of chlorinated products produced, and their environmental effects are also unknown (although the paper quotes a study that shows that they have a greater binding affinity to the oestrogen receptor than bisphenol-A, it is not clear what this means in terms of effects). It is not therefore possible to determine whether the formation of these substances is of concern based on the currently available data. However, this might be considered further in any risk reduction strategy. The draft EU risk assessment of sodium hypochlorite (EC, 2002) also addresses chlorinated products formed in paper mills to some extent.

including Professor Oehlmann, will be set up to define the requirements. In parallel with this, the way in which the results will be used, including assessment factors, should be discussed and agreed.

If the effect on snails is not reproducible at low concentrations, the current "conservative" PNEC based on the fish sperm development LOEC will need to be reconsidered. In view of the uncertainties expressed in the results by certain experts, a specific investigation of the effects of bisphenol-A on sperm cell development in fathead minnows is required, to address the concerns with regard to the existing study.

It is recognised that these uses give a risk using the "conservative" PNEC. Although there is considerable uncertainty associated with the validity of this PNEC, these uses will be examined during the development of the risk reduction strategy for those uses identified as posing a risk regardless of the PNEC used (see below). The impact of emission reduction consequent to any proposed control measures will also need to be considered.

3.3.1.2 Sediment

For bisphenol-A the sediment concentrations and the sediment PNECs are both derived from the corresponding PEC and PNEC values from water using the equilibrium partition method. The PEC/PNEC ratios will therefore be the same as the surface water and the same conclusions will apply. It should be noted that some of the measured levels in sediments are higher than the estimated PNEC, although the measured data are currently limited in scope. The measured sediment levels appear higher than expected from measured concentrations in water, although the water concentrations are not specifically related to the sediment samples. This will be considered further when the aquatic effects assessment is refined.

3.3.1.3 Wastewater treatment plants

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

A $PNEC_{microorganisms}$ of 320 mg/l is derived from the available toxicity data and compared with the PEC_{stp} in **Table 3.11**. The PEC/PNEC ratios are all less than 1 indicating that there is no concern for microorganisms in wastewater treatment plants due to the production and use of bisphenol-A.

This applies to production and all use areas.

3.3.2 Terrestrial compartment

There are not thought to be any direct releases of bisphenol-A to soil. Therefore, the PECs in soil are due to application of sewage sludge containing bisphenol-A and via aerial deposition. The short lifetime of bisphenol-A in the atmosphere and its low volatility mean that atmospheric deposition contributes only a small amount to soil concentrations. Therefore site-specific PECs in soil have only being calculated when the fate of sludge is unknown or it is known to be applied to agricultural soil. Where sludge is disposed of via landfill or incineration a PEC_{soil} has not been calculated and the assessment for the regional scenario will be taken as relevant.

There is no information on the degradation of bisphenol-A in soil and so soil degradation rates have been estimated from surface water degradation rates. Similarly there are no toxicity data for terrestrial species and the $PNEC_{soil}$ has therefore been calculated from the $PNEC_{water}$ of 1.6 μg/l using the equilibrium partitioning method. The $PNEC_{soil}$ is calculated as 23 μg/kg wet weight.

Table 3.12 compares the $PNEC_{soil}$ with the $PEC_{agricultural\ soil}$.

Table 3.12 PEC/PNEC ratios for bisphenol-A in soil

	PEC agricultural soil averaged over 30 days (mg/kg wet wt)	PEC/PNEC ratio
Site-specific		
Epoxy resin production (ER 4)	0.463	20
Generic Scenarios		
Phenoplast cast resin processing	0.0199	0.87
Thermal paper recycling	3.14	136
PVC – Inhibitor during production process	4.59	200
PVC – Anti-oxidant during processing	0.0022	0.10
PVC – Preparation of additive packages	0.175	7.61
PVC – Anti-oxidant in plasticiser production	0.027	1.17
PVC – Plasticiser use	0.0015	0.065
Regional		
Regional	$9.7 \cdot 10^{-5}$	0.004

If the "conservative" aquatic PNEC of 0.1 μg/l is used to estimate the PNEC for the terrestrial compartment (1.3 μg/kg wet weight), then all use areas give PEC/PNEC ratios above one. The predicted regional concentration is below this lower PNEC value, indicating no risk on the regional scale.

Result

Conclusion (i) There is need for further information and/or testing.

This applies to the following uses of bisphenol-A:

- epoxy resin production,
- phenolplast cast resin processing,*
- thermal paper recycling,
- use as an inhibitor in the production of PVC,
- use as an anti-oxidant in PVC processing,*
- preparation of additive packages for PVC production,
- use as an anti-oxidant in the production of plasticisers for use in PVC processing,
- use as a plasticiser in PVC processing.*

This conclusion applies to all uses of bisphenol-A for which emissions via sewage sludge application are known to occur or for which no information is available and they are assumed to occur. The majority of these uses may pose a risk when using the higher of the two PNECs (derived from the aquatic PNEC of 1.6 μg/l); those marked with * are only identified as posing a risk when the conservative PNEC is used.

The PNECs are derived from those for the water compartment using the equilibrium partitioning method. These could in principle be revised by testing terrestrial organisms, but it is uncertain which tests could usefully be performed. Standard terrestrial toxicity tests on plants and microorganisms may not be the most appropriate, especially in view of the possible importance of endocrine disrupting effects at low concentrations. There may also be dosing problems in soil in view of the relatively rapid degradation of bisphenol-A. At this stage it is therefore proposed to await the outcome of the aquatic testing before considering whether any terrestrial data could usefully be gathered (especially as there may be grounds for read across of data for snails). The conclusions for the terrestrial compartment for these uses will therefore remain as conclusion (i). In addition, the UK Department of Environment, Food & Rural Affairs is conducting research into endocrine disruption in the earthworm *Eisenia andrei* and bisphenol-A is one of the test compounds. The project aim is to develop molecular markers of exposure to, and population level effects of, endocrine disruption for use in field and laboratory studies. It is expected that this work will provide relevant information to a timescale compatible with that of the aquatic tests.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This applies to the following uses:

- bisphenol-A production,
- thermal paper manufacture.

This conclusion is reached for these uses because they have no reported releases to the terrestrial environment.

3.3.3 Atmosphere

Bisphenol-A has a limited release to the atmosphere. In this assessment the only significant local emissions identified are those from production sites, and these give rise to low estimated concentrations in the air. Emissions through volatilisation from PVC articles in use are also considered in the assessment. Once released bisphenol-A is degraded by the reaction with hydroxyl radicals, and very low concentrations are estimated on the regional scale. There are few monitoring data for bisphenol-A in air, but these also indicate low concentrations. There are no known biotic or abiotic effects of bisphenol-A in the atmosphere. However, in view of the expected low atmospheric concentrations, any potential biotic effects are unlikely to be of concern. Because of its low volatility and short lifetime in the atmosphere long-range transport is unlikely to occur. Similarly transport from the troposphere to the stratosphere is also unlikely. Bisphenol-A is not a known ozone depleter or greenhouse gas, and nor is it thought to contribute to low-level ozone formation.

Result

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This applies to production and all use areas.

3.3.4 Secondary poisoning

Based upon measured BCF data bisphenol-A is not thought to bioaccumulate in fish.

In Section 3.2.4 a PNECoral of 33 mg/kg food was derived for the secondary poisoning scenario. The concentration of bisphenol-A in fish and earthworms for predators has been estimated using the EUSES program and the fish bioconcentration factor of 67. The resultant PEC/PNEC ratios are detailed in **Table 3.13**.

Table 3.13 PEC/PNEC ratios for fish and earthworms

	Concentration in fish from surface water for predators (mg/kg)	PEC_{fish}/ $PNEC_{oral}$	Concentrations in earthworms from agricultural soil for predators (mg/kg)	$PEC_{earthworms}$ / $PNEC_{oral}$
Site-specific				
Bisphenol-A production	0.017	0.0005	$3.3 \cdot 10^{-3}$	0.0001
Epoxy resin production	0.041	0.0012	0.6	0.018
Thermal paper production	0.034	0.001	$6.7 \cdot 10^{-4}$	$2 \cdot 10^{-5}$
Generic scenario				
Phenoplast cast resin processing	0.047	0.0014	0.026	0.0008
Thermal paper recycling	6.32	0.19	4.06	0.12
PVC – Inhibitor during production process	7.7	0.23	5.93	0.18
PVC – Anti-oxidant during processing	0.011	0.0003	$3.36 \cdot 10^{-3}$	0.0001
PVC – Preparation of additive packages	0.30	0.009	0.23	0.0069
PVC – Anti-oxidant in plasticiser production	0.052	0.016	0.036	0.011
PVC – Plasticiser use	0.0098	0.0003	$2.5 \cdot 10^{-3}$	$7.6 \cdot 10^{-5}$

If the measured bioconcentation factor if 144 for the freshwater clam is used to estimate concentrations in food for predators feeding on aquatic organisms, the PEC/PNEC ratios are increased by a factor of 2.15. All the ratios are still below 1.

Result

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This applies to production and all use areas. However, a revision of the PNECoral value will be considered if additional information on the interpretation of mammalian developmental data becomes available as a result of further studies being conducted for the human health assessment.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 Occupational exposure

4.1.1.1.1 General introduction

Definitions and sources

In this document, unless otherwise stated, the term exposure is used to denote personal exposure as measured or otherwise assessed without taking into account the effect of any personal protective equipment (PPE) which might be in use. This definition permits the effects of controls other than PPE to be assessed and avoids the problem of trying to quantify the actual protection provided by PPE in use.

The general discussion sections summarise the important issues arising from the exposure assessments and brings together measured exposure data and predictions from the EASE (Estimation and Assessment of Substance Exposure) model. EASE is a general-purpose predictive model for workplace exposure assessments. It is an electronic, knowledge based, expert system which is used where measured exposure data are limited or not available. The model is in widespread use across the European Union for the occupational exposure assessment of new and existing substances.

All models are based upon assumptions. Their outputs are at best approximate and may be wrong. EASE is only intended to give generalised exposure data and works best in an exposure assessment when the relevance of the modelled data can be compared with and evaluated against measured data.

EASE is essentially a series of decision trees. For any substance, the system asks a number of questions about the physical properties of the substance and the circumstances of its use. For most questions, the EASE user is given a multiple-choice list from which to select the most appropriate response. Once all the questions have been answered, the exposure prediction is determined absolutely by the choices made. EASE can be used to estimate inhalation and dermal exposure - dermal exposure is assessed as the potential exposure rate to the hands and forearms (a total skin area of approximately 2,000 cm^2). The dermal model is less developed than the inhalation model, and its outputs should be regarded as no more than first approximation estimates.

The output ranges generated by EASE for inhalation exposure relate to steady-state conditions, and estimate the average concentration of the substance in the atmosphere over the period of exposure. The model will not directly predict short-term exposures, but predictions of values for these circumstances are possible by interpreting and modifying the output data using professional judgement. Although short-term exposures may be predicted by EASE in this way, such modifications to the model output should be regarded with caution.

Where real exposure data are not available or scant, EASE has been used to predict exposures. Details of the reasoning behind any assumptions made during the course of EASE predictions are made clear in the relevant sections.

Overview of exposure

The total number of persons occupationally exposed to bisphenol-A is not known, but due to its widespread use in epoxy resins and polycarbonate it is expected to be thousands. However, the exposure is likely to be negligible in many cases as the residual bisphenol-A in epoxy resins and polycarbonate is low.

Most of the data used in this assessment have been supplied by industry, either directly or through trade organisations. The HSE has no bisphenol-A exposure data on its NEDB (National Exposure Database) and no data were available from any of the other competent authorities. There are little data available from published papers although two were found relating to 1) use of epoxy resin-based paint and 2) the use of epoxy resin-based powder paints.

The occupational exposure to bisphenol-A is discussed in 10 sections:

- manufacture of bisphenol-A,
- manufacture of PC,
- manufacture of articles from PC,
- manufacture of epoxy resins and moderated epoxy resins,
- use of bisphenol-A in PVC manufacture,
- manufacture of liquid epoxy paints, lacquers and powder coatings,
- use of epoxy resin-based powder coatings, paints and lacquers,
- manufacture of thermal papers,
- manufacture of tin plating additive,
- manufacture of tetrabrominated flame retardants (TBBA).

Some uses of bisphenol-A have been identified but not discussed in the following sections as these uses do not apply in the European Union or because information on some of the minor uses was not available. These include tyre manufacturing, brake fluid manufacturing, polyols/polyurethane manufacturing and polyamide processing.

In a number of instances, companies supplying information stated that personal protective equipment and/or respiratory protective equipment was used. However, unless stated otherwise in the text, details of the type were not provided.

The industry from which the highest inhalation exposures were reported was the bisphenol-A manufacturing industry with 8-hour TWAs ranging from "none detected" to 23.3 mg/m^3. Reasonable worst-case scenarios have been estimated using the 90^{th} percentile. This has been calculated where there is sufficient data. Where insufficient data has been submitted, professional judgement has been used to estimate the 90^{th} percentile. A reasonable worst-case 8-hour TWA for bisphenol-A manufacturing has been estimated at 5 mg/m^3.

Short-term exposures varied considerably, ranging from "none detected" to 43.6 mg/m^3. Generally, short-term exposures rarely exceeded 10 mg/m^3.

Dermal exposure ranged from 0 to 5 mg/cm^2/day (EASE estimation). Bag filling and maintenance activities gave rise to the highest estimates for dermal exposure. A reasonable worst-case scenario would be 5 mg/cm^2/day.

Occupational exposure limits

Table 4.1 Occupational exposure limits for bisphenol-A

Country	8-hour TWA exposure limit (mg/m³)	Source
Germany	5 (inhalable)	List of MAK and BAT values 1997
Holland	5 (respirable)	The National MAC-list 1999
USA*	5	Proposed WEEL - AIHA

* This information was provided by personal communication and no information was available with respect to whether the limit would be for inhalable or respirable dust.

These limits are provided for information and not as an indication of the level of control of exposure achieved in practice in workplaces in these countries.

4.1.1.1.2 Manufacture of bisphenol-A

Measured data

There are six sites in the EU where bisphenol-A is manufactured.

Bisphenol-A is manufactured in enclosed systems, breached for sampling during production and during filling of the product into bags, bulk bags and silos. It is during these activities and during maintenance of the production plant that the potential for exposure to bisphenol-A arises. As the bisphenol-A is generally produced as granules, prills or flakes; the potential for exposure is reduced.

The manufacturers of bisphenol-A have undertaken personal sampling. However, there is no consistency of approach regarding what has been sampled and how the analysis has been carried out. Some companies have sampled for respirable dust, others for inhalable dust, some have analysed the dust for bisphenol-A, others haven't and some companies have provided results using a variety of sampling and analysis techniques. One company explained that initially inhalable bisphenol-A had been sampled and analysed, but as the levels were so low they stopped analysing the inhalable dust. As long as the results remained well within the relevant occupational exposure limit and the conditions on the plant did not change inhalable dust would continue to be collected to monitor exposure and control. The occupational exposure limits quoted differ in the fraction of particulate the limits apply to. It is not known why the occupational exposure limits differ.

Data were supplied by all the manufacturers of bisphenol-A in the EU. For reasons of anonymity, the companies have each been referred to as Company A, B, C and D. This approach has been adopted for all companies contributing information to this risk assessment document.

Industry data

Company A manufactures bisphenol-A. This company reported personal sampling results for respirable dust during material sampling and during filling of the product into bags, bulk bags and silos during the period 1988 to 1992. They also reported some results for total inhalable dust at one of the filling stations. Specific analysis of respirable dust for bisphenol-A from samples taken at two filling stations was also carried out during 1998.

Sampling of the product is carried out once per shift and takes approximately 3 minutes to complete. The product as prills is collected in polyethylene bags. During sampling protective clothing, shoes, gloves, safety glasses and a helmet are worn. No short-term sampling results were provided. The results of personal exposure monitoring give a range of exposures to respirable dust of 0.04 mg/m^3 to 0.86 mg/m^3 8-hour TWA, with an arithmetic mean 8-hour TWA of 0.36 mg/m^3.

One operator works at each filling station, and filling is carried out for the whole shift. There is local exhaust ventilation at the silo-filling station. Operators wear personal protective equipment including overalls, shoes, gloves, safety glasses and a helmet. The results of sampling for respirable particulate at all filling stations gave a range of 0.1 mg/m^3 to 5.01 mg/m^3 8-hour TWA, with an arithmetic mean of 0.82 mg/m^3.

Four samples for total inhalable particulate were also reported for one of the filling stations. The range of results was 0.42 to 1.61 mg/m^3, with an arithmetic mean of 1.1 mg/m^3. The company has recently analysed respirable particulate samples, collected during filling operations, for bisphenol-A. Three results were reported for bulk bag filling with a range of 0.21 to 1.79 mg/m^3, with an arithmetic mean of 0.81 mg/m^3. Three results were also reported for silo truck filling which gave a range of less than 0.5 to 1.61 mg/m^3. This gives an arithmetic mean of 0.89 mg/m^3 for this activity. No details of the sampling and analysis methods were made available.

At another site, the company reported that sampling for total inhalable particulate has been carried out between 1993 and 1996. Results ranged from less than 0.1 to 6.0 mg/m^3, 8-hour TWA. Fifteen results were reported, twelve of these being less than 1 mg/m^3. One result of 1.9 mg/m^3 was from a worker who carried out product sampling during the sampling period. Product sampling at this plant was carried out two to three times per shift, with each task taking approximately four minutes to complete. Personal protective equipment is worn, including overalls, shoes, gloves safety glasses and a helmet. Respirators are also made available. The other two results of 2.6 mg/m^3 and 6.0 mg/m^3 were obtained from workers who were reported to have carried out technical work on the installation. Technical work has been interpreted to be maintenance work. When such work is carried out on the installation breathing apparatus as well as protective clothing is worn. No further details of the type of work carried out during these sampling periods were provided. Details of the methods of sample collection or analysis were not provided. Further sampling was carried out in 1997 and 1998. During 1997, 8 samples for total inhalable particulate were collected for a range of activities. The results ranged from less than 0.1 to 0.9 mg/m^3, with an arithmetic mean of 0.38 mg/m^3, 8-hour TWA. In 1998, a total of 8 samples were collected for inhalable bisphenol-A. The results ranged from less than 0.13 to 1.61 mg/m^3, 8-hour TWA with a mean of 0.3 mg/m^3.

Sampling results from maintenance activities during 1998 and 2000 have been reported by Company A. In 1998, four results ranging from less than 0.05 to 0.62 mg/m^3 were obtained. In 2000 a further four sample results were obtained. These ranged from less than 0.35 to less than 0.62 mg/m^3. These results were for total inhalable particulate. The samples were analysed gravimetrically.

Company B manufactures bisphenol-A in granules and flakes. This company reported personal sampling results during packaging operations and during rework of bisphenol-A. The results ranged from 0.002 mg/m^3 to 7.5 mg/m^3 during packaging and from 0.002 mg/m^3 to 23.3 mg/m^3 during reworking of bisphenol-A. The company reported that a local exhaust ventilation system would be fitted to control dust exposure during the first quarter of 2001. The method of sampling used was air drawn at 2 l/min through a preweighed filter, which was subsequently reweighed to determine the total inhalable particulate fraction collected. It is unlikely however that this

method could reliably measure to 0.002 mg/m^3, so the lower result should be treated with some caution.

It was reported by the company that high results were obtained during packaging where individuals had taken part in cleaning operations, during which respiratory protective equipment is used. During reworking of material operators use disposable overalls and respiratory protective equipment.

Company C manufactures bisphenol-A in prills. This company provided sampling results from personal air sampling for total inhalable particulate which were then analysed for bisphenol-A using a NIOSH method. Both task-specific and 8-hour TWA results were provided. No details were provided about how the tasks were undertaken. The highest results were obtained during operation of a bagging machine in 1990. Two short-term measurements for this activity gave results of 14 and 15 mg/m^3. However, it should be noted that these two results were for total inhalable particulate rather than specific bisphenol-A measurements. Subsequent sampling exercises indicated that bisphenol-A accounted for between 9 and 91% of the total inhalable dust. 8-hour TWAs for bag filling during 1996 and 1997 between 0.02 and 0.93 mg/m^3 were reported by the company to have been collected following modifications to the plant. These results were for inhalable bisphenol-A, not for total inhalable particulate as in the earlier sampling. Measurements for maintenance of a screw conveyor gave results between 0.8 and 1.35 mg/m^3 for the duration of the task. Results for 8-hour TWAs for similar maintenance activities of 0.52 to 1.35 mg/m^3 were reported. No details were provided of the way tasks were carried out or about the use of engineering controls. Information was provided about the use or otherwise of respiratory protective equipment but not about other personal protective equipment. Results for short-term task-specific samples are shown in **Table 4.3**.

Company D manufactures bisphenol-A. They identified three tasks during which there was a potential for exposure to bisphenol-A; sampling, truck loading and bagging. The sampling task only takes place twice per 8-hour shift and takes 3 minutes to complete. The company reported results of personal monitoring for bisphenol-A dust for plant operators and maintenance personnel. No further details about specific tasks undertaken during the personal sampling periods were provided. Sampling was carried out by drawing in air through a filter. Analysis of the bisphenol-A was carried out by dissolving the collected dust in methanol and analysing the samples using HPLC. No further details about the sampling and analysis were provided.

Data were also obtained from the American Society of the Plastics Industry (SPI) from five production facilities. The results are full-shift samples covering a range of activities. No details were provided about work patterns or how activities were carried out. Almost all results reported were less than 1 mg/m^3, 8-hour TWA. Only one company reported any results above 1 mg/m^3. Given the other results reported by that company, 8-hour TWAs above 1 mg/m^3 would appear to be unusual. The sampling and analysis methods were not reported.

It was also reported by SPI that short-term task-specific sampling had been carried out by one company. A range of results of none detected to 0.96 mg/m^3 was reported for activities including sampling, maintenance and cleaning. No further details were reported.

All the results have been summarised in **Table 4.2** and**Table 4.3**.

The industry data provided indicate that during normal operations on a bisphenol-A manufacturing facility, 8-hour TWAs for inhalable bisphenol-A rarely exceed 5 mg/m^3. Results that exceeded this figure were usually where cleaning or maintenance had taken place during the measurement period. Short-term task-specific data are limited but indicate that exposure rarely exceeds 10 mg/m^3. High results obtained during bagging activities at one company have been reduced substantially following modifications to the bagging plant.

Modelled data

Inhalation

Only information supplied by one of the manufacturers was detailed enough to allow any modelling of inhalation exposure. The information supplied was used to provide a generic estimation of exposures across similar manufacturing sites.

Sampling was identified as a source of exposure for plant operators at most plants. This was reported to be carried out once or twice per shift and takes about three minutes to complete. The EASE scenario that best suits this activity is low dust techniques (bisphenol-A is usually in form of prills or flakes) and no LEV. (It was reported that there was no LEV present during this activity). This gave an exposure range to bisphenol-A of 0 to 5 mg/m^3. It is likely that sampling at other plants is not vastly different.

Filling silos was reported by one company to be a full-shift activity. The EASE scenario which best fits this activity is low-dust techniques with LEV present. This gives a predicted exposure range of 0 to 1 mg/m^3, which can be taken as representing an 8-hour TWA as this activity is continued for a whole shift.

The EASE scenario which best suits bulk bag filling as reported is low dust techniques with no LEV present. This gives a predicted exposure range of 0 to 5 mg/m^3, which can be taken to be representative of an 8-hour TWA if the activity is full shift. The use of the parameter "no LEV present" is because of the uncertainty surrounding the use of LEV at all bagging activities.

The range of predicted inhalation exposures seem to confirm the results obtained from industry, with 8-hour TWA exposures rarely exceeding 5 mg/m^3. Where 90th percentiles have been calculated for exposure data, they did not exceed 2 mg/m^3, but calculation of 90th percentiles was not possible for all data, so the figure of 5 mg/m^3 was reached using professional judgement based on the calculated 90th percentiles and interpretation of the other exposure data available. The figure of 5 mg/m^3, 8-hour TWA will therefore be taken forward as a reasonable worst case for risk characterisation.

For short-term exposure the calculation of 90th percentiles was not possible, so a reasonable worst-case exposure level for short-term exposure of 10 mg/m^3 was reached using professional judgement.

Table 4.2 Summary table of occupational exposures during manufacture of bisphenol-A

Work activities	No of samples	Type of sample	Range 8-h TWA (mg/m^3)	Arithmetic mean 8-h TWA (mg/m^3)	90th percent. 8-h TWA (mg/m^3)	Source
Sampling and filling (1988-1992)	24	resp. part.	0.04 to 5.01	0.72	1.23	Industry
Filling big bags (1998)	3	inhalation BPA	0.21 to 1.79	0.81	1.61	Industry
Filling silo tankers (1998)	3	inhalation BPA	less than 0.5 to 1.61	0.89		Industry
Various (1998)	8	inhalation BPA	0.13 to 0.62	0.3		Industry
Various (1997)	8	TIP	less than 0.1 to 0.9	0.38	1.79	Industry
Various (1993-1996)	15	TIP	less than 0.1 to 6	0.94		Industry
Filling (1988-1992)	4	TIP	0.42 to 1.61	1.1		Industry
Packaging	9	TIP	0.002 to 7.5	1.1	n/a	Industry
Reworking	8	TIP	0.002 to 23.3	7.9	n/a	Industry
Plant operator	12	TIP	less than 0.1 to 0.8	0.3	n/a	Industry
Plant operator	7	inhalation BPA	0.21 to 1.04	not known	n/a	Industry
Maintenance	3	inhalation BPA	0.52 to 1.35	not known	n/a	Industry
Maintenance (1998-2000)	8	*BPA	less than 0.05 to 0.62		n/a	Industry
Charging big bags (1996-1997)	5	inhalation BPA	0.02 to 0.93	0.35	n/a	Industry
Plant operator	13	*BPA	0.02 to 2.13	0.61	2.12	Industry
Maintenance operator	2	*BPA	0.04 to 2.08	1.06		Industry
Product sampling	n/a	inhalation BPA	0 to 5	n/a		EASE
Silo filling	n/a	inhalation BPA	0 to 1	n/a		EASE
Bulk bag filling	n/a	inhalation BPA	0 to 5	n/a		EASE
Various	not known	*BPA	nd to 2.6	not known		SPI (USA)

* particulate fraction collected not known
TIP total inhalable particulate
Resp. part respirable particulate
Inhalation BPA inhalable bisphenol-A

Table 4.3 Task-specific occupational exposure measurements during manufacture of bisphenol-A at Company C

Task	No of samples	Type of sample	Range (mg/m^3)	Arithmetic mean (mg/m^3)
Bagging machine operator (1990)	2	TIP	14 to 15	14.5
Cleaning	1	TIP	4.5	na
Bulk car loader	3	TIP	0.3 to 2.5	1.33
Plant operator	10	inhalation BPA	0.21 to 1.2	not known
Maintenance of screw conveyor	3	inhalation BPA	0.8 to 1.35	1.11
Charging of big bags	5	inhalation BPA	0.13 to 9.5	not known

TIP total inhalable particulate
Inhalation BPA inhalable bisphenol-A

Dermal

As bisphenol-A is manufactured in largely enclosed processes, the only opportunities for dermal exposure arise during sampling activities, bagging or filling operations, or during technical work (maintenance) on the plant itself. Enough information was available to allow EASE modelling of dermal exposure to be carried out on sampling and filling operations.

For sampling, the most appropriate EASE scenario was non-dispersive use, direct handling with incidental contact, where incidental refers to one significant contact in a shift, for example, spilling bisphenol-A whilst taking a sample. This results in a prediction of 0 to 0.1 mg/cm^2/day, although on most days no such accidental contacts will occur. It is estimated that the equivalent of 420 cm^2 of skin may be exposed during sampling activities. Operators are understood to wear gloves and other protective equipment during sampling activities. PPE, properly selected and worn will significantly reduce exposure.

Bagging and other filling operations are carried out for the duration of the shift. The most appropriate EASE scenario for this activity was direct handling with extensive contact. This results in a prediction of 1 to 5 mg/cm^2/day. It is estimated that the equivalent of 420 cm^2 of skin may be exposed during bagging and other filling activities. Operators are understood to wear PPE including gloves. PPE, properly selected and worn will significantly reduce exposure.

It was not possible to carry out EASE modelling of maintenance activities as there was insufficient information available.

Based on the EASE data a reasonable worst case for dermal exposure during sampling activities of 0.1 mg/cm^2/day has been taken forward for risk characterisation. For bagging and other filling activities a reasonable worst case of 1 mg/cm^2/day has been taken forward for risk characterisation.

4.1.1.1.3 Manufacture of PC

Measured data

Two companies manufacture polycarbonate (PC). Where information was available, it was reported that bisphenol-A enters the plant in a closed system. At some sites the bisphenol-A enters the plant from the bisphenol-A manufacturing plant on-site as a solution into a closed system. At sites where solid bisphenol-A is used this is transferred pneumatically in a closed system. The closed system is reported by one of the companies to be strictly operated due to the necessity of controlling exposure to other hazardous substances. There is reported to be no opportunity for exposure to bisphenol-A on this plant since the bisphenol-A will always be in solution and due to the way in which the plant is operated. Product sampling takes place via a closed loop system so there is no opportunity for exposure. Once the polycarbonate polymer is formed it is reported by industry that there is a maximum of 100 ppm residual bisphenol-A within the polymer but this is reported to be bound into the matrix of the polymer. Personal dust sampling was carried out in 1990-1991 in the area where the extruded PC is chopped into granules and bagged, so the results refer to PC dust. At the same plant between 1993 and 1996 personal sampling for total inhalable particulate (TIP) was carried out. Results from 1998 are also reported from the same site. The results are expressed as total respirable particulate, and only the mean was reported. In order to demonstrate that there was no bisphenol-A within the dust in this area, a further personal air sample was taken in 2000 and analysed specifically for

bisphenol-A. The result was less than 0.001 mg/m^3, 8-hour TWA. The results of the sampling exercises are tabulated below.

Table 4.4 Occupational exposure to dust during manufacture of PC

Task	No of samples	Particulate fraction sampled	Range 8-h TWA (mg/m^3)	Mean 8-h TWA (mg/m^3)	Range of BPA assuming max 100 ppm in PC (mg/m^3)	90th percentile 8-h TWA BPA assuming max 100 ppm in PC (mg/m^3)
Plant operator Extrusion and bagging area	4	Respirable particulate	0.07 to 0.27	0.2	$7 \cdot 10^{-6}$ to $2.7 \cdot 10^{-5}$	n/a
Plant operator Extrusion and bagging area	16	TIP	0.1 to 1.1	0.43	$1 \cdot 10^{-5}$ to $1.1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
Plant operator Extrusion and bagging area	Not known	Respirable particulate	Not known	Less than 0.1	Less than $1 \cdot 10^{-5}$ (mean)	n/a
Plant operator Extrusion and bagging area	1	Bisphenol-A	Less than 0.001	n/a	Less than 0.001	n/a

TIP Total inhalable particulate

The SPI supplied some data obtained from manufacturers in the USA. There were a total of six results, all of which were personal samples. However none were full-shift samples. The sample durations ranged from 20 to 185 minutes; the exposures ranged from "nd" (none detected) to less than 0.64 mg/m^3. It is assumed that no bisphenol-A was found on any of the samples and the differences relate to the length of the different sampling periods. The results are presented in **Table 4.5**. The exposures measured relate to specific tasks, but there is no further information which would allow the estimation of an 8-hour TWA. These results seem to confirm reports from industry that exposure to bisphenol-A during PC manufacture is unlikely. No information on sampling methods was provided. Due to the lack of information relating to sampling methods and limits of detection, it was felt that the reasonable worst-case scenario for short-term exposure should be set at 0.5 mg/m^3.

Table 4.5 Short-term task-specific occupational exposure during manufacturing of PC in the USA

Operator task	Sample duration (min)	BPA exposure (mg/m^3)
Rail car disconnection	85	less than 0.15
Rail car disconnection	20	less than 0.64
Rail car hook-up	185	less than 0.07
Rail car disconnect	20	less than 0.64
Rail car hook-up, switch compartments	60	less than 0.22
Injection moulding operator	143	nd

Modelled data

Inhalation

EASE was used to model exposure to bisphenol-A during the bagging of PC granules. The parameters used were inhalable dust, low dust techniques (as the extruded PC is chopped into granules), with LEV present. This results in an exposure range of 0 to 1 mg/m^3. Taking into account that the maximum residual bisphenol-A in the PC is 100 ppm, this results in an exposure range of 0 to $1 \cdot 10^{-4}$ mg/m^3. This EASE range is lower than the latest result provided by industry.

A reasonable worst-case exposure of $1 \cdot 10^{-3}$ mg/m^3 8-hour TWA has been concluded based on the latest exposure result and further information reported by industry and the result of the EASE modelling.

Dermal

There was reported to be no possibility of dermal exposure during the manufacturing of PC. The only possibility of dermal contact is during the bagging of PC granules. Even this is a largely automated process with little opportunity for contact. The EASE parameters used were non-dispersive use, direct handling with intermittent contact. This resulted in a range of 0.1 to 1 mg/cm^2/day. Given that the residual bisphenol-A content of PC is 100 ppm, this results in an exposure range of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mg/cm^2/day. It is estimated that approximately 420 cm^2 of skin may be exposed during this activity.

4.1.1.1.4 Manufacture of articles from PC

Bisphenol-A polycarbonate is an extremely stable polymer and any residual bisphenol-A present in polycarbonate is retained very effectively within the polymer matrix. There was no information available from manufacturers of articles from polycarbonate. However as the PC would not be heated to a temperature any greater than that used by the PC manufacturers for extrusion, the results from the scenario on PC manufacture can be used. Therefore a reasonable worst-case scenario for PC manufacture would be $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA.

The loading of PC granules from big bags would provide the only opportunity for dermal exposure. As this activity is similar to the loading of big bags carried out by the manufacturers of PC, EASE estimates of dermal exposure for that activity have been used to estimate potential dermal exposure during loading activities during manufacture of articles from PC. This gives an estimated exposure range of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mg/cm^2/day. It is estimated that 420 cm^2 of skin may be exposed during this activity.

4.1.1.1.5 Manufacture of epoxy resins and epoxy modified resins

Measured data

There are a number of companies within the EU which manufacture epoxy resins, or modify epoxy resins from larger batch manufacturers to suit their own requirements. All the companies who responded to a request for information identified the charging of reactors with bisphenol-A as the main source of exposure, with the possibility of exposure during maintenance activities being identified by some companies. Once the bisphenol-A has been incorporated into the resin, the potential for exposure is negligible as the majority of the bisphenol-A is reacted. Residual

amounts of bisphenol-A in epoxy resin depend on whether the resin is a liquid or a solid. The figure of 300 ppm has been put forward as representative of residual bisphenol-A in epoxy resin (personal communication, APME). Most residual bisphenol-A is understood to be trapped within the resin matrix (personal communication).

Company E manufactures epoxy resins. They use between 2.5 to 4.5 ktonnes bisphenol-A per annum. Higher molecular weight grades of epoxy resins are used for can coatings, while the lower molecular weight grades are used in powder coatings. The bisphenol-A is delivered by container from the bisphenol-A manufacturer holding approximately 23 tonnes into a closed process. Three activities were identified where the potential for exposure to bisphenol-A exists; delivery (twice per week), changing of filter socks (every two years) and calibration of the weigh vessel (every five years). The company reported that once the bisphenol-A is transferred into the hopper there is no opportunity for exposure as the bisphenol-A is incorporated into resin and once reacted there is a negligible percentage of residual unreacted bisphenol-A. The company also stated that there had been no reported problems of ill-health associated with bisphenol-A.

The bisphenol-A arrives in a road container lined with plastic. The plastic liner extends to a tun dish and pipework so that the transfer point is fully enclosed. The bisphenol-A prills are transferred by negative pressure and circulating nitrogen to a hopper. The transfer takes about two hours to complete. The driver and one member of the company's personnel are involved. The driver slits the top of the plastic liner to allow the prills to flow. He reported occasional redness on his arm following a delivery. During the delivery the driver wears a disposable overall, gloves and respiratory protective equipment. The operator also wears RPE and a disposable overall. There is some escape of bisphenol-A dust from the top of the road container during the transfer.

Three maintenance personnel are involved in the work to change the filter socks. These filters are in the system to separate the circulating nitrogen from bisphenol-A dust once the bisphenol-A has been dropped into the storage hopper. These filters need replacing approximately every two years and this task takes about six hours to complete. The maintenance personnel wear disposable overalls, eye protection, gloves and RPE.

The weigh vessel requires recalibration every five years. In order for this to take place the vessel has to be completely emptied. This entails the last 0.5 tonnes of bisphenol-A being emptied into drums, which are then sealed and disposed of. This activity takes about 1.5 hours and involves two operators. The company reported that the operators wear RPE, gloves and disposable overalls.

The company had not undertaken any personal exposure measurements during any of the above activities.

Four companies who make modified epoxy resins for can coatings for lining food and beverage containers reported information on use and occupational exposure. All four companies identified charging of reactor vessels with bisphenol-A as the main or only source of exposure to bisphenol-A. One company reported quality control sampling of the bisphenol-A as a potential source of exposure. The charging process was reported by the companies to last between 15 and 30 minutes depending on the size of the charge and whether the bisphenol-A was charged from 25 kg bags or bulk bags. Details of the exact methods of charging vessels were not available.

All four companies had carried out some exposure sampling during charging operations. Insufficient details were provided by three of the four to allow 8-hour TWAs to be calculated.

Company F had carried out an instantaneous dust reading close to the charging point. The result was reported to be less than 1 mg/m^3. The use of this result to estimate operator exposure should be treated with extreme caution as it is a static and instantaneous reading.

Company G had carried out sampling during a number of charging operations, but had not analysed samples for bisphenol-A. Samples were reported by the company to have been collected in accordance with MDHS 14/2. The results ranged from 0.32 to 17.5 mg/m^3. It is known that charging takes between twenty and thirty minutes to complete once per shift, which gives a range of 8-hour TWAs of 0.02 to 1.09 mg/m^3, as it was reported that there were no other sources of exposure to bisphenol-A.

Company H had not carried out analysis of samples for bisphenol-A, but had conducted sampling during charging operations. Individual results were not reported but all results were reported to be less than 3 mg/m^3.

Company I reported having conducted sampling using a passive sampling method during charging activities. No further details of the sampling and analysis were reported, but results were all below 0.5mg/m^3. These results would need to be treated with caution as passive sampling has not been reported as a sampling technique for bisphenol-A elsewhere.

All four companies reported the use of LEV and PPE during charging operations.

The results of short-term sampling during charging are summarised in **Table 4.6**. Only one data set allowed for the calculation of a 90th percentile. This was calculated at 10 mg/m^3.

Table 4.6 Summary of short-term, task-specific occupational exposures during manufacture of epoxy resins

Company	Activity	No of samples	Type of sample	Range (mg/m^3)	90th percentile (mg/m^3)
Company F	Charging reactor	1	Static, instantaneous	1*	
Company G	"	12	TIP	0.32 to 17.5	10
Company H	"	Not known	TIP	less than 3	
Company I	"	Not known	**BPA	less than 0.5*	

* results should be treated with caution - see text
** no information relating to fraction of dust collected

A large quantity of data were supplied by the SPI, from the 1970s to the mid-1990s. Although job types were supplied, there were no details of how tasks were performed or the availability of personal protective equipment. Results included both full-shift and task-specific samples. Task-specific samples were not time-weighted to a 15-minute period but varied depending on the length of time the task took to complete.

All the results for 8-hour TWAs were below 2.8 mg/m^3, with the mean 8-hour TWAs, with the exception of results for maintenance activities, below 0.3 mg/m^3. The 90th percentile for all 8-hour TWAs reported was 0.7 mg/m^3.

Results for short-term task-specific sampling ranged from none detected to 43.6 mg/m^3. The results were not annotated, so although it is known that the highest result was for a process operator over a 16-minute period, there are no details about the task undertaken during that period. The mean short-term results all fall below 5 mg/m^3. 90th percentiles have been calculated for each activity reported, with the highest 90th percentile of 11.76 mg/m^3.

No details of sampling methods were provided. The results can be found in **Tables 4.7** and **Table 4.8**.

Table 4.7 8-hour TWA occupational exposure to bisphenol-A during epoxy resin production in the USA

Activity	No of samples	Type of sample	Range of 8-h TWA (mg/m^3)	Mean 8-h TWA (mg/m^3)	90th percentile 8-h TWA (mg/m^3)
Loading/unloading	26	not known	nd to 0.99	0.18	0.7
Bagging/palletising	37	not known	nd to 2.8	0.25	
Process operators	25	not known	less than 0.1 to 1.1	0.26	
Equipment technician	6	not known	less than 0.1	less than 0.1	
Maintenance	2	not known	0.37 to 1.2	0.8	

Table 4.8 Short-term task-specific occupational exposure to bisphenol-A during epoxy resin production in the USA

Activity	No of samples	Type of sample	Range of results (mg/m^3)	Mean result (mg/m^3)	90th percentile (mg/m^3)
Loading/unloading	11	not known	0.003 to 3.2	0.77	1.7
Bagging/palletising	23	not known	nd to 3.87	0.55	0.64
Process operators	25	not known	nd to 43.6	3.96	11.76
Equipment technician	1	not known	less than 0.1	less than 0.1	n/a
Maintenance	8	not known	nd to 0.69	0.38	n/a

Modelled data

Inhalation

Inhalation exposure was modelled for the unloading of the road car in the bulk manufacture of epoxy resins. This task would be similar for other bulk manufacturers of epoxy resins so the EASE data estimated could indicate exposures in similar circumstances in other companies. The EASE scenario which best fitted the situation was exposure to inhalable dust using low dust techniques with LEV present. The predicted dust exposure range was 0 to 1 mg/m^3. As it is known that this task takes two hours to complete and would be the only source of exposure to the personnel involved, an 8-hour TWA can be calculated. The estimated 8-hour TWA range for this activity is 0 to 0.25 mg/m^3.

Inhalation exposure was also modelled for the changing of filter socks and for recalibration of the weigh vessel at Company E. These maintenance activities were chosen for modelling as they are defined maintenance activities and sufficient information was available about the tasks to make modelling possible. The results illustrate the range of possible exposures to bisphenol-A during maintenance activities generally.

The EASE scenario best suited to the changing of the filter socks is dry manipulation of non-fibrous, readily aggregating, inhalable dust in the absence of LEV. The predicted exposure range is 0 to 5 mg/m^3. The maintenance personnel are reported to wear disposable overalls, gloves, eye protection and respiratory protective equipment. PPE, properly selected and worn will significantly reduce exposure.

The EASE scenario best suited to the emptying of the weigh vessel to allow its recalibration is dry manipulation of non-fibrous, readily aggregating, inhalable dust in the absence of LEV. The predicted exposure range is 0 to 5 mg/m^3. The task is reported to take two operators approximately one and a half hours to complete. The maintenance personnel are reported to wear disposable overalls, gloves, eye protection and respiratory protective equipment during this activity. PPE, properly selected and worn will significantly reduce exposure.

It is estimated that a reasonable worst-case 8-hour TWA would be 0.7 mg/m^3, taking into account the sampling data reported and the estimations using EASE.

A reasonable worst-case exposure for short-term, task-specific exposure would be 11 mg/m^3, based on the two highest 90th percentiles (10 and 11.76 mg/m^3) calculated from exposure data.

Dermal

No information was available for dermal exposure. The EASE model was used to estimate the potential for dermal exposure to bisphenol-A during charging of reactors, which takes between 3 minutes to 30 minutes depending on the amount being charged and the type of bag it is supplied in. The EASE scenario used was non-dispersive use, direct handling with intermittent contact. This gave an estimated dermal exposure of 0.1 to 1 mg/cm^2/day. No consideration has been taken of the fact that it was reported that personal protective equipment is used by the operators during charging of reactors. PPE, properly selected and worn will significantly reduce exposure. It is estimated that an area of skin equivalent to 420 cm^2 may be exposed.

EASE was also used to estimate dermal exposure during the two maintenance activities identified at Company E. The EASE scenario best suited to the changing of the filter socks at Company E is direct handling, non-dispersive use with intermittent contact. This gives a predicted exposure range of 0.1 to 1 mg/cm^2/day. Operators were reported to wear PPE. PPE, properly selected and worn will significantly reduce exposure. It is estimated that an area of skin equivalent to 840 cm^2 may be exposed during this activity.

The EASE scenario that best suits the emptying of the weigh vessel is direct handling, non-dispersive use with intermittent contact. This gave a predicted exposure range of 0.1 to 1 mg/cm^2/day. PPE was reported to be worn. PPE, properly selected and worn will significantly reduce exposure. It is estimated that an area of skin equivalent to 840 cm^2 may be exposed during this activity.

The reasonable worst-case dermal exposure on the basis of the EASE data is estimated to be 1 mg/cm^2/day.

4.1.1.1.6 Use of bisphenol-A in PVC manufacture

No information was received directly from the PVC industry. However, it is known that bisphenol-A is added during the polymerisation stage to control polymer length, as a stabiliser at the compounding stage, and as an anti-oxidant in the production of plasticisers used in PVC production. The amount of bisphenol-A used by these manufacturing sites is relatively small, ranging from ~2 tpa to about 90 tpa and its use is currently being phased out altogether.

It is reported (CEFIC) that bisphenol-A is generally supplied to these companies in bags or intermediate bulk containers (IBCs) and is added to reactor vessels in much the same way as in other industries discussed in this risk assessment e.g. thermal paper producers, can coatings producers. In some applications it may be added directly into calendering equipment. No further

information is available. As the process is similar to those mentioned above, the EASE figures for inhalation exposure and dermal exposure have been used to estimate a reasonable worst-case scenario for this use. For inhalation EASE predicts an exposure range of 0 to 1mg/m^3 for the activity. This is equivalent to an 8-hour TWA of 0 to 0.04 mg/m^3. For dermal exposure, EASE predicts an exposure range of 0 to 0.1 mg/cm^2/day.

A reasonable worst-case scenario for 8-hour TWA based on EASE is 0.1 mg/m^3 and 1 mg/m^3 for short-term exposure. A reasonable worst-case scenario of 0.1 mg/cm^2/day has been estimated for dermal exposure using EASE data. It is estimated that an area of skin equivalent to 420 cm^2 may be exposed during this activity.

4.1.1.1.7 Manufacture of liquid epoxy paints, lacquers and powder coatings

Epoxy paints

The potential for exposure to bisphenol-A during manufacture of liquid epoxy paints is negligible. Liquid epoxy resins, used to manufacture the paints, contain approximately 10 ppm residual bisphenol-A (personal communication), most of which is trapped within the resin matrix. Given this information the manufacture of liquid epoxy paint is unlikely to give rise to exposure to bisphenol-A.

Powder coatings

HSE occupational exposure data

Data were available for powder coating manufacture in HSE's NEDB (National Exposure Database). The data were for total inhalable particulate. These data were collected at plants that manufacture polyester paints which do not contain bisphenol-A. However, it is thought that the manufacturing techniques are not materially different and that the range of exposures to inhalable particulate will be similar across all powder paint manufacturing plants. The highest exposures were found for activities such as weighing and milling, although high exposures were found for all activities measured (weighing, mixing, extrusion, milling and packing and cleaning).

As the percentage of residual bisphenol-A in epoxy resins is ~300 ppm, with most of the residual bisphenol-A trapped within the epoxy resin matrix, a worst-case estimate of bisphenol-A exposure can be made.

The NEDB contained 28 results for total inhalable particulate during the manufacture of powder paints. These results are summarised in **Table 4.9**. These results were used to calculate a range of exposures to bisphenol-A during the manufacture of powder paints, given that the residual bisphenol-A in epoxy resin may be up to 300 ppm (personal communication - APME). The calculated results presented do not take into account the fact that most of the residual bisphenol-A in an epoxy resin is bound into the matrix, or that the powder paint contains substances other than the epoxy resin. This is because it is reasonable to assume that there will be some exposure to the epoxy resin itself prior to mixing, and this should be reflected in the reasonable worst case taken forward for risk characterisation.

Industry data

The epoxy-based coating powder manufacturing industry has supplied some personal exposure data. A total of 210 measurements were carried out, although the raw data was not reported. The range of personal exposures across four companies for all activities was 0.3 to 10 mg/m^3, 8-hour TWA for total inhalable particulate. Exposure to bisphenol-A has been estimated based on 300 ppm in the epoxy resin. The industry supplied data is lower than the HSE data. This may reflect recent improvements in control, although this is not clear from the information supplied. The results are in **Table 4.9**.

Table 4.9 Occupational exposure to bisphenol-A during manufacture of powder paints

No. of samples	Range of TIP results 8-hTWA (mg/m^3)	90th percentile TIP result 8-h TWA (mg/m^3)	Range of calculated exposures to BPA 8-h TWA (mg/m^3)	90th percentile calculated exposure to BPA 8-h TWA (mg/m^3)	Source
28	1.1 to 64	28	$3.3 \cdot 10^{-4}$ to 0.02	0.008	HSE
210	0.3 to 10	Not known	$9 \cdot 10^{-5}$ to $3 \cdot 10^{-3}$	Not known	Industry

The SPI were able to provide two results from the manufacture of powder coatings in the USA. Both personal samples were of 130 minutes duration. Although job titles were given there were no details of tasks covered by the sampling period, nor details of the sampling method provided. The powder handler had an exposure of 0.3 mg/m^3; no bisphenol-A was detected from the sample worn by the "Tape man". It is difficult to assess these short-term results as there are only two, but they do not appear to contradict the calculated results.

On the basis of the calculated results and the published results it is estimated that a reasonable worst-case 8-hour TWA would be 0.01 mg/m^3. A reasonable worst-case short-term exposure of 0.3 mg/m^3 has been estimated based on the results from SPI.

Modelled dermal exposure

Exposure to particulate during the manufacture of powder paints is reported to be generally poorly controlled, with often significant dermal exposure where personal protective equipment was not being worn. The use of dry brushing and the use of compressed air during cleaning activities are widely reported, as is poor control during activities such as milling and mixing. The EASE scenario which best fits these activities is non-dispersive use, direct handling with extensive contact. EASE estimates an exposure range of 1 to 5 mg/cm^2/day. As the bisphenol-A content of epoxy resins is so low (~300 ppm), the EASE prediction can be further refined to give an exposure range of $3 \cdot 10^{-4}$ to $1.5 \cdot 10^{-3}$ mg/cm^2/day. The figure of $1.5 \cdot 10^{-3}$ mg/cm^2/day is taken to be a reasonable worst-case dermal exposure. It is estimated that an area of skin equivalent to 1,300 cm^2 may be exposed during these activities.

4.1.1.1.8 Use of epoxy resin-based paints, lacquers, and powder coatings

Epoxy paints and lacquers

The potential for exposure to bisphenol-A during use of epoxy paints and lacquers is negligible. Liquid epoxy resins contain approximately 10 ppm residual bisphenol-A, most of which is trapped within the resin matrix. Epoxy resin-based paints may contain up to 40% epoxy resin.

Given the very low residual bisphenol-A in an epoxy resin, use of epoxy paint is unlikely to give rise to exposure to bisphenol-A.

Published occupational exposure data

One published paper has been found which details a US occupational hygiene survey where sampling for bisphenol-A was undertaken (NIOSH, 1984). Two part epoxy resins were used to encapsulate inductors in an electronics plant in the USA. No bisphenol-A was detected.

Powder paints

It is estimated that between five and ten thousand companies within the UK alone use powder paint technology, although it is thought that polyester-based paints are more widely used than epoxy resin-based powder paints.

The epoxy resins used are lower molecular weight solids, containing only a trace of bisphenol-A in the product (Peltonen et al., 1986a). The coating powders may contain up to 40% epoxy resin. The parts can be coated by dipping in a fluidised bed of powder or by electrostatic spraying. The paint is then cured by heating in an oven at a temperature of about 200^{o}C.

HSE occupational exposure data

The HSE's NEDB contains 53 results for total inhalable particulate exposure during the use of polyester powder paints. It is not thought that exposure to powder paint differs with the type of powder paint used. The results ranged from 0.2 to 131 mg/m^3, with high results being found for all the tasks measured (spraying, loading and cleaning). Even with such high results for total inhalable particulate, exposure to bisphenol-A would be negligible given the low levels of bisphenol-A in the powder paint. The range of exposure to bisphenol-A has been calculated using the figure for residual bisphenol-A in the epoxy resin powder (300 ppm) and the maximum figure for epoxy resin content in the coating powder, 40%. The results are summarised in **Table 4.10**.

Table 4.10 Occupational exposure to bisphenol-A during powder painting

No. of samples	Range of exposure to TIP (mg/m^3)	Range of exposure to BPA (mg/m^3)	90th percentile exposure to TIP (mg/m^3)	90th percentile exposure to BPA (mg/m^3)	Source
53	0.2 to 131	$2.4 \cdot 10^{-5}$ to 0.02	32	0.005	HSE

Published data

Only one published paper from the USA containing occupational exposure data for exposure to bisphenol-A during powder painting was found (NIOSH, 1979). A limited number of both personal and static samples for a variety of contaminants, including bisphenol-A, were collected during both application of powder paint using a fluidised bed, and spraying the parts. Local exhaust ventilation was in use in one plant (spray painting), although the results and observations reported indicate that the LEV was not working adequately. There was a history of skin, eye and mucous membrane irritation in several employees working in and around the powder paint dipping and spraying operations. The results of sampling are summarised in **Table 4.11**.

In Plant 1 (dip painting), there is one operator at the fluidised bed. His job is to knock the conveyor chain taking the parts from the fluidised bed to the curing oven in order to remove

excess paint from the part prior to curing. Each cycle takes about 3.5 minutes to complete. There is no LEV at this plant.

Table 4.11 Occupational exposure to bisphenol-A during powder coating operations

Area	Personal/static	Type of sample	Result 8-hour TWA (mg/m^3)
Plant 1	Dip painter	Total inhalable	0.005
	Dip painter	"	0.004
	Dip painter	Respirable	0.005
	Static on parts dipper hanger	Total inhalable	0.006
	"	"	0.005
	"	Respirable	0.005
	"	Total inhalable	0.005
Plant 2	Spray painter	Total inhalable	1.039
	"	"	1.063
	"	"	0.503
	"	"	0.243
	"	"	0.259
	"	"	0.173
	"	Respirable dust	0.008
	"	"	0.101
	"	"	0.026
	"	"	0.131
	"	"	0.052
	Touch up painter	Total inhalable	0.007
	"	Respirable	0.003
	Material handler	Total inhalable	0.003
	"	"	0.004
	Static outside spray station	"	0.042
	"	"	0.011
	"	"	0.007
	"	Respirable	0.002
	Static at wheel balancing area	Total inhalable	0.001
	"	"	0.004

In Plant 2 (spray painting), two spray paint operators spray parts moving along a conveyor in a ventilated booth. The parts then enter a curing oven for about 20 minutes.

The results for the range of exposures for spray painters were reported to be 0.173 to 1.063 mg/m^3 8-hour TWA (6 samples), when total inhalable dust samples were analysed for bisphenol-A and 0.008 to 0.131 mg/m^3 8-hour TWA when respirable dust samples were analysed for bisphenol-A. The range of exposure for dip painters was 0.004 to 0.005 mg/m^3 8-hour TWA

(2 samples), when total inhalable dust samples were analysed for bisphenol-A. Only one respirable dust sample was analysed for a dip painter and the result was 0.005 mg/m^3 8-hour TWA, the same as the total inhalable dust results.

Modelled inhalation inhalation data

EASE was used to estimate inhalation exposure for both dip painting and spray painting. There were only three dip painting results available so EASE was used to augment these results. The EASE parameters used were non-dispersive use, dry manipulation, without LEV. The EASE range estimated was 5 to 50 mg/m^3. When it is taken into account that there is a maximum of 120 ppm bisphenol-A in the coating powder, the exposure range becomes $6 \cdot 10^{-4}$ to $6 \cdot 10\text{-}^3$mg/m^3. The three real results obtained are within this range. Therefore a reasonable worst-case exposure of 0.005 mg/m^3 for this activity is estimated.

For spray painting the parameters used to estimate the exposure range were the same, as although LEV is usually present it often does not work effectively and EASE does not allow for distinction between effective and ineffective LEV. The estimated exposure range is therefore $6 \cdot 10^{-4}$ to $6 \cdot 10^{-3}$ mg/m^3. The 90th percentile of calculated exposures lies at the top end of this range. Taking into account the results reported from the US, professional judgement has been used to estimate a reasonable worst-case exposure of 0.5 mg/m^3.

Modelled dermal exposure data

As no dermal exposure data are available, EASE was used to model exposure. The parameters used were wide-dispersive use, direct handling with extensive contact. These parameters were felt to be representative as during dip painting there was no LEV and although there was LEV at the spray painting operation it was inadequate to control dermal exposure. The conditions described indicated extensive contact as clothing was observed to be visibly contaminated with the powder paint. The predicted exposure range was 5 to 15 mg/cm^2/day. Given that the bisphenol-A content in the coating powder is up to 120 ppm, the predicted exposure range becomes $6 \cdot 10^{-3}$ to $1.8 \cdot 10^{-3}$ mg/cm^2/day. A reasonable worst-case dermal exposure is estimated to be $1.8 \cdot 10^{-3}$ mg/cm^2/day. It is estimated that an area of skin equivalent to 1300 cm^2 may be exposed during these activities.

4.1.1.1.9 Manufacture of thermal papers

Several companies in Europe manufacture thermal papers using bisphenol-A. All the companies which responded to the request for information identified the loading of bisphenol-A into mixing vessels as the only source of potential exposure. Two companies had undertaken exposure monitoring but only one of these had specifically monitored for bisphenol-A.

Industry data

Company J reported that they had undertaken sampling specifically for bisphenol-A during the charging of bisphenol-A pellets from a bulk bag into a metal container, from which the bisphenol-A is transported within a closed system to a vessel containing a polymer solution. The operator has to couple the bulk bag to a filler cap on the metal container. This task takes approximately ten minutes to complete and may be carried out once or twice per shift. The company sampled for 1 hour, using a filter and dust sampling pump, pulling air at 2 litres per minute. The filter was subsequently analysed for bisphenol-A. The result was less than 1 mg/m^3. As this is the only time when exposure to bisphenol-A arises an 8-hour TWA can be calculated.

Using the worst case when the charging task would be carried out twice in one shift the 8-hour TWA would be less than 0.25 mg/m^3. A calculated short-term exposure (15 min) gave a result of less than 4 mg/m^3.

The company also reported the use of local exhaust ventilation, and that operators wear gloves, respirators and aprons.

Company K reported that they had undertaken dust monitoring during the charging of bisphenol-A into mixing vessels. Results were reported as being significantly below the MAK value. No further details were provided. The company also stated that gloves, aprons and dust masks were used by the operators and that LEV is in use at the loading point. Loading of bisphenol-A into mixing vessels would normally potentially expose three operators for approximately 10 minutes once or twice a day.

Modelled data

Inhalation

The EASE model was used to estimate inhalation exposure during the loading of bisphenol-A into vessels during the manufacture of thermal paper additive. The EASE scenario which best suits this activity is the use of low dust techniques in the presence of LEV. This resulted in a predicted exposure range of 0 to 1 mg/m^3. As it was reported that the task lasts 10 minutes and is carried out twice per shift an 8-hour TWA can be calculated. This gives a predicted 8-hour TWA range of 0 to 0.04 mg/m^3.

The modelled result is lower than that reported by the company. It is difficult to assess the validity of the reported result as there was only one result. It is reported as a "less than" figure, indicating that no bisphenol-A was detected on the sample and that the reported result is a function of the limit of detection of the method and the relatively short sampling period. Taking into account the scant data available, an estimation of a reasonable worst-case 8-hour TWA of 0.1 mg/m^3 was made.

Dermal

The EASE model was used to predict the dermal exposure during the charging of bisphenol-A into mixing vessels. The EASE scenario which best suits this activity is non-dispersive, direct handling with incidental contact, resulting in an exposure range of 0 to 0.1 mg/cm^2/day. The operators were reported to wear PPE. PPE, properly selected and worn will significantly reduce exposure. A reasonable worst-case dermal exposure is 0.1 mg/cm^2/day. It is estimated that an area of skin equivalent to 420 cm^2 may be exposed during this activity.

4.1.1.1.10 Manufacture of tin plating additive

Company G uses about 125 tonnes per annum bisphenol-A in the manufacture of tin plating additives. These additives go into tin plating baths treating tin to be used for packaging food and beverages. The manufacturing process is a batch process, with bisphenol-A being added to the reactor vessel manually. Twenty five 25 kg bags of bisphenol-A are charged into the vessel for each batch. There is usually one batch made per day. Two employees are involved in this process. It is estimated that charging takes about 5 minutes and is the only point at which the operators are exposed to bisphenol-A. The operators wear overalls, safety glasses, safety boots and helmet, disposable dust/organic vapour respirators and PVC gauntlets when charging the

vessel. There is also LEV above the charging point and from within the vessel itself. The only other potential for exposure arises during maintenance of the LEV, which is carried out every three months and is subject to a permit to work system where the potential for contamination is considered. No occupational exposure data were available.

Modelled data

Inhalation

Sufficient detail of the manufacturing process in Company G was available to model potential exposure. The EASE scenario which best fits the actual process is low dust techniques, a non-fibrous dust with LEV present. With an exposure time of 5 minutes per shift, this gives a short-term exposure (15 minutes) of 0 to 0.33 mg/m^3. As this is the only period in the shift where the potential for exposure to bisphenol-A exists, an 8-hour TWA range has been calculated at 0 to 0.01 mg/m^3. These predictions do not take into account the wearing of respiratory protective equipment which was reported by Company G. The EASE result which gave rise to these estimations was an exposure range of 0 to 1 mg/m^3.

An estimation for a reasonable worst-case 8-hour TWA has been made based on the EASE data. This figure is 0.05 mg/m^3 8-hour TWA. An estimation of a reasonable worst-case 15-min TWA of 0.3 mg/m^3 has been made.

Dermal

Dermal exposure to bisphenol-A was calculated during charging of the reactor vessel in Company G. The EASE scenario used was non-dispersive, direct handling with incidental contact. EASE gave a predicted exposure range of 0 to 0.1 mg/cm^2/day. The use of PVC gauntlets and overalls was reported by the company. PPE, properly selected and worn will significantly reduce exposure. A reasonable worst-case dermal exposure of 0.1 mg/cm^2/day has been estimated based on the EASE data. It is estimated that an area of skin equivalent to 420 cm^2 may be exposed during this activity.

4.1.1.1.11 Manufacture of tetrabrominated flame retardants (TBBA)

One company reported the use of bisphenol-A in the manufacture of TBBA. Bisphenol-A is delivered to the site in bulk tanker. The bisphenol-A is charged to site storage vessels by a pneumatic system using nitrogen. Once the transfer of bisphenol-A has taken place the pipeline is purged with nitrogen prior to disconnection to prevent exposure. The connection and disconnection of the transfer pipework takes about 10 minutes to complete. Bisphenol-A is transferred to the reaction vessel via a closed system with pneumatic transport. The manufacturing process also takes place in a closed system, so there is no opportunity for worker exposure. On completion of the manufacturing process a sample is taken from a sample loop with local exhaust ventilation. It was reported by the producer that the bisphenol-A content in the final product is about 3 ppm. It is unlikely that exposure to bisphenol-A would occur during sampling as the TBBA, which may contain up to 3 ppm bisphenol-A is in solvent solution, so the content of bisphenol-A will be lower than 3 ppm, and the process is controlled by use of LEV. It was reported that there is potential for worker exposure during maintenance and cleaning operations. Planned maintenance and cleaning takes place once per year. During these activities it is reported that personal protective equipment is worn. It was stated that safety shoes, helmet, safety glasses, gloves and respiratory protective equipment are worn when cleaning or handling a

spillage. Details of the type of PPE worn were not specified. In the event of a problem within the closed system it is reported by the Company that bisphenol-A would be emptied out of the system into bulk bags under LEV. One sampling result of 1-2 mg/m^3, total inhalable particulate (TIP) was reported. However, this sample was a static sample taken over a 24-hour period in the packaging area where the finished product is packaged. It is therefore unlikely to represent worker exposure to bisphenol-A.

Modelled data

EASE was used to estimate exposure to bisphenol-A during packaging of the final product into bulk bags. The parameters used were inhalable dust, dry manipulation with LEV present. This resulted in an exposure range of 2 to 5 mg/m^3. However the bisphenol-A content of the finished product, TBBA, is reported by the Company to be 3 ppm. This results in a predicted exposure range of $6 \cdot 10^{-6}$ to $1.5 \cdot 10^{-5}$ mg/m^3. The length of time spent on this activity was not reported by the company so it was not possible to determine a time-weighted average exposure. It is therefore assumed that this activity is continued for 8 hours and the 8-hour TWA range for this activity is $6 \cdot 10^{-6}$ to $1.5 \cdot 10^{-5}$ mg/m^3. The reasonable worst-case exposure is $1.5 \cdot 10^{-5}$ mg/m^3 8-hour TWA.

EASE was used to estimate the dermal exposure to BPA during packaging of the final product into bulk bags. The parameters used were direct handling, non-dispersive use with intermittent contact. This gave an estimated exposure range of 0.1 to 1 mg/cm^2/day. However, the content of BPA in the final product is reported to be 3 ppm, which when taken into account gave a dermal exposure range of $3 \cdot 10^{-7}$ mg/cm^2/day to $3 \cdot 10^{-6}$ mg/cm^2/day. The reasonable worst-case dermal exposure is $3 \cdot 10^{-6}$mg/cm^2/day. It is estimated that an area of skin equivalent to 420 cm^2 may be exposed during this activity.

EASE was not used to estimate inhalation or dermal exposure during potential spillages or maintenance as insufficient information was available and the circumstances in which exposures of this type could occur are likely to be variable.

4.1.1.1.12 General discussion on inhalation exposure

The results on which this assessment is based can be found summarised in **Table 4.12** (8-hour TWAs) and **Table 4.13** (short-term exposures).

This discussion follows the order in which the previous sections have appeared.

The bisphenol-A manufacturing process is largely an enclosed system with breaches for product sampling, product bagging and tanker/silo filling and some maintenance activities. Product sampling is a short-term activity typically lasting about 3 to 5 minutes, and may be carried out once or twice per shift. There were no short-term sample results available so EASE was used to estimate exposures during this activity giving a three-minute exposure range of 0 to 5 mg/m^3 and a short-term exposure level of 0 to 1 mg/m^3. Short-term results for bagging gave results of 14 and 15 mg/m^3, although these results are reported not to reflect the current occupational exposure. Data provided by SPI (USA) gave short-term task-specific results between nd and 0.96 mg/m^3. A reasonable worst-case scenario for short-term exposures is 10 mg/m^3.

8-hour TWA exposures for operators varied widely, both in the way they were sampled and analysed, and in the range of the results reported. Many operators measured total inhalable particulate or respirable dust, with some samples being analysed specifically for bisphenol-A. The results ranged from none detected (nd) to 23.3 mg/m^3 8-hour TWA. Product bagging and

tanker/silo filling were reported to be full-shift activities. Exposures for these activities were generally below 5 mg/m^3. All the EASE results predicted exposure ranges below 5 mg/m^3 for the above activities. The highest results were obtained where maintenance activities or cleaning were carried out during the sampling period, although information regarding the types of tasks carried out was not available. Sampling results for more recent maintenance activities (1998-2000) ranged from less than 0.05 to 0.62 mg/m^3. A reasonable worst-case scenario for 8-hour TWA for manufacturing activity would be 5 mg/m^3.

It was reported that there was little or no opportunity for exposure to bisphenol-A during the manufacture of polycarbonate, as the bisphenol-A entered the plant as a solution and was piped directly into a closed system. However, four respirable dust samples for PC dust had been collected in 1990-1991, although they were not analysed for bisphenol-A. Further dust sampling was undertaken from 1993 to 1996. These were for TIP and were not analysed for bisphenol-A. These results ranged from 0.1 to 1.1 mg/m^3. The 90th percentile for these figures was 1.0 mg/m^3. It was reported by industry that there is a maximum of 100 ppm residual bisphenol-A in the PC polymer. Taking this into account, the reported results range from $7 \cdot 10^{-7}$ to $1.1 \cdot 10^{-4}$ mg/m^3, 8-hour TWA with a 90th percentile of $1 \cdot 10^{-4}$ mg/m^3, 8-hour TWA. In 2000, the same company took a personal sample to confirm that there was no exposure to bisphenol-A in the PC manufacturing plant. The sample was analysed for bisphenol-A. The result was less than $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA. EASE modelling resulted in a range of 0 to $1 \cdot 10^{-4}$ mg/m^3, 8-hour TWA. A reasonable worst-case scenario for this activity would be $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA. There is reported to be no opportunity for exposure to bisphenol-A during the manufacture of articles from polycarbonate, due to the stability of the polymer, and the retention of any residual bisphenol-A within the polymer matrix. As the manufacturing process does not use any higher temperatures than those used for extrusion in the PC manufacturing industry, the same results have been used to represent exposure in the manufacture of articles from PC. The reasonable worst-case scenario is therefore $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA. A number of responses from companies manufacturing epoxy resins and modified epoxy resins highlighted the charging of vessels with bisphenol-A prills or flakes as the main source of exposure in this industry. Short-term exposures during this activity ranged from 0.32 to 17.5 mg/m^3, with 8-hour TWAs of up 1.2 mg/m^3. A reasonable worst-case scenario would be an 8-hour TWA of 0.7 mg/m^3. A reasonable worst-case scenario for short-term exposure would be 11 mg/m^3.

The use of bisphenol-A in PVC manufacture is being phased out. As handling of bisphenol-A is considered to be similar to industries such as thermal paper manufacturing, the EASE data for that scenario were used to generate data for PVC manufacturing. A reasonable worst-case scenario was estimated to be 0.1 mg/m^3 8-hour TWA. A short-term reasonable worst-case exposure is estimated to be 1 mg/m^3.

Manufacture of liquid epoxy resin-based paints is not reported to be a source of significant exposure to bisphenol-A given the very low (10 ppm) quantity of residual bisphenol-A in the uncured epoxy resin, most of which would be retained within the resin matrix.

The residual amount of bisphenol-A in epoxy resins for powder paints is reported to be about 300 ppm. Calculations made using this figure and total inhalable particulate exposure measurements from the HSE's NEDB, gave an estimated exposure of up to 0.02 mg/m^3, 8-hour TWA. Industry supplied data for personal exposure across all activities ranging from 0.3 to 10 mg/m^3, 8-hour TWA for total inhalable particulate. This is calculated to give a range of personal exposures to bisphenol-A of $9 \cdot 10^{-5}$ to $3 \cdot 10^{-3}$ mg/m^3. Given that the amount of residual bisphenol-A in powder paints is likely to be lower than that calculated, a reasonable worst-case scenario of 0.01 mg/m^3 8-hour TWA has been estimated. A short-term reasonable worst-case estimate of 0.3 mg/m^3 has been made based on data from SPI

Exposure to total inhalable particulate during the use of powder paints has been reported to be across a higher range than for manufacturing. The percentage of bisphenol-A in the coating powder is up to 40%. The estimated range of 8-hour TWAs is up to 0.02 mg/m^3. Actual measured exposure results were reported in a NIOSH paper. The range of 8-hour TWAs reported was 0.003 to 1.063 mg/m^3. A reasonable worst-case scenario for an 8-hour TWA is estimated to be 0.5 mg/m^3 for spraying coating powders and 0.005 mg/m^3 for dip-painting.

Thermal paper manufacturers reported only one exposure result for bisphenol-A, which was lower than the limit of detection for an hour-long sample. An 8-hour TWA calculated from this result gave a figure of less than 0.25 mg/m^3. Enough information was available to allow EASE estimations to be made. The estimated range predicted was 0 to 0.04 mg/m^3. A reasonable worst-case scenario for an 8-hour TWA for this industry is estimated to be 0.1 mg/m^3. A reasonable worst-case scenario for short-term exposure would be 4 mg/m^3.

Small quantities of bisphenol-A are used in the manufacture of tin plating additives. No exposure data were available but sufficient information was supplied to allow an EASE prediction to be made. This gave an exposure range of 0.02 to 0.05 mg/m^3 8-hour TWA, with the only source of exposure identified being the charging of the reactor vessel with bisphenol-A. A reasonable worst-case scenario would be an 8-hour TWA of 0.05 mg/m^3.

One company is currently manufacturing TBBA using bisphenol-A. No exposure data were available, but EASE was used to estimate exposure during the packaging process. This gave an estimated exposure range of $6 \cdot 10^{-6}$ to $1.5 \cdot 10^{-5}$ mg/m^3 8-hour TWA.

In summary, 8-hour TWAs rarely exceeded 5 mg/m^3 in bisphenol-A manufacturing facilities, and rarely exceeded 0.5 mg/m^3 in the other industries discussed. Short-term exposures could reach as high as 43.6 mg/m^3, but were more usually less than 10 mg/m^3.

Table 4.12 Summary table of occupational exposure data (8-hour TWA) used in this exposure assessment

Work activities	No of samples	Type of sample	Range 8-hour TWA (mg/m^3)	Mean 8-hour TWA (mg/m^3)	90th percentile 8-hour TWA (mg/m^3)	R W S exposure inhal. BPA (mg/m^3)	Source
Bisphenol-A manufacturing							
Sampling and filling (1988-1992)	24	respiration part.	0.04 to 5.01	0.59	1.23		Industry
Filling big bags (1998)	3	inhalation BPA	0.21 to 1.79	0.81	1.61		Industry
Filling silo tankers (1998)	3	inhalation BPA	less than 0.5 to 1.61	0.89			Industry
Various (1998)	8	inhalation BPA	0.13 to 0.62	0.3			
Various (1997)	8	TIP	less than 0.1 to 0.9	0.38	1.79		
Various (1993-1996)	15	TIP	less than 0.1 to 6	0.94			Industry
Filling (1988-1992)	4	TIP	0.42 to 1.79	1.1			
Packaging	9	TIP	0.002 to 7.5	1.1	n/a		Industry
Reworking	8	TIP	0.002 to 23.3	7.9	n/a		Industry
Plant operator	12	TIP	less than 0.1 to 0.8	0.3	n/a		Industry
Plant operator	13	*BPA	0.02 to 2.13	0.61	2.12		Industry
Maintenance operator	2	*BPA	0.04 to 2.08	1.06			Industry
Plant operator	7	inhalation BPA	0.21 to 1.04	not known	n/a		Industry
Maintenance	3	inhalation BPA	0.52 to 1.35	not known	n/a		Industry
Maintenance (1998-2000)	8	*BPA	Less than 0.05 to 0.62		n/a		Industry
Charging big bags	5	inhalation BPA	0.02 to 0.93	0.35	n/a		Industry
Various	not known	*BPA	nd to 2.6	not known	n/a		SPI (USA)
Operator incl. sampling	n/a	inhalation BPA	0 to 0.03	n/a	n/a		EASE
Product silo filling	n/a	inhalation BPA	0 to 1	n/a	n/a		EASE
Bag filling	n/a	inhalation BPA	0 to 5	n/a	n/a	5	EASE

Table 4.12 continued overleaf

Table 4.12 continued Summary table of occupational exposure data (8-hour TWA) used in this exposure assessment

Work activities	No of samples	Type of sample	Range 8-hour TWA (mg/m^3)	Mean 8-hour TWA (mg/m^3)	90th percentile 8-hour TWA (mg/m^3)	R W S exposure inhal. BPA (mg/m^3)	Source
PC manufacturing							
Plant operator	4	respiration part (BPA)	0.07 to 0.27 ($7 \cdot 10^{-7}$ to $2.7 \cdot 10^{-5}$)	0.2 ($2 \cdot 10^{-5}$)	n/a	$1 \cdot 10^{-3}$	Industry
Plant operator	16	TIP (BPA)	0.1 to 1.1 ($1 \cdot 10^{-5}$ to $1.1 \cdot 10^{-4}$)	0.43 ($4.3 \cdot 10^{-5}$)	$1 \cdot 10^{-4}$		Industry
Plant operator	not known	respiration part (BPA)	not known	Less than 0.1 (less than $1 \cdot 10^{-5}$)	Not known		Industry
Plant operator	1	inhalation BPA	n/a	Less than $1 \cdot 10^{-3}$	n/a		Industry
Plant operator	n/a	inhalation BPA	0 to 1 · 10-4	n/a	n/a		EASE
Manufacture of articles from PC							
Plant operator	n/a	inhalation BPA	0 to 1 · 10-4	n/a	n/a	$1 \cdot 10^{-3}$	EASE / Industry
Epoxy resin manufacturing							
Charging reactors	not known	various	less than 0.01 to 1.09	not known		0. 7	Industry
Various	96	*BPA	less than 0.1 to 2.8	0.24	0.7		SPI (USA)
Container unloading	n/a	inhalation BPA	0 to 0.25	n/a			EASE
Use of bisphenol-A in PVC manufacture							
Charging reactors	n/a	inhalation BPA	0 to 0.04	n/a	n/a	0.1	EASE
Manufacture of epoxy resin-based paints, lacquers, and coating powders							
Coating powders manufacturing							
Various	28	inhalation BPA	$3.3 \cdot 10^{-4}$ to 0.02 (calc)	0.01	0.008	0.01	HSE
Various	210	inhalation BPA	$9 \cdot 10^{-5}$ to $3 \cdot 10^{-3}$	not known	not known		Industry

Table 4.12 continued overleaf

Table 4.12 continued Summary table of occupational exposure data (8-hour TWA) used in this exposure assessment

Work activities	No of samples	Type of sample	Range 8-hour TWA (mg/m^3)	Mean 8-hour TWA (mg/m^3)	90th percentile 8-hour TWA (mg/m^3)	R W S exposure inhal. BPA (mg/m^3)	Source
Use of epoxy resin-based paints, lacquers and coating powders							
Coating powders use							
Spraying, loading, cleaning	53	inhalation BPA	$2.4 \cdot 10^{-5}$ to 0.02 (calc)	$1.6 \cdot 10^{-3}$	0.005	0.5	HSE
Spray painters	6	inhalation BPA	0.173 to 1.063	0.6			NIOSH
Spray painters	n/a	Inhalation BPA	$6 \cdot 10^{-4}$ to $6 \cdot 10^{-3}$	n/a	n/a		EASE
Dip painters	2	inhalation BPA	0.004 to 0.005	0.0045		0.005	NIOSH
Dip/spray painters	7	respiration BPA	0.003 to 0.131	0.04			NIOSH
Dip painters	n/a	inhalation BPA	$6 \cdot 10^{-4}$ to $6 \cdot 10^{-3}$	n/a	n/a		EASE
Thermal paper manufacturing							
Charging reactor	1	inhalation BPA	less than 0.25	less than 0.25		0.1	Industry
Charging reactor	n/a	inhalation BPA	0 to 0.04	n/a			EASE
Manufacture of tin plating additive							
Manufacture of tin plating additive - charging vessel	n/a	inhalation BPA	0.02 to 0.05	n/a		0.05	EASE
Manufacture of TBBA							
Packaging final product	n/a	inhalation BPA	$6 \cdot 10^{-6}$ to $1.5 \cdot 10^{-5}$	n/a		$1.5 \cdot 10^{-5}$	EASE

TIP total inhalable particulate
Inhal. BPA inhalable bisphenol-A
(BPA) calculated BPA concentration in particulate
Inhalation Part inhalable particulate
Resp. part. respirable particulate

Table 4.13 Summary table of short-term, task specific occupational exposures to bisphenol-A used in this exposure assessment

Work activities	No of samples	Range (mg/m^3)	Mean (mg/m^3)	RWS exposure (mg/m^3)	Source
Bagging machine operator 1990	2	14 to 15	14.5	10	Industry
Bisphenol-A manufacturing Various	15	nd to 0.96	not known		SPI (USA)
PC manufacturing Connecting bisphenol-A chargepoint	6	nd to less than 0.64	0.29	0.5	SPI (USA)
Epoxy resin manufacture-charging reactor	12	0.32 to 17.5 (inhalable dust)	1.52	11	Industry
Epoxy resin manufacture various	68	nd to 43.6	1.81		SPI (USA)
Manufacture of coating powders	2	Nd to 0.3	0.15	0.3	SPI (USA)
Use of bisphenol-A in PVC manufacture – charging reactors	n/a	0 to 1	n/a	1	EASE
Thermal paper manufacture charging reactor	1	less than 4	less than 4	4	Industry

4.1.1.1.13 General discussion on dermal exposure

The results of dermal exposure predictions can be found in **Table 4.14**.

Dermal exposure to bisphenol-A can occur during manufacturing and use of bisphenol-A. During manufacturing operators can come into contact during product sampling and during bag filling and other filling operations. Using the EASE model, dermal exposure during sampling was estimated to be in the range 0 to 0.1 $mg/cm^2/day$. Exposure is likely to be towards the lower end of the range as the activity takes less than five minutes to complete. It is estimated that 420 cm^2 of skin may be exposed during this activity.

Filling operations are full-shift activities, so the potential for dermal exposure is greater. The EASE estimation gave a range of 1-5 $mg/cm^2/day$. The operators are reported to wear personal protective equipment, including gloves. PPE, properly selected and worn will significantly reduce exposure. A reasonable worst-case exposure is 1 $mg/cm^2/day$. It is estimated that 420 cm^2 of skin may be exposed during this activity.

The only potential for dermal exposure during PC manufacturing was during the bagging of PC granules. The EASE estimation gave a range of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ $mg/cm^2/day$.

The same exposure range was used to estimate exposure during the manufacture of articles from PC, when loading PC granules from the big bags to the extruder.

The main source of exposure identified during epoxy resin manufacturing was the charging of reactors. The EASE estimation gave a range of 0.1 to 1 $mg/cm^2/day$. The use of PPE during this task was reported. PPE, properly selected and worn will significantly reduce exposure.

Estimations of dermal exposure during two maintenance activities were carried out using EASE as an illustration of the potential dermal exposures during general maintenance activities. The EASE prediction gave a range of 0.1 to 1 $mg/cm^2/day$ for both activities.

For PVC manufacturing, a reasonable worst-case scenario of 0.1 mg/cm^2/day has been estimated for dermal exposure using EASE data. It is estimated that an area of skin equivalent to 420 cm^2 may be exposed during this activity.

EASE was used to predict dermal exposures during the manufacture and use of epoxy resin-based powder coatings. Although controls are generally poorer in these industries, the potential for exposure is lower due to the small amount of residual bisphenol-A in the epoxy resin (approximately 300 ppm). The range of dermal exposure predicted using EASE during epoxy resin-based powder coating manufacture was $3 \cdot 10^{-4}$ to $1.5 \cdot 10^{-3}$ mg/cm^2/day. The figure of $1.5 \cdot 10^{-3}$ mg/cm^2/day is taken to be a reasonable worst-case dermal exposure. The range estimated using EASE for powder coating application was $6 \cdot 10^{-4}$ to $1.8 \cdot 10^{-3}$ mg/cm^2/day given a maximum bisphenol-A content of 120 ppm. Charging reactors was the only activity identified by the thermal paper manufacturers and the tin plating additive manufacturers where the potential for dermal exposure arises. This activity takes about 5 to 10 minutes per shift. EASE was used to estimate a range of dermal exposure. The range predicted was 0 to 0.1 mg/cm^2/day.

Dermal exposure during bag filling of TBBA was estimated using EASE. The range predicted, taking into account the fact that there is only 3 ppm BPA in the final product, is $3 \cdot 10^{-7}$ mg/cm^2/day to $3 \cdot 10^{-6}$ mg/cm^2/day.

In summary, dermal exposure was estimated to be highest during filling operations during bisphenol-A manufacture, which is a full-shift activity. The estimated range of exposures were the same for charging reactors and maintenance activities during epoxy resin manufacturing, but these tasks were shorter lived, so exposures are likely to be lower. The lowest dermal exposure range predicted was for PC manufacturing, which has a very low percentage of residual bisphenol-A.

Table 4.14 Summary table of estimated dermal exposures using EASE

Work activities	Extent of area of dermal contamination	Range of dermal exposures (mg/cm^2/day)	RWS for dermal exposure (mg/cm^2/day)
Bisphenol-A manufacturing			
Product sampling	420	0 to 0.1	0.1
Bag filling/other filling operations	420	1 to 5	1
Manufacture of PC			
Bag filling of PC granules	420	$1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
Manufacture of articles from PC			
Loading PC granules from big bags	420	$1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
Epoxy resin manufacturing			
Charging reactors	420	0.1 to 1	1
Maintenance - changing filter socks	840	0.1 to 1	1
Maintenance - emptying weigh vessel	840	0.1 to 1	1
Use of bisphenol-A in PVC manufacture			
Charging reactors	420	0 to 0.1	0.1
Manufacture of coating powders			
Manufacturing	1,300	$3 \cdot 10^{-4}$ to $1.5 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$

Table 4.14 continued overleaf

Table 4.14 continued Summary table of estimated dermal exposures using EASE

Work activities	Extent of area of dermal contamination	Range of dermal exposures (mg/cm²/day)	RWS for dermal exposure (mg/cm²/day)
Use of coating powders			
Powder coating	1,300	$6 \cdot 10^{-4}$ to $1.8 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$
Thermal paper manufacturing			
Charging reactors	420	0 to 0.1	0.1
Manufacture of tin plating additive			
Charging reactor	420	0 to 0.1	0.1
Manufacture of TBBA			
Bag filling	420	$3 \cdot 10^{-7}$ to $3 \cdot 10^{-6}$	$3 \cdot 10^{-6}$

4.1.1.2 Consumer exposure

As indicated in Section 2.1, the EU usage of bisphenol-A is estimated to be approximately 690,000 tonnes/year. The largest quantities are used in the production of polycarbonates and epoxy resins, which have many applications in consumer goods, such as food contact containers, adhesives and protective coatings. A description of the major uses of bisphenol-A is given in Section 2.2.2.

In these consumer applications, bisphenol-A is contained within or generated from a polymer matrix. Potential consumer exposure can therefore arise only under conditions where residual monomer in the polymer matrix becomes available for exposure or where breakdown of the polymer occurs, to generate additional monomer which is available for exposure. Under certain conditions, for example, at elevated temperature or extreme pH, hydrolysis of the polymer may occur, resulting in the regeneration of bisphenol-A from the polymer and thus increasing the amount of bisphenol-A which may be available for exposure. The products that are likely to have the potential for the highest exposure of consumers to bisphenol-A are those that are used in applications which involve direct contact with foodstuff. These include food and beverage containers which have epoxy resin internal coatings, and polycarbonate tableware and bottles, such as those used for infant formula milk. Exposure to bisphenol-A arising from use of these products is determined by the migration of bisphenol-A from the polymer into the food with which it is in contact, under the particular conditions of use. Migration of bisphenol-A from these products into food or beverages stored in them may occur if conditions are created which allow hydrolysis of the polymer during food or beverage storage or if there is residual monomer in the polymer. Consumption of the food or beverage will then result in ingestion of bisphenol-A. Inhalation and dermal exposure is considered to be negligible.

Other relatively minor sources of consumer exposure to bisphenol-A that are considered in this consumer exposure assessment arise from its use in dental fissure sealants and in epoxy-based surface coatings and adhesives. The use of bisphenol-A in dental fissure sealants will result in oral exposure. For epoxy-based surface coatings and adhesives, the main route of exposure is dermal.

Other uses of bisphenol-A, such as in printing inks and thermal paper, are considered to result in negligible potential for consumer exposure in comparison with the other sources considered and therefore will not be addressed further in this assessment.

4.1.1.2.1 Bisphenol-A polycarbonate

Food contact applications

There are many applications of polycarbonates which involve direct contact with food. These include returnable beverage bottles, infant feeding bottles, tableware such as plates and mugs and food-storage containers. These main uses will be considered in this exposure assessment. A number of studies have been conducted which investigate the potential consumer exposure to bisphenol-A as a result of using these products. These studies have addressed the potential for exposure to residual bisphenol-A contained within the polycarbonate and have also explored the conditions which are necessary to initiate hydrolysis of the polymer to generate bisphenol-A which is then available for migration. Summaries of the relevant studies are given below.

A well reported, preliminary study to investigate the migration of bisphenol-A from commercially available polycarbonate baby feeding bottles was conducted by Earls et al. (2000). The study is unpublished but the full report was available to the rapporteur. The study was conducted using 21 new unused polycarbonate bottles purchased from retail outlets, and 12 used (reported to be <1-2-year-old) polycarbonate bottles obtained from staff using the on-site crèche of the research facility. The approximate length of use to the nearest year and the method of cleaning/sterilisation of the used bottles were recorded.

The study was designed to simulate realistic worst-case conditions for baby bottle cleaning, feed preparation and storage. The solutions used in the study to simulate normal bottle contents were those proposed in the draft European standard for childcare articles: "Drinking Equipment, Part 2 – Chemical requirements and tests". Boiling water was used to represent milk, and 3% (v/v) glacial acetic acid solution, fruit juice.

All bottles were pre-washed using a steam steriliser. Boiling water (100 ml) or 3% acetic acid solution prepared with fresh boiling water (100 ml), was added to freshly cleaned/sterilised bottles. The bottles were sealed immediately and placed at 1-5°C (refrigerator) for 24 hours. On removal from the fridge the bottles were heated to approximately 40°C by immersion in boiling water. A 1 ml sample of the water or acetic acid solution was then taken for bisphenol-A analysis. Analysis was carried out using diode array high performance liquid chromatography (HPLC). The method had a detection limit of 10 ppb (10 μg/l).

There was no detectable migration of bisphenol-A into either water or acid from any of the new polycarbonate bottles. For the used bottles bisphenol-A was detected in water and/or acetic acid samples from five of the twelve bottles, the levels measured ranging from 20-50 ppb (20-50 μg/l). However, there was no apparent correlation between the concentrations of bisphenol-A measured in the bottle contents and estimated age of the bottle, cleaning method or simulant used in the test.

Overall, the results indicate that migration of bisphenol-A from used polycarbonate bottles into the bottle contents can occur under the realistic worst-case conditions of this study. The results suggest that migration of bisphenol-A leading to detectable levels in the bottle contents occurs only in previously used bottles, with up to 50 ppb (50 μg/l) detected in the bottle contents. However, no conclusions can be drawn with respect to the conditions of use or cleaning and the degree of leaching of bisphenol-A from used bottles.

Mountfort et al. (1997) conducted a study into the potential degradation of polycarbonate baby bottles during sterilisation with consequent release of bisphenol-A. The residual bisphenol-A content in the polycarbonate matrix of twenty-four brands of plastic baby feeding bottles was

determined. The report states that there was no correlation between the molecular weight of the polymer and residual bisphenol-A concentration. It was also found that the residual content of bisphenol-A may vary between different batches of the same brand of bottle. The brand that had the greatest concentration of residual bisphenol-A, when measured in the initial analysis, was selected for further study. Bottles of this brand were newly purchased in quantity, to ensure that all bottles had the same batch code.

To mimic general use of the bottles, migration of bisphenol-A into the bottle contents following three methods of sterilisation was investigated (chemical, dishwashing and steam). Chemical sterilisation involved hypochlorite sterilant used according to manufacturers' instructions. Baby bottles were immersed in sterilant for 2 hours at ambient temperature and then rinsed three times with water, the rinse water being retained for analysis. Infant feed formula, prepared according to manufacturers' instructions, was then added to the rinsed bottle, shaken for 15 seconds, and heated in a microwave for 30 seconds. The feed was then allowed to stand for 20 min and a sample was taken for analysis. This process (sterilisation and feed preparation) was then repeated and samples were taken for analysis after 3, 10, and 20 cycles. After every fourth sterilisation the bottle was left to stand in sterilisation solution overnight. Dishwashing sterilisation followed the same procedure, with the bottles sterilised in a domestic dishwasher using a proprietary detergent. The rinsings from the machine were collected for analysis. Steam sterilisation again used the same procedures, and the bottles were steamed for approximately 9 min. Following steam sterilisation bottles were allowed to cool before filling with feed and the residual water in the steam steriliser was kept for analysis. Triplicate bottle samples were used for each procedure. Bottles containing distilled water were also kept at 40°C for 10 days and the water analysed for bisphenol-A content. The samples were analysed using HPLC methods with a limit of detection at 30 ppb (0.03 mg/kg).

After twenty cycles of chemical sterilisation, steam sterilisation or dishwasher washing, bisphenol-A was not detected in the feed or in the bottle rinsings. No detectable levels could be found in the distilled water samples stored at 40°C for 10 days. Thus, in this study, as in the previous study, no detectable bisphenol-A is found in newly purchased bottles.

The above study was extended by Mountfort (1997). Based on the initial stage of the above study where the residual bisphenol-A content of the bottles was determined, three were chosen for further study: the bottles containing the highest, median and a low level of bisphenol-A monomer. A further twenty bottles of each brand were purchased, ensuring they had the same batch code (the batch codes were different from those tested originally). Analysis revealed that residual bisphenol-A content varied between batches. The bottles were subjected to the same testing regimes as those described above, with the limit of detection for bisphenol-A in infant formula and in fruit juice at 0.03 mg/kg, and the limit of detection for dishwasher rinsings lower at 0.0012 mg/kg (due to a preconcentration step by evaporation). Up to fifty repeat cycles were tested after chemical, steam, or dishwasher sterilisation. Bisphenol-A was not found in any formula or fruit juice samples (<0.03 mg/kg) or in bottle rinsings (<0.0012 mg/kg). Bottles were also tested before and after twenty cycles, for migration into distilled water held in the bottle for ten days at 40°C; there was no measurable migration into the water.

In an unpublished study by Hanai (1997), for which only brief details are available, the migration of bisphenol-A from infant feeding bottles was investigated. The study included six commercially available infant feeding bottles (age not stated). The bottles were filled with purified water (26ºC) and allowed to stand at room temperature for five hours or filled with hot (95ºC) water and allowed to stand at room temperature overnight, after which time samples were

taken for analysis. Analyses were performed using gas chromatography/mass spectroscopy (GC/MS), with a detection limit of 2 ppb.

No detectable levels of bisphenol-A were found in water samples from bottles filled at 26ºC and analysed after five hours. Levels ranging from 3.1-55 ppb were detected in overnight samples of water from bottles filled at 95ºC. These latter values are consistent with those reported by Earls et al. (2000) for used bottles.

Simoneau et al. (2000) carried out a study looking at the migration of bisphenol-A from baby bottles into various food simulants. Information from this study is limited as the only details are from a poster presentation. The migration of bisphenol-A from 48 intact bottles from the same batch was measured in various simulants (3% aqueous acetic acid, 10% or 95% aqueous ethanol, olive oil, water or methanol 100%) and time/temperature conditions, with horizontal shaking used to simulate worst-case scenarios. The bottles were half filled with the simulant, then agitated on a horizontal shaker at 140 cycles/min, at 50°C. The simulant was evaporated, redissolved in ethanol (15%) and analysed by reverse phase HPLC and fluorescence detection; the detection limit was 10 ppb.

Results show that there was no detectable migration of bisphenol-A observed for water, 3% aqueous acetic acid, 10% aqueous ethanol or olive oil. However migration was detected in methanol (100%), which decreased over time, indicating that the simulant was having a degradation effect on the bottle, and ethanol (95%); the latter gave consistent results. Following this initial study, the authors undertook a larger European study using 95% ethanol as the simulant. For the European study, 163 bottles were purchased from supermarkets and pharmacies from all Member States. The results for this study showed migration levels ranging from non-detectable to 110 ppb. This value is approximately 2-fold higher than the highest values measured in other studies. However, migration was only detected using 95% ethanol as the food simulant; no migration was detected when simulants representative of the normal bottle contents were used–water or 3% acetic acid. Therefore the results from this study are not considered to be representative of the normal conditions of use of these bottles.

A study undertaken by Kawamura et al. (1998) investigated the migration of bisphenol-A from polycarbonate products. The study looked at the migration of bisphenol-A from children's tableware and infant feeding bottles. The study included 14 samples of new, unused tableware. Four of the samples tested (rice bowl, mug, soup cup and dish) were products that had been recalled from the Japanese market because the plastic contained residual amounts of bisphenol-A and other phenols in excess of 500 ppm. The remaining 10 samples represented products that are commercially available in Japan (4 infant feeding bottles, 3 mugs, 2 rice bowls and 1 measuring cup).

Various test conditions were employed during this study. In most cases, samples were washed in water prior to testing, although some samples were tested without washing. Four food simulants were used: n-heptane, water, 4% acetic acid and 20% ethanol solution. Some tests were conducted with the simulant at elevated temperature (60 or 95°C). A boiling treatment was included, in which the item was placed in boiling water for five minutes. For some samples, migration tests were repeated up to five times, and the sample was thoroughly washed in water prior to each repeat test. Migration of bisphenol-A into the food simulant was measured usually after 30 minutes contact time of the product with the food simulant. The solutions were analysed for bisphenol-A by HPLC, with a quantification limit of 0.6 ppb (the detection limit was not stated, but is assumed to be three tenths of the quantification limit, i.e. 0.2 ppb).

For the four samples of products that had been removed from the market, migration of bisphenol-A led to a resultant concentration in the food simulant of 40 ppb (40 μg/kg) or less for all tests under a range of conditions. Migration was greatest when n-heptane was used as the food simulant. For the six samples of commercially available products, migration of bisphenol-A resulted in concentrations in the food simulant of 5 ppb or less for three samples, with no detectable levels recorded for the other samples.

The results of the repeat migration tests indicated that migration rate decreased with repeated testing. For example, a repeat test conducted on one of the samples of products withdrawn from the market, showed an initial level of 27 ppb bisphenol-A in food simulant, falling to 1.5 ppb after five test cycles. Similarly, in tests where the product sample was not washed before testing, migration of bisphenol-A led to concentrations in the food simulant which were 2-7-fold higher (up to 38 ppb) compared with the results for a comparable product sample which had been washed prior to testing. These results suggest that bisphenol-A which is available for migration is present at the surface of the samples, and thus is removed by washing.

In relation to the infant feeding bottles, the only test condition under which there were any detectable levels of bisphenol-A in the bottle contents was when boiling water was used as the food simulant. In an unwashed bottle filled with boiling water and kept at room temperature, 3.9 ppb bisphenol-A was detected in the water analysed after 30 minutes. In washed bottles, the highest detectable level of bisphenol-A was 0.7 ppb, measured in water following microwave heating of the bottle for 10 minutes. In a test in which the bottle was filled with boiling water and kept at room temperature for 24 hours, 0.5 ppb was detected in the water analysed after 24 hours, but no detectable levels were found after 30 minutes.

Overall, this paper shows bisphenol-A migration from polycarbonate tableware products into food simulants, with concentration levels of up to 40 ppb being recorded from products no longer sold and levels of up to 5 ppb from commercially available products. The highest level of bisphenol-A found in the contents of infant feeding bottles was 3.9 ppb, measured in an unwashed bottle, filled with boiling water. The study suggests that migration potential decreases with washing and continued use of the product.

Biles et al. (1997) conducted a study for the determination of bisphenol-A in reusable polycarbonate food-contact plastics and its migration to food simulating liquids. The food simulants used were water, ethanol solutions (8%, 10%, 50% and 95%) and Miglyol® (a fractionated coconut oil). HPLC and GC/MS were used for analysis of bisphenol-A in the polycarbonate and in food simulants. The limit of detection in ethanolic simulants and water was 2 ppb (2 μg/l), and for fruit juices, infant formula and Miglyol® was 100 ppb (100 μg/l). Baby bottles and a training cup were purchased, representing at least six different manufacturers, and water carboys (19 l capacity) were also obtained for analysis.

Four migration experiments were performed on the baby bottles, representing “exaggerated”, “repeat use”, “typical use”, and “more extreme typical use”, with extreme time and temperature conditions. Under “exaggerated” conditions, samples of the bottles were cut, placed in contact with food simulant, heated to 65°C in an oven and agitated. Samples of food simulant were removed for analysis every 24 hours for a total of 10 days. Under “repeat use” conditions, pieces of bottle were placed with food simulant in a glass vial. These were placed in an oven at 100°C for 30 min, the simulant removed, the sample and vial rinsed with ethanol, then the vial filled with fresh simulant. This process was repeated for four 30 min cycles. Under “typical use” conditions, a whole bottle was filled with either infant formula or apple juice and refrigerated at 4°C for up to 72 hours. Samples of liquid were removed for analysis at regular intervals; 1 g of the threaded portion of the bottle was removed for analysis of residual bisphenol-A in the

polycarbonate. For the "more extreme typical use" conditions, pieces of a bottle were placed in a vial with 20ml of water or 10% ethanol/water solution. The vials were heated in an oven at 100°C for 30 min and then cooled to room temperature. A sample of the bottle contents was removed before the vials were placed in a refrigerator (4°C) and further samples were taken after 48 and 72 hours. Migration of bisphenol-A into distilled water stored in water carboys for up to 39 weeks was also determined (temperature of experiment not stated).

Levels of bisphenol-A in the food or food simulant were presented as a percentage of the residual level measured in the plastic; actual concentrations were not reported. Under "typical use" conditions, using whole bottles and thus most representative of normal use, bisphenol-A was not found at detectable levels in either infant formula or in fruit juice. Under "exaggerated use" conditions, migration was found in excess of the residual bisphenol-A measured in the bottles, suggesting that breakdown of the polymer had occurred. For "repeat use" conditions, the levels of bisphenol-A in the food simulant were found to decrease significantly after the initial use. Under the "more extreme" conditions any migration occurred during the 100°C sterilisation step, with little or no bisphenol-A migration during the 72-hour refrigeration. For water stored in a carboy, it was found that migration increased as contact time increased. Under conditions where migration occurred, the concentration of bisphenol-A in food simulants ranged from 13-368% of the bisphenol-A residue levels determined in the plastic.

Howe and Borodinsky (1998) completed a study of the potential exposure to bisphenol-A from food contact use of polycarbonate resins. The study was conducted in accordance with procedures developed by the US Food and Drug Administration (1995), and used their recommended food simulating solvents, time and temperature conditions.

The tests were designed to simulate the heating of food in the container under conditions that would mimic expected shelf life. Tests were carried out on discs prepared from an equal blend of three commercial food-contact grade polycarbonate resins provided by three US manufacturers.

The analyses of the samples were carried out using HPLC, with a detection limit reported to be equivalent to 5 ppb. Each set of tests was performed in triplicate. Tests were carried out on polycarbonate samples using water, 10% ethanol and Miglyol® as the simulants, kept at 100ºC for 6 hours; on samples using water, 3% acetic acid and 10% ethanol at 49ºC for 6 hours, 101 hours and 240 hours; and samples using Miglyol® as the simulant, kept at 49ºC for 6 hours, 96 hours and 240 hours. All of the samples provided results below the limit of detection of the method used in this study.

A 1998 Japanese study was carried out in Yokohama, to determine the migration of bisphenol-A from polycarbonate tableware used in schools. The study was unpublished with no test report available. The only information on this study was taken from a press release. A total of 186 tableware samples were included in the study. Two methods were used to evaluate bisphenol-A migration: one involved the use of food samples (water, soup, olive oil) and the other used 4 food simulants (n-heptane, water, 20% ethanol and 4% acetic acid). No information is given on the method of analysis of bisphenol-A, nor the detection limits of the method.

The food or food simulants were placed in new and used (1-3 years use) dishes. To mirror actual use, the dishes were sterilised after washing, in a hot air sterilising chamber at 85ºC for 30 minutes. No information was provided on the length of time the food was in contact with the tableware, or on the temperature of the food or food simulant. In tests using food samples, no bisphenol-A was detected in any sample, from either new or used dishes. For tests using food simulants, bisphenol-A was detected within a range of 0.6-1.0 ppb, from eight out of a total of sixty tableware samples.

Limited information is available for an unpublished study, which investigated the relationship between migration of bisphenol-A from polycarbonate plastic containers and temperature (Takahashi, 1998). The only information available is from an excerpt of a Japanese publication, the original of which cannot be traced and therefore it is not possible to verify the reliability of this information. Two infant feeding bottles and one mug were filled with 200 ml of water and heated to 25°C, 50°C, 76°C or 95°C. The levels of bisphenol-A in the water were determined after 30 minutes. Migration of bisphenol-A resulted in levels in the water which increased with water temperature. For the mug, levels of bisphenol-A in the water after 30 minutes were reported to increase from 0.056 ppb at 50°C to 0.76 ppb at 95°C; for the bottles, levels were reported to be 0.0008-0.018 ppb at 50°C and 0.8-2.0 ppb at 95°C. No results are quoted for tests at 25°C. The levels of bisphenol-A quoted in this study are quite low in comparison with limits of detection that have been reported for other more reliable studies and thus raises questions about their validity. Overall, no conclusions can be drawn from this study.

A study to investigate the migration of bisphenol-A into milk from returnable polycarbonate bottles was conducted by Bayer AG (1999c). Only a summary of this study is available. Milk samples taken from 24 returnable polycarbonate bottles obtained from a dairy were the subject of the study. The bottles were in regular use and were reported to be on average on the fifth return cycle. Twenty-four milk-filled bottles were stored for one month at refrigerator temperature (2-8°C) prior to analysis. (The milk was presumably sterilised, although this is not stated in the summary report). The milk samples were analysed by HPLC with a fluorescence detector and a detection limit of 1 ppb (1 μg/kg). Analysis of the milk showed no detectable migration of bisphenol-A.

Summary of studies of migration from polycarbonate

There is relatively limited good quality information on the levels of bisphenol-A in food and drink resulting from migration from polycarbonate tableware. A small number of studies have been conducted to measure bisphenol-A concentrations in the contents of polycarbonate infant feeding bottles. Two of these studies have measured levels of up to about 50 ppb (50 μg/l; 0.05 mg/kg assuming a density of 1 g/ml) bisphenol-A in the food simulant contents of used bottles, in tests which represent realistic worst-case exposure conditions. This value will be used as the basis for calculating consumer exposure for this scenario.

In relation to polycarbonate tableware and food storage containers, a number of well-reported studies have found no detectable levels of bisphenol-A in the food or drink contents of the tableware. Where detectable migration levels have been reported, the data derive from reports of limited detail and reliability and in studies in which food simulants have been used; migration into actual foodstuffs has not been detected. The highest reported level of bisphenol-A in food simulants detected as a result of migration from polycarbonate tableware is 5 ppb (5 μg/kg; $5 \cdot 10^{-3}$ mg/kg). Although there is some uncertainty about the reliability of this value, it will be used as the basis of calculating consumer exposure for this scenario.

Using these values, estimates of daily ingestion of bisphenol-A can be calculated. **Table 4.15** shows the estimates of daily ingestion for infants, arising from the use of polycarbonate feeding bottles. Estimates are derived for infants aged 1-2 months and 4-6 months. The estimates for daily intake of milk are taken from MAFF (1998).

Table 4.15 Estimates of infant ingestion of bisphenol-A from the use of polycarbonate feeding bottles

Age of baby	Daily intake of milk (l)	Concentration of bisphenol-A in milk (μg/l)	Daily ingestion of bisphenol-A (μg/day)
1 – 2 months	0.699	50	35
4 – 6 months	0.983	50	50

These values of 35 μg/day (0.035 mg/day) for a 1-2 month baby and 50 μg/day (0.05 mg/day) for a 4-6 month baby will be taken forward to the risk characterisation.

For exposure arising from the use of polycarbonate tableware, the most realistic scenario is considered to be that of a young child, for whom the total daily food and drink intake may be taken from polycarbonate tableware. The total daily intake of food and drink for a young child (1.5-4.5 years) is estimated to be 2 kg. This value is based on UK data for the consumption of solid and liquid food by young children, and represents the 97.5^{th} percentile consumption (HMSO, 1995). Therefore, assuming that the concentration of bisphenol-A in the foodstuff is 5 μg/kg ($5 \cdot 10^{-3}$ mg/kg), total daily ingestion of bisphenol-A is 10 μg (0.01 mg/day). This value will be taken forward to the risk characterisation.

4.1.1.2.2 Bisphenol-A epoxy resins

Bisphenol-A based epoxy resins are formulated with curing agents to yield high-performance crosslinked coatings. Heat cured epoxy coatings are, due to their favourable properties such as toughness, adhesion and chemical resistance, used as protective linings for metal sanitary cans to maintain the quality of canned food and beverages.

Epoxy resins with differing molecular weights have different applications. High molecular weight epoxy resins are used in heat cured protective interior coatings for food and beverage containers; liquid and low molecular weight epoxy resins are typically used in ambient cured industrial protective coatings, adhesives, floorings or fillers. The majority of exterior coating applications are industrial and therefore negligible consumer exposure is expected. However, there are some consumer applications for these products and therefore these scenarios will be addressed in this exposure assessment.

Food contact applications

Epoxy resins are used as binders in protective linings in food and beverage cans and in wine storage vats. Migration levels from epoxy coatings are governed by a variety of parameters such as coating composition, coating weight, curing conditions, sterilisation time and temperature and type of foodstuff. Carbonated soft drinks are the predominant type of beverage distributed in cans. These cans are typically filled at room temperature, and stored at or below room temperature. Canned foods are mostly sterilised at high temperatures, up to 135°C. The sterilisation time will vary, with shorter residence times for higher temperatures. Typically, sterilisation at 120°C is performed for 90 minutes. The canned foods are subsequently stored at room temperature.

Approximate coating weights for typical beverage cans are 250 mg/330 ml (1.06 mg/cm^2) for a tinplate can and 125 mg/330 ml for an aluminium can; for food cans, coating weight may vary between 0.4 and 2.5 mg/cm^2 (Nehring Institute, 1998).

A number of studies which have investigated migration of bisphenol-A from epoxy resin coated cans and a single study of migration into wine vats, are available and are summarised below.

A study of the migration of bisphenol-A from epoxy resin into canned food is available (Howe et al., 1998; The Society of the Plastics Industry, 1995). The study was conducted in two phases (at different points in time) to investigate bisphenol-A migration from a total of 18 cans selected as being representative of the US market. The samples fell into three categories, "2-piece" beverage/beer cans (n = 3), "2-piece" food cans (n = 5) and "3-piece" food cans (n = 10), with coating formulations containing the maximum levels of bisphenol-A-epoxy in commercial use. Testing was conducted to simulate or exaggerate the most severe conditions of actual use, in accordance with US FDA recommendations. Solutions of 10% and 95% ethanol were used as food simulants to mimic aqueous based food or drink and fatty foodstuffs respectively; high temperature treatment was used to simulate pasteurisation of can contents. Various testing regimes were performed, each involving an initial high temperature phase in which cans were filled with 10% or 95% ethanol heated to 66-120°C and maintained at this temperature for 30 minutes or 2 hours, followed by a 10-day period during which the can temperature was maintained at 49°C.

The ethanol solution from each can was analysed for bisphenol-A content after the initial temperature phase (i.e. after 30mins or 2hrs) and after 10 days. In the first phase of the study, analysis of these ethanol solution samples was carried out using HPLC with fluorescence detection. The second phase of the study used HPLC and GC/MS. For each phase, the method had a detection limit of 5 ppb.

The first phase of the study showed no detectable levels of bisphenol-A in ethanol solutions taken from the beer/beverage cans, at either time point. Analysis of ethanol solutions from the "2-piece" and "3-piece" food cans gave results ranging from no detectable levels of bisphenol-A, to levels up to 120 ppb. The average concentration of bisphenol-A in the ethanol solution samples from each type of food was 63 ppb.

This initial phase of the study brought to light the possibility that other substances may be interfering with the analysis of bisphenol-A, resulting in higher than expected results. Thus, the second phase, identical in protocol to the first, was undertaken to establish the analytical methods and to remove the possibility of interference. This second phase gave results ranging from no detectable bisphenol-A to 77 ppb in the ethanol solution sampled from food cans from the same manufacturers as those used in the first study and 12–94 ppb for additional cans included from other manufacturers.

Overall, this study shows that under conditions that simulate the pasteurisation process used during the canning of food or drink, some migration of bisphenol-A into the contents of food cans can occur; no migration into the contents of drinks cans was detected. The second phase of the study, which is considered to provide more reliable measurements of bisphenol-A, showed that levels up to 94 ppb (94 μg/kg) are detectable in food can contents.

The UK Food Standards Agency (FSA) conducted a survey of bisphenols in canned food (FSA, 2001). The study was carried out to establish whether migration of bisphenol-A occurs into retail samples of canned food in the UK. This study looked at migration into the actual food contents rather than using a food stimulant. The study used the following canned samples, with numbers of each sample type in parentheses: vegetables (10); beverages (11); fish in aqueous media (10); soup (10); desserts (5); infant formulae (4); fruit (2); pasta (5) and meat products (5). Three cans of each sample, with the same batch number, were purchased from retail outlets in the south of England. The samples were weighted so that approximately eighty percent were from

supermarkets, which included around forty percent "own brand" foods to reflect consumer shopping habits.

A method for determining bisphenol-A in the food was developed using gas chromatography-mass spectroscopy (GC/MS) with a detection limit of 0.002 mg/kg (2 ppb), where the bisphenol-A was acylated using acetic anhydride after isolation from the food by solvent extraction methods. Samples were spiked with known amounts of standard, blank samples were used and calibration curves constructed to check the validity of the results obtained. Where bisphenol-A was quantified at 0.007 mg/kg (7 ppb) or greater then the result was checked by searching for the presence of a m/z ion at 213 on the mass spectroscopy spectrum.

Bisphenol-A was detected at up to 0.07 mg/kg (70 ppb) in 37 of the 62 samples, and at 0.35-0.42 mg/kg (350-420 ppb) in one sample. The remaining samples showed no detectable levels of bisphenol-A. Four different types of infant formula were tested and none of these showed any detectable limits of bisphenol-A. Canned beverage samples tested also showed no detectable levels of bisphenol-A. The sample that showed the highest level of bisphenol-A content was a sample of canned meat. The FSA has since been informed that the manufacturer of this particular product has already replaced the coating system that resulted in the high level of contamination, by a system that is more compliant with minimising potential bisphenol-A migration. Therefore the highest level of bisphenol-A detected in canned foods from this survey will be taken as 0.07 mg/kg (70 ppb).

Kawamura et al. (1999) in a well reported study, presented information looking at the migration of bisphenol-A from can coatings into drinks. The drink samples consisted of a total of 47 canned beverages: coffee (13), black tea (9), other teas (8), alcoholic beverages (10) and soft drinks (7), all the samples were purchased in Tokyo. The study investigated the bisphenol-A content of the commercial canned beverages and the effect of the can coating material and storage at 60°C on migration. Solid phase extraction was used to extract the samples for testing. The samples were tested using gas-chromatography/mass spectrometry GC/MS and Fourier transform infra-red spectroscopy (FT-IR) with attenuated total reflectance crystal attached (IR-ATR).

The contents of the drinks cans were first tested for the presence of bisphenol-A. To test for effects due to elevated storage temperatures, the cans were emptied and thoroughly rinsed with water. An amount of simulant corresponding to the volume of the can contents was heated to the test temperature, placed in the container and covered in aluminium foil. Migration tests were then run for 30 minutes at 95°C using water and acetic acid (4%) as simulants and for 30 minutes at 60°C with ethanol (20%). If n-heptane was used the test was run for one hour at 25°C. The limit of detection was 2.0 ng/ml (0.002 μg/ml; $2 \cdot 10^{-3}$ mg/l; $2 \cdot 10^{-3}$ mg/kg) for the drink samples or 1.0 ng/ml (0.001 μg/ml; $1 \cdot 10^{-3}$ mg/l; $1 \cdot 10^{-3}$ mg/kg) for the food simulants used.

For coffee beverages, 11 of the 13 samples showed concentrations in the beverage of 3.3-213 ng/ml (average ~50ng/ml; 0.05 mg/kg). For black tea beverages, bisphenol-A was detected at 8.5-90 ng/ml (0.009-0.09 mg/l) in 4 of the 9 samples. Other tea samples showed concentrations of 3.7-22 ng/ml (0.004-0.02 mg/l) in five of the eight samples tested. Alcoholic beverages showed only one detectable level at 13 ng/ml (0.01 mg/l) in sake; heating of the sake for 30 min did not increase the amount of bisphenol-A detected. Bisphenol-A was not detected in the seven soft drink samples.

Given that the canned coffee and tea drinks which show a high bisphenol-A content are sometimes sold from storage at 55-60°C in automatic vending machines in Japan and because of variations in the degree of contact with the different materials coating the cans, further tests were carried out. A coffee and tea specimen were stored for 4 weeks in an incubator at 60°C in the

upright position with the lid facing up and in an inverted position with the lid facing down. There was no significant difference in the amount of bisphenol-A found to migrate from storage under these conditions. Migration tests were also carried out on empty cans from the same specimens using the water, acetic acid, ethanol and n-heptane simulants as described previously. No detectable bisphenol-A was found in n-heptane, and no more than 2.5 ng/ml (0.003 mg/l) was found in the water, acetic acid or ethanol.

A study was carried out to investigate potential xenoestrogens including bisphenol-A released from lacquer coatings in food cans (Brotons et al., 1995). The samples consisted of twenty different brands of canned foods purchased in supermarkets in Spain and the USA. (The cans were originally packed in Brazil, France, Spain, Turkey and the United States). The liquid was taken from cans containing foods such as green beans, corn, peas and mushrooms and analysed for bisphenol-A. For canned fatty food types such as condensed soup, the contents were emptied and the cans refilled with bidistilled water. The cans were then autoclaved for 25 minutes at 125°C. For some cans, this process was repeated. Extracts of the water from the cans then analysed to determine estrogenic activity; analysis of bisphenol-A content was also undertaken.

Samples of the can contents (the original liquid content of the can or distilled water following autoclaving) were analysed by HPLC and mass spectroscopy. The detection limits of the methods were not given. Levels of bisphenol-A found in samples of the contents from the cans ranged from not detectable to 4.2–22.9 μg/can. In cans filled with water and autoclaved, bisphenol-A was measured in the water from some cans undergoing repeated autoclaving.

In this study the weight of simulant/food contents containing the highest amount of bisphenol-A (22.9 μg) was 0.3 kg, resulting in a concentration of 80 μg/kg canned food (80 ppb).

Overall, three studies provide consistent evidence for migration of bisphenol-A from epoxy resin linings of food cans into the can contents. Two of these studied migration under conditions which represent the sterilisation process which would normally occur. Migration of bisphenol-A in these studies results in levels of up to about 70-90 ppb (70-90 μg/kg) in the can contents, from studies using fatty foods or simulants which mimic fatty foods. As migration is likely to be greatest into fatty foods, these results are considered to be representative of realistic worst-case conditions. Rounding this up, a value of 100 ppb (100 μg/kg; 0.1 mg/kg) bisphenol-A in the contents of a typical food can will be used in the calculation of total daily ingestion of bisphenol-A in this scenario. This value will be taken forward to the risk characterisation. For alcoholic beverage cans, the only detectable levels were found in sake. As this alcoholic beverage is not relevant to the EU market, this result will be disregarded for the purposes of this risk characterisation. For soft drink/beverage cans, results from three studies indicate no detectable bisphenol-A in the can contents using analytical methods with a minimum detection limit of 2 ppb (2 μg/kg; 0.002 mg/kg). Given that the pasteurisation conditions applied to beverage cans for soft and alcoholic drinks is less ‘severe’ (in terms of temperature and time), and given that the epoxy resin lining of beverage cans is thinner than that of food cans it is considered reasonable to assume that the results of this study, showing no migration from beverage cans, can be generally applied. Therefore no value for bisphenol-A in canned soft or alcoholic beverages will be taken forward for risk characterisation.

In addition, one study has found detectable levels of bisphenol-A in hot canned beverages (canned tea and coffee). Highest levels were found in the contents of canned coffee, with an average level of 50 ng/ml (50 ppb; 0.05 mg/l). The availability of these beverages in the EU is unknown. However, consumption is likely to be low. In view of this, and given that consumption of canned food will dominate the risk characterisation, this value will not be taken forward.

A study into the migration of constitutive monomers, including bisphenol-A, from epoxy resins used as coating materials for wine vats was carried out by Larroque et al. (1989). The study investigated the influence of different factors on the migration of the monomers and also the extent of migration. Different wine simulants were used to account for factors which could influence migration, such as pH and alcoholic strength. Experiments were carried out at room temperature. The wine samples were analysed using gas chromatography with a flame ionisation detector and HPLC with a fluorimetric detector, with a detection limit of 200 ppb (0.2 mg/l).

Three series of experiments were performed. In series 1, three types of epoxy based coatings were manually applied (100 μm layer) to glass media and allowed to set before testing. In this series, the base epoxy resin and hardening agent were used in equal quantities as recommended by manufacturer. The wine simulant was in contact with the resin for about 4 years. In series 2, the resin was applied in the same way, but an excess or deficiency of hardening agent was used. Contact time was 1 year. Series 3 used the same resins in the correct ratio of base to hardening agent, applied mechanically onto aluminium plates (1 mm layer). Contact time was about 3 years.

Results for bisphenol-A migration were expressed in terms of mg bisphenol-A migrant/kg coating. The results for studies in series 1 showed the greatest levels of bisphenol-A migration, in the range ~30-160 mg bisphenol-A/kg resin after 4 years. In series 2, migration of bisphenol-A was unaffected by the amount of hardener used. Levels of bisphenol-A determined after 1 year was ~1-13 mg bisphenol-A/kg resin. In series 3, migration was generally below the limit of detection. However, detectable levels of bisphenol-A were found in two of the wine simulants, 20% aqueous alcohol and an alcoholic solution containing tannic acid. Migration into these two simulants was 0.7 and 1.8 mg bisphenol-A/kg resin.

The authors calculate that based on a level of bisphenol-A migration of 100 mg/kg resin, then for a 1,500 l vat lined with 10 kg resin, the amount of bisphenol-A in the wine will be 650 ppb (650 μg/l).

No other information is available on this exposure scenario. Given the conditions of the single study available (newly applied resin, with extended contact time), it is likely that the level of migration and resultant estimated levels of bisphenol-A in the wine contents of the vat will be over-estimated, although it is not known to what extent.

An alternative exposure assessment has been supplied by industry based on the following assumptions, that the coating contains ca. 50% epoxy resin and that all the bisphenol-A will leach out. The calculation for a 1,000 l container is as follows:

1,000 liter container	(10 · 10 · 10 dm)
- Area: 6 · 10 · 10 =	600 dm²
- Coating thickness	250 μm
- Density varying from	1.4-2.2 g / cm³
- Resulting in weight of coating per dm²	3.5-5.5 g
- Binder % in coating	30-50 %
- Resulting in binder per dm²	1.75 g
- 600 · 1.75 = binder	1,050 g
- Max 10 ppm BPA in the binder 10.5 mg BPA / 1,000 =	0.01 mg / kg
- 0.01 mg BPA per liter wine 10 ppb BPA per liter wine	

However, given that this approach is based on purely theoretical calculations, at this stage, the measured data will be used for the purposes of risk characterisation, although it must be recognised that this will be a very worst-case estimate of exposure. In addition, the use of epoxy-lined wine vats in the EU is limited – the majority of wine is reported to be stored in uncoated stainless steel vats. The value of 650 ppb (650 μg/l) wine will be used as the basis for the calculation of total daily ingestion of bisphenol-A for this scenario.

Summary of food contact applications

Table 4.16 provides the estimates of daily ingestion of bisphenol-A, as a result of food contact applications of epoxy-resins. Intake for adults is based on consumption of one bottle (0.75 l) of wine per day and consumption of all other food and drink from canned sources. Based on UK data, the estimate of total daily food and drink consumption for an adult is 4.5 kg (of which 2 kg is expected to be water); this represents the 97.5th percentile of consumption (HMSO, 1990). Of the 2.5 kg of food consumed daily, there is uncertainty in relation to the average consumption of canned food. In the absence of reliable data for canned food consumption an estimate of consumption must be made.

There are two possible approaches to this: one approach is to estimate average *per capita* consumption based on industry data for EU food can production and the EU population. Using this approach, the average number of cans consumed per person per day is:

$20.6 \cdot 10^9$ cans produced per year/$377 \cdot 10^6$ = 54 cans per person per year
= 0.1 cans per person per day

The second approach would be to base the consumption figure on data recommended by the Scientific Committee on Food (SCF). The recommendation by the SCF is '…that a person may consume daily up to 1 kg of food in contact with the relevant food contact material' (see http://europa.eu.int/comm/food/fs/sc/scf/out82_en.pdf). It is not stated whether this 1 kg relates to a 90th percentile or higher of the population of eaters.

From these two alternative approaches, the SCF recommendation gives a more conservative estimate of 1 kg canned food consumption per person per day and this value will be used in the risk characterisation. A combined adult intake for consumption of wine and all other food is also given.

Intake is also calculated for infants aged 6-12 months, for whom a high quantity of food may come from canned products. Intake is calculated for young children, in the age group 1.5-4.5 years. This age group has been chosen to represent the group with the highest potential food intake per kg bodyweight. In calculating bisphenol-A intake for infants, estimated intake of canned food is based on UK survey data, which indicated that the 97.5th percentile daily consumption of canned foods of the type which could contain a source of bisphenol-A, for this age group (including baby foods) is 0.375 kg (FSA, 2001; HMSO, 1992). In calculating intake for young children, there is no reliable information on canned food intake. The only information available is an estimated daily intake of food and drink, again based on 97.5 percentile values obtained from UK data (HMSO, 1995). Therefore, for the purposes of risk characterisation, a value of 2 kg for total intake is assumed. It should however be noted that as this intake includes drink and assumes that all food could come from sources resulting in bisphenol-A exposure, it will result in an overestimate of actual intake, although the degree of overestimation is unknown.

Table 4.16 Estimates of daily ingestion of bisphenol-A from food contact applications of epoxy-resins

Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of bisphenol-A in wine (μg/l) or food (μg/kg)	Daily ingestion of bisphenol-A (μg/day)
Wine	0.75	650	500
Canned food (infant 6-12 months)	0.375	100	40
Canned food (young child 1.5-4.5 years)	2	100	200
Canned food (adult)	1.0	100	100
Canned food + wine (adult)	0.75 l wine 1.0 kg food	650 μg/l wine 100 μg/kg food	600

The value of 500 μg/day (0.5 mg/day) for ingestion of bisphenol-A resulting from consumption of wine will be carried forward to the risk characterisation.

The values of 100 μg/day (0.1 mg/day) for an adult, 200 μg/day (0.2 mg/day) for a young child and 40 μg/day (0.04 mg/day) for an infant, for ingestion of bisphenol-A resulting from the consumption of canned food will be carried forward to the risk characterisation as a worst-case scenario. In addition, a combined adult intake of 600 μg/day (0.6 mg/day), for consumption of wine in addition to food, will be carried forward.

4.1.1.2.3 Other applications

Marine antifouling paints

Marine antifouling paints are used in the consumer sector for the protection and decoration of yachts and boats. The paints are applied by brush or roller. In the UK these paints are typically applied once per year. There are some measured data on consumer exposure arising from the brush application of these products (Garrod et al., 2000).

Using estimates of exposure to the paint product, potential exposure to bisphenol-A can be calculated based on its content in the product. Information from industry indicates that the maximum level of epoxy-resin in such paints is 40% (w/w). Liquid epoxy-resins used to manufacture the paints contain approximately 10 ppm residual bisphenol-A (Section 4.1.1.1.7). The amount of marine antifouling paint used per event ranges from 1.75-5 litres, with a median value of 4 litres (Garrod et al., 2000).

Calculations of bisphenol-A exposure as a result of brush application of antifouling paints are detailed in the table below, based on a paint containing 40% epoxy-resin and a residual level of 10 ppm bisphenol-A in the resin. The estimates of total inhalation and total dermal exposure to the product are based on Garrod et al. (2000). These estimates do not include any contribution to exposure from cleaning the application equipment. Exposure occurs via the inhalation and dermal routes. With respect to inhalation exposure brushing or rolling generates an aerosol. Although exposure to bisphenol-A vapour would be low in these applications (because of low vapour pressure), exposure to bisphenol-A in an aerosol is possible.

Table 4.17 Estimates of exposure to bisphenol-A for application of marine anti-fouling paints

Application method/PPE	Application time (hours)	Total airborne concentration of product (mg · m^{-3})	Total dermal exposure to paint (mg)	Inhalation exposure to BPA [1),2)] (μg)	Dermal exposure to BPA [1)] (μg)
Brushing/T-shirt and shorts, no gloves	1.5	0.04	7,335	$3 \cdot 10^{-4}$	29
Brushing/gloves and overalls	1.5	0.04	140	$3 \cdot 10^{-4}$	0.6

1) Assuming 40 % resin in paint, 0.001 % (10 ppm) residual bisphenol-A in resin
2) Assuming a breathing rate of 10 m^3 in 8 hours

The values of $3 \cdot 10^{-4}$ μg ($3 \cdot 10^{-7}$ mg) for inhalation exposure and 29 μg (0.03 mg) for dermal exposure to bisphenol-A per event, resulting from brush application of paint without protective clothing will be taken forward to the risk characterisation.

Wood varnish

There are no data on consumer exposure arising from the application of wood varnish. However, measured data are available for the professional application of wood preservatives (Garrod et al., 2000). Given that the application methods are similar, these data are likely to be representative of the exposure arising from the application of wood varnish and therefore have been used to derive consumer exposure estimates for this scenario. As before, estimates of exposure to bisphenol-A are calculated on the basis of its content in the product; resin content is 40% w/w, with a residual level of bisphenol-A in the resin of 10 ppm. Exposure occurs via the inhalation and dermal routes. The amount of wood varnish used per event ranges from 1.0-8.5 litres, with a median value of 4 litres (Garrod et al., 2000).

Estimated exposures for brush application of wood varnish, with and without protective clothing (PPE) are given in the table below. The estimates of total inhalation and total dermal exposure to the product are based on Garrod et al. (2000). With respect to inhalation exposure brushing or rolling generates an aerosol. Although exposure to bisphenol-A vapour would be low in these applications (because of low vapour pressure), exposure to bisphenol-A in an aerosol is possible.

Table 4.18 Estimates of exposure to bisphenol-A for brush application of wood varnish

PPE	Application time (hours)	Total airborne concentration of product (mg · m^{-3})	Total dermal exposure to paint (mg)	Inhalation exposure to BPA [1,2)] (μg)	Dermal exposure to BPA [1)] (μg)
None (T-shirt and shorts)	2.5	1.63	903	0.02	3.6
Gloves and overalls	2.5	1.63	44	0.02	0.18

1) Assuming 40 % resin in varnish, 0.001 % (10 ppm) residual bisphenol-A in resin
2) Assuming a breathing rate of 10 m^3 in 8 hours

The values of 0.02 μg ($2 \cdot 10^{-5}$ mg) for inhalation and 3.6 μg (0.0036 mg) for dermal exposure to bisphenol-A per event, for brush application without the use of protective clothing and gloves, will be carried forward to the risk characterisation.

Wood fillers

Bisphenol-A is present in some wood fillers sold for consumer use. Information provided by industry indicates that a typical product on the market contains approximately 20% of epoxy resin. In the UK, approximately 800 kg of wood filler is sold per year.

Wood fillers sold in the UK are designed to be kneaded by hand before use. The product instructions recommend that gloves be worn during kneading, to prevent skin contact. However the use of appropriate gloves cannot be assumed. Therefore exposure estimates for handling wood filler are given for this scenario with and without the use of gloves. In this scenario, it is assumed that the product contains 20% resin with a residual bisphenol-A content of 10 ppm. Exposure occurs to the hands only.

Table 4.19 Estimates of dermal exposure to bisphenol-A for handling wood filler

PPE	Surface area exposed (cm^2) [1]	Volume of product on skin/gloves (cm^3) [2]	Mass of product on skin (mg) [3, 4]	Dermal exposure to BPA [5] (μg)
None	215	4.3	4,300	9
Gloves	215	4.3	215	0.4

1) Assuming 20% of the total surface area of both hands (1075 cm^2) is potentially exposed
2) Assuming 20% of product is transferred to the skin, to form a layer 0.1 cm thick
3) Assuming product density is 1 g/cm^3
4) Assuming glove efficiency is 95%
5) Based on 20% resin in wood filler, 0.001 % (10 ppm) residual bisphenol-A in resin

The value of 9 μg (0.009 mg) bisphenol-A per event, resulting from the handling of wood filler without gloves, will be taken forward to the risk characterisation.

Adhesives

Epoxy resin based adhesives are available to consumers. These adhesives are sold in "2-pack" systems. Potential dermal exposure to residual bisphenol-A in the epoxy resin can therefore arise from consumer use of these 2-pack products. In 2-pack adhesives, residual bisphenol-A content is less than 1 ppm. Based on a residual level of 1 ppm bisphenol-A in the adhesive, dermal exposure to bisphenol-A arising from the use of adhesives is calculated to be 0.014 mg per event (see **Table 4.20**).

Table 4.20 Estimates of dermal exposure to bisphenol-A for handling adhesive

PPE	Surface area exposed [1] (cm^2)	Volume of product on skin/gloves [2] (cm^3)	Mass of product on skin [3] (mg)	Dermal exposure to BPA [4] (μg)
None	54	1.1	1,075	1

1) Assuming 5% of the total surface area of both hands (1,075 cm^2) is potentially exposed
2) Assuming 20% of product is transferred to the skin, to form a layer 0.1 cm thick
3) Assuming product density is 1 g/cm^3
4) Assuming 1 ppm (0.0001%) residual bisphenol-A in resin

4.1.1.2.4 Dental fissure sealant

Bisphenol-A is a component of restorative materials such as fissure sealant, used in dentistry. It is not an active ingredient in any dental sealant or composite, but derivatives of bisphenol-A used in dentistry include bis-glycidyldimethacrylate (bis-GMA) and bisphenol-A-dimethyl acrylate (bis-DMA). Bisphenol-A may be present as an impurity in these substances, or may be formed as a result of degradation. It has been demonstrated that bisphenol-A can be released from sealants which contain bis-DMA but not those containing bis-GMA (Schmalz et al., 1999). Sealants consist of an organic resin matrix, whereas resin based composites (or fillings) consist of an organic resin matrix with an inorganic filler. According to information from the British Dental Association, filled composites would result in substantially less exposure than sealants, possibly because they contain proportionally less resin. Most sealants contain only bis-GMA.

Consumer exposure occurs during the polymerisation process following application of the resin. The resin matrix is initially present as a fluid monomer that is converted into a rigid polymer by a free radical initiated addition. Once applied to tooth cavities, composites and sealants are polymerised *in-situ*; the polymerisation reaction may be initiated chemically or by photo-initiation using UV or visible light. The degree of formation of oligomers into polymers varies depending on the composition of the resin and its distance from the tooth surface. Conversion of 60-75% is expected with most common composites. Lower levels of conversion may be associated with greater migration of free components from the composites (Ferracane and Condon, 1990).

The available studies which investigate bisphenol-A migration from dental fissure sealants and composites are summarised below.

Hamid and Hume (1997a) studied the release of components from resin pit and fissure sealants *in vitro*. The fissure systems of ten extracted molar teeth were filled with sealant, cured and then placed in separate containers of distilled water (stored at 4°C). Each sample was moved to fresh containers of water at defined times (4, 14, 43, 144 and 432 min; 1, 3 and 10 days), and the water samples analysed using HPLC, with a limit of detection of 70-90 ppb. Bisphenol-A was not detected in any samples.

A study into the cytotoxicity and leaching of substances from four light-cured resin based pit and fissure sealants was carried out by Geurtsen et al. (1999). Only the results for the leaching tests are reported here. Due to complexities involved with HPLC separations, the authors used gas chromatography/mass spectrometry (GC/MS) to study the leaching of components from sealants, or where decomposition had already occurred, GC/MS was used to study the decomposition products. The limit of detection was not quoted.

For each sealant product, fifteen equally sized samples (5mm diameter; surface area 19.63 mm^2) were prepared according to the manufacturers' instructions, and polymerised for 60 s in glass moulds. Three specimens from each product were placed in 5ml of distilled water for 24 hours, after which time GC/MS analysis was carried out on the water to determine migration products. All water samples were tested twice and the mean error did not exceed 10%.

Various sealant components were identified in the water analysed after 24 hours, including co-monomers, initiators and co-initiators. However no bisphenol-A, bis-GMA or UEDMA was detected in any of the water samples analysed. The results from this study support the data published by Hamid and Hume (1997).

Nathanson et al. (1997) studied the *in vitro* elution of leachable components from dental sealants. Seven commercially available light-cured pit and fissure sealants were studied. Samples were cured using a method to mimic clinical conditions and then weighed and transferred to a test-

tube for analysis. Samples were then placed in 95% ethanol for four minutes. The ethanol was analysed for bisphenol-A using HPLC; no limit of detection was quoted. No detectable levels of bisphenol-A were found in any of the samples; bis-DMA was found in two of the samples.

An abstract by Moon et al. (2000) reports the leaching of components, including bisphenol-A, from dental pit and fissure sealants. The purpose of the study was to identify and quantify bisphenol-A, triethyleneglycol dimethacrylate (TEGDMA), UEDMA and bis-GMA that could be released from seven commercially available resin based pit and fissure sealants. Sealants were cured in a 1·5 mm acrylic mould and were placed in 75% ethanol or artificial saliva for 1, 7, 14, 21 and 28 days, after which time the ethanol or saliva was analysed for migration products by HPLC. No detection limits were given.

In artificial saliva, only TEGDMA migrated out of the sealant. All components analysed for, except bisphenol-A, were found in ethanol or saliva at relatively high concentrations at the early time point, and so the levels detected were not linearly related to immersion time. In 75% ethanol the amount of bisphenol-A detected varied with sealant product and was reported to range between 0.023–2.790 μg/mg. It is not clear from the abstract whether these values refer to μg per mg ethanol solution or per mg of sealant material. There was no correlation between the concentration of bisphenol-A and bis-GMA. Bis-GMA did not degrade to bisphenol-A during the 28-day immersion period. The authors conclude that the 75% ethanol solution penetrates the resin matrix, maximising the migration of resin components.

Schmalz et al. (1999) conducted a study to investigate bisphenol-A content of resin monomers and related degradation products in dental sealants. The aim of the study was to analyse the bisphenol-A content of different fissure sealant resin monomers and the release of bisphenol-A under hydrolytic conditions. The monomers studied were bis–DMA and bis-GMA. The study was divided into four parts. In part 1, the bisphenol-A content of pure bis-GMA, bis-DMA and bisphenol-A diglycidylether (BADGE; an intermediate product of bis-GMA synthesis) was analysed. In the second part, bis-GMA and bis-DMA were subjected to chemical hydrolysis at a pH range of 0–11. In the third part, bis-GMA and bis-DMA were subjected to hydrolysis by porcine liver esterase, and in the fourth part, bis-GMA and bis-DMA were placed in unstimulated pooled saliva of six healthy subjects with no history of periodontal disease or recent restorations.

Bisphenol-A was analysed by HPLC using tetrahydrofuran/methanol/0.1M phosphoric acid in a ratio of 2:1:1 (v/v/v) as the mobile phase. The detection limit was reported as $1\text{-}10^4$ ppm. As this is a range, it is presumably a typographical error and the actual detection limit is unclear, although detection limits for some individual elements of the study were also reported. Detection was by a spectrofluorophotometer, with excitation wavelength set at 275 nm, and emission 300 nm.

Part 1 of the study showed that bis-GMA and BADGE samples from one manufacturer had no detectable amounts of bisphenol-A (detection limit $\leq$ 2 ppm) whilst bis-DMA and BADGE-monomer samples from a second manufacturer showed detectable levels ranging from 4 to 155 ppm of bisphenol-A. In part 2 of the study, no bisphenol-A (detection limit $\leq$ 1%) was found in bis-GMA after hydrolysis at pH 0-11. For bis-DMA, bisphenol-A was detected in samples at pH 11, with 99.8% conversion of bis-DMA into bisphenol-A. Using porcine liver esterase, there was no conversion of bis-GMA to bisphenol-A, however bis-DMA converted, with a conversion of 82.5%. In samples placed in saliva, again, there was no detectable conversion of bis-GMA. After 24 hours, 81.4% of bis-DMA was converted to bisphenol-A.

The results of this study show that no bisphenol-A could be identified in commercially available bis-GMA monomers at the given detection limits. However, bisphenol-A was found as a contaminant in BADGE and bis-DMA. In tests to investigate the degradation of bis-DMA and

bis-GMA to bisphenol-A, under hydrolytic conditions, only bis-DMA was found to degrade under conditions of chemical (pH) and biological (esterases, saliva) hydrolysis.

The authors conclude that based on these results, where bisphenol-A is reported to migrate from dental sealants, this may be attributed to the bis-DMA content of the sealant, which may degrade to bisphenol-A under hydrolysis conditions; bisphenol-A is not expected to be released from fissure sealants based on bis-GMA, if pure monomer is used.

A study was undertaken to look at the estrogenicity of resin-based composites and sealants used in dentistry (Olea et al., 1996). The study was initiated due to concern that bisphenol-A monomer migrates from the resin and can be swallowed. To determine bisphenol-A and related compounds in saliva, eighteen healthy male and female dental patients (aged 18-25; average age 20) were asked to spit into a flask for one hour before and one hour after the application of a sealant to a molar. Approximately 50 mg sealant was applied across the surface of 12 molars for each subject. The sealant contained both bis-GMA and bis-DMA. HPLC (using an acetonitrile-based mobile phase) and GC/MS methods were then used to analyse the samples of saliva for bisphenol-A; no limit of detection was quoted.

The amount of bisphenol-A measured in samples of saliva collected after treatment ranged from 90-931 μg. The volume of saliva collected in this period was not given. However, based on the average rate of saliva production for passive mouthing of 0.5 $ml.min^{-1}$ (i.e. 30 ml in 1 hour), this would result in a concentration of 3-31 μg/ml (3-31 ppm) bisphenol-A in these saliva samples. Composite components, including bisphenol-A, were not observed in any of the saliva samples collected before treatment, with the exception of one subject. This subject had been treated 2 years previously with a sealant, and analysis of the pre-treatment saliva from this subject showed the presence of bisphenol-A (66.4 μg) and bis-DMA (49.2 μg). The results from this subject were excluded from the authors' analysis, although no explanation for this exclusion is provided. Migration of bisphenol-A and bis-DMA was found to vary between commercial composites and batches. Residual bisphenol-A in saliva after curing ranged from 0.1 to 2% of the 50 mg applied to the tooth surface 1 hour after treatment.

Lewis et al. (1999) completed a study into the identification and characterisation of estrogen-like components in commercial resin-based dental restorative materials. The purpose of the study was to analyse a broad spectrum of resin-based dental products for the presence of estrogen-like compounds, specifically bisphenol-A and bis-DMA.

Twenty-eight commercial resin-based dental restorative materials including those previously examined by Olea et al. (1996) were included in the study. The materials included restorative composites as well as sealants. Materials containing a filler phase (0.5 g) were placed in a centrifuge tube with 5.0 ml of spectroscopic grade acetonitrile (40 min/12,000%g). The supernatant liquor (2 ml) was withdrawn for analysis stock. Unfilled products (0.5 g) were placed into glass vials with acetonitrile and agitated to cause dissolution. The samples were analysed using HPLC, with the detection window set to 208nm, using an acetonitrile based mobile phase. This mobile phase allowed good resolution between bis-DMA and ethoxylated bis-GMA (which elute near one another). All analyses were compared against standard samples of bisphenol-A and bis-DMA. Resolution of less than 1 μg/ml of the standard materials was obtained.

In order to obtain adequate separation of major and minor components, very long run times were used to prevent overlap of eluted peak areas. The plots of standard component elutions were isolated and superimposed upon a plot of commercial product. If the presence of one of the standard composites was suggested, further analysis of the residue (obtained by evaporation) was

performed using total attenuated reflectance infrared (ATR-IR) the deposit was analysed and compared to a reference absorption pattern.

When analysis was performed by HPLC, two sealant products showed peaks with similar elution times to those of bisphenol-A and therefore were further analysed using ATR-IR. However, the presence of bisphenol-A could not be verified using IR. Thus, using HPLC and IR techniques, bisphenol-A was not found in any of the sealant products analysed. In comparing these results with those reported by Olea et al. (1996), in which bisphenol-A was reported to migrate from the same dental sealant products, the authors suggest that given the difficulties in analytical resolution, it is possible that TEGDMA or other components present in bis-GMA may have been mistakenly identified as bisphenol-A in the Olea et al. (1996) study.

Arenholt-Bindslev et al. (1999) completed a study of time-related bisphenol-A content and estrogenic activity in saliva samples collected after placement of two types of dental fissure sealant (A and B), each with a different monomer composition, based either on bis-DMA (sealant A), or bis-GMA (sealant B). Only the results of the bisphenol-A analysis are presented here.

Eight male volunteers (healthy, 20-23-year-old) with no prior history of placement of fissure sealants or composite resin fillings had four molars sealed, using standard procedures, with either of the two sealant products (four people per product). The amount of sealant applied to each person was 38 ± 3 mg (i.e. ~ 10 mg per tooth). Pre-treatment saliva (5 ml) was collected whilst the patients were fasting. Fissure sealants were placed and saliva samples (5 ml) were collected in glass vials immediately, 1 hour and 24 hours after placement of the fissure sealant. The bisphenol-A content of the saliva samples was determined by HPLC, using a spectrofluorometer with an excitation wavelength of 275 nm and emission wavelength of 300 nm. The detection limit for this study was 0.1 ppm and the quantification limit was 0.3 ppm. In samples collected immediately after placement of sealant A (based on bis-DMA), the bisphenol-A levels varied within a range of 0.3-2.8 ppm (average 1.43 ppm). Bisphenol-A was not detected in saliva samples collected at the later time points. Saliva samples taken after placing sealant B (based on bis-GMA) showed no detectable levels of bisphenol-A at any time point.

Although one sealant product showed measurable levels of bisphenol-A in saliva, the levels measured were much lower than the levels reported by Olea et al. (1996). The authors of this study suggest that the amount of sealant used in the two studies (38 ± 3 mg compared to 50 mg) is one factor which could have contributed to this difference in results. They also note that in the Olea et al. (1996) study, no TEGDMA was identified from the HPLC profiles. The authors suggest that the presence of TEGDMA would have been expected and therefore it is possible that elution of TEGDMA could have confounded the analysis of bisphenol-A. They also suggest that some of the bis-DMA monomer in the sealant used by Olea et al. (1996) may have been converted to bisphenol-A by the esterases found in saliva. In the present study, bisphenol-A was only found in the sealant A (based on bis-DMA) in saliva samples collected immediately after placement of the sealant.

A study into the pharmacokinetics of bisphenol-A released from a dental sealant was carried out by Fung et al. (2000). The study determined the rate and time course of bisphenol-A released from a commercial dental sealant (understood to contain bis-DMA) when applied at a “dose” of 8 mg (one tooth) or 32 mg (four teeth) to 40 healthy adults. The subjects recruited, 40 adults (18 men and 22 women, 20–55 years of age), did not have any previous history of pit and fissure sealant or composite resin restorations. Saliva (30 ml) and blood (7 ml) samples were collected from all subjects immediately before sealant placement (baseline) and at one hour, three hours, 1 day, 3 days and five days after sealant placement.

The low "dose" group (7 men, 11 women) received a single application of 8 mg dental sealant on one surface, whilst subjects in the high 'dose' group (11 men, 11 women) received a total dosage of 32 mg of sealant. The sealant was applied using typical methods. After the five days two subjects from the high-dose group had one sealant missing from a molar. Each subject provided 20–30 ml of saliva (subject was asked to expectorate into a 50 ml plastic container for 30 mins) and 5.5-7.0 ml of blood one hour before dental resin sealant placement. The same procedure was carried out for collecting saliva and blood at each time point post treatment.

HPLC was used to analyse the bisphenol-A content of the samples using a fluorescence detector set at 278 nm excitation and 315 nm emission (215 nm also quoted in this paper which is believed to be a typographical error somewhere). The detection sensitivity was 5 ppb or 5 ng/ml for bisphenol-A. The elution profiles were compared against standards of TEGDMA, bis-DMA, bisphenol-A and bis-GMA.

Bisphenol-A was detected in some saliva samples (5.8 – 105.6 ppb) collected at 1 hour and 3 hours, however bisphenol-A was not detectable beyond 3 hours or in any of the blood specimens. For the one and three hour samples, the bisphenol-A concentration in the high-dose (32 mg) group was significantly greater than in the low-dose (8 mg) group.

The concentration of bisphenol-A in saliva reported in this study is more that 250 times less than the values quoted by Olea et al. (1996). The source of the bisphenol-A in this study is uncertain, it may be present as an impurity or from enzymatic degradation of bis-DMA. This study suggests that when bisphenol-A is released orally from sealant, it may not be absorbed systemically; or that the quantity adsorbed is very small and below the detection limit; or bisphenol-A absorbed into systemic circulation is metabolised.

Pulgar et al. (2000) completed a study into the determination of bisphenol-A and related aromatic compounds released from bis-GMA based composites and sealants by high performance liquid chromatography. The aim of the study was to determine aromatic components eluted by *in vitro* polymerised bis-GMA based composites and sealants, to investigate how pH modifications effect the leaching of the components, and to assess their presence prior to polymerisation. It was found that bisphenol-A, bis-DMA, BADGE and bis-GMA amongst others leached from composites and sealants before and after polymerisation.

HPLC was used for analysis with an UV detector at 280 nm; the mobile phase was acetonitrile based. GC/MS was used to confirm these results. Reference standards were used for bisphenol-A, bis-GMA, bisphenol-A ethoxylate (EPBA) bis-DMA, bisphenol-A propoxylate (PBPA) and BADGE. The detection and quantification limits were calculated in accordance with 10 concordant measurements of standard solutions for each of the products analysed: bisphenol-A, 0.20 mg/ml.

Polymerised/non-polymerised samples were studied at pH 1, 7, 9 and 12. The temperature used for these conditions was 37ºC. Three samples of each commercial product were analysed under these eight physico-chemical conditions. Samples consisting of composite (100 mg) and sealant (50 ng) were used for non-polymerised systems and composite (100 mg) for polymerised samples.

Bisphenol-A was detected in all of the commercial samples studied before and after polymerisation, the other components were found in the sample to varying degrees. All samples showing detectable levels of bis-DMA were also positive for the presence of bis-GMA, BADGE and bisphenol-A. For bisphenol-A, migration increased as the pH became more alkaline. The maximal amount of bisphenol-A migration during the study was 1.8 μg/mg dental material.

The authors state that the composites and sealants are unstable, and that to some extent, depending on the aggressiveness of the medium, it is always possible to detect elution of

monomers, oligomers and precursors. This study confirms the leaching of bisphenol-A and other aromatic compounds from one sealant, and new data on bisphenolic monomers leaching from seven other composites currently in dentistry.

Noda et al. (1999) carried out a study using HPLC to analyse dental resin composite components. The purpose of this study was to establish a reliable method to detect residual bisphenol-A in uncured dental resin. The study was initiated because of differences in the detection of bisphenol-A from dental sealants reported in previously published studies (Hamid and Hume, 1997; Nathanson et al., 1997, 1998; Olea et al., 1996). The method used HPLC and fractionation of compounds for molecular analysis.

The initial phase of the study was conducted to establish a suitable mobile phase for the HPLC to separate TEGDMA and bisphenol-A peaks. This solvent/mobile phase was acetonitrile. All procedures were carried out in glass vessels in order not to contaminate samples with components which could potentially leach from plastic. Five dental resin composites were tested. The detection limit for bisphenol-A was determined to be 0.1 μg/ml solute extracted. The concentration of bisphenol-A found in the uncured resins ranged from 0.1–2.2 ng/mg raw resin. Samples of uncured resin composite (500 mg) were dissolved in acetonitrile (500 ml) and analysed using HPLC. It was found that using aqueous solutions as the mobile phase in the HPLC analysis did not separate bisphenol-A and TEGDMA, and it was concluded that a non-aqueous based mobile phase, such as acetonitrile, should be used to ensure separation.

A study was completed by Richardson (1997), on the assessment of adult exposure and risks from components and degradation products of composite resin dental materials. This study was generic in nature and did not relate to any specific commercial resin product. Modelled estimates of exposure to particular components of dental materials were derived. The exposure values calculated were 0.41 ± 0.31 μg/kg-day for bis-DMA, 0.02 ± 0.017 μg/kg-day for formaldehyde and $3.3 \cdot 10\text{-}5 \pm 2.5 \cdot 10\text{-}5$ μg/kg-day for methacrylic acid. There was no discussion or inclusion in this study of the migration of bisphenol-A.

A study into the *in vitro* cytotoxicity of resin-containing restorative materials after ageing in artificial saliva has been reported by Wataha et al. (1999). However, no analysis of bisphenol-A migration was performed. Although bisphenol-A was used as a positive control in this study, there is no information on release of bisphenol-A from dental sealants.

Summary of dental fissure sealant studies

A number of studies have been conducted looking at the release of bisphenol-A from commercially available dental sealants under a variety of exposure conditions. The information suggests that release of bisphenol-A is most likely only under conditions where degradation of the parent monomer (bis-DMA or bis-GMA) could occur. The data also suggest that degradation of bis-GMA does not occur and therefore only those sealants which contain bis-DMA are likely to release bisphenol-A.

Three studies have shown the release of bisphenol-A into the saliva of humans following placement of dental sealant. The results of these three studies provide somewhat different estimates of bisphenol-A concentration in saliva measured 1 hour post treatment (5.8 - 105.6 ppb, 3-31 ppm or 0.3-2.8 ppm). However, it appears possible that the higher estimates of bisphenol-A concentration in saliva may overestimate the actual concentrations which could be expected to arise following dental treatment, as a result of interference in the analytical method used to determine bisphenol-A.

Given the uncertainties surrounding the reliability of the higher estimates of bisphenol-A concentration in saliva, the concentration of bisphenol-A in saliva following dental treatment is considered to more likely to be in the range 0.3-3 ppm. This concentration of saliva was measured at 1 hour post treatment. When saliva samples were analysed for bisphenol-A concentration at time points later than 1 hour post treatment, in two studies, no measurable levels were detected. This suggests that any exposure to bisphenol-A as a result of dental treatment will be an acute event.

4.1.1.2.5 Household papers

Vinggaard et al. (2000) completed a study looking at the identification and quantification of estrogenic compounds in recycled and virgin paper for household use, determined by an in vitro yeast estrogen screen and chemical analysis. The purpose of the study was to assess the migration of estrogenic compounds from paper manufactured for household use (kitchen rolls). The study used twenty different brands of kitchen rolls, nine of which were made from recycled paper with 80-100% recycled fibres, the remaining eleven from 'virgin' paper. All samples were purchased from Danish retail shops. Only the results of the chemical analysis for bisphenol-A are reported here.

The paper samples were subjected to solvent extraction for one hour followed by chemical analysis and quantification for detection of a variety of compounds by: gas chromatography/mass spectrometry GC/MS (limits of detection between <4.5-5 pg/μL of injection volume, limits of quantification were 5-20 pg/μL, corresponding to between 1-4 μg/kg of paper); gas chromatography/Fourier transform infra-red/mass spectroscopy GC/FTIR/MS (the detection limit estimated to be 1 μg/mL, corresponding to 0.2 mg/kg of paper); and gas chromatography/flame ionisation detection GC/FID. Extracted samples were sent in coded form to laboratories performing the analysis.

Chemical analysis showed that extracts from six of the nine recycled papers contained levels of bisphenol-A ranging from 0.55-24.1 mg/kg (ppm) of kitchen roll. Extracts from the majority of virgin papers contained negligible or no bisphenol-A; one sample had levels of 0.12 mg/kg (ppm).

The migration of bisphenol-A from virgin papers was negligible and the migration found from recycled papers was measured under extreme experimental conditions, i.e. when extracted with methanol (100%) and ethanol (95%), thus the results are not considered to be representative of everyday household use. Therefore this scenario will not be considered for risk characterisation.

4.1.1.3 Humans exposed via the environment

Table 3.8 from the environment section has been repeated here (**Table 4.21**) and gives the predicted environmental exposures to bisphenol-A and the daily human doses arising from releases from production, processing and manufacture of bisphenol-A, epoxy resins, PVC and thermal paper, and for releases at the regional level.

It can be seen that the daily human intake via the environment based upon typical human consumption and inhalation rates at the regional level is $1.78 \cdot 10^{-5}$ mg/kg/day and the highest local exposure (use as an inhibitor in PVC production) is 0.059 mg/kg/day. These two figures will be taken forward into the risk characterisation.

Table 4.21 Concentrations for indirect exposure of humans via the environment

	Concentration in drinking water (mg/l)	Concentration in wet fish (mg/kg)	Concentration in plant roots (mg/kg)	Concentration in plant leaves (mg/kg)	Concentration in milk (mg/kg wet weight)	Concentration in meat (mg/kg wet weight)	Concentration in air (mg/m^3)	Total daily intake (mg/kg day)
Site-specific								
Bisphenol-A production	$3.93 \cdot 10^{-4}$	0.027	$1.49 \cdot 10^{-3}$	1.96	$2.64 \cdot 10^{-3}$	$8.35 \cdot 10^{-3}$	$3.61 \cdot 10^{-4}$	0.0338
Epoxy resin production	0.012	0.074	0.3	0.065	$4.85 \cdot 10^{-5}$	$1.53 \cdot 10^{-4}$	$2.08 \cdot 10^{-10}$	$3.22 \cdot 10^{-3}$
Thermal paper production	$8.86 \cdot 10^{-4}$	0.06	$1.4 \cdot 10^{-4}$	$3.14 \cdot 10^{-5}$	$1.02 \cdot 10^{-6}$	$3.21 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$1.25 \cdot 10^{-4}$
Generic scenarios								
Phenoplast cast resin processing	$1.29 \cdot 10^{-3}$	0.0875	0.013	$2.81 \cdot 10^{-3}$	$2.98 \cdot 10^{-6}$	$9.42 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$3 \cdot 10^{-4}$
Thermal paper recycling	0.187	12.6	2.03	0.441	$4.45 \cdot 10^{-4}$	$1.41 \cdot 10^{-3}$	$2.08 \cdot 10^{-10}$	0.0448
PVC – Inhibitor during production process	0.227	15.4	2.97	0.643	$6.01 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$	$2.08 \cdot 10^{-10}$	0.0591
PVC – Anti-oxidant during processing	$2.19 \cdot 10^{-4}$	0.0148	$1.51 \cdot 10^{-3}$	$3.28 \cdot 10^{-4}$	$4.45 \cdot 10^{-7}$	$1.41 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$4.46 \cdot 10^{-5}$
PVC – Preparation of additive packages	$8.8 \cdot 10^{-3}$	0.595	0.114	0.0246	$2.3 \cdot 10^{-5}$	$7.3 \cdot 10^{-5}$	$2.08 \cdot 10^{-10}$	$2.27 \cdot 10^{-3}$
PVC – Anti-oxidant in plasticiser production	0.0014	0.0964	0.0173	0.00374	$3.63 \cdot 10^{-6}$	$1.15 \cdot 10^{-5}$	$2.08 \cdot 10^{-10}$	$3.58 \cdot 10^{-4}$
PVC – Plasticiser use	$1.88 \cdot 10^{-4}$	0.0127	$1.1 \cdot 10^{-3}$	$2.39 \cdot 10^{-4}$	$3.62 \cdot 10^{-7}$	$1.15 \cdot 10^{-6}$	$2.08 \cdot 10^{-10}$	$3.64 \cdot 10^{-5}$
Regional	$1.14 \cdot 10^{-4}$	$7.74 \cdot 10^{-3}$	$1.96 \cdot 10^{-4}$	$4.37 \cdot 10^{-5}$	$1.85 \cdot 10^{-7}$	$5.86 \cdot 10^{-7}$	$2.08 \cdot 10^{-10}$	$1.78 \cdot 10^{-5}$

4.1.1.4 Combined exposure

The worst-case combined exposure would be someone exposed via the environment near to a PVC production plant, and who is also exposed via food contact materials as described in Section 4.1.1.2.

The exposures for these component parts are presented below. The maximum combined exposure from these sources is 31 mg/kg/day for both the regional and local scenarios. Exposure is dominated by the occupational exposure.

Table 4.22 Components of combined exposure

Source of exposure	Exposure (mg/kg/day)
As a consumer: (oral exposure via food and wine)	$9 \cdot 10^{-3}$
Indirect exposure via the environment: Regional Local	 $1.78 \cdot 10^{-5}$ 0.06
Total: Regional Local	 $9 \cdot 10^{-3}$ 0.069

The value of $9 \cdot 10^{-3}$ for consumer exposure is based on an adult consumer receiving exposure via canned food and wine. The values of $1.78 \cdot 10^{-5}$ and 0.06 for regional and local environmental exposure, respectively have been taken from **Table 4.21**. The main route of exposure from environmental sources is the oral route. The average body weight of 70 kg has been assumed.

4.1.2 Effects assessment: Hazard identification and dose (concentration) - response (effect) assessment

There are two grades of bisphenol-A and the purity of each varies according to the intended use. Bisphenol-A used to manufacture bisphenol-A polycarbonate is of very high purity, typically 99.9%. Bisphenol-A used to produce epoxy resins is less pure, typically 97.4% (Dow Chemical Company, 1985). Few of the toxicity studies reported the grade of bisphenol-A tested and it is difficult to assess the extent (if any) to which the purity may influence the toxicological properties.

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

There are three main studies available, all of which have evaluated the toxicokinetics of bisphenol-A in a single species, the rat (Pottenger et al., 1997a; 1997b; Knaap and Sullivan, 1966). The methodological details of these studies are summarised in the following paragraphs, and the results described under the subheadings for absorption, distribution, metabolism and elimination. These studies have primarily used the oral and parenteral route of exposure; there are no data available for the dermal or inhalation routes.

In a recent well conducted study (Pottenger et al., 1997a; 2000), Fischer F344 rats (5 per sex per dose) fitted with in-dwelling jugular cannulae were administered a single dose of 10 or 100 mg/kg ^{14}C-labelled bisphenol-A by oral gavage, intraperitoneal (i.p.) or subcutaneous (s.c.) injection. The ^{14}C label was on the second position of the propane group. Unchanged bisphenol-A and radioactivity were determined in blood and plasma samples taken 0.08, 0.25, 0.5 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24, 48, 72, 96, 120, 144 and 168 hours post-dosing. Radioactivity was determined in urine and faecal samples collected every 12 or 24 hours. At 7 days post-dosing, animals were sacrificed and selected tissues (brain, liver, kidneys, peri-renal fat, gonads, uterus, and skin) and remaining carcass were analysed for radioactivity. Examination of metabolic products was also conducted by HPLC on selected urine and faecal samples.

In a further study by the same group (Pottenger et al., 1997b; 2000), F344 rats (3 per sex per dose per sacrifice time) were administered a single dose of 10 or 100 mg/kg ^{14}C-labelled bisphenol-A by oral gavage, i.p. or s.c. injection. Again, the ^{14}C label was on the second position of the propane group. For each dose level and route, animals were sacrificed at two time points corresponding to peak plasma radioactivity (early sacrifice time) and when unchanged bisphenol-A could no longer be detected in the blood (later sacrifice time), as determined approximately from the initial study. For oral administration, the early sacrifice time was 5-15 minutes and the later sacrifice time was 45 minutes in males and 18 hours in females (see Section 4.1.2.6). Following sacrifice, structural identification of plasma metabolites was determined by three methods; extracted ion chromatography retention time match, co-chromatography, and mass spectrum ion fragmentation pattern match.

In an older study (reported by Union Carbide Corporation, 1962; Knaap and Sullivan, 1966), 120 mg ^{14}C-labelled bisphenol-A (labelled on the propyl group) was formulated in 900 mg propylene and administered to groups of 4 male rats as a single oral dose of approximately 800 mg/kg. Three such experiments were conducted, to determine: the radioactivity in faecal and urine samples collected over 8 days post dosing; exhaled $^{14}CO_2$ up to four hours post dosing; radioactivity in intestines and carcass over 8 days post dosing; the recovery of unchanged

bisphenol-A from urine and faecal samples; and examination of metabolic products in the urine and faeces by gas chromatography and ion exchange chromatography.

A single study in mice is available (Taylor et al., 1999). This briefly reported study, available as an abstract only, investigated the potential for bisphenol-A (tritium-labelled) to bioaccumulate in the blood serum of pregnant animals. This study consisted of two experiments, with mice receiving single and/or multiple doses of bisphenol-A by the oral route. However, it is reported that, due to the low levels of radiolabelled bisphenol-A used (up to 20 μCi/dose), radioactivity in blood serum could not be determined by GC. Instead, a HPLC peak tentatively identified as bisphenol-A was bioassayed for oestrogenic activity using MCF-7 breast cancer cells (see Section 4.1.2.9.1). Cell proliferation, and hence oestrogenic activity, was seen by the authors as confirming the peak to be bisphenol-A. The study is not described here in further detail, as the measurement technique used, and limited information provided, mean no reliable conclusions can be drawn from the data.

Absorption

In the initial Pottenger et al. (1997a) study, 14-16% and 24-28% of the radioactive label were recovered in the urine of males and females, respectively over 7 days following oral administration. In the Knapp and Sullivan (1966) study, 28% of the radioactive label was recovered in the urine in males over 8 days following oral administration. These results indicate that significant absorption of bisphenol-A had taken place.

In the initial Pottenger et al. (1997a) study, peak concentrations of parent compound in the blood were observed 15 minutes after oral administration for all dose groups except low-dose males, where bisphenol-A could not be detected at even the earliest sampling time (5 minutes). Peak concentrations of radioactivity in the plasma were observed 5 minutes post dosing in low-dose females, and 15 minutes post dosing in low-dose males and high-dose animals of both sexes. The presence of bisphenol-A in the blood so soon after oral administration indicates a rapid absorption from the gastrointestinal tract.

Some disproportionate ($\geq$22 and 57 fold) increases in average peak values of parent compound in blood relative to dose group were observed in males and females, respectively. Disproportionate values were observed in males, as the parent compound was not detected in the blood of low-dose animals at any sample time. In addition, these increases were also due to the wide variation in observed values at the top dose (e.g. at 5 minutes post dosing, values ranged from 0.28-1.87 μg/g in females and 0-0.32 μg/g in males). However, average peak plasma radioactivity was found to increase approximately linearly with dose, for all routes of exposure. In addition, area-under-the curve (AUC) values for parent compound and plasma radioactivity, showed an approximately proportional increase with dose for all three routes of exposure, indicating that absorption of bisphenol-A is linear across the dose levels used.

In an attempt to quantify the proportion of the dose absorbed from the gastrointestinal tract following oral administration, the percentages of total radioactivity recovered in the urine and faeces over 7 days were compared for the oral and parenteral routes. Similar faecal values for the oral and parenteral routes could indicate that parent compound in the faeces had been extensively absorbed before its excretion. Faecal elimination values were similar across all three exposure routes for low- and high-dose males (80-83% and 74-82%, respectively, for all exposure routes). However, greater variation in faecal elimination for different exposure routes was seen in low- and high-dose females (54-72% and 52-69%, respectively); oral administration resulted in the greatest proportion of the dose eliminated in the faeces. Therefore although these data do not clearly indicate the extent of absorption following oral exposure, they suggest that absorption

may be extensive. Consideration was therefore given to the time taken for radioactivity to appear in the faeces following oral administration. The average gastrointestinal transit time for F344 rats is 12-18 hours, yet following oral administration, over 50% of the faecal elimination occurred after 24 hours post dosing. Thus, overall, the data suggest that there is extensive absorption following oral administration.

In the second Pottenger et al. (1997b) study, the observed peak plasma radioactivity values were, overall, in agreement with the data reported in the initial study; females demonstrated higher values than males for total plasma radioactivity. In this second study, peak plasma radioactivity in males at the top dose and in females at both doses was approximately twice and half the value observed in the initial study, respectively. All other values were similar between the two studies. Again, the observed differences are considered as a limitation of the data; lower values in peak plasma radioactivity in females are probably a reflection of the use of only one early sample time in the second study.

Data are also available from a study investigating the conversion of bisphenol-A to bisphenol-A glucuronide in rat fetus and testes (Miyakoda et al., 2000). Though the complete study details are described below, only data relating to absorption into the blood are presented here; further information on distribution to the fetus and testes is presented in the section “Distribution”.

A single oral dose of 10 mg/kg bisphenol-A (unlabelled) was administered to pregnant Wistar rats on day 19 of gestation and to 10 week old male Wistar rats (number of animals not reported). Fetuses were removed from pregnant females 1 hour after dosing, and blood samples were taken and testes were removed from male rats after 1, 3 and 8 hours. Blood and tissue samples were treated with or without β-glucuronidase for 2 hours prior to extraction and acetylation of bisphenol-A and its measurement by gas chromatography-mass spectrometry. One hour after administration, approximately 90% of the bisphenol-A was present as bisphenol-A glucuronide in plasma (mean concentration of 590 ppb). Bisphenol-A was seen to steadily decrease in blood plasma. However, the glucuronide was observed to decrease in plasma at 3 hours (by approximately 45%) then return to the initial concentration at 8 hours. The authors suggest that this increase may possibly be due to enterohepatic circulation of bisphenol-A. Overall, the data indicates extensive absorption of bisphenol-A from the gastrointestinal tract.

A recent study has also investigated the toxicokinetics of bisphenol-A in pregnant F344 rats (Takahashi and Oishi, 2000). Again, the complete study details are described below but only data relating to absorption are presented here.

Dams (number not reported) were administered a single dose of 1,000 mg/kg bisphenol-A (unlabelled) by oral gavage on day 18 of gestation. Blood samples were taken 10, 20, 30 and 40 minutes and 1, 2, 4, 6, 12, 24 and 48 hours post-dosing, and 2-6 dams were sacrificed at each of these time points. Maternal kidneys and liver were removed along with 8-12 fetuses per time point. Bisphenol-A content was determined in blood, tissues and fetuses by HPLC and spectrophotometry, and comparing the peak area obtained with that of a reference concentration of pure (95%) bisphenol-A.

Bisphenol-A was detected in blood 10 minutes after dosing and reached a maximum concentration after 20 minutes (0.007%, of the administered dose per gram of blood). The concentration of bisphenol-A was then seen to decrease, and after 6 hours was 2% of the maximum. This study further indicates that bisphenol-A is rapidly absorbed following oral administration.

A further study investigating absorption of bisphenol-A in female DA/Han rats following a single i.v and oral dose is available (Upmeier et al., 2000). Only details for oral administration (a relevant exposure route) are presented below.

Female rats (number not reported) were administered 10 or 100 mg/kg bisphenol-A by gavage. Blood samples ($\geq$3) were taken 0.5, 1.5, 3, 6, 8 and 48 hours post-dosing for rats administered 10 mg/kg, and after 10, 20, 30 and 45 minutes, 1.5, 2, 3, 4, 6, 8, 24, 32 and 48 hours for rats administered 100 mg/kg. Bisphenol-A was isolated from blood samples and analysed by GC-MS.

Bisphenol-A was detected in blood plasma at the earliest sampling time in both dose groups. At 10 and 100 mg/kg, maximum bisphenol-A plasma levels were observed 1.5 (31 ng/ml plasma) and 0.5 hours (150 ng/ml) post-dosing, respectively. Bisphenol-A levels gradually declined, although a further increase was seen with peaks in concentration being observed at 6 (40 ng/ml) and 3 hours (134 ng/ml) for 10 and 100 mg/kg, respectively. Observed intermittent rises in individual animal plasma concentrations were considered to be characteristic of enterohepatic circulation. After 48 hours bisphenol-A in plasma was at or below the detection limit (12 ng/ml) in both dose groups. Thus, this study also indicates that bisphenol-A is rapidly absorbed following oral administration. There is also evidence of enterohepatic circulation.

Preliminary data have been received on a recently completed *in vitro* dermal absorption study with bisphenol-A (*In Vitro* Technologies, 2001). Human dermatomised skin samples (with an area of 63.6 mm^2 per sample), obtained from 3 donors, were placed into diffusion chambers and 5 (3.18 mg/ml) or 50 mg/cm^2 (31.8 mg/ml) ^{14}C-labelled bisphenol-A in ethanol was delivered to each skin sample (6 samples/donor/dose level). It was not reported where on the molecule the label was. After the ethanol had evaporated, the bisphenol-A was re-suspended in artificial sweat. Receptor fluid, which consisted of a balanced salt solution containing albumin, was then collected over 0-1, 1-2, 2-4, 4-8, 8-12, 12-18 and 18-24 hours and radioactivity measured (method not stated in this draft report). At 24 hours, radioactivity on the skin surface (obtained from recovery swabs), in the stratum corneum and the "lower" skin layer was determined.

The mean cumulative % of total radioactivity in receptor fluid following application of 5 mg/cm^2 bisphenol-A at 1, 2, 4, 8, 12, and 18 hours ranged from mean values of 0.046-0.063%, 0.060-0.078%, 0.096-0.14%, 0.57-1.22%, 1.80-3.49% and 5.38-11.30%, respectively. For 50 mg/cm^2 application dose, mean values ranged from 0.027-0.115%, 0.044-0.171%, 0.109-0.245%, 0.491-0.835%, 1.52-2.13% and 3.88-4.58 %. These values are not corrected for recovery of the administered dose, although recovery was generally high. At 24 hours, the mean total recovered dose for all three donors was 19.7-29.9% at the skin surface, 2.31-2.69% in stratum corneum, 51.9-57.5% in the "lower" skin layer and 11.6-26.1% in receptor fluid, following application of 5 mg/cm^2. For 50 mg/cm^2, mean values were 28.4-38.6% in the "top" skin layer, 3.88-8.38% in stratum corneum, 38.8-55.3% in the "lower" skin layer and 6.97-9.12% in receptor fluid.

This study did not include tritiated water as a marker for skin integrity. Although skin integrity was not directly determined in this study, the pattern of results suggests that integrity was lost after 4-8 hours. Therefore, the data obtained after this time are considered to be unreliable for determination of dermal absorption. The only reliable data available are those for the (cumulative) % of the total dose in receptor fluid at 8 hours, which were 0.57-1.22% and 0.491-0.835% following application of 5 and 50 mg/cm^2, respectively. No measurements of the radioactivity in the skin at this time point were made and thus there is no information on the percentage of the applied dose that may be present in the skin and available for subsequent absorption. However, the concentrations in the receptor fluid will be in dynamic equilibrium

with the concentrations in the lower skin layers. The low concentrations of radioactivity found in the receptor fluid at up to 8 hours, when the skin was undamaged, should be indicative of the concentrations likely to be in the lower skin layers at these time points. At 24 hours, when the skin was damaged, the ratio between receptor fluid levels and lower layer levels was in the range of 1:2 up to 1:8. If it is assumed that the higher ratio applies to the data after 8 hours of exposure, it is reasonable to predict that up to about 10% of the applied dose would be present in the lower skin layers. Overall, based on the available information, it is concluded that dermal absorption of bisphenol-A is in the region of 10%.

There are no direct toxicokinetics studies on bisphenol-A following inhalation exposure. However, on the basis of the observed decreases in absolute liver and kidney weight in a rat 90-day inhalation study (see Section 4.1.2.6.1) and bisphenol-A's high partition coefficient, it would be assumed that significant absorption via the respiratory tract would occur. These data do not allow a quantitative assessment of the absorption of bisphenol-A for the inhalation route.

Distribution

A detailed investigation of the distribution of bisphenol-A has not been conducted. However, some data are available, including data from studies primarily investigating the transfer of bisphenol-A from pregnant rats into fetuses.

In a well reported series of studies, Snyder et al. (2000) investigated the metabolism, distribution and excretion of bisphenol-A. The complete study details are described below, but only the data relating to distribution are described here.

Two groups of four lactating CD rats received 100 mg/kg [Ring-^{14}C]-labelled bisphenol-A by gavage on day 14 post-delivery. For one group, milk and blood samples were collected at 1 hour post-dosing; for the second group, milk, blood and tissue samples were collected at 8 hours. Prior to sample collection, animals were anesthetized and given i.p. injections of oxytocin to facilitate milk release. ^{14}C content was determined in all samples by scintillation counting. ^{14}C content was also determined in liver, abdominal and subcutaneous fat, kidneys, lungs, intestines and intestinal contents and carcass.

The second group of animals was returned to their pups immediately following dosing (10 pups per dam). Groups of 4 pups per litter were killed at 2 and 4 hours post-dosing of the dams, and the remaining pups were killed at 6 hours. Radioactivity in the carcass of these pups was determined.

Two additional lactating CD rats were also administered 100 mg/kg [Ring-^{14}C]-labelled bisphenol-A by gavage, and returned to their pups. Pups were killed 24 hours post-dosing and ^{14}C content determined in carcasses from 10 animals. Oxytocin was administered to dams prior to collection of milk, blood and tissue samples at 26 hours.

In the dams, 84, 77 and 27% of the administered ^{14}C label was recovered in the milk, blood, plasma, tissues and carcass after 1, 8 and 26 hours, respectively. The greatest % of the ^{14}C dose was seen in the intestine and intestinal contents at 1 (83%), 8 (75%) and 26 hours (26%). Minimal levels were seen in milk (0.0031%), blood (0.0059%), plasma (0.0106%), carcass (0.64%) and subcutaneous fat (0.0024%) at 1 hour; levels in these tissues further decreased with time. A fraction of the ^{14}C dose was seen in the liver at 1 (0.38%), 8 (0.74%) and 26 hours (0.14%). Peaks were seen in the kidney and lungs at 8 (0.021%) and 26 hours (0.0011%) respectively. Radioactivity amounting to less than 0.01% of the administered dose was detected in pup carcasses at 2–24 hours. The quantity of label tended to increase with time (0.0049, 0.0070, 0.0061 and 0.0082% at 2, 4, 6 and 24 hours, respectively). The source of this slight

increase in ^{14}C was suggested to be via the mother's milk. Overall, the data from Snyder et al. (2000) provide evidence of limited systemic distribution of bisphenol-A and limited transfer of bisphenol-A to pups via the breast milk.

In a briefly reported study (Miyakoda et al., 1999), pregnant Wistar rats (number not reported) were administered a single dose of 10 mg/kg bisphenol-A (unlabelled) by oral gavage on day 19 of gestation. Maternal blood samples were taken 1, 3 and 24 hours post-dosing, at which time an unstated number of dams were sacrificed and fetuses removed. Bisphenol-A was extracted from blood and fetus samples and acetylated prior to its measurement by gas chromatography-mass spectrometry.

Maximum concentrations of bisphenol-A were observed 1 hour post dosing: 34 ppb and 11 ppb in maternal blood and fetuses respectively. At 3 hours, bisphenol-A concentrations decreased in maternal blood and fetuses to approximately 3 ppb and 4 ppb respectively. At 24 hours a slight increase was observed in fetuses (to approximately 8 ppb). This increase was not discussed by the authors. Overall, the study provides evidence of limited distribution of bisphenol-A to the fetus.

Additional data on the distribution of bisphenol-A are available from a recent Miyakoda et al. (2000) study (see under "Absorption" for methodology). In this study, no significant difference in bisphenol-A concentration was detected between untreated and β-glucuronidase-treated fetal extracts. In treated males, one hour after administration, approximately 90% of the bisphenol-A was present as bisphenol-A glucuronide in testes (mean concentration of 160 ppb). Bisphenol-A was seen to slightly increase in testes (from approximately 20 ppb at 1 hour to 50 ppb at 8 hours) and bisphenol-A glucuronide to decrease.

No reliable conclusions can be drawn from the results observed in rat fetuses as the data can be interpreted to suggest that either bisphenol-A does not cross the placental barrier or bisphenol-A has crossed the placental barrier but UDP-glucuronosyltransferase (which catalyses the glucuronidation of bisphenol-A) is not present in the fetus. The study indicates that distribution to the testes did occur. However, when comparing the concentration of bisphenol-A and bisphenol-A glucuronide in the testes and in blood plasma (see under 'Absorption') up to 8 hours post-dosing, it cannot be reliably determined whether bisphenol-A and/or bisphenol-A glucuronide can pass through the testicular barrier.

Takahashi and Oishi (2000) also investigated the toxicokinetics of (unlabelled) bisphenol-A in pregnant rats (see under 'Absorption' for methodology). Bisphenol-A was detected in the liver and kidney from the dam and the fetus 10 minutes after dosing and reached maximum concentrations after 20 minutes; 0.083%, 0.017% and 0.004% of the administered dose per gram of liver, kidney and fetal tissue, respectively. The concentration of bisphenol-A was then seen to decrease, and after 6 hours was 5% of the maximum in the liver, kidneys and fetus. The concentration of bisphenol-A in the fetus was observed to decrease in almost the same manner as that in maternal blood. This study provides further evidence that limited distribution of bisphenol-A to the fetus occurs.

Further data from a repeated dose toxicity study in pregnant rats described in Section 4.1.2.6.1 and the DNA adduct study described in Section 4.1.2.7.3 show that bisphenol-A reaches the liver following oral administration. This is supported by the metabolism data which indicate that first pass metabolism occurs following oral administration (see Section 4.1.2.5) and that there is subsequent distribution of a metabolite(s). In an *in vivo* micronucleus study, a decrease in the ratio of polychromatic to normochromatic erythrocytes was observed following oral exposure, suggesting that bisphenol-A or a metabolite was bioavailable to the bone marrow (see Section 4.1.2.7.3). The repeated dose toxicity study in the dams also indicates that limited

distribution to the fetus can occur; this is supported by a study which investigates the transfer of bisphenol-A and/or its metabolites from lactating dams to pups (see Section 4.1.2.6). Thus, overall, distribution of the parent compound and/or metabolite(s) that is taken up can occur, but from the limited evidence available, the extent of distribution appears to be limited.

Metabolism

In the Pottenger et al. (1997a) study, samples of urine taken 0-12 hours post-dosing, and faeces taken 0-72 hours post-dosing, were analysed by HPLC to identify the radiolabelled entities present. The major form of radioactivity in the urine and faeces following oral administration was identified as the glucuronide conjugate and parent compound, respectively. In the faeces, 71-75% and 61-63% of the administered oral dose was recovered as parent compound in males and females, respectively. This was 86-99% of the faecal radioactivity. In the urine, 8-10% and 19-20% of the administered dose was recovered as the glucuronide conjugate in males and females (69-87% and 57-68% of the total urinary radioactivity), respectively. The authors also reported a metabolite present in all faecal samples whose mass spectral characteristics appeared to be that of the sulphate conjugate, and accounted for 4-5% of the administered dose in males and 2-4% in females.

In the second Pottenger et al. study (1997b), extracted ion chromatography, co-chromatography and mass spectrum ion fragmentation pattern analysis identified the major plasma metabolite in males and females at the early sacrifice time as the glucuronide of bisphenol-A, following oral, i.p. and s.c. administration (early sacrifice times were 5-15 minutes, 15-30 minutes and 45 minutes-1 hour for the oral, i.p and s.c routes, respectively). No substantial differences in glucuronide levels were observed between doses following oral administration. The fraction of plasma radioactivity identified as the glucuronide at the early sacrifice time following oral administration was 91% and 76% in males and 96% and 87% in females in the low and high-dose groups, respectively. Corresponding levels of parent compound were 2% and 8% in males, and 4% and 7% in females. These data indicate rapid metabolism of the parent compound to the glucuronide. Parent compound at the early sacrifice time following i.p and s.c administration were 27-51% and 66-76%, respectively. The substantially greater values for unchanged parent compound observed following i.p. and s.c. administration indicate that first pass metabolism occurs following oral administration.

At the later sacrifice time following oral administration (45 minutes in males and 18 hours in females), 100% of the plasma radioactivity was in the form of the glucuronide in low-dose males and females, compared to 68% in high-dose males and 98% in high-dose females. Parent compound was present in high-dose males and females at 11% and 2%, respectively. A possible explanation for the presence of parent compound at the later sacrifice time would be enterohepatic circulation; intestinal cleavage of conjugates would lead to parent compound still being detected at much later time points. At the later sacrifice times for i.p. (8-72 hours) and s.c (18-72 hours) administration, parent compound (3%) was only detected in males in both i.p. dose groups.

In this later Pottenger et al. study (1997b), sulphate conjugates of bisphenol-A were not detected in the plasma at either sacrifice time following oral administration but were tentatively identified in plasma and urine of the i.p group by ion fragmentation pattern; no standard was available to confirm the structural identity of the metabolite.

In the Knapp and Sullivan study (1966), problems were encountered in the analysis of bisphenol-A metabolites in urine and faecal samples collected over 8 days following oral administration. Thus, the results are of limited value quantitatively. However, the results do

show that the major urinary metabolite appeared to be the glucuronide of bisphenol-A and only negligible levels of parent compound were detected in the urine. In the faeces, approximately one third of the material was identified as parent compound, one third was identified as a possible hydroxylated metabolite (on the basis that it showed the same retention time upon gas chromatography analysis as bisphenol-A diacetate) and one third could not be identified by the analytical techniques employed.

A study by Snyder et al. (2000) investigated the excretion and metabolism of bisphenol-A in F344 and CD rats. The complete study details are described below but only results relating to metabolism are presented here. In one study, two groups of four lactating F344 and CD rats were administered a single dose of 100 mg/kg [Ring-^{14}C]-labelled bisphenol-A by oral gavage on day 14 post-delivery and placed in glass metabolism cages for 144 hours, after which time animals were killed. Urine and faeces were collected every 24 hours for analysis of ^{14}C content. Analyses of selected urine and faecal samples were undertaken by HPLC and NMR.

The major radioactive peak observed in the urine of CD and F344 rats at all collection times (24-96 hours) was determined to be bisphenol-A glucuronide and accounted for 81-89% of the radioactivity. The level of radioactivity associated with unchanged bisphenol-A ranged from 2.2-4.6% for CD rats and 5.8–10% for F344 rats. In both strains, unchanged bisphenol-A was the major peak found in faeces at 24 and 48 hours (data not shown) and accounted for 98% of the radioactivity.

Part of a further study by Snyder et al. (2000) was to determine the potential metabolites in the plasma and milk of lactating female CD rats over 26 hours following a single dose of 100 mg/kg [Ring-^{14}C]-labelled bisphenol-A by oral gavage on day 14 post-delivery (see "Distribution" for methodology). The major radioactive peak observed in plasma and milk in females at 1, 8 and 26 hours was identified by retention time to be bisphenol-A glucuronide. Unchanged bisphenol-A was also detected in the plasma of 4/10 females and in the milk of 2/10 females (no further details provided).

Elsby et al. (2001) investigated the modulatory effects of human and rat liver microsomal metabolism on the oestrogenicity of bisphenol-A in a well reported series of experiments. The metabolic aspects of these studies are reported below (see Section 4.1.2.9.1 for studies investigating endocrine modulating activity).

The metabolism of bisphenol-A was determined in primary cultures of hepatocytes from adult female Wistar rats. Hepatocytes were incubated with 0, 100 or 500 μM bisphenol-A for 2 hours, after which time reactions were terminated. Analysis of metabolites was by LC-MS. Incubation with 500 μM bisphenol-A yielded one major metabolite identified as bisphenol-A glucuronide, and two minor metabolites identified as 5-hydroxy bisphenol-A and bisphenol-A sulphate. Bisphenol-A glucuronide was the only metabolite formed during incubation of 100 μM bisphenol-A with hepatocytes. Thus, the major metabolite of bisphenol-A observed in rat hepatocytes *in vitro*, and in rats *in vivo*, is bisphenol-A glucuronide. In addition, the formation of 5-hydroxy bisphenol-A and bisphenol-A sulphate only at the higher concentration (500 μM) suggests that saturation of glucuronidation can occur in rat hepatocytes *in vitro*.

The oxidation of bisphenol-A was determined in liver microsome preparations from immature female Wistar rats and humans (Elsby et al., 2001). Incubations containing 1 mg of human or immature rat microsomal protein and 0 or 200 μM bisphenol-A were stored for 30 minutes. Reactions were initiated by the addition of NADPH. Analysis of metabolites was performed by GC-MS. A single metabolite, identified as 5-hydroxy bisphenol-A, was observed following incubation of bisphenol-A with human and rat liver microsomes.

In a further study by Elsby et al. (2001), the metabolism of bisphenol-A to the glucuronide was determined in liver microsome preparations from both immature (21-25-day-old) female Wistar rats (n = 8) and humans. Microsomal preparations were prepared from human livers obtained from 4 males (24–57-year-old) and from 4 females (35–65-year-old). Human microsomal protein (500 μg) was incubated with 0–1,000 μM bisphenol-A for 30 minutes. Immature rat microsomal protein (50 μg) was incubated with 0–1,000 μM bisphenol-A for 10 minutes. Reactions were initiated by the addition of uridine diphosphate glucuronic acid (UDPGA). Analysis of metabolites was performed by HPLC.

The mean maximum rate of metabolism (Vmax) for bisphenol-A glucuronidation was 5.9 and 5.2 nmol/min/mg of protein for pooled human male and female livers respectively. The mean substrate concentration to give half the maximum rate of metabolism (Km) was determined to be 77.5 μM in males and 66.3 μM in females. Thus, no significant difference in metabolic capacity was observed between human male and female liver microsomes *in vitro*. In immature female rat microsomes, Vmax and Km were 31.6 nmol/min/mg of protein and 27.0 μM, respectively. Statistically significant differences were observed between the Vmax of glucuronidation for human and immature female rat liver microsomes. Thus, in this *in vitro* study, human liver microsomes did not glucuronidate bisphenol-A as extensively as rat liver microsomes.

Sipes (2001) comprehensively investigated the metabolism of bisphenol-A in liver microsome preparations in a series of experiments. In these studies, ^{14}C-bisphenol-A is labelled on the second position of the propane group. The results of these studies generally agree with those observed by Elsby et al. (2001).

Initially, Sipes (2001) confirmed that incubation of bisphenol-A with rat microsomes (from male F344 rats) resulted in one primary metabolite, bisphenol-A glucuronide. Next, the rate of bisphenol-A metabolism to the glucuronide was determined in liver microsome preparations from groups of 4 male and 4 female Sprague Dawley and F344 rats. Microsomes (2.5 mg/ml protein) were incubated with ^{14}C-bisphenol-A and 500 μM bisphenol-A for 0-15 minutes. Reactions were initiated by the addition of UDPGA. Bisphenol-A was separated from the glucuronide by HPLC and the collected peaks quantified by scintillation counting.

Similar rates of glucuronidation of bisphenol-A were observed between sexes and strains; approximately 20 and 50% of the bisphenol-A had been glucuronidated after 2 and 10 minutes, respectively.

Studies were then undertaken by Sipes (2001) to determine precise estimations of Km and Vmax and thus calculate the intrinsic metabolic clearance of bisphenol-A (clearance = Vmax/Km) by liver microsomal preparations from male and female rats (4 per sex), mice (4 per sex) and humans. A comparison was also undertaken between Sprague Dawley and F344 rats; the effects of age and pregnancy on metabolic clearance were also determined in Sprague Dawley rats. For humans, clearance was determined in commercially obtained pooled samples of male (n = 15) and female (n = 15) livers, as well as individual liver samples from 5 males (aged 36–56) and 3 females (28–61).

Microsomal protein (1.25 mg/ml) was incubated with 2.5–2,000 μM ^{14}C-bisphenol-A for 2 minutes. Reactions were initiated by addition of UDPGA, and clearance (glucuronidation) of bisphenol-A by microsomal protein was calculated. It was observed that at bisphenol-A concentrations > 500 μM the system appeared to be saturated, as the formation of bisphenol-A glucuronide decreased.

Clearance in adult (77-day-old) Sprague Dawley rats was calculated to be 1.9 ml/min/mg in males and 1.3 ml/min/mg in females. In comparison, clearance was 1.0 and 1.7 ml/min/mg in adult F344 males and females respectively. In adult CF-1 mice, clearance was 3.0 ml/min/mg in males and 1.3 ml/min/mg in females. In humans, clearance was 0.9 and 0.4 ml/min/mg in the pooled male and female liver samples respectively, and in individual human samples averaged 0.5 ml/min/mg in males and 0.3 ml/min/mg in females.

In general, there appears to be no marked difference in clearance between adult Sprague Dawley and F344 rats. However, a clear sex difference was observed in CF-1 mice. Clearance in humans was lower than that observed in rats (Sprague Dawley and F344) and mice.

To investigate the effects of age, in addition to the investigations in adult Sprague Dawley rats described above, clearance was also determined in 4- and 21-day-old rats (n = 4 per sex) as well as from fetuses on day 19 of gestation (n = 8 per sex). The clearance rate of bisphenol-A was also determined in the mothers (n = 4) from which the fetuses were removed. The clearance rate in 21-day-old males and females was 2.7 and 2.4 ml/min/mg, respectively, compared to 1.2 and 2.6 ml/min/mg in 4-day-old animals and 0.9 and 0.7 ml/min/mg in fetuses. The rate in the mothers of the fetuses was 2.6 ml/min/mg.

Thus, in Sprague Dawley rats, clearance of bisphenol-A in 4 and 21-day-old animals was similar to or exceeded that in adults (77-day-old). Clearance in fetuses was lower than that seen in older animals. This finding may be due to saturation of glucuronidation capacity at lower concentrations in the fetus compared with the adult. A difference in the clearance of bisphenol-A was also observed between pregnant and non-pregnant (77-day-old) Sprague Dawley rats. However, the considerable individual variation observed among female samples (pregnant and non-pregnant) prevent any reliable conclusions being drawn on whether there is a significant difference in clearance of bisphenol-A between pregnant and non-pregnant females.

Sipes (2001) also determined the binding of bisphenol-A to hepatic microsomal protein in adult male Sprague Dawley rats (n = 3). Hepatic microsomes were incubated with 2.6-1202 μM ^{14}C-bisphenol-A for 15 minutes and binding to microsomal protein determined by scintillation counting. It was observed that approximately 30% of the ^{14}C-bisphenol-A present in microsomal fractions is unbound and available for formation of an enzyme substrate complex with glucuronyl transferase. When metabolic constants were determined assuming 30% of the total mass of bisphenol-A was available for glucuronidation, the intrinsic clearance increased from 2.3 to 7.2 ml/min/mg.

Preliminary studies have been undertaken to identify the glucuronyl transferase isoform responsible for the metabolism of bisphenol-A in the rat (Sipes, 2001). However, no reliable conclusions can be drawn from the data.

Sipes (2001) determined the metabolism of bisphenol-A in the S9 fraction from the livers of male and female F344 rats, and male homogeneous Gunn rats (which are deficient in the UGT isoform that conjugates bilirubin). S9 fractions were incubated with 500 μM ^{14}C-bisphenol-A for 0–30 minutes. Metabolites were analysed by HPLC.

Similar to the incubations with microsomes described above, bisphenol-A incubated with S9 formed a single metabolite, bisphenol-A glucuronide, in both rat strains. Reaction rates for glucuronidation were similar in male and female F344 rats. However, the rate was greater in male Gunn rats compared with male F344 rats; after 10 minutes approximately 40% of the bisphenol-A had been converted to the glucuronide in Gunn rats compared to approximately 10% in F344 rats.

The metabolism of bisphenol-A by primary cultures of hepatocytes from rats (Sprague Dawley, F344 and Gunn), mice (CF-1) and humans of both sexes (n = 2-4) was investigated (Sipes, 2001). Hepatocytes were incubated with 0-20 μM ^{14}C-bisphenol-A for up to 12 hours. Metabolites were analysed by HPLC and GC-MS.

Bisphenol-A was completely metabolised when incubated with human or rat hepatocytes for 6-12 hours (no data were presented for mice). Bisphenol-A glucuronide was produced by hepatocytes from all species and strains, along with bisphenol-A sulphate and/or a di-conjugate bisphenol-A glucuronide/sulphate in some species. Metabolic profiles (mean values) obtained in rats, mice and humans are presented below in **Table 4.23**.

Table 4.23 Metabolite formation (%) obtained in isolated hepatocytes from rat, mouse and human liver

Species	Sex	Bisphenol-A glucuronide	Bisphenol-A sulphate	Di-conjugate bisphenol-A glucuronide/ sulphate
Rat (F344)	M	30	1	70
	F	86	0	10
Rat (Sprague Dawley)	M	54	0	30
	F	100	0	0
Rat (Gunn)	M	92	0	9
	F	62	0	1
Mouse (CF-1)	M	100	0	0
	F	86	0	0
Human	M	80	0*	1**
	F	77	0*	3**

* The sulphate was observed in 1 (out of 3) female and 1 (out of 2) male hepatocyte preparations at 2 and 7.5 %, respectively. These samples were excluded from the calculation of the mean metabolite production. The sulphate was not observed in any other hepatocyte preparation.

** The di-conjugate was observed in 1 female sample at 43 % and 1 male sample at 15 %. These samples were excluded from the calculation of the mean metabolite production. Levels of 1-4 % were observed in all other samples.

The results in humans indicated a similar metabolic pattern seen in rat and mouse species with a bi-phasic kinetic profile, suggesting a high affinity UGT is involved in metabolism at low concentrations and high capacity UGT at high concentrations.

Sipes (2001) also investigated concentration dependent metabolite formation. Liver weight was determined in 4 male and 4 female rats (F344 and Sprague Dawley) and mice (CF-1), and one human sample (female). Hepatocyte preparations were then incubated with various concentrations of bisphenol-A for 10 minutes. No further details were provided.

A biphasic kinetic profile was observed in all species. Vmax values for the second phase of metabolism were calculated to be 0.39, 0.46, 0.5 and 0.27 nmol/min/$0.5 \cdot 10^6$ cells in female Sprague Dawley and F344 rats, CF-1 mice and human respectively. No significant difference was observed in Vmax values between males and females.

Using the number of hepatocytes per gram of liver reported by Kedderis and Held (1996) and the determined average liver weights, Sipes (2001) extrapolated bisphenol-A metabolism rates from hepatocytes to intact livers in rats, mice and humans. The capacity of the liver to metabolise bisphenol-A was estimated to be 61.8 and 46.5 μmol/h, 79.9 and 54.5 μmol/h and 23.6 and

13.8 μmol/h in male and female F344 and Sprague Dawley rats and CF-1 mice, respectively. The rate in the human female was determined to be 8 mmol/h.

The biphasic kinetic profile in hepatocytes from rats, mice and humans suggest a high affinity UGT is involved in metabolism at low concentrations and a high capacity UGT at high concentrations. The rate of bisphenol-A metabolism in human hepatocytes was less than that seen in rats and mice, though the overall capacity of the liver was observed to be greater in human than in rat and lowest in the mouse. This calculation does not take into account the true *in vivo* hepatic situation where not all cells may express the same metabolic capacity, where hepatic size is related to body size and physiological parameters such as blood flow may be important. Furthermore, the calculations are based on limited kinetic data, particularly human females, and do not allow for individual variability in expression of enzyme activity.

Additional data on the metabolism of bisphenol-A are available from *in vivo* and *in vitro* studies on the interaction of bisphenol-A with DNA (see Sections 4.1.2.7.1 and 4.1.2.7.3), and are supported by results from a chemical photodecomposition study (see Section 4.1.2.5.1).

In vivo, two major and several minor adducts were detected in DNA extracted from the liver of rats administered bisphenol-A by the oral or i.p route (Atkinson and Roy, 1995a). The chromatographic mobility of the two major adducts was the same as those observed when bisphenol-A was incubated with purified rat DNA in the presence of a peroxidase or microsomal P450 activation system (Atkinson and Roy, 1995a; 1995b). The chromatographic mobility of these major bisphenol-A-DNA adducts closely matched that of two adducts formed from the interaction of bisphenol O-quinone with purified rat DNA or deoxyguanosine 3'-monophosphate, and their formation appeared to be inhibited by the presence of known inhibitor(s) of cytochrome P450. As supporting evidence for the possibility of metabolism of bisphenol-A to bisphenol O-quinone, free radicals were formed following the irradiation of bisphenol-A with UV light (Peltonen et al., 1986b); the formation of bisphenol O-quinone from bisphenol-A would produce a free radical as an intermediate step (bisphenol semiquinone). Taking all these additional data into account, it seems likely that bisphenol-A may be metabolised by cytochrome P450 to bisphenol O-quinone.

Overall, the data show that bisphenol-A mainly undergoes first pass metabolism to form the glucuronide conjugate. However, there is some evidence to suggest that limited oxidation of bisphenol-A to bisphenol O-quinone by cytochrome P450 may occur. Metabolism may also occur by bisphenol-A entering the enterohepatic circulation.

Comparative *in vitro* studies of metabolism suggest some quantitative differences in the rate of metabolism between rats, mice and humans. In general, human liver samples show slower rates of glucuronidation compared with either rats or mice. Estimates of overall liver metabolic capacity suggest that human liver may have greater metabolic capacity than either rats or mice and that capacity is lowest in the mouse. However, these estimates are based on limited kinetic data and therefore are of uncertain reliability.

Elimination

In the Pottenger et al. (1997a) study, bisphenol-A disappeared rapidly from the blood, and could not be detected in low and high-dose males 5 and 45 minutes post-dosing, respectively. In females, negligible levels were observed 45 minutes and 18 hours post dosing in the low and high-dose group, respectively, demonstrating a significant sex difference in the rate of clearance from the blood between the sexes. There is insufficient information available to help resolve the

apparent sex difference. No such sex difference was observed in the plasma, where radioactivity was absent in plasma samples 72 hours after administration in all dose groups.

The total recovery of radioactive label over 7 days following oral administration was 96-98% in both sexes at both dose levels. Of these totals, elimination of the radioactive label in the urine was 14-16% in the male and 24-28% in the female dose groups. A clear sex difference in total urinary elimination was observed across dose levels, with females excreting approximately twice as much radioactivity than males. This sex difference was also observed following parenteral administration with 13-15% and 21-34% of the total radioactivity being excreted in the urine in males and females, respectively. Again, there is insufficient information available to help resolve the apparent sex difference. Elimination in the faeces was 81-82% in males and 69-72% in females. The major route of elimination following parenteral dosing was also via the faeces; 74-83% in males and 52-64% in females, respectively.

The majority (82-96%) of the radioactive dose was excreted by 72 hours post dose for the oral, i.p and s.c routes of administration. Both urinary and faecal excretion appeared to be a zero order process from 24-72 hours post dosing for the oral, i.p. and s.c. routes of administration. This suggests an initial saturation of excretion, or excretion being limited by the rate of absorption. Following this initial period, excretion became a first order process. The percentage of the oral dose recovered in tissues and carcass 7 days post-dosing by gavage was minimal; 0.03% in low-dose animals and up to 0.35% in high-dose animals. The only tissues with quantifiable levels of radioactivity were the liver and kidney, with <0.02% of the administered dose. The low amount of radioactivity retained in the tissues suggests that the potential for bioaccumulation may be limited. This is confirmed by the results of a repeated dose toxicity study in pregnant rats (see Section 4.1.2.6.1).

The average gastrointestinal transit time for F344 rats is 12-18 hours, yet over 50% of the faecal elimination occurred after 24 hours post dosing following oral administration. Small amounts of parent compound were still detected in the faeces at 72 hours and more. A possible explanation for the presence of parent compound at these later sampling times is intestinal cleavage of the conjugate, and a significant role for enterohepatic circulation, as parent compound was no longer quantifiable in blood at these later sampling times. Though these data indicate extensive absorption following oral administration they do not allow a quantitative determination of absorption to be made.

Similar results to those obtained for oral administration were observed following i.p. and s.c. administration; the major route of excretion was in the faeces and females excreted approximately twice as much radioactivity in the urine than males.

In a study by Snyder et al. (2000) investigating the toxicokinetics of bisphenol-A in lactating F344 and CD rats (see under "Absorption" for methodology), 93% of the administered dose was recovered for both strains. For CD rats the % radioactivity recovered was 21% in urine, 70% in faeces and 1.4% in carcass. Corresponding values in F344 rats were 42, 50 and 1.1%, respectively. Although the recovery of ^{14}C was identical in both species with no difference in the % dose recovered in the carcasses, urinary excretion of ^{14}C in F344 rats was twice that seen in CD rats.

In a poorly reported study available in an abstract form only (Fennell et al., 2000), female Fischer 344 rats were observed, over 6 days, to excrete more radioactivity in urine and less in faeces compared with female Sprague Dawley rats, following administration of 100 mg/kg ^{14}C-labelled bisphenol-A by gavage. Both strains were reported to have similar radioactivity profiles, with F344 rats excreting more parent compound. No further details are available.

Although this study suggests a strain difference in elimination of bisphenol-A, the limited reporting means that no reliable conclusions can be drawn from this study.

In a poorly reported developmental study available in abstract form only (Gould et al., 1998a), a concentration-related secretion of bisphenol-A into maternal milk was detected by gas chromatography and mass spectroscopy (see Section 4.1.2.9.3). The transfer of bisphenol-A and its metabolites from lactating dams to pups was determined in another study reported in abstract form only (Fennell et al., 2000). In lactating Sprague Dawley rats administered 100 mg/kg ^{14}C-labelled bisphenol-A by gavage, radioactivity recovered in the milk was 0.95, 0.63 and 0.26 μgram equivalents/ml milk at 1, 8 and 26 hours after dosing, respectively. Radioactivity in pup carcasses ranged from 44-78 μg equivalents/kg. Bisphenol-A glucuronide was reported as the major metabolite in milk. No further details are available. Although the limited details available limit the value of these studies, the results do suggest qualitatively that bisphenol-A and/or its metabolites can be excreted in milk.

In the Knaap and Sullivan study (1966), urine and faeces were collected over 8 days following oral administration of bisphenol-A. In three similar experiments, 86-93% of the radioactive dose was recovered; average recoveries were 28% in the urine and 56% in the faeces. The majority of the administered dose was excreted by 48 hours. No radioactivity was detected in exhaled CO_2 when rats were placed in a metabolism chamber for 4 hours immediately after dosing. ^{14}C residues were not detected in samples of intestine and carcass that were analysed 8 days post dosing. Therefore, within the confines of this early study, the results obtained are in agreement with those of the recent well conducted Pottenger et al. (1997a) study.

4.1.2.1.2 Studies in humans

In a generally well reported study, Schaefer et al. (2000) investigated concentrations of xenoestrogens (including bisphenol-A), antiandrogens, phytoestrogens, mycotoxins and organochlorines in human endometrium and body fat (reference tissue). Samples of endometrium and body fat were obtained between 1995 and 1998 from 23 women (age 34-51 years) undergoing hysterectomy for uterine myoma. It is reported that questionnaires ascertained there was no occupational exposure to the chemicals investigated. Bisphenol-A was extracted from the samples and measured by GC-MS. The detection limit for bisphenol-A was reported as 1-20 μg/kg (wet weight), depending on the tissue analysed (no further details reported).

Bisphenol-A was detected in 1/23 samples of endometrium at a concentration of 13 μg/kg (giving a median of <1 μg/kg). Bisphenol-A was not detected in the 21 samples of body fat analysed. Thus, bisphenol-A was only detected in endometrium in a single case. Consequently, it is considered that overall there is no evidence from this study to suggest that bisphenol-A accumulates in either human endometrium or body fat.

In a study which was reported in abstract form only (Takada et al., 1999), levels of bisphenol-A and nonylphenols were measured in three human umbilical cords obtained from a hospital. Bisphenol-A was detected in the umbilical cords (1.6-0.4 ng/g wet tissue), but was also present in the blank control. No further details are available. Given the limited reporting and presence of bisphenol-A in the blank control, no reliable conclusions can be reached from this study.

In a further study reported as an abstract (Kuribayashi et al., 1999), bisphenol-A, nonylphenol and octylphenol were measured in 3 human umbilical cords (3 samples per cord) obtained from a hospital. Significant levels of bisphenol-A (defined as values exceeding the blank by a factor of

2) were observed in 6 of the 9 samples; 0.2-2.0 ng/g wet weight. However, as stated for Takada et al. (1999), the limited reporting and presence of bisphenol-A in the blank control again mean that no reliable conclusions can be drawn from the study.

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

The limited data available in humans, from a single study, indicate that bisphenol-A does not accumulate in endometrium or body fat (the only tissues tested). In experimental animals, toxicokinetic data are available from three oral studies in a single species, the rat and from an *in vitro* dermal absorption study, using human skin. These studies provide the basis for a general understanding of the main features of the toxicokinetic profile. Following oral administration, absorption from the gastrointestinal tract is rapid and extensive, although it is not possible to reliably quantify the extent of absorption. Following dermal exposure, the available data suggest that there is limited absorption, in the region of about 10% of the applied dose. Bisphenol-A was removed rapidly from the blood, and metabolism data indicate extensive first pass metabolism following absorption from the gastrointestinal tract. A clear sex difference was observed in the clearance of parent compound from the blood. In females parent compound was present in the blood at much later sampling times. There are no data available to explain why this sex difference was observed. In view of this first pass metabolism, the bioavailability of unconjugated bisphenol-A is probably limited following oral exposure, at no more than 10-20% of the administered dose. Limited data are available for the distribution of bisphenol-A following oral administration: an *in vivo* DNA adduct study shows that bisphenol-A reaches the liver, an *in vivo* micronucleus study suggests that bisphenol-A or a metabolite reaches the bone marrow, a limited toxicokinetic study suggests that bisphenol-A or a metabolite reaches the testes, and a repeated dose study in pregnant rats suggests that bisphenol-A reaches the liver of both the dam and fetus. However, because of first pass metabolism, it is likely that the distribution and bioavailability of unconjugated bisphenol-A is limited following oral exposure. There is also evidence of enterohepatic circulation occurring.

The major metabolic pathway in rats involves glucuronide conjugation; limited sulphate conjugation may also occur. Approximately 10% and 20% of the administered dose was recovered in the urine as the glucuronide metabolite in males and females, respectively. There are no data available to explain why this sex difference was observed. Comparative *in vitro* studies of metabolism suggest some quantitative differences in the rate of metabolism between rats, mice and humans. In general, human liver samples show slower rates of glucuronidation compared with either rats or mice. Estimates of overall liver metabolic capacity suggest that human liver may have greater metabolic capacity than either rats or mice and that capacity is lowest in the mouse. However, these estimates are based on limited kinetic data and are therefore of uncertain reliability. *In vitro* data in rats also indicate that fetuses do not metabolise bisphenol-A as extensively as immature and adult animals. In addition, data from cell free systems and *in vivo* studies on the interaction of bisphenol-A with DNA, supported by a chemical photodecomposition study, suggest that limited oxidation of bisphenol-A to bisphenol O-quinone by cytochrome P450 may occur.

The major route of excretion is via the faeces with the urinary route being of secondary importance: over 7 days post dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post dose. A sex difference was also observed in elimination, with females excreting approximately twice as much radioactivity in the urine (24-28%) than males (14-16%). Again, there are no data available to explain why this sex

difference was observed. In addition, a strain difference was observed in elimination, with female F344 rats excreting approximately twice as much radioactivity in the urine than female CD rats. Data from a number of studies suggest limited excretion of bisphenol-A in the milk. However, the data do not allow a reliable quantitative determination to be made.

The first pass metabolism and extensive and rapid elimination of bisphenol-A suggest that the potential for transfer to the foetus and bioaccumulation may be limited. This is supported by data from toxicokinetic studies in pregnant rats that suggest limited distribution of bisphenol-A to the foetus, but no evidence for accumulation, and results from a repeated dose study in pregnant rats which show limited distribution to the fetal liver, with no evidence to indicate accumulation in the liver, the only organ tested.

There are no data on the toxicokinetics of bisphenol-A following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Inhalation exposure

In a well conducted study (Nitschke et al., 1985a), groups of 10 male and 10 female Fischer F344 rats were exposed, whole body, to bisphenol-A dust (polycarbonate grade) at concentrations of 0 or 170 mg/m^3 (the highest attainable concentration) for 6 hours. The mass median aerodynamic diameter (MMAD) of the test substance was 3.9 micrometers (μm), and the exposure concentration used was the highest attainable by the investigators with the test system used. Half the animals were necropsied on the day following exposure and the rest on day 14. Microscopic examination was limited to the respiratory tract (nasal turbinates, larynx, trachea and lungs) and associated tissues. No deaths occurred and therefore the LC_{50} value for rats is > 170 mg/m^3. No gross signs of toxicity were observed. At necropsy, "slight" inflammation of the epithelium lining of the anterior portion of the nose and "slight ulceration" of the oronasal duct were reported in 5/5 males and 4/5 females exposed to 170 mg/m^3 and sacrificed on day 2. No exposure-related effects were observed in animals necropsied on day 14. It is concluded that bisphenol-A is of relatively low acute inhalation toxicity.

Oral exposure

Rats

The acute oral toxicity of bisphenol-A has been investigated in a number of gavage studies. In the most recent study, which was well conducted, Sprague-Dawley rats (5 per sex per group) received 2,000 or 5,000 mg/kg of bisphenol-A (Hazleton Laboratories, 1985). No deaths occurred at 2,000 mg/kg. At 5,000 mg/kg all the females and 1/5 males died. Clinical signs of toxicity observed at 2,000 and 5,000 mg/kg on the day of dosing included lethargy and

prostration. Hunched posture and piloerection were also observed at 5,000 mg/kg after dosing. At necropsy, pale livers and/or haemorrhaging of the GI tract were observed in animals that had died during the study. The LD_{50} for males and females combined was approximately 5,000 mg/kg, although females appeared generally more sensitive than males.

Studies in F344 rats were conducted as part of an investigation of bisphenol-A for the National Toxicology Programme (NTP, 1982). Rats (5 per sex per group) were given 2,150, 3,160, 4,640 or 6,810 mg/kg of bisphenol-A. Mortalities were 0, 1, 3 and 5 in males, and 1, 4, 2 and 5 in females, respectively. The calculated LD_{50} values were 4,100 mg/kg in males and 3,300 mg/kg in females. No other aspects of the study were reported.

In briefly reported studies, rat LD_{50} values from 3,200 to 5,660 mg/kg have been given (Jones, 1968, Mellon Institute of Industrial Research, 1946, 1948, 1965). No further information was supplied.

In an old and briefly reported range finding study, evaluating the acute oral toxicity of both the recognised grades of bisphenol-A in rats (2 per sex per group), no animals died at 1,000 mg/kg but both animals died at 2,000 mg/kg for both grades. (Dow Chemical Company, 1957). In this study, the small number of animals used and lack of experimental details preclude any meaningful conclusions about the acute toxicity of bisphenol-A.

Mice

In a study conducted using $B6C3F_1$ mice (NTP, 1982), LD_{50} values in males and females were 5,200 and 4,100 mg/kg, respectively. No other aspects of the study were reported.

A mouse LD_{50} value of 1,600 mg/kg was reported (Jones, 1968). No further information was supplied. Given the complete lack of experimental details of this relatively old study and, on comparison with the findings reported by the NTP, this relatively low LD_{50} value is not considered to be a reliable indicator of the acute oral toxicity of bisphenol-A in mice.

Rabbits

In a briefly reported study, a rabbit LD_{50} value of 2,230 mg/kg was obtained (Mellon Institute of Industrial Research, 1948). No other information was reported.

Overall, these results indicate that bisphenol-A is of low acute oral toxicity in rats, mice and rabbits.

Dermal exposure

Rabbits

The acute dermal toxicity of bisphenol-A has been investigated in two relatively poorly reported studies. In the first, 2,000 mg/kg of bisphenol-A, as a 10% solution in propylene glycol (PEG) was administered to a group of 15 rabbits and resulted in the death of three animals (Mellon Institute of Industrial Research, 1948). In the second study, 6,400 mg/kg of bisphenol-A, as a 40% solution in dimethylsulfoxide (DMSO), killed 1/4 rabbits following a 24-hour exposure period. (Mellon Institute of Industrial Research, 1965). Although neither study report presented any further information, the consistent findings lead to the conclusion that bisphenol-A is of low acute dermal toxicity with a LD_{50} >2,000 mg/kg in rabbits.

4.1.2.2.2 Studies in humans

During open heart surgery, "marked" haematuria was observed in two children (both were 3-year-old girls) at the onset of cardiopulmonary bypass (Larson et al., 1977). A chemical contaminant was found in the bypass apparatus that was identified as containing bisphenol-A and reaction products of bisphenol-A with ethylene oxide. In subsequent investigations, crystalline bisphenol-A was found to show no haemolytic effect *in vitro*. Taking into account also the complex and non-quantifiable nature of exposure to chemicals by the subjects in this study, it is concluded unhelpful in the evaluation of bisphenol-A toxicity.

4.1.2.2.3 Summary of acute toxicity

No useful information is available on the effects of single exposure to bisphenol-A in humans. Oral LD_{50} values beyond 2,000 mg/kg are indicated in the rat and mouse, and dermal LD_{50} values above 2,000 mg/kg are evident in the rabbit. Few details exist of the toxic signs observed or of target organs. For inhalation, a 6-hour exposure to 170 mg/m^3 (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed. These data indicate that bisphenol-A is of low acute toxicity by all routes of exposure relevant to human health.

4.1.2.3 Irritation

4.1.2.3.1 Skin

Studies in animals

A recent well conducted study is available investigating the skin irritation potential of bisphenol-A. The only other data are from very briefly reported, non-conventional studies. No individual or mean numerical scores are provided in the reports of these older studies.

In the recent well reported study (Leuschner, 2000a), three rabbits received a 4-hour application of 500 mg bisphenol-A moistened with water under a semi-occlusive dressing. Skin reactions were noted at 1, 24, 48 and 72 hours after patch removal. No signs of erythema or oedema were observed at any of the time-points. Therefore, bisphenol-A was observed to have negligible skin irritation potential in this study.

It was reported in a previous study (Mellon Institute of Industrial Research, 1946; 1965) that "moderate to marked capillary injection" in the skin was observed in rabbits with a 40% solution of bisphenol-A in DMSO. In contrast, "slight capillary injection" was reported with a 10% solution in PEG. No further details are available. Jones (1968) reported "slight irritation" in guinea pigs following a 24-hour exposure to a 40% solution of bisphenol-A in a mixture of acetone and corn oil.

A briefly reported study compared the skin irritation potential of both recognised commercial grades of bisphenol-A (Dow Chemical Company, 1957). For each grade, ten consecutive applications of the undiluted material to rabbit skin produced signs of "slight" or "slight to moderate" irritation. It is not clear how many rabbits were employed. No further details are available.

Studies in humans

The only information available is from written company correspondence that stated, "production personnel involved in the bagging of this material (bisphenol-A) have experienced a frequency of skin rash" (Dow Chemical Company, 1957) and "finely divided materials may settle on the skin, particularly if the person is sweating, and cause a burning or tingling sensation" (Du Pont, 1962). The reliability of these statements is unclear and 'skin rash' could be related to the skin sensitisation endpoint, rather than irritation.

A recent well conducted animal study has shown that bisphenol-A is not a skin irritant. The human data are so poorly reported that no reliable conclusions can be reached from them on the skin irritation potential of bisphenol-A.

4.1.2.3.2 Eye

Studies in animals

A recent well conducted study is available investigating the eye irritation potential of bisphenol-A. The only other data available are from very briefly reported studies employing a limited number of animals. No individual or mean numerical scores are provided in the reports of these older studies.

In the well conducted study (Leuschner, 2000b) three rabbits received a single instillation of 0.1 g bisphenol-A into the eye. The eyes were examined 1, 24, 48 and 72 hours after instillation and daily thereafter up to 28 days. Scattered or diffuse areas of corneal opacity, irritation to the iris and conjunctival redness were observed in all animals after 1 hour. Chemosis was observed in one animal from 1 hour until day 6, and in the remaining two animals at 24 hours only. Corneal opacity and irritation to the iris persisted in a single animal to day 28. In the remaining two animals, no effects were observed on the eye by day 14. Whitish deposits, which the author reports were probably pus, were also observed in two animals from 72 hours to day 5 post-instillation. The persistence of corneal opacity and irritation of the iris to the end of the observation period (day 28) indicate that bisphenol-A has the potential to cause serious damage to the eyes.

In studies comparing the two commercial grades of bisphenol-A (Lockwood, 1984a; 1984b) it was reported that instillation of 0.1 g of bisphenol-A (polycarbonate grade) into a rabbit's eyes resulted in "slight" conjunctival irritation, and "moderate" clouding of the cornea with "slight" reddening of the iris. No irritation was observed at 7 days post-instillation. When the eye was washed (it was not stated how long after instillation this was) no signs of irritation were observed. The second commercial grade of bisphenol-A similarly produced "moderate" discomfort, "moderate" redness and swelling of the conjunctiva, and "moderate" clouding of the cornea with vascularisation and reddening of the iris in the washed and unwashed eye. It was stated that the washed eye 'healed' within 7 days and the unwashed eye within 20 days post-instillation. Overall, these briefly reported studies indicate, qualitatively, that bisphenol-A has the potential to cause eye irritation.

Carreon (1982) reported that instillation of undiluted bisphenol-A (amount not stated) into a rabbit's eyes caused "very slight" discomfort, "slight" conjunctival redness and swelling, transient reddening of the iris and "very slight" transient corneal haziness. All signs of irritation were "essentially absent 48 hours following exposure." Given the very limited reporting of

methodological details, this study does not detract from the findings reported by Lockwood (1984a; 1984b) or Leuschner (2000b).

In older studies which were also very poorly reported, it was stated that a 5% solution of bisphenol-A in PEG produced "major corneal damage", and a 1% solution produced "trace injuries" to rabbit eyes (Mellon Institute of Industrial Research, 1946). It was also reported that instillation of 0.5 ml of a 5% solution of bisphenol-A in DMSO produced "severe corneal necrosis" and a 1% solution produced "trace injuries" (Mellon Institute of Industrial Research, 1965). Given the very limited details available for both these studies, the reliability of the findings reported is difficult to assess, and no useful conclusion about the magnitude of the eye irritation potential of bisphenol-A can be reached from them.

Studies in humans

The only information available is from written company correspondence. Du Pont (1962) stated, "(the workers) experienced eye or nasal irritation." Also, information from "bisphenol-A manufacturers" stated that "workers complained of eye and throat irritation when exposed to bisphenol-A dust" (US Environmental Protection Agency, 1985). However, the absence of any exposure details means that these anecdotal findings are of little value in determining the eye irritation potential of bisphenol-A.

4.1.2.3.3 Respiratory tract

Studies in animals

In an acute inhalation toxicity study (see Section 4.1.2.2.1; Nitschke et al., 1985a) and repeat exposure studies (see Section 4.1.2.6.1; Nitschke et al., 1985b; 1988), local inflammation effects were observed in the upper respiratory tract of rats after a single exposure to 170 mg/m^3 bisphenol-A dust for 6 hours and repeated exposures to 50 mg/m^3 and above for 2 and 13 weeks, respectively. These studies provide evidence that bisphenol-A has the potential to cause respiratory irritation.

Steinhagen et al. (1987) studied the sensory irritation potential of bisphenol-A dust on respiration rate in male mice and rats using the Alarie assay. The reporting of this study is available as an abstract only, and states that the animals were exposed, head only, to 39-820 mg/m^3 bisphenol-A (MMAD 0.72 to 1.13 μm) for 15 minutes. At 152 mg/m^3 and above, decreased respiration rates were observed in both species. The RD_{50} values were 684 and 959 mg/m^3 in mice and rats, respectively. Without further details, the precise mechanism of the decreased respiratory rates in this study cannot be established and the significance of these values for human health is unclear.

Studies in humans

The only information is from anecdotal written company correspondence (see Section 4.1.2.3.2), reporting that workers complained of "respiratory irritation" (US Environmental Protection Agency, 1985; Du Pont, 1962). Shell Oil Company (1984) has reported a survey in which a questionnaire was administered to 9 employees. The survey also included observation of individuals at work (details not provided) and it was reported that bisphenol-A appeared to cause "respiratory irritation".

In the absence of further details, it is not possible to further explore the reliability of these statements. However, qualitatively, they do suggest that bisphenol-A dust has the potential to cause respiratory irritation.

4.1.2.3.4 Summary of irritation

Limited human anecdotal information of uncertain reliability is available from written industry correspondence suggesting that workers handling bisphenol-A have in the past experienced skin, eye and respiratory tract irritation. It cannot be determined whether the reported skin reactions were related to skin sensitisation or irritation. However, a recent well conducted animal study clearly shows that bisphenol-A is not a skin irritant. A recent well conducted animal study shows that bisphenol-A is an eye irritant; effects persisted until the end of the study (day 28 post-instillation) in 1 of 3 rabbits. Overall, taking into account the animal and human evidence, bisphenol-A has the potential to cause serious damage to the eyes.

Slight and transient nasal tract epithelial damage was observed in rats exposed to bisphenol-A dust at 170 mg/m^3 for 6 hours. Slight local inflammatory effects in the upper respiratory tract were observed in rats exposed to 50 mg/m^3 and 150 mg/m^3 of bisphenol-A in 2 and 13 week repeat inhalation studies, but were not observed at 10 mg/m^3 in the same studies. Increased duration of exposure did not increase the severity of the response at 50 and 150 mg/m^3. Taken together with anecdotal human evidence, these data suggest bisphenol-A has a limited respiratory irritation potential.

4.1.2.4 Corrosivity

The data available and summarised above show that bisphenol-A is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Skin

Studies in animals

The skin sensitisation potential of bisphenol-A has been investigated in several studies. Generally, the studies are only briefly reported with omission of the observations at induction and with no information on the use of positive controls. Also, the description of challenge results is provided in summary form only.

In a maximisation study, 15 guinea pigs were employed in the treated and control groups (Thorgeirsson and Fregert, 1977). At induction, 5% bisphenol-A was injected intradermally and topically. At challenge, 1% bisphenol-A was applied, and produced no skin responses in treated or control animals (six epoxy resins were also applied; see below). Hence a negative result was obtained. It was stated that the topical irritancy of chemicals for induction was determined by 24-hour patch testing (no further information provided). However, since no reliable details were given to justify the choice of concentrations used, it is possible that induction and challenge concentrations were not maximised. Consequently, this apparently negative study does not provide completely reliable evidence for the lack of sensitisation potential.

As an additional part to this study, maximisation tests were conducted on six epoxy resins of diglycidyl ether-bisphenol-A type with average molecular weights (MW) of 340-1,850. Control and treated groups employed 20 guinea pigs for each epoxy resin. The induction and challenge concentrations were the same as those used with bisphenol-A. At challenge, the other epoxy resins and bisphenol-A were also applied to the control and treated animals to determine if cross sensitisation between the epoxy resins and bisphenol-A could occur. Skin responses were observed in ≥30% animals induced and challenged with epoxy resins MW 340-1,280. It is reported (data not shown) that animals sensitised to epoxy resins did not react to bisphenol-A, and vice-versa. As stated previously, since the bisphenol-A induction and challenge concentrations may not have been maximised, the negative results of this cross challenge study cannot be regarded as being conclusive.

In the first of two guinea pig closed-patch tests reported by Procter & Gamble Company (1969), an unstated concentration of bisphenol-A was applied topically, for 6 hours once weekly for 3 weeks, to a test group of 20 guinea pigs. The study included a control group of 20 guinea pigs but it was not reported how these animals were treated over the induction period. Two weeks after the final induction treatment, a challenge concentration of 25% bisphenol-A was applied to both the test and control groups. No skin responses were observed in any animals at challenge, although it was not reported when challenge reading(s) were taken. In the second part of the study, a further guinea pig closed-patch test was conducted involving 16 and 10 guinea pigs in treated and control groups, respectively. For induction of the test group, an unstated concentration of bisphenol-A was applied for 4 hours once weekly over 3 weeks. Again, no details are provided on the treatment of control animals during induction. Two weeks after the final topical induction a challenge concentration of 50% bisphenol-A was applied to both groups. It is not reported when challenge reading(s) were taken. Skin responses were seen in 2/16 (12.5%) test animals and 0/10 control animals. Given the absence of full experimental details for the induction and challenge steps in these tests, interpretation is compromised. However, the second study suggests that bisphenol-A may possess a limited skin sensitising potential.

Two further results are available. Union Carbide Corporation, (1947) reported that 2/12 guinea pigs were “weakly sensitised” to bisphenol-A in a modified Landsteiner test. No further details are available. Jones (1968) reported very briefly another guinea pig test in which bisphenol-A was applied as a 1% solution in a mixture of acetone, dioxane and guinea-pig fat (details not specified). Apparently, no sensitisation response was observed in any of the animals. However, these studies are considered of no value given the limited reporting and use of non-standard test methods.

Studies in humans

There are anecdotal reports of skin inflammation being observed in workers exposed to pure bisphenol-A (see Section 4.1.2.3.1), but the limited information provided means that the nature of these skin reactions cannot be ascertained. However, there are a number of case reports of individuals showing skin problems following exposure to resin acids derived from bisphenol-A who have shown sensitivity to bisphenol-A at challenge in patch tests. The following representative examples show that although bisphenol-A has been found to elicit an allergic response in already sensitised individuals, it is not entirely clear if it can act as an inducer itself. In many cases it seems that the explanation of the findings lies in cross-sensitisation to bisphenol-A resulting from primary sensitisation to epoxy resins that are derived from bisphenol-A.

A 53-year-old man with no past personal or family history of skin disease developed dermatitis of the right hand and on his nose after 5 years of working with various liquid waxes (Freeman and Warin, 1984). In patch tests, positive reactions were observed with only 2 out of 10 waxes,

and these were the only ones to contain bisphenol-A as an ingredient. In further tests, he was found to respond positively to 1% bisphenol-A. The patch test results suggest that the initial causative agent may have been the bisphenol-A present in two of the waxes.

In a very briefly reported case study, both a 17-year-old woman and 25-year-old man developed dermatitis on the feet that corresponded to the area in contact with the plastic sandals they wore (Srinivas et al., 1989). Patch testing was performed with 30 different potential plastic and glue allergens, but apparently a positive result was seen only with bisphenol-A (1%). Bisphenol-A was demonstrated to be present in the footwear. Again, either free bisphenol-A may have caused these allergic responses or these constitute further cases of cross sensitisation arising consequent to initial sensitisation to bisphenol-A-derived epoxy resins in plastics.

A 65-year-old woman who wore dentures developed symptoms of burning mouth and burning tongue (van Joost et al., 1988). In patch tests, positive results were observed with a bisphenol-A-derived epoxy resin standard and to bisphenol-A itself. In explaining the results, the authors attributed the sensitisation to bisphenol-A-derived epoxy resins used frequently for denture repair procedures.

A 44-year-old woman who had worn PVC gloves for 4 years developed glove-shaped hand eczema (Estlander et al., 1999). Patch tests indicated that the gloves were responsible for the skin responses and that the woman was sensitised to both bisphenol-A (1%) and colophony resin acids (20%) present in the glove. However, the woman had suffered from bouts of eczema and hand dermatitis throughout her life, confounding interpretation of the results.

A 42-year-old woman who had worked as a dental assistant for 4 years developed hand dermatitis (Jolanki et al., 1995). In patch tests, the 2 dental composite resins she had worked with produced weak reactions. Both these dental composite resins were analysed and found to contain bisphenol-A, to which the woman was also found to be sensitised. A positive patch test was also recorded for formaldehyde, and this was present in one of the resins. Consequently, it is unclear if bisphenol-A was the cause of dermatitis in this instance.

Between 1974 and 1976, 16 patients (aged 18-65-year-old) at a clinic had dermatitis on the thighs where their trouser pockets, all made of polyamide, had contact with the skin (Grimalt and Romaguera, 1981). Dermatitis was stated to have occurred after 2 to 12 months of contact. None of the patients were atopic although 5 had atopy antecedents. In patch tests, positive responses to bisphenol-A and pieces of trouser pocket were seen 6/16 patients. A further 5 patients responded to the trouser material only. These findings suggest that the positive responses to bisphenol-A were most likely a consequence of cross sensitisation. However, it is unclear exactly what materials had been used in the manufacture of, or were present in, the polyamide pockets and therefore further interpretation of these results is not possible. Also in this study a group of 50 volunteers, apparently without dermatitis but for whom no further details were given, were tested with bisphenol-A to determine whether the patch test concentration used was irritating. No irritant responses to bisphenol-A were seen. However, the results observed in these 50 volunteers do not assist in the interpretation of this study and, overall, no conclusions about the skin sensitisation potential of bisphenol-A can be drawn from this study.

Six patients (aged 23-52-year-old) who had worked for 1-5 years in a plant manufacturing bisphenol-A-derived epoxy resins developed allergic contact dermatitis on the hands, arms, legs or face (van Joost et al., 1990). In patch tests, positive reactions to a bisphenol-A-derived epoxy resin standard (MW 385), and to bisphenol-A itself were observed in 3/6 and 1/6 patients, respectively.

Among 99 dermatitis patients from 8 factories, 78 responded in patch tests to an epoxy resin of MW approximately 150 (Krajewska and Rudzki, 1976). The molecular weight of this epoxy resin does not coincide with that of an epoxy resin based on bisphenol-A. However, the workers had exposure to bisphenol-A-derived epoxy resins in the factory and this seems the most likely cause of their skin problem. In further tests, the 78 patients were tested with 2% bisphenol-A itself and positive reactions were observed in 13/78 instances.

As the following representative examples typically show, bisphenol-A itself has also given positive patch test results on challenge in workers from a number of different occupational settings. These include: an 18-year-old man employed for 1 week sanding archery bows made of fibre glass (Gaul, 1960); a 55-year-old man who had worked 15 years in a textile plant employed in the recovery of synthetic wool fibres (Romaguera et al., 1981); a 26-year-old woman who had worked for 2 years assembling the plastic cases of hearing aids (Romaguera et al., 1986); and a 22-year-old man who had worked for 4 years manufacturing motor vehicles (Hayakawa et al., 1985). In all these cases, positive patch tests to bisphenol-A-derived epoxy resin were also observed and, in the case studies reported by Romaguera et al. (1981 and 1986) and Hayakawa et al. (1985), positive results were obtained with formaldehyde and bisphenol-F, respectively. It is not possible to attribute any of these cases of dermatitis to skin sensitisation induced by bisphenol-A itself.

In addition to those examples described above relating to bisphenol-A-derived epoxy resins, bisphenol-A may elicit sensitising responses following exposure of individuals to other structurally similar substances. For example, a 34-year-old woman with allergic eczema of the neck caused by a hair lotion that did not contain bisphenol-A still responded in a patch test to bisphenol-A (Fregert and Rorsman, 1960).

In addition to these patch test studies showing positive patch tests with bisphenol-A, there are many similar studies in the literature that report negative findings (Prens et al., 1986; Jolanki et al., 1987; van Joost, 1988; Holness and Nethercott; 1989 and 1993; Jolanki et al., 1990; Kanerva et al., 1991; Moura et al., 1994; Patussi et al., 1995; Angelini et al., 1996).

Overall, this mixed pattern of results from human studies suggests there is some potential of bisphenol-A either to cause sensitisation or to trigger sensitisation reactions in individuals exposed to resins containing bisphenol-A.

There are a small number of reports suggesting that individuals responding in patch tests to bisphenol-A may also respond to other chemicals. Bruze and Zimerson (1985) and Jolanki et al. (1990) have both reported an apparent cross-sensitisation response with bisphenol-F, present in some phenol-formaldehyde resins. In contrast, Fregert and Rorsman (1961) found that dimethyldi-(4-hydroxyphenyl)-silane produced positive patch responses in four previously unexposed dermatitis patients who had previously been diagnosed hypersensitive to bisphenol-A (cause of sensitisation to bisphenol-A is not stated). Fregert and Rorsman (1962) found that dermatitis patients responding to bisphenol-A after showing sensitivity to bisphenol-A-derived epoxy resins were also sensitive to stilboesterols.

Photosensitisation

In addition to these studies on skin sensitisation potential, several studies have examined the ability of bisphenol-A in the presence of UV light to produce photosensitising responses. These studies were conducted in response to a clinical report by Allen and Kaidbey (1979) that photoallergic contact dermatitis occurred in workers using a bisphenol-A-derived epoxy resin (see following section).

In a mouse ear-swelling test, a dose group of 6 ICR mice were pre-treated with *Corynebacterium parvum* as immunological adjuvant, and then received topical application of 0.02 ml 1% bisphenol-A on the rear shaven flank (Maguire, 1988). Following this the application site was irradiated with UV-B and then UV-A to complete the induction phase of the test. Control animals (number not stated) received neither bisphenol-A nor UV exposure in this phase. At challenge, both groups of mice received 0.01 ml of 1% bisphenol-A on the left ear followed by UV-A irradiation to both ears. The right ear then received 0.01 ml of bisphenol-A. Allergenic responses were measured by the increase in ear thickness 24 hours after challenge, compared to thickness in the same ear before challenge. The left ears of "several" mice from the treated and control groups were removed after the 24-hour measurements for a microscopic examination.

There was a statistically significant increase in mean thickness of the left ears of animals in the test group following challenge. No significant increase in mean thickness was observed in the right ear. In the control group, no significant increase in swelling was observed in either ear. Histopathology showed no inflammation in the ears of control animals, whereas oedema and a monocellular infiltrate were observed in the left ears of treated animals. This demonstrated that the observed increase in left ear thickness could not be explained as a straightforward skin sensitisation reaction. Furthermore the lack of ear swelling in the control group demonstrated the absence of a phototoxic response to bisphenol-A and UV under the challenge conditions. Overall, it can be concluded that a photosensitisation response had been found in test animals. This conclusion was supported by the histopathological findings.

A similar BALB/c mouse ear-swelling test consisted of four groups of 6-8 animals, designated photosensitisation, skin sensitisation, vehicle/radiation control and phototoxicity groups (Gerberick and Ryan, 1990). Mice in the photosensitisation, skin sensitisation and vehicle/radiation control groups were pre-treated with the immunological adjuvant cyclophosphamide. At induction, animals in the photosensitisation and skin sensitisation groups received a daily application of 50 ml of a 20% bisphenol-A on the rear shaven flank for 3 consecutive days. The vehicle/radiation group received vehicle only. Following the last application, only animals in the photosensitisation and vehicle/radiation group were irradiated with UV-A and then UV-B. Animals in the phototoxicity group were not treated at induction. Animals in the photosensitisation, skin sensitisation and phototoxicity groups were challenged with 8 ml of a 10% bisphenol-A solution on each ear. The vehicle/radiation group received vehicle only. All animals except those of the skin sensitisation group were then irradiated with UV-A and then UV-B. The photosensitisation reaction was measured by the increase in ear thickness 24 hours after challenge compared to thickness in the same ear before challenge.

A statistically significant increase in mean ear swelling was observed in animals in the photosensitisation group when compared to the skin sensitisation, vehicle/radiation and phototoxicity control groups. The results demonstrate that the increase in ear thickness is due to photosensitisation, and not skin sensitisation, phototoxicity or the vehicle under the conditions of photochallenge.

In addition to the mouse ear swelling test performed by Maguire (1988) as described above, three mechanistic tests were also reported. They were all reported in summary form only and all focused on the mouse ear swelling test.

In the first mechanistic study, 3 groups of Swiss-Webster mice (6 mice per group) were pre-treated with cyclophosphamide as an adjuvant, and the same topical application of bisphenol-A (concentration not stated) at induction. The first group was additionally irradiated with UV-A and UV-B, the second with UV-A only and the third was left with no specific UV exposure. The animals were then all treated with *C. parvum*. A fourth group of mice, acting as a

phototoxicity control, was not treated at induction. At challenge, all mice received bisphenol-A solution (concentration not stated) to both ears and then irradiation of the left ear with UV-A. A statistically significant increase in mean ear thickness was observed 24 hours after challenge only for those mice that had received both UV-A and UV-B. This demonstrates that both UV-A and UV-B are required during induction to demonstrate a photoallergic response.

In the second study, Balb/C·A/J mice (number not stated) pre-treated with cyclophosphamide, received topical application of 5% bisphenol-A solution on the rear shaven flank for 2 consecutive days followed by exposure to UV-A and UV-B. They were then injected with *C. parvum* to complete the induction phase. Two days later, animals were sacrificed and the lymphoid cells from the regional lymph nodes injected into a group of mice (number not stated) pre-exposed whole body to X-rays to diminish immunocompetence. One hour after this transfer of cells, the mice were challenged with bisphenol-A solution (concentration not stated) and exposed to UV-A. On measuring ear thickness after challenge (it is not reported how long after) it was found that the recipient mice were UV-A photosensitive to bisphenol-A, showing the cellular transmission of allergenicity by lymphoid cells.

In the third study, a photosensitisation response was observed at challenge in a group of mice (number not stated) that had been treated with 1% bisphenol-A, irradiated with UV-A and UV-B and treated with *C. parvum* at induction. Photosensitisation was not observed at challenge in the phototoxicity control group, which were not treated at induction. A week after the initial challenge, the left ears were again exposed to UV-A and allergic reactions measured by the increase in ear thickness after a further 24 hours. After this rechallenge with UV-A only, a statistically significant increase in mean ear thickness was observed in treated animals, demonstrating photosensitivity. In separate experiments (data not reported), Maguire stated that DNFB (a control sensitiser, not photoallergenic) -induced mice, whose rechallenged sites were similarly exposed to UVA, had no ear thickening. The results of this study show a specific and persistent light reactivity following photosensitisation to bisphenol-A. However, the duration of this light induced reactivity, without further exposure to bisphenol-A, is unknown.

Overall, the studies involving exposure of mice to UV light together with supporting mechanistic data suggest that bisphenol-A can induce a photosensitising reaction that appears to be mediated by the immune system. However, it is noted that the test methods employed have not been fully validated. Peltonen et al. (1986b) have suggested that bisphenol-A photosensitising potential may be due to the formation of free radicals by UV mediated photodecomposition, and that these radicals may react with macromolecules to form an antigen responsible for the observed photosensitisation.

Allen and Kaidbey (1979) reported, in detail, photosensitisation in eight workers who had been exposed during winter months to dense fumes of an epoxy resin mixture based on bisphenol-A (88.5% bisphenol-A-epichlorohydrin epoxy resin) during heating of the mixture with gas burners. Two months later, in the spring, all 8 workers (who it is assumed, were still being exposed to the resin) noted the sudden onset of stinging, burning and erythema following a 10-15-minute exposure to direct sunlight. The symptoms were limited to areas previously exposed to the hot resin fumes.

Photopatch tests (with UV-A only) were conducted on these individuals with a bisphenol-A-derived epoxy resin standard, with the particular epoxy resin mixture used at work and with bisphenol-A (0.01-1%) itself. A solvent control treated site was also included. The tests involved duplicate applications of substance to the lower back under an occlusive dressing for 24 hours, after which one set was irradiated with UV-A. Responses were evaluated at the end of irradiation, and then 24, 48 and 72 hours later. In addition, standard patch tests were performed

for both the epoxy resins (standard and mixture) and, in a control group of eight volunteers, all the test substances were assayed for phototoxicity.

In the photopatch tests, positive skin reactions were observed in 4/8 workers with both the epoxy resin mixture and the epoxy resin standard, and all 8 workers exhibited a positive reaction to 0.01, 0.1 or 1% bisphenol-A. Three of the four individuals responding to the epoxy resins appeared to be conventionally sensitised, as they also responded positively to both the epoxy resins (standard and mixture) in the patch tests without UV light exposure. No skin reactions were observed at any of the other sites or in the control group at non-irradiated sites. Overall, these findings showed that bisphenol-A-derived epoxy resins and/or bisphenol-A have the potential to cause skin responses when individuals are also exposed to UV light.

In a poorly reported study, a "photosensitive-like flare-up" on the face neck and upper chest was observed in 7 patients with a contact dermatitis to polyamide trouser pockets (Grimalt and Romaguera, 1981). A standard series of potential photosensitising substances, including bisphenol-A (1%) was tested for sensitising and photosensitising potential in each patient. Six of the patients responded to bisphenol-A in the standard patch test and one patient responded in the photopatch tests. It was reported that wavelengths of 320-340 nm intensified the reaction. It is not known whether the patient with the positive photopatch was one of 6 patients who gave a positive patch test with 1% bisphenol-A. No other data were provided and, although the initial exposures were not fully characterised, this study is viewed as supporting evidence for the photosensitisation potential of bisphenol-A (or epoxy resins).

4.1.2.5.2 Respiratory tract

No data are available.

4.1.2.5.3 Summary of sensitisation

There are several reports of patients with dermatitis responding to bisphenol-A in patch tests. However, it is unclear whether bisphenol-A or related epoxy resins were the underlying cause of the hypersensitive state. Anecdotal information indicates skin inflammation in workers handling bisphenol-A, although given the uncertain reliability of this information no conclusions can be drawn from it. In animals, a skin sensitisation test performed to current regulatory standards is not available. The available studies are negative, but the test reports lack detail and no reliable justifications were given for the choice of concentrations used. In the study using the highest challenge concentration, 50% in a guinea pig closed-patch test, a sensitisation rate of 12.5% was obtained. It is possible that the concentrations used in all the available studies were not maximised and a greater response might have been obtained with higher induction and challenge concentrations. Based on the findings from the most robust study, bisphenol-A may possess a skin sensitisation potential, albeit a limited one. Bisphenol-A in the presence of UV light can also elicit skin responses in humans, and reproducible positive results for photosensitisation have been obtained in mouse ear swelling tests. Mechanistic studies in mice have suggested this is an immune-mediated process. Therefore, examination of the available human and experimental animal studies leaves the picture somewhat unclear as to whether one or more of the following are properties of bisphenol-A; (1) orthodox skin sensitisation (2) photosensitisation (3) bisphenol-A eliciting a response in people previously skin sensitised to another substance (e.g. epoxy resins).

Overall, it is clear that skin reactions can be a potential consequence of repeated skin exposure in humans. Thus, taking all of these data available into account, bisphenol-A is considered capable of producing skin sensitisation responses in humans. There are no data from which to evaluate the potential of bisphenol-A to be a respiratory sensitiser.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

Inhalation exposure

Rats

In a dose-finding study (Nitschke et al., 1985b), Fischer 344 rats (20 per sex per group) were exposed whole-body to 0, 10, 50 or 150 mg/m^3 bisphenol-A dust for 6 hours/day, 5 days/week for 9 exposures. The top concentration (150 mg/m^3) was close to the maximum attainable concentration. The MMAD for all exposures was in the range 2.6 to 6.2 μm. Half the animals were sacrificed 1 day after the final exposure and the remainder 29 days after the final exposure. Routine haematology, biochemical investigations and urinalysis were performed. Macroscopic and microscopic pathology examinations of tissues were performed (including reproductive organs, i.e. testes, epididymis, prostate, seminal vesicle, coagulating gland, ovary, uterus, cervix and vagina) in animals at 0 and 150 mg/m^3 sacrificed 1 day after the final exposure. For all other animals only the respiratory tract and associated tissues were analysed. There were no treatment-related deaths and no significant body weight findings. During the exposure period, reddish staining was observed around the nose of animals at 50 mg/m^3 and above. Females at 150 mg/m^3 also exhibited perineal soiling. No treatment-related haematological, urinalysis or biochemical effects were observed. At histopathology, no systemic toxicity was observed. Very slight to slight inflammation and hyperplasia of the epithelial lining of the anterior portion of the nasal cavity were observed in animals at 50 and 150 mg/m^3. The nasal epithelium changes were reversible, as they could not be detected in animals sacrificed 28 days later. No effects were observed at 10 mg/m^3 in this dose-finding study, with slight, transient nasal epithelium inflammation being the only effect seen at 50 and 150 mg/m^3.

In a follow-up 90-day study (Nitschke et al., 1988), Fischer 344 rats (30 per sex per dose) were exposed whole-body to 0, 10, 50 or 150 mg/m^3 bisphenol-A dust for 6 hours/day, 5 days/week for 13 weeks. The MMAD for all exposures was in the range 2.2 to 5.2 μm. Ten animals per sex per exposure were sacrificed 1 day, 4 weeks and 12 weeks after the final exposure. Routine haematology and biochemical investigations were conducted. Animals at 0 and 150 mg/m^3 sacrificed 1 day after the final exposure were subject to a full necropsy (including reproductive organs). For all other animals at all exposure levels a limited number of tissues were analysed, which included the nasal tissues and any visible lesions.

No treatment-related deaths were observed. During the 13-week exposure period, a very slight to moderate amount of reddish staining was observed around the nose of animals at 50 mg/m^3 and above. Very slight perineal soiling was also observed in almost all animals at 50 mg/m^3 and above. This was also evident in 2/10 females at 10 mg/m^3. At the end of the exposure period at 150 mg/m^3, mean body weight gain was reduced by 5% in males and 11% in females. Absolute liver and kidney weights were also decreased in females at 150 mg/m^3 by 8% and 10%, respectively. There were no significant bodyweight or organ weight changes at 10 or 50 mg/m^3.

No treatment-related differences in haematology or clinical chemistry were observed. At necropsy, increased caecal size, due to distension of the caecum with food, was observed in all animals at 50 and 150 mg/m^3. Very slight to slight hyperplasia and slight to "subchronic" inflammation of the anterior portion of the nasal cavity were observed in all animals at 50 and 150 mg/m^3. Slight decreases (6%) in body weight gain, increased caecal size in 5/10 males and very slight nasal epithelium hyperplasia and inflammation were seen in animals at 150 mg/m^3 in the 4 week recovery group. No effects were observed at 10 or 50 mg/m^3 in the 4-week recovery group. No changes related to bisphenol-A were detected in any of the 12-week recovery groups. No effects on reproductive organs were seen in any group. The authors attributed the distension of the caecum as a toxicological consequence of ingestion of bisphenol-A due to grooming and/or clearance from the respiratory tract. The observed very slight perineal soiling in 2/10 females at the low dose is not considered toxicologically significant, in the absence of other evidence of toxicity at this dose. The NOAEL for this study is 10 mg/m^3, with minimal inflammation of the anterior nasal cavity epithelium being produced at 50 and 150 mg/m^3. There was no evidence of toxicity at any site other than the upper respiratory tract.

In a study report obtained as a translation, rats (strain, sex and number not specified) were exposed daily for 4 hours to 0 or 15-86 mg/m^3 (mean 47 mg/m^3) bisphenol-A for 4 months (Stasenkova et al., 1973). No particle size data were provided. At the end of the exposure period, a statistically significant decrease in body weight gain, reduced hippuric acid excretion in the urine, reduced ascorbic acid content in the liver and kidneys, and increased liver and kidney weights were observed in treated animals. At necropsy, "morphological changes" were observed in the liver, kidney and lungs, but no further information was provided. No conclusions can be reached from this relatively poorly reported study.

In another poorly reported study (Gage, 1970), 4 male Alderley Park rats were exposed five times to a 'saturated atmosphere' of bisphenol-A for 6 hours. There was no quantification of this exposure level. No signs of toxicity were observed, and no gross or macroscopic changes were reported at necropsy. However, no further details are available and in view of the very limited information provided no useful conclusion can be reached from this study.

Oral exposure

In some dietary studies, the doses administered were reported in ppm only. Therefore, the following default values (taken from Gold et al., 1984). have been applied to convert ppm to mg/kg bw. In these calculations, it is assumed that 1,000 ppm in the diet represents 1 g bisphenol-A per kg diet.

Table 4.24 Default values for dose calculations

Species	Sex	Body weight (kg)	Food intake (g/day)	Water intake (ml/day)
Rat * (lifetime studies)	M F	0.5 0.35	20 17.5	25 20
Rat (other studies)	M F	0.2 0.175	20 17.5	25 20
Mouse *	M F	0.03 0.025	3.6 3.25	5 5

* These values were included in the "Guidelines for inclusion of potency considerations in setting specific concentration limits for carcinogens in Annex I of Directive 67/548/EEC" by the Commission working group on the classification and labelling of dangerous substances.

Rats

In a 2-week study (NTP, 1982), F344 rats (5 per sex per group) were fed 0, 500, 1,000, 2,500, 5,000 or 10,000 ppm bisphenol-A in the diet. Using the default values given in **Table 4.24**, daily intakes of bisphenol-A are estimated to have been 0, 50, 100, 250, 500 and 1,000 mg/kg in males and females. No deaths were observed. Compared to controls, mean body weight gain was reduced by 60% or more in males at 2,500 ppm and above, and by 40% or more in females at 5,000 ppm and above. No other aspects were investigated.

In a briefly reported study (General Electric, 1976a), CD rats (5 per sex per group) were fed 2,000, 4,000, 8,000 or 12,000 ppm bisphenol-A for 2 weeks. Bisphenol-A exposure in the diet resulted in mean dose levels of 234, 496, 936 and 1,348 mg/kg in males and 242, 506, 971 and 1,454 mg/kg in females. Pre-treatment measurements served as controls. Dose-related decreases in body weight were seen in males at 4,000 ppm and above and in females at 8,000 ppm and above. Very slight decreases in food consumption were observed in male rats at 8,000 and 12,000 ppm. No treatment-related macroscopic findings were observed at necropsy. The very limited range of observations made and the lack of a control group prevent any useful conclusion being reached from this study.

In a poorly reported study available as an abstract only (Ohsako et al., 1999), groups of male rats (number and strain not reported) were administered 6 daily doses of 2 ng/kg-200 mg/kg bisphenol-A by gavage. The study also included a control group. Testicular weight and daily sperm production were determined in rats up to 36 days after the first dose. Protein levels were also determined in testicular cytosol. The authors report that the low effect level in this study was 20 μg/kg bisphenol-A, which decreased daily sperm production 36 days after the first dose. Increasing dose levels of bisphenol-A were reported not to affect the magnitude of the decrease in daily sperm production i.e. there was no dose-response relationship. In controls, the authors report that testicular weight and daily sperm production were observed to increase from 8 to 36 days after the first dose. In testicular cytosol, the expression of several proteins was reported to be "considerably affected" by treatment with bisphenol-A. No further details are available. The limited details provided in this briefly reported abstract mean that no reliable conclusions can be drawn from the data.

In a study which is reported in abstract form only (Chahoud et al., 1999), a group of six dams (strain not reported) were administered 50 mg/kg bisphenol-A in 2% Mondamin solution by gavage on days 6-20 of gestation. Dams were sacrificed 15 minutes after the last dose and blood and liver samples obtained from the dams and foetuses. The presence of bisphenol-A in liver and plasma was determined by gas chromatography-mass selection detection. In the dams, concentrations of 71.1 and 0.11 mg/kg bisphenol-A were detected in the liver and plasma, respectively. Corresponding values in the fetus were 0.14 and 0.04 mg/kg bisphenol-A. No other aspects were investigated.

In a well reported study (Takahashi and Oishi, 2001), male F344 rats (8 per dose group) were fed 0, 0.25, 0.5 and 1% bisphenol-A in the diet (equivalent to 0, 235, 466 and 950 mg/kg/day) for 44 days. Animals were killed at the end of the dosing period, blood samples taken to determine testosterone levels and macroscopic and microscopic pathology examinations performed on the reproductive organs (i.e. testes, preputial gland, epididymides, prostate and seminal vesicles with coagulation gland).

Compared to controls, a statistically significant decrease in body weight gain was observed at 466 (13%) and 950 mg/kg (18%) at the end of the dosing period. No effect was seen on testosterone levels. A statistically significant and dose-related decrease in absolute ($\geq$22%) and

relative liver weight (≥10%) was seen at 466 mg/kg and 950 mg/kg, compared to controls. Although statistically significant increases (≥8%) were seen in relative kidney weights at 235 mg/kg and above these were not dose-related, and absolute kidney weights were similar to controls. Compared to controls a statistically significant decrease in both absolute and relative weight of preputial gland was seen at 235, 466 and 950 mg/kg (26, 36 and 38%, and 22, 26 and 25%, respectively). A statistically significant decrease in absolute (45%) and relative (32%) dorsal and lateral prostate gland weight was seen at 950 mg/kg. A statistically significant decrease in absolute seminal gland weight at 950 mg/kg (47%) was not observed after adjustment for body weight.

At necropsy, degeneration of seminiferous tubules (i.e. a decrease in diameter), arrest of spermatogenesis and a decrease in elongated spermatids was observed at 235 mg/kg and above. The incidence of these effects, which were not seen in control animals, increased with dose. Disorganisation of late spermatids in stages I-VI was seen from 235 mg/kg, along with occasional sloughing at 466 and 950 mg/kg and nuclear pyknosis at 950 mg/kg. Disappearance of step 19 spermatids was observed at 235 mg/kg and above. Investigation of the spermatogenic stages revealed a decrease in the number of seminiferous tubules in stages I-VI at 235 (59%), 466 (70%) and 950 mg/kg (53%) compared to controls. Statistically significant increases were seen in stages IX-XI and XII-XIV at 235, 466 and 950 mg/kg (243, 416 and 296%, and 219, 257 and 195%, respectively). No significant effect was observed on stages VII-VIII.

Therefore, in this study, a reduction in weight of several reproductive organs and testicular toxicity was observed from 235 mg/kg. Although these effects on the reproductive organs have not been seen in any other robust repeated dose toxicity study in rats or mice (including a 2-year study in F344 rats), the severity of effects were generally observed in a dose-related manner. In addition, the data are supportive of adverse effects on fertility (a reduction in litter size) seen in fertility studies in both Sprague Dawley rats (there being no data in F344 rats) and CD-1 mice (see Section 4.1.2.9.2).

In a briefly reported 90-day study (NTP, 1982), groups of F344 rats (10 per sex per group) were fed 0, 250, 500, 1,000, 2,000 or 4,000 ppm bisphenol-A in the diet for 13 weeks. Using default values, daily intakes of bisphenol-A are estimated to have been 0, 25, 50, 100, 200 and 400 mg/kg in males and females. No treatment-related deaths were observed. At the conclusion of the study, body weight gain was reduced by 18-28% in males and 11-28% in females at 1,000 ppm and above compared to controls. The size of this decrease was dose-related in females but not in males. Food consumption was not affected at any dose. It was not reported whether routine haematology or biochemical investigations were performed. A thorough necropsy of tissues (which included reproductive organs i.e. testes, prostate, seminal vesicles, ovaries and uterus) revealed that the only treatment-related effects observed were “hyaline masses” in the bladder lumen of 30-60% of male rats from 250 ppm, and caecal enlargement in 60-100% of animals in each treatment group with the exception of females at 250 ppm. No inflammatory changes or other mucosal abnormalities were detected when the caecal walls were examined histologically. No other signs of systemic toxicity were reported at necropsy. The authors provided no further information or discussion on the hyaline masses, though it is noted that they were observed only in males and were seen in the absence of any clear adverse effects on the kidney. Additionally, they were not observed in any other repeated dose toxicity study, or carcinogenicity bioassay, in rats. Therefore, the hyaline masses are not considered treatment-related but a chance finding. Thus, in this 90-day study, the only effect observed in animals at 500 ppm (50 mg/kg) was caecal enlargement. At higher doses the only effect seen was a decrease in body weight gain (>10%), but it is not clear if this is due to lack of palatability or a toxic effect.

In a well reported dietary study (Til et al., 1978), Wistar rats (15 per sex per group) were fed 0, 100, 500 or 2500 ppm bisphenol-A for 90 days. Bisphenol-A exposure in the diet resulted in mean dose levels (as calculated by the authors) of 0, 7, 37, and 182 mg/kg in males and 0, 7, 37 and 185 mg/kg in females, respectively. Male rats were sacrificed on day 91-92, and females on day 92 and 95. Routine haematology and biochemical investigations were performed at sacrifice. Urinalysis was performed on urine collected from 10 rats/sex/group during the 16 hours prior to sacrifice. At necropsy, animals in the control and top dose groups were subject to a complete and thorough gross and histopathological examination (which included reproductive organs).

A statistically significant decrease in mean body weight gain was observed in males (17%) and females (9%) at the top dose, and females at the mid dose (7%) compared to controls. Clinical chemistry examination revealed a statistically significant decrease in fasting blood glucose levels in females at the mid dose and above (12%) and in males at the top dose (8%). A statistically significant decrease in creatinine levels was observed in males at the mid dose and above (15-16%), and an increase in total white blood cell counts was observed in females at the top dose (31%). No other treatment-related haematological, urinalysis or biochemical effects were observed. In terms of organ weights, at the top dose there was a statistically significant increase in the mean relative weight of the brain in males (15%) and females (11%), the kidneys (8%) in females and the testes (13%) in males compared to controls. At necropsy, the only effects observed were enlarged caeca in 2 males at the mid dose and 7 males and 9 females at the top dose, and alopecia in 1 female at the low dose, 3 females at the mid dose and 6 females and 1 male at the top dose.

The changes in the caecum and skin were not accompanied by any microscopic changes. However, as no accompanying microscopic changes were observed in the skin, and alopecia was not observed in any other repeated dose toxicity or carcinogenicity study in rats, this is not considered to have been treatment-related, but rather a chance finding. Additionally, the decreases in fasting glucose level and creatinine level and increases in total white blood cell counts were seen without any accompanying histopathological effects and are therefore regarded as being toxicologically insignificant. Thus, in this 90-day study, the only effect observed in animals at 500 ppm (37 mg/kg) was caecal enlargement, with decreases in body weight gain (9-17%) and associated increases in relative organ weights (8-15%) being the only additional effects observed at 2,500 ppm. Again, it is not known if the decrease in body weight gain is due to a toxic effect or lack of palatability.

In a relatively old and briefly reported dietary study, Sherman rats (5 per sex per group) received 0, 2, 8, 30, 150 or 520 mg/kg/day bisphenol-A for 90 days (Mellon Institute of Industrial Research, 1948). No effects were observed on appetite, body weight gain, body length, fatness (g/mm body length) and liver and kidney organ weights. At necropsy, no gross changes were noted in the tissues examined; liver, kidney, small intestine, spleen and testicles. However, no detailed histopathological examination was conducted. The restricted scope of this study means that it is of limited value.

Further information on repeated dose toxicity can be derived from a well conducted and reported multigeneration study (Tyl et al., 2000, see Section 4.1.2.9.2 for full details of this study, including information on findings in the reproductive organs). Groups of Sprague Dawley rats (30/sex/dose) were administered bisphenol-A in the diet at approximately 0, 0.001, 0.02, 0.3, 5, 50 or 500 mg/kg over three generations. The F_0 males and females were exposed for 15 and 18 weeks, respectively. The F_1 and F_2 generations were exposed from birth to 18 weeks of age in males, and 21 weeks in females. The F_3 generation was exposed from birth to about 13 weeks of age (males and females).

Evidence of general toxicity was seen in adults of all generations, although there were no treatment-related deaths or clinical signs of toxicity. At 500 mg/kg/day, body weight gain was consistently reduced throughout the exposure period in comparison with controls, across all generations. Terminal body weights were reduced by ≥22% in males and ≥13% in females. At 50 mg/kg/day, a statistically significant decrease in body weight gain was seen occasionally during the exposure period: by 7% in F_0 females at the end of the pre-breed, mating and gestation periods, 6-7% in F_1 males at the end of the pre-breed and mating period and 11-12% in F_2 males at the end of the pre-breed and mating period. At sacrifice, a statistically significant reduction in terminal body weight gain was seen in F_1 males and females (6%) and F_2 males (12%) at 50 mg/kg/day. Food consumption (g/kg/day) was variable at 500 mg/kg/day in all generations throughout the exposure period. However, these changes were not consistent; increases in feed consumption and decreases in food efficiency were not always statistically significant. Decreases were seen in several absolute non-reproductive organ weights at 500 mg/kg/day, which is likely to be due to the significant decreases observed in body weight gain. Statistically significant increases were seen in several relative organ weights at 500 mg/kg/day; paired kidney weights in F_0-F_3 males (≥11%), paired adrenal weights in F_1-F_3 males (≥19%), pituitary weights in F_1-F_3 males (≥17%), spleen weights in F_1-F_3 females (≥11%) and brain weights in F_0-F_3 males and females (>11%). Increases were seen in other organ weights but were not consistent; they were only observed in 1 or 2 generations. No consistent changes in relative organ weights were seen at 50 mg/kg/day. Histopathological examination revealed renal tubule degeneration of the kidney in female F_0 (4/13), F_1 (8/11) and F_2 (7/13) animals at 500 mg/kg/day. Renal tubule degeneration was not seen in F_3 females or control animals. Chronic inflammation of the liver was also seen in females, and males, but with no convincing dose-response relationship. These data are tabulated below.

Males

		Dose level (mg/kg/day)						
Gen	Finding	0	0.001	0.02	0.3	5	50	500
F_0	Chronic liver inflammation	0/30	0/14	0/12	1/13	0/14	0/12	2/11
F_1	Chronic liver inflammation	1/30	0/10	0/10	0/10	0/10	0/10	1/10
F_2	Chronic liver inflammation	11/30	3/10	3/10	2/10	2/10	3/11	2/10
F_3	Chronic liver inflammation	4/30	0/10	1/11	1/10	1/10	3/10	3/10

Females

		Dose level (mg/kg/day)						
Gen	Finding	0	0.001	0.02	0.3	5	50	500
F_0	Chronic liver inflammation	0/30	0/12	0/12	0/12	0/14	1/12	3/13
F_1	Chronic liver inflammation	3/30	0/10	0/10	3/10	1/10	1/10	3/11
F_2	Chronic liver inflammation	3/30	1/11	0/10	2/12	2/11	2/12	5/13
F_3	Chronic liver inflammation	4/30	1/10	1/10	1/10	4/10	1/10	1/10

No consistent treatment-related effects were seen in F_0, F_1, F_2 or F_3 animals at 5, 0.3, 0.02 or 0.001 mg/kg/day bisphenol-A.

To summarise, a dose-dependent trend was not apparent for chronic liver inflammation in all generations/sexes and is thus considered to be background variation (the incidence in bisphenol-A treated animals being comparable to that seen in controls) and not treatment-related. Likewise, decreases in body weight gain were only occasionally seen at 50 mg/kg/day and, in addition, with the exception of F_2 males where a decrease of 12% was observed, the decreases in body weight gain were <10% and not considered to be biologically significant. The no effect level for repeated exposure effects in this multigeneration study is 50 mg/kg/day, based on consistent and significant reductions in body weight gain in both sexes and renal tubule degeneration in females only, at 500 mg/kg/day.

In a 2-year bioassay (NTP, 1982), groups of F344 rats (50 per sex per group) were fed 0, 1,000 or 2,000 ppm bisphenol-A in the diet for 103 weeks. This corresponded to mean doses of 0, 74 and 148 mg/kg in males and 0, 74 and 135 mg/kg in females. Animals were observed twice daily, weighed every 2 weeks for the first 13 weeks and monthly thereafter, and subject to a complete and thorough gross and histopathological examination (which included reproductive organs) either at death or at the end of the study.

No gross signs of toxicity were observed in bisphenol-A treated animals. The survival rates in treated animals were not significantly different from untreated animals throughout the study. At the end of the study, the mean body weights were reduced by 4% and 9% in low- and high-dose males and 6% and 11% in low and high-dose females compared to controls. A reduced feed intake was observed in both sexes compared to controls; 7% and 12% in low and high-dose males and 17% and 28% in low- and high-dose females, respectively. No treatment-related non-neoplastic histopathological changes (including caecal enlargement) were observed at necropsy. The slight decreases (<10%) in body weight gain seen at the low dose are not considered to be toxicologically significant. Hence, the NOAEL in this 2 year study is 1,000 ppm (74 mg/kg), with only minimal toxicity observed at 2,000 ppm; a decrease in body weight gain (>10%) which, in the absence of any clinical or histopathological sign of systemic toxicity, could be due to a lack of palatability as much as a toxic effect.

Mice

In a study in females only (NTP, 1985a), CD-1 mice (8 per group) were dosed orally by gavage with 0, 120, 250, 500, 1,000, 1,500 or 2,000 mg/kg bisphenol-A in corn oil for 10 consecutive days. Animals were sacrificed two days after the final dose. Deaths were observed in 6 and 7 females at 1,500 and 2,000 mg/kg, respectively. The most common outward sign of toxicity at 120 and 250 mg/kg was rough coat. At 1,000 mg/kg lethargy and piloerection were also observed, and at 1,500 mg/kg and above ataxia, comatose behaviour, dyspnea, vocalisation, hunched back and hypersensitivity were observed. No significant effect was observed on body weight gain or relative liver weights in bisphenol-A treated animals compared to controls. However, no detailed histological examination was conducted, limiting the value of this study in females.

In a 2-week study (NTP, 1985b), groups of CD-1 mice (8 per sex per group) were fed 0, 0.31, 0.62, 1.25, 2.5 or 5.0% bisphenol-A in the diet. Using default values, daily intakes of bisphenol-A are estimated to have been 0, 372, 744, 1,500 and 3,000 mg/kg in males, and 0, 403, 806, 1,625, and 3,250 mg/kg in females. At the top dose, 6 male and 6 female mice died during the dosing period. Compared to controls, a statistically significant decrease in mean body weight gain was observed in males (≥11%) at 2.5% and above, and in the two surviving females (31%)

at 5.0%. Dehydration, dyspnea, lethargy and ptosis were observed in males and females at 2.5%. In addition, piloerection, diarrhoea and moribundity were observed in animals at the top dose. No other aspects were investigated.

Takao et al. (1999a) investigated the effect of bisphenol-A on male reproductive tract development in 5-week-old mice. Further experimental details beyond those provided in the published report were obtained from the author, and these additional data have been incorporated into the summary below.

Groups of 7 C57BL/6 mice received 0, 0.5 or 50 μg/ml bisphenol-A in drinking water for 4 or 8 weeks. Daily intakes of bisphenol-A are calculated to have been approximately 0, 0.14 and 12.7 mg/kg/day and 0, 0.11 and 10.4 mg/kg/day over 4 and 8 weeks respectively. Animals were sacrificed after exposure, and plasma levels of testosterone, corticosterone, and luteinizing hormone (LH) measured by commercially available RIA and EIA. Although the kit used to determine LH was reported by the authors to detect mouse LH, it was also specific for the rat. One testis per mouse was taken for macroscopic and microscopic examination.

No treatment-related effects were observed on body weight gain, testes and spleen weight in this study after either a 4- or 8-week exposure period. Compared to controls, a dose related decrease in testosterone was seen in bisphenol-A treated animals following 4 and 8 weeks exposure. This decrease was statistically significant in animals receiving 10.4 mg/kg/day for 8 weeks; approximately 2 pg/ml testosterone compared to 20 pg/ml in controls. No significant effect on corticosterone or luteinizing hormone levels were seen in bisphenol-A treated animals at 4 or 8 weeks. At necropsy, multinucleated giant cells containing more than 3 nuclei were seen in the seminiferous tubules of the testes of mice receiving bisphenol-A for 8 weeks; 1.8 cells/263 seminiferous tubes and 0.2 cells/283 seminiferous tubes at 10.4 and 0.11 mg/kg/day, respectively. These multinucleated cell values were determined from a single section per animal with no differentiation of cell types (i.e. spermatogonia, spermatocytes etc). No multinucleated giant cells were seen in control animals at 4 and 8 weeks, or in bisphenol-A treated animals after 4 weeks.

The toxicological significance of the observed large reduction in testosterone in the absence of any other hormonal changes (specifically an associated increase in LH) is unclear. It is also noted that the small number of micronucleated giant cells seen in the seminiferous tubules have not been reported in any other repeated dose or fertility study in mice (or rats). It is considered likely that any such effects would have been detected in the many animals examined in the recent GLP rat 2-generation and multigeneration studies. Therefore, the unspecified multinucleated giant cells seen in seminiferous tubules are not considered treatment-related but a chance finding. Thus, overall, in light of the methodological uncertainties and apparent inconsistencies in respect of the hormone level changes, it is difficult to draw any meaningful conclusions from this report.

In a briefly reported dietary study (NTP, 1982), groups of $B6C3F_1$ mice (10 per sex per group) were fed 0, 5,000, 10,000, 15,000, 20,000 or 25,000 ppm bisphenol-A in the diet for 90 days. Using default values, daily intakes of bisphenol-A are estimated to have been 0, 600, 1,200, 1,800, 2,400 and 3,000 mg/kg in males and 0, 650, 1,300, 1,950, 2,600 and 3,250 mg/kg in females. No treatment-related deaths were observed. Compared to controls, mean body weight gain was reduced by 14% or more in males at 15,000 ppm and above, and by 17% or more in females at 5,000 ppm and above. The magnitude of the reduced body weight gain was not dose-related in either sex. At necropsy (which included examination of the reproductive organs), multinucleated giant hepatocytes were observed in males in all bisphenol-A treated groups with an incidence and severity that were dose-related (observed in 9/10 males compared with

0/10 females at the top dose). No further details were provided. In view of the observed treatment related multinucleated giant hepatocytes in male mice a no effect level cannot be identified in this study.

In a 13-week study (Furukawa et al., 1994), which was translated from Japanese, groups of B6C3F$_1$ mice (10 per sex per group) were fed 0, 0.2, 0.5, 1.0, 2.0 or 4.0% bisphenol-A in the diet. Although the doses were presented in mg/kg it was reported that the food intake per animal was 5.2-9.6 g/animal/day. This is much too high a value and hence it seems likely that there has been a miscalculation in the daily feed intake. Therefore, default values have been used, and daily intakes of bisphenol-A estimated to have been 0, 240, 600, 1,200, 2,400 and 4,800 mg/kg in males and 0, 260, 650, 1,300, 2,600 and 5,200 mg/kg. Animals were observed once daily and subject to a complete and thorough gross and histopathological examination either at death or the end of the study. Routine haematology investigations were performed after the 13-week exposure period.

Two males died at 0.2% and two females died at 4.0%. The deaths at 0.2% are not considered to be treatment-related as no deaths were observed at higher dose levels. Compared to controls, a statistically significant decrease in body weight gain was observed in males ($\geq$24%) and females ($\geq$10%) at 2.0% and above. Statistically significant decreases in the number of erythrocytes, haemoglobin content and haematocrit values (all 18-19%), and increase in the number of platelets (36%), were observed in males at 4.0%. In females, a statistically significant decrease in the number of erythrocytes (9-18%) and haematocrit values (9-19%) were observed at 0.5% and above, and haemoglobin content was decreased (12-17%) at 1.0% and above. The magnitude of these decreases in females was not dose-related.

Statistically significant changes were observed in absolute liver weights and brain weights in males and females at 2.0% and above compared to controls, but the magnitude of these changes were either not dose-related or $\leq$10% with the exception of a decrease in brain weight (17%) in females at 4.0%. Statistically significant increases in absolute and relative kidney weight (>30%) and decreases in ovary weights (>38%) were observed in females at 4.0% and at 2.0% and above, respectively. At necropsy, atrophy and vacuolation of myocytes in the heart, and fibrous osteodystrophy were observed in both sexes at the top dose. Fibrous osteodystrophy and atrophy of myocytes was seen in a single male at 2.0%. Cystic dilation, degeneration or regeneration of renal tubules and focal fibrosis were observed in males and females at 1.0% and above. Histological enlargement of hepatocytes was not seen, but multinucleated hepatocytes were observed in the livers of males (8/8) and females (1/10) at 0.2% and above. Both the frequency and severity of the multinucleated giant hepatocytes incidence increased in males and females with dose up to 1.0%, and then generally decreased thereafter. Increased extramedullary haematopoiesis in the spleen was observed in both sexes at 2.0% and above. However, the miscalculation in the daily feed intakes limit the value of this study and prevent any reliable conclusions to be made about the exact dose at which effects were seen.

In a 2-year bioassay (NTP, 1982), groups of 50 male and 50 female B6C3F$_1$ mice were fed 0, 1,000 or 5,000 ppm, and 0, 5,000 or 10,000 ppm bisphenol-A in the diet, respectively. Weekly feed consumption and body weight data were not provided. Therefore, using default values, daily intakes of bisphenol-A are estimated to have been 120 and 600 mg/kg in males, and 650 and 1,300 mg/kg in females. Animals were observed twice daily and subject to a complete and thorough gross and histopathological examination (which included reproductive organs) either at death or at the end of the study.

No significant differences in survival were observed between treated and untreated animals throughout the study. Body weight gains in high-dose males and females, and low-dose females

were stated to be lower than control values, but no further information was given. Food consumption was reported to be similar among the groups, but excessive spilling of feed meant feed consumption could not be precisely evaluated. At necropsy, a treatment-related increase in multinuclear giant hepatocytes were observed in males; 1/49, 41/49 and 41/50 in the control, low and high-dose groups, respectively. It was reported that these giant cells appeared to contain 6 to 20 nuclei. These cells were only observed in females at the top dose: 2/48. At the low dose, the only treatment-related effects observed were multinuclear giant hepatocytes (with no associated increase in liver tumours) in 41/49 males, and an unstated reduced body weight gain in females. Therefore, LOAELs of 120 mg/kg in males and 650 mg/kg in females are identified in this 2-year study.

Dogs

In a briefly reported dose finding study (General Electric, 1976b), groups of 2 beagle dogs were fed 2,000, 4,000, 8,000 or 12,000 ppm bisphenol-A for 2 weeks. Bisphenol-A exposure in the diet resulted in mean dose levels of 49, 88, 281 and 293 mg/kg in males and 50, 137, 262 and 278 mg/kg in females. Pre-treatment measurements served as controls. No outward signs of toxicity, changes in body weight gain or food consumption were observed. At necropsy slight focal mucosal congestion and haemorrhage of the gastrointestinal tract was observed in several dogs. The author reports that such lesions can occur spontaneously in untreated animals; and these lesions were not observed in the subsequent 90-day study. In view of the small group sizes and the limited information provided no reliable conclusions can be reached from this study.

In a subsequent 90-day study (General Electric, 1976c), groups of beagle dogs (4 per sex per dose) were fed 0, 1,000, 3,000 or 9,000 ppm bisphenol-A. Bisphenol-A exposure in the diet resulted in mean dose levels of 0, 28, 74 and 261 mg/kg in males and 0, 31, 87 and 286 mg/kg in females. Blood and urine samples were obtained from all dogs for analysis prior to dosing and 1, 2 and 3 months into the study. At necropsy, only the top dose animals were subject to histopathological examination (which included testes, prostate, uterus and ovaries among the tissues examined). No outward signs of toxicity or treatment-related changes in body weight gain, food consumption, ophthalmoscopy, haematology, biochemistry or urinalysis were observed. The only treatment-related effect observed was an increase in relative liver weight of 18% and 26% at the top dose in males and females, respectively. No significant increase in focal mucosal congestion and haemorrhage of the gastrointestinal tract was observed in bisphenol-A treated animals in this study. The NOAEL for this study is approximately 80 mg/kg, with only increases in relative liver weight being observed at approximately 270 mg/kg.

Dermal exposure

There are no data available.

4.1.2.6.2 Studies in humans

No data are available.

4.1.2.6.3 Summary of repeated exposure

No useful information on the effects of repeated exposure to bisphenol-A in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in rats following exposure to 50 mg/m^3 (6 hours/day, 5 days/week for 13 weeks). There was no significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m^3. A NOAEL of 10 mg/m^3 was identified in rats in this 13-week study.

Dietary studies in rats produced a decrease in body weight gain and minor changes in the weights of several organs at higher doses probably of no toxicological significance, especially given the absence of other related pathological findings. However, in one study in male rats, reductions in the weight of several reproductive organs and testicular toxicity was seen following dietary exposure to 235 mg/kg for 44 days. A NOAEL was not established from this study. Although these effects on the reproductive organs have not been seen in any other robust repeated dose toxicity study in rats or mice (including a 2-year study in F344 rats), the severity of effects was generally dose-related and therefore cannot be disregarded. The only other finding was an inconsistent observation of caecal enlargement in some 90-day studies. The caecal enlargement was observed at 25 mg/kg and above and was without any associated histological abnormalities. In addition, it was not observed in a 2-year study at doses up to about 140 mg/kg or a multigeneration study at doses up to 500 mg/kg/day. Consequently, this is not regarded as a toxicologically significant observation of relevance to humans. A NOAEL of 74 mg/kg has been established for rats from a 2-year study.

Dietary studies in mice indicated that the liver is a target organ in this species, with changes being observed in the size and nucleation state of hepatocytes in 2-year and 90-day studies. The incidence and severity of these treatment-related multinuclear giant hepatocytes was greater in males than in females, and it was not possible to identify a no effect level for males. The effect was observed at all dose levels used in males from 120 mg/kg. In females, a no-effect level of 650 mg/kg was identified for these cellular changes in the 2-year study. The only other findings in mice were significant reductions in body weight gain at dose levels of approximately 650 mg/kg/day and above. Thus, LOAELs of 120 mg/kg in males for multinuclear giant hepatocytes and 650 mg/kg in females for a reduction in body weight gain of unknown magnitude, were identified in a 2-year study.

In a 90-day dietary study in dogs, a no effect level of approximately 80 mg/kg was identified, with increases in relative liver weight being the only other finding observed at approximately 270 mg/kg: in the absence of histopathology this finding is of doubtful toxicological significance.

There are no animal data available for repeated dermal exposure.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies *in vitro*

Unless otherwise stated, Aroclor- induced rat liver S9 was used as the metabolic activation system in the tests described in this section.

Cell free systems investigating DNA adduct formation

The interaction of bisphenol-A with DNA has been investigated using a ^{32}P-postlabelling method.

Atkinson and Roy (1995b) detected 1 major and 7 minor adducts following a 2-hour incubation of bisphenol-A with purified rat DNA in the presence of a peroxidase activation system. In control reactions of DNA in the absence of peroxidase enzyme or bisphenol-A no adducts were detected. In a follow-up study, adducts with the same chromatographic profile as the major adduct and one of the minor adducts obtained in the initial study were also detected following incubation of bisphenol-A with purified rat DNA in the presence of a microsomal cytochrome P450 activation system. The formation of these adducts appeared to be inhibited by the presence of known inhibitor(s) of cytochrome P450 (Atkinson and Roy, 1995a). These two studies have shown that bisphenol-A has the potential to react with isolated DNA, but only following activation by oxidative metabolism.

Cell free systems investigating microtubule disruption

In vitro studies have suggested bisphenol-A may have aneugenic activity in cultured mammalian calls (see below). Consequently, the possibility of an effect of bisphenol-A on microtubule formation in a non-physiological, cell-free system was investigated (Pfeiffer et al., 1996). Microtubule proteins were purified from bovine brain and incubated with 0 or 50-200 μM bisphenol-A in the absence of metabolic activation for 30 minutes. The ability of the proteins to form microtubules in this system was then assayed spectrophotometrically. The polymerisation of microtubule proteins was inhibited by bisphenol-A in a dose-related manner, by up to approximately 60% with 200 mM bisphenol-A compared to controls.

In a similar study, purified microtubule proteins were incubated with 0 or 20-200 mM bisphenol-A, and the morphology of the microtubules formed was examined by electron microscopy (Pfeiffer et al., 1997). The frequency of altered microtubules (characterised as "spiral structures") increased with the concentration of bisphenol-A. Also, these altered microtubules remained unchanged on cooling to 4^{o}C, whereas those in control cultures disappeared.

The data from these studies show that in a crude cell free system, bisphenol-A disrupts microtubule formation.

Studies in bacteria

In a well conducted Ames test with pre-incubation (Haworth et al., 1983), *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 were exposed to bisphenol-A at concentrations up to 333.3 μg/plate in the presence and absence of metabolic activation (Aroclor induced rat and hamster liver S9). At 333 μg/plate slight and complete clearance of the background lawn was observed in all strains with (rat and hamster liver S9) and without metabolic activation, respectively. No increase in the number of revertants was seen in any of the tested cultures. Controls gave results that confirmed the validity of this test. The negative result was confirmed by independent experiment. This conclusion is in agreement with the assessment of these data by Tennant et al. (1986; 1987).

In a smaller scale, non-regulatory test (Dean and Brooks, 1978), *S. typhimurium* TA 1538 and *Escherichia coli* strains WP2 and WP2*uvra* were exposed with pre-incubation to bisphenol-A at concentrations up to 1.0 mg/ml (concentration in μg/plate not stated) in the presence and absence

of metabolic activation. Cytotoxicity, as evidenced by a ≥50% decrease in the number of spontaneous revertants, was observed at 0.5 mg/ml in WP2*uvra* with and without metabolic activation and TA 1538 without metabolic activation. No cytotoxicity was observed in WP2 with and without metabolic activation. No increase in the number of revertants was seen in any of the tested cultures. An independent experiment using a plate incorporation protocol and the performance of the controls confirmed the validity of this test.

Bisphenol-A has also been tested in two Ames tests using direct plate incorporation. In a well conducted experiment (Schweikl et al., 1998), *S. typhimurium* TA97a, TA98, TA100 and TA102 were incubated with up to 500 μg/plate bisphenol-A with and without metabolic activation. No increase in the number of revertants was seen in any of the tested cultures. Cytotoxicity, as evidenced by a ≥50% decrease in the number of spontaneous revertants, was observed in strains TA 100 and TA102 with and without metabolic activation, and in TA97a and TA98 without metabolic activation only. Controls gave results that confirmed the validity of this test. The negative results were confirmed by independent experiment.

JETOC (1996) published data for an Ames test using *S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100. In addition, a test was performed using E. coli WP2uvrA. These strains were exposed to bisphenol-A at concentrations up to 1,250 μg/plate in the presence and absence of metabolic activation. No further data concerning the performance of the test were given. No increase in the number of revertants was seen in any of the tested cultures. Cytotoxicity, as evidenced by a ≥50% decrease in the number of spontaneous revertants was observed in all strains, with and without metabolic activation, at 313 μg/plate bisphenol-A and above. Positive controls gave results that confirmed the validity of this test. The negative results were confirmed by independent experiment.

In an Ames study (Takahata et al., 1990) translated from Japanese, *S. typhimurium* strains TA 97, TA 98, TA 100 and TA 102 were exposed to bisphenol-A at concentrations up to 5,000 μg/plate in the presence and absence of metabolic activation. No increase in the number of revertants was seen in any of the tested cultures. Cytotoxicity, reported as a "lethal effect", was observed with and without metabolic activation, at 500 μg/plate bisphenol-A in strains TA 98 and TA 102, and at 1,000 μg/plate in strains TA 97 and TA 100. Positive controls gave results that confirmed the validity of this test. It is not stated whether a second independent experiment was conducted.

Studies in fungi

In an assay to detect mitotic gene conversion (Dean and Brooks, 1978), negative results were obtained when *Saccharomyces cerevisiae* strain JD1 was exposed to bisphenol-A at concentrations up to 0.5 mg/ml (concentration in μg/plate not stated) in the presence and absence of metabolic activation. No signs of toxicity were observed. A positive control was used with metabolic activation and produced marked increases in the revertant counts. In this limited study, there was no evidence of mutagenicity in fungi.

Regulatory studies in mammalian cells

In a gene mutation test conducted for the US National Toxicology Program (Myhr and Caspary, 1991), mouse lymphoma L5178Y cells (*tk* locus) were exposed in soft agar to 5-60 μg/ml bisphenol-A for 4 hours (48-hour expression period) with and without Aroclor-induced rat liver S9 in the first of two experiments. Marked cytotoxicity was seen at the highest concentration with and without metabolic activation and there was no evidence of mutagenic activity in any of

the treated cultures. The positive control substances with and without activation gave clear increases in mutation frequency but, in view of recent criticisms from some experts with experience of this assay (Moore et al., 1999a), it is noted that these responses were of a relatively low magnitude. It is possible that "small colonies" were not counted optimally in this study. The lack of a mutagenic response to bisphenol-A was confirmed in a repeat experiment with and without activation. The authors also stated that "highly toxic treatments in other experiments failed to cause increases in mutant frequency." In conclusion, this study gave a negative result in accord with the standards commonly employed for assessment. However, the possibility that colony counting in this soft agar method was not optimised for detecting the widest possible range of induced mutations cannot be excluded.

Bisphenol-A was also assayed in a mouse lymphoma test by 2 laboratories in Japan as part of a multi-centre trial in that country to evaluate the utility of this system to detect clastogens and spindle poisons (Honma et al., 1999; data provided by Prof. J. Parry, personal communication). Using the microtitre method, in both the laboratories cells were exposed to bisphenol-A for 3 hours with and without hepatic S9 from rats induced with phenobarbital and 5,6-benzoflavone. The tests included a 45-hour expression period. Each laboratory conducted one main experiment with test concentrations selected on the basis of cytotoxicity measured in a preliminary test. In laboratory "A", dose-related, statistically significant increases in mutation fraction were seen with and without S9 at concentrations (up to 55.6 mg/ml bisphenol-A) that did not show marked cytotoxicity. In laboratory "B", there were no significant dose-related increases seen without S9 up to a marked cytotoxic dose of 60 mg/ml bisphenol-A. In the presence of S9, there were no dose-related increases up to cytotoxic concentrations of bisphenol-A (relative total growth at 55 mg/ml bisphenol-A was 14%). However, with extreme cytotoxicity (2% relative total growth at the top concentration of 60 mg/ml bisphenol-A), there was a statistically significant increase in mutation fraction with S9. There were no observations among the control cultures to suggest that the test system in the second laboratory had been less sensitive than the first. Given the non-reproducibility of the positive findings in the first study, the authors of the large-scale trial declared the result "inconclusive".

A critical analysis of all the data from the Japanese trial described above has recently been reported by Moore et al. (1999b). Taking into account their extensive experience of the assay, this latter group of authors concluded that bisphenol had actually given a negative result with S9 and an "inconclusive" result without S9. Consequently, it is concluded that the Japanese trial provides some support for the negative mouse lympohoma test findings reported by the NTP (see above). There remains a slight concern, however, that the conditions without activation have not been tested robustly.

In a further gene mutation assay, Chinese hamster V79 cells (*hprt* locus) were exposed to 0.1 and 0.2 mM bisphenol-A for 24 hours (96-hour expression period) without metabolic activation only (Schweikl et al., 1998). A 79% decrease in relative cell growth was observed at 0.2 mM. Negative results were obtained in two independent experiments, and the positive control showed the system was performing appropriately. The significance that can be attached to this negative result is limited partly because the level of cytotoxicity observed was less than that normally expected (i.e. 90%), but also by the few doses tested and the lack of information on the cytotoxicity at the lower doses. Also the absence of cultures exposed in the presence of an exogenous metabolic activation system limits the value of this study.

In a chromosome aberration study reported by Ivett et al. (1989) and Tennant et al. (1986; 1987), Chinese hamster ovary (CHO) cells were exposed in two separate experiments to 30-50 μg/ml bisphenol-A for 2 hours with metabolic activation and 20-40 μg/ml for 8 hours without metabolic activation. Cells were harvested at 11 hours with metabolic activation and 21 hours

without metabolic activation. In the first test with metabolic activation, an increase in the percentage of metaphases with chromosome aberrations from bisphenol-A treated cultures was observed only at the top dose in the presence of cytotoxicity; 14% at 50 μg/ml compared to 3% in controls. In these high-dose cultures, it was stated that cell confluence was reduced by approximately 70%. In the second test, no significant increases were observed in with metabolic activation; only 3% of cells at the highest dose had aberrations. No significant increases in aberrations were observed without metabolic activation with bisphenol-A evidently being tested up to "toxic levels." The positive controls produced clear increases in chromosome aberrations. Overall, this study is adjudged to have given a negative result, since the observed increase in the first experiment was not reproducible. However, the significance attached to this negative study is limited by the lack of details about cytotoxicity in the tests without activation.

In a sister chromatid exchange (SCE) study reported by Ivett et al. (1989) and Tennant et al. (1986; 1987), CHO cells were exposed in two separate experiments to 30 - 50 μg/ml bisphenol-A for 2 hours with metabolic activation and 0.8-25 μg/ml for 26-34 hours without metabolic activation. A 20% or greater increase in SCEs was the criterion used for a positive result in this study. A clear negative result was obtained with metabolic activation. Without activation, a 22% increase in SCEs was observed at 8 μg/ml, the highest dose scored in the first test. In the second test, which scored cells at 15-25 μg/ml, no increase in SCEs was seen. Cytotoxicity (cell cycle delay) was reported at 8 μg/ml, but no further information was provided. Clear increases in SCE's were observed with the positive controls. Overall, this study is considered negative, as the increase in SCE frequency seen in the first test without metabolic activation was not reproducible. Though again, the lack of details on cytotoxicity mean negative result cannot be regarded as completely reliable.

In a poorly reported rat hepatocyte unscheduled DNA synthesis (UDS) assay, bisphenol-A did not induce UDS in primary rat hepatocytes derived from F344 males (Tennant et al., 1986). No further details are available.

Non-regulatory studies in mammalian cells

A number of studies have been conducted by the same laboratory using Syrian hamster embryo (SHE) cells (see below). It has been suggested that these cells possess a representative spectrum of enzymes involved in oxidative and peroxidative metabolism (Pienta, 1996) and so assays using SHE cells have not included an exogenous metabolism system. In view of this, the tests have been regarded here as "non-regulatory."

A well reported gene mutation study using SHE cells (Na^+/K^+ *ATPase* and *hprt* locus) was conducted by Tsutsui et al., (1998). Cells were exposed to 25-200 μM bisphenol-A for 48 hours. The positive control benzo(a)pyrene produced the appropriate responses at both loci. No increase in mutations at either locus was observed in the presence of bisphenol-A. This negative result supports the findings in the gene mutation tests with mouse lymphoma and V79 cells.

A chromosome aberration study using SHE cells was conducted by Tsutsui et al. (1998). Cells were exposed to 25-200 μM bisphenol-A for 6 hours and harvested 18 hours after treatment and 200 metaphases scored per dose group. The results were presented only briefly, and it was stated that no statistically significant increases in chromosome aberrations were observed. Since the results were not provided in detail, a critical assessment of these findings cannot be made, and this negative result cannot be regarded as completely reliable.

In a test for aneuploidy and polyploidy, SHE cells were exposed to 25-200 μM bisphenol-A for 48 hours before harvesting (Tsutsui et al., 1998). It is not reported whether a positive control was

used. In this system, there was significant toxicity (at least 50% reduction in cell growth) at 100 μM. At 200 μM cell growth was inhibited completely. On scoring 100 metaphases per dose group, no statistically significant increases in the number of diploid or tetraploid cells were observed. In contrast, statistically significant increases in cells with a chromosome number within 1-3 of the diploid number (2N = 44) were observed at concentrations of 50 μM and above. It is noted that there was not a clear dose-related trend. In an additional test, cells were exposed to 0 or 200 μM bisphenol-A for 72 hours. A clear increase in the frequency of cells with chromosome number within 1-3 of 2N was observed. There is a possibility that these findings could have been an artefact of the metaphase preparation technique, but no changes were seen in controls. Consequently, it is concluded that this study appears to have demonstrated *in vitro* aneuploidy induction by bisphenol-A.

The mutagenicity of five bisphenols, including bisphenol-A, was further investigated in SHE cells by Tsutsui et al. (2000). This study investigated the activity of bisphenol-A in a gene mutation assay at the Na^+/K^+ ATPase and *hprt* loci, a chromosome aberration assay and an assay for aneuploidy and polyploidy. The methodology was the same as that described previously by Tsutsui et al. (1998), but only a single dose level of 100 μM bisphenol-A was used in all these assays and, in the chromosome aberration study, an additional exposure period of 24 hours, along with 48 hours, was used prior to harvest. Again, it was not reported whether a positive control was used. Treatment with bisphenol-A caused an inhibition of cell growth, by approximately 40 %. In the presence of bisphenol-A, no increase in gene mutations was observed at either locus, and no statistically significant increase was seen in chromosome aberrations (data not shown) or the number of diploid or tetraploid cells. However, a statistically significant increase was seen in cells with a chromosome number within 1-3 of the diploid number (2N = 44). The *in vitro* aneuploidy induction observed with bisphenol-A in this study is consistent with that observed by Tsutsui et al. in their earlier study.

In a non-standard test, Pfeiffer et al. (1997) exposed Chinese hamster V79 cells to 200 μM bisphenol-A in DMSO for 6 hours without metabolic activation. The basis for the selection of this dosing regime was not stated, but bisphenol-A was found to have a dose-relate effect on the metaphases per 1,000 cells in another experiment. The highest dose employed was 200 μM and this produced a clear, marked arrest of mitosis. In a cytotoxicity test involving trypan blue exclusion, a comparable treatment reduced the % of viable cells from 98.4% (untreated and solvent controls) to 92.8%. However, the total number of cells present after treatment with DMSO (57%) and bisphenol-A (26%) was decreased relative to the untreated culture (100%). For the scoring of micronuclei, cells were washed and then fixed in methanol. The number of micronucleated cells was determined and then, using CREST antikinetochore antibodies, micronuclei containing whole chromosome/chromatids (CREST-positive) were distinguished from micronuclei containing fragments (CREST-negative). Bisphenol-A resulted in a significant increase in CREST-positive micronuclei in three independent experiments. The mean number (for the 3 experiments combined) of these micronuclei observed in bisphenol-A treated cells was 117/3,000 compared to 8/3,000 in controls. There were no significant increases in CREST-negative micronuclei. The percentage of CREST positive micronucleated cells with 1 or 2 micronuclei was 80% and 10%, respectively. Similar experiments were performed with cells incubated for 12 or 24 hours. In both these cases, bisphenol-A apparently gave a clear induction of CREST-positive micronuclei (data not shown). Overall, this study reports an apparent aneugenic effect of bisphenol-A in cultured mammalian cells in the absence of exogenous metabolic activation. In additional experiments, Pfeiffer et al. (1997) investigated the effect of bisphenol-A (200 μM for 6 hours) on mitosis and microtubule formation in Chinese hamster V79 cells. Cultures were prepared as in the micronucleus test, but the cells were stained with tubulin

antibodies, allowing examination of the cytoplasmic and spindle microtubules by fluorescence microscopy. Bisphenol-A caused a significant reduction of the cytoplasmic microtubule complex in virtually every cell, leading to condensed staining, with short fibres around the cell nucleus. In metaphase cells, the mitotic spindle was no longer visible and diffuse tubulin was surrounded by chromosomes in an irregular arrangement. This provides further evidence to suggest that bisphenol-A is aneugenic *in vitro*.

The effect of bisphenol-A exposure without metabolic activation (100-200 μM for 3-24 hours) on microtubule formation in Chinese hamster V79 cells was also investigated by Ochi (1999). As in the study of Pfeiffer et al. (1997), cells were stained with microtubule antibodies allowing examination of cytoplasmic and spindle microtubules by fluorescence microscopy. In mitotic cells, aberrant spindles, such as tripolar and multipolar, were observed in cells exposed to 100 μM and above. An increase in the number of γ-tubulin signals (a component of microtubule organising centres) in mitotic cells was seen with bisphenol-A. This increase coincided with that of aberrant spindles, but was not dose-related (925-1,420% and 435-1,950% following exposure to 100-200 μM bisphenol-A for 6 and 12 hours, respectively). In interphase cells, bisphenol-A had no effect on the microtubule network or the incidence of γ-tubulin. The incidence of multipolar division was investigated in cells at telophase. In the presence of bisphenol-A, abnormal cytokinesis was seen, with cells dividing into 3 daughter cells (multipolar division). Compared to controls, the incidence increased approximately 6 and 32 fold at 100 and 150 μM bisphenol-A, respectively. These results and those of Pfeiffer et al. (1997) provide further evidence to suggest that bisphenol-A is aneugenic *in vitro*.

A chromosome aberration study using an epithelial-type rat liver cell line (RL1) was conducted by Dean and Brooks (1978). In the first test, RL1 cells were exposed to bisphenol-A at concentrations of 10-30 μg/ml for 24 hours. No exogenous metabolic activation system was added because the investigators believed the RL1 cells to be metabolically competent. Concentrations of 20-30 μg/ml were previously determined to inhibit cell growth by 50%. No increase in chromosomal aberrations was observed. This was confirmed in a second test conducted at 30 μg/ml only, with a 24-hour exposure time. The positive controls produced clear increases in chromosome aberrations. The significance that can be attached to this negative result is limited by the use of only one harvest time and by the absence of cultures exposed in the presence of an exogenous metabolic activation system (the metabolic competency of RL1 cells was not established).

The ability of bisphenol-A to induce DNA single strand breaks in primary rat hepatocytes was investigated by an alkaline elution method (Storer et al., 1996). Cells were exposed to 0.2-0.5 nM bisphenol-A for 3 hours and then harvested. Cells irradiated with gamma radiation served as the positive controls. A dose-related increase in DNA single strand breaks was observed with bisphenol-A, with the criteria for a positive result (a $\geq$3.0 fold increase in the elution slope) being seen at 0.4 nM. However, cytotoxicity was observed from 0.2 nM bisphenol-A. Thus, in this study, bisphenol-A was only observed to induce DNA damage at concentrations that produced cytotoxicity.

The interaction of bisphenol-A with DNA in SHE cells was investigated by ^{32}P-postlabelling (Tsutsui et al., 1998). Cells were exposed to 50-200 μM bisphenol-A for 24 hours. It is not reported whether the study employed a positive control. Two main adduct spots were revealed chromatographically at 50 μM and three at 100 μM and above. These adducts were not observed in controls. A clear dose-related trend in adduct formation was observed with bisphenol-A. This study shows that *in vitro* bisphenol-A is capable of interactions with DNA. However, the three main adduct spots were not further characterised.

4.1.2.7.2 Studies in Drosophila

In a *Drosophila melanogaster* sex-linked recessive lethal assay (Foureman et al., 1994), male flies were fed a 5% sucrose solution containing 0 or 10,000 ppm bisphenol-A for 72 hours. Males were then mated 1:3 with Basc females. Females were replaced every 2-3 days to make a total of 3 broods. The offspring were scored for wild-type F2 males. A mortality rate of 1% was observed in exposed males. No mutagenic effect was observed. For insect systems such as this, there is too little comparative data with mammalian cells and the relevance of findings to the mammalian *in vivo* system is uncertain (Aardema et al., 1998).

4.1.2.7.3 Studies *in vivo*

Studies in somatic cells

In a well conducted micronucleus assay (Shell Oil Company, 1999), groups of ICR mice (5 per sex per dose per sampling time) received a single oral dose of 0, 500, 1,000 or 2,000 mg/kg bisphenol-A. Bone marrow was sampled at 24 hours. At the high dose and in the control group, bone marrow was also sampled at 48 hours. Clinical signs of toxicity in the treated animals at 500 mg/kg and above included lethargy and piloerection. Compared to the negative controls, the mean ratios of polychromatic to normochromatic erythrocytes (P/N ratios) were reduced in all of the treatment groups. The decreases were by 15-24% at 24 hours and 26-34% at 48 hours. Although there were no dose-related trends in P/N ratio, the observation that the values from treated animals were consistently lower than controls suggests that bisphenol-A was having an effect and that it was bioavailable to the bone marrow following oral administration in this study. No increases in the incidence of micronuclei were observed following bisphenol-A treatment, but the positive control, cyclophosphamide, gave a clear increase in the frequency of micronuclei. Overall, it is concluded that bisphenol-A did not express genotoxic activity *in vivo* in this standard study. This provides reassurance that the aneugenic potential of bisphenol-A seen *in vitro* is not expressed *in vivo*.

DNA adduct formation has been investigated in rat liver by ^{32}P-postlabelling (Atkinson and Roy, 1995a). Groups of four CD rats were administered a single dose of 0 or 200 mg/kg bisphenol-A by i.p. injection and killed 4, 8, 24, 48 and 72 hours post injection. Further groups of 5 rats were administered 0 or 200 mg/kg bisphenol-A by gavage daily for 4, 8, 12 and 16 days; it was not reported when these animals were sacrificed. Two major and several minor adducts were detected following both exposure regimes. No adduct spots were observed in unexposed controls. Following i.p. administration, the half life of one of the major adducts ("adduct A") was between 8 and 12 hours. The level of the other adduct ("adduct B") was not affected up to 72 hours post injection. The opposite was observed following gavage; adduct B decreased after 8 days of exposure (with a half life of approximately 11 days) while adduct A was not affected. The chromatographic mobility of these two major adducts appeared to match that seen for adducts detected when bisphenol-A was reacted with purified DNA following peroxidase-mediated activation (see above). However, there was no further characterisation of these adducts. It is concluded that bisphenol-A is capable of producing DNA adducts *in vivo*.

Studies in germ cells

Bisphenol-A has been tested for mutagenic activity in a dominant lethal assay (Bond et al., 1980). In this study, which is reported in abstract form only, male Sprague Dawley rats received 5 daily intraperitoneal injections of 85 mg/kg bisphenol-A. The abstract states that 85 mg/kg was

the maximum tolerated dose in this study, and that there was no difference between control and treated animals in relation to potential dominant lethal findings. However, as no additional details are available the reliability of this negative result cannot be analysed further.

4.1.2.7.4 Studies in humans

No data are available.

4.1.2.7.5 Summary of mutagenicity

No human data regarding mutagenicity are available. However, bisphenol-A appears to have demonstrated aneugenic potential *in vitro,* positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems there is information that shows bisphenol-A disrupts microtubule formation. Bisphenol-A has been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. However, some deficiencies in the conduct of these studies have been noted and the negative results cannot be taken as entirely conclusive. Bisphenol-A does not appear to be anuegenic *in vivo*, since a recently conducted, standard mouse bone marrow micronucleus test has given a negative result. Bisphenol-A was negative in a briefly reported dominant lethal study in rats but, given the limited details provided, this is not regarded as an adequate negative result. The only other data in somatic cells *in vivo* are from a ^{32}P-postlabelling assay, which showed that bisphenol-A is capable of producing DNA adduct spots in rat liver following oral administration. These adduct spots were not characterised fully.

Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies (see below), it does not appear that bisphenol-A has significant mutagenic potential *in vivo*. Any aneugenic potential of bisphenol-A seems to be limited to *in vitro* test systems and is not of concern. The relevance of the finding that bisphenol-A can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

Inhalation exposure

There are no data available.

Oral exposure

Bisphenol-A has been tested in an NTP carcinogenicity bioassay using F344 rats and B6C3F$_1$ mice (NTP, 1982), as described below.

Rats

Groups of 50 male and 50 female rats were fed 0, 1,000 or 2,000 ppm of bisphenol-A for 103 weeks. These doses were equivalent to approximately 0, 74 and 148 mg/kg in males and 0, 74 and 135 mg/kg in females. Animals were observed twice daily, weighed every 2 weeks for the first 13 weeks and monthly thereafter, and were all subjected to a thorough gross and microscopic examination either at death or at the end of the study.

Throughout the study, survival rates in the treated groups were not significantly different from controls, with 23/50, 30/50 and 27/50 control, low-dose, and high-dose males surviving to the end of the study. Corresponding survival rates among females were 35/50, 35/50 and 37/50. Body weight gain in both dose groups of exposed males and females was lower than control animals throughout the study. At the end of the study, the mean body weights were reduced, relative to controls, by 4% and 9% in low and high-dose males, and 6% and 11% in low and high-dose females, respectively. A reduced feed intake was also observed in both sexes. At the end of the study, the overall mean feed was reduced by 7% and 12% in low- and high-dose males, and 17% and 28% in low- and high-dose females, respectively. There were no further significant non-tumour toxicological findings.

The increased incidence of leukaemias (histopathological type not further defined) in males was 13/50 in controls, 12/50 at low dose and 23/50 at top dose; and similarly in females: 7/50, 13/50 and 12/50. Only in top dose males was the increased incidence statistically significant when compared to concurrent controls. However, no statistically significant trend or incidence in leukaemias was observed in males after adjustment for intercurrent mortality by lifetime analysis. Leydig cell tumours were observed in males with incidence rates of 35/49, 48/50 and 46/49 in control, low-dose and high-dose groups, respectively. It is noted that this type of benign tumour is frequent in elderly F344 rats from control groups and that the statistically significant increased incidences were within the historical limits of the laboratory (mean incidence of 88%). Therefore, the Leydig cell tumour findings are considered unlikely to be treatment-related. Also in males, the incidence of mammary gland fibroadenomas was 0/50, 0/50 and 4/50. However, the incidence of these benign tumours at the top dose was not statistically significant compared to controls, and no similar increases were observed in female rats (8/50, 8/50, 5/50). Consequently, these mammary tumours are regarded as a chance finding, unrelated to bisphenol-A exposure. Overall, it is concluded that bisphenol-A has not produced a toxicologically significant increased incidence in tumours in this well conducted study in rats.

Mice

Mice were fed bisphenol-A in the diet for 103 weeks. Groups of 50 male and 50 female mice were fed 0, 1,000, 5,000 and 0, 5,000, 10,000 ppm of bisphenol-A, respectively. Weekly food consumption and body weight data were not provided. Therefore, using default values (see **Table 4.24**, Section 4.1.2.6.1), daily intakes of bisphenol-A are estimated to have been 120 and 600 mg/kg in males, and 650 and 1,300 mg/kg in females. Body weight gains in high-dose males and females and low-dose females were stated to be lower than control values throughout the study, without a similar decrease in food consumption. Animals were observed twice daily and were all subjected to a thorough gross and microscopic examination either at death or at the end of the study.

No significant differences in survival were observed between bisphenol-A treated and untreated animals throughout the study. In males, 42/49, 37/50 and 38/50 animals survived to the end of the study in the control, low-dose and high-dose groups, respectively. Corresponding survival rates among females were 39/50, 37/48 and 41/48. At histopathology, the only statistically

significant non-neoplastic finding was an increased incidence of multinucleated giant hepatocytes in males (1/49, 41/49, 41/50). These cell types were only observed in females at the top dose (2/48), and were not associated with an increased incidence in liver tumours in either sex (see below).

In male mice, the incidence of lymphomas was 2/49, 8/50 and 5/50 in controls, low- and high-dose groups, respectively. The incidence in leukaemias in male mice was 0/49, 1/50 and 0/50. The lymphoma tumour frequency observed was marginally statistically significant at the low dose ($p = 0.049$) compared to controls, but there was no dose-response overall. In females, there were no increased incidences of lymphomas (11/50, 8/48, 8/48) or leukaemias with dose (2/50, 2/48, 0/48). The increase in low-dose males is regarded as a chance finding, unrelated to bisphenol-A exposure. There were no significant tumour findings in any other tissue. In this well conducted study, bisphenol-A was not carcinogenic to $B6C3F_1$ mice.

Dermal exposure

No data are available.

Other related studies

Cell transformation was evaluated in Syrian hamster embryo (SHE) cells in 2 laboratories (Jones et al., 1988). In the first laboratory, SHE cells were exposed to 10 - 60 μg/ml of bisphenol-A. Toxicity, as determined by a ≥50 decrease in relative cloning efficiency, was observed from 40 μg/ml. A single transformed colony was observed at 40 μg/ml. However, this finding was not reproducible in two further experiments. In the second laboratory, SHE cells were exposed to 2-30 μg/ml bisphenol-A. No toxicity was observed. A single transformed colony was observed at 30 μg/ml. Again, this result was not reproducible in a second experiment, and did not exceed the laboratory's background level of transformation by >3 (the criterion of the study's authors for a positive response). Overall, bisphenol-A is considered to have given a negative response in these cell transformation assays.

In a further study, SHE cells were exposed to 25 to 200 μM bisphenol-A for 48 hours and harvested 7 days later (Tsutsui et al., 1998). No toxicity was observed. A statistically significant increase in the number of morphologically transformed colonies was observed from 50 μM. However, the incidence of transformed colonies was seen to decrease with dose; <0.01%, 0.01%, 0.11%, 0.08% and 0.06% at 0, 25, 50, 100 and 200 μM, respectively. A positive response was observed with the tumour promoter benzo(a)pyrene. Overall, this study is considered to have given an equivocal response, as the finding of increased morphological transformation in treated cultures was not dose-related.

Cell transformation was also investigated by Tsutsui et al. in a more recent study (Tsutsui et al., 2000). The methodology was the same as that described in their earlier study, but only a single dose level of 100 μM bisphenol-A was used. No signs of toxicity were observed. A statistically significant increase in the number of morphologically transformed colonies (0.20% vs 0% in controls) was observed at 100 μM. Although a positive control was included (benzo(a)pyrene) the incidence of transformed colonies was not determined. Thus, although an increase in morphological transformations was seen, the use of only one dose level of bisphenol-A combined with the absence of positive control data mean that no reliable conclusions can be drawn from this study.

In a further poorly reported cell transformation study, bisphenol-A apparently showed no activity in Balb/c 3T3 cells, in the absence or presence of rat hepatocytes. No further details are available (Tennant et al., 1986).

4.1.2.8.2 Studies in humans

No information is available.

4.1.2.8.3 Summary of carcinogenicity

There are no human data contributing to the assessment of whether or not bisphenol-A is carcinogenic. In animals, a dietary carcinogenicity study in two species is available: F344 rats and $B6C3F_1$ mice. A small increased incidence of leukaemias was seen in male and female F344 rats along with increases in the frequency of mammary gland fibroadenomas in male rats. These increases were not statistically significant, were slight and in a strain prone to these tumours. An increased incidence in benign Leydig cell tumours seen in male rats was within historical control limits. In mice, a small increased incidence in lymphomas was observed in males, but was not statistically significant and there was no dose-related trend. No increased incidence in any tumour type was observed in female mice. Overall, all of these tumour findings in rats and mice are not considered toxicologically significant. Consequently, it is concluded that bisphenol-A was not carcinogenic in this study in both species. No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, bisphenol-A did not exhibit properties that raise concern for potential carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m^3 in a 13 week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m^3. Taking into account all of the animal data available the evidence suggests that bisphenol-A does not have carcinogenic potential.

4.1.2.9 Toxicity to reproduction

4.1.2.9.1 Studies investigating endocrine modulating activity

Recent interest in the endocrine modulating potential of bisphenol-A extends from studies to determine whether *Saccharomyces cerevisiae* produced oestrogens. Krishnan et al. (1993) discovered that the yeast-conditioned media showed the presence of a substance that competed with [^{3}H]oestradiol for binding to oestrogen receptors from rat uterus. This substance was identified as bisphenol-A, which was thought to have leached out of polycarbonate flasks during the autoclaving of distilled water; the water was for use in media preparation. Following on from this discovery, the oestrogenic potential of bisphenol-A has been investigated in a number of studies, using either cell free systems, recombinant yeast, oestrogenic sensitive MCF-7 human breast cancer cells or the rodent uterotrophic response assay. None of these assays has been validated as an internationally accepted test method, although the MCF-7 and uterotrophic assays have been established for a number of years as standard assays for oestrogenic activity. It should be noted that the significance to human health of any oestrogenic activity detected in these assays has yet to be established.

Cell free systems

In a briefly reported study (Olea et al., 1996), the relative binding affinity of bisphenol-A to oestrogen receptors was investigated. Cytosol from immature female rat uteri was incubated in medium containing various concentrations of bisphenol-A and 3 nM [^{3}H]17β-oestradiol, for 16 hours. In this assay the relative binding affinity of bisphenol-A to oestrogen receptors was approximately 4 orders of magnitude lower than that for 17β-oestradiol.

In a briefly reported study (Maruyama et al., 1999), the binding affinity of bisphenol-A to oestrogen receptors in an oestrogen-responsive rat pituitary cell line, MtT/E-2, was investigated. Cytosol from MtT/E-2 cells was incubated with various concentrations of bisphenol-A and [^{3}H]–oestrogen. The binding affinity of bisphenol-A to oestrogen receptors was approximately 4 orders of magnitude lower than that for oestrogen.

In a briefly reported study using rat uterine cytosol, the relative binding affinity of bisphenol-A to oestrogen receptors was approximately 3 orders of magnitude lower than that for 17β–oestradiol (Feldman and Krishnan, 1995).

In a further briefly reported study, the relative binding affinity of bisphenol-A for oestrogen receptors was investigated by Dodge et al. (1996). Protein from MCF-7 cell lysates was incubated with various concentrations of bisphenol-A and 0.5 nM [^{3}H]17β-oestradiol, for 18 hours. In this assay, the relative binding affinity of bisphenol-A to oestrogen receptors was approximately 2 orders of magnitude lower than that for 17β-oestradiol.

Recently, it has been found that the rat, mouse and human oestrogen receptor exists as two subtypes, ERα and ERβ. The relative binding affinity of bisphenol-A to the receptors was investigated using human ERα and ERβ protein in insect cell extracts attached to the wells of microtitration plates (Kuiper et al., 1998). Following adhesion, receptor proteins were incubated with various concentrations of bisphenol-A and [^{3}H]17β-oestradiol for 18 hours. The relative binding affinity of bisphenol-A for both the α and β oestrogen receptors was approximately 4 orders of magnitude lower than that for 17β-oestradiol.

In a study reported as an abstract only (Zacharewski and Matthews, 2000), the ability of bisphenol-A and bisphenol-A glucuronide to compete with [^{3}H]17β-oestradiol for binding to α and β oestrogen receptors was investigated in 3 different preparations; mouse uterine cytosol, a bacterially expressed glutathione-S-transferase (GST)-ER fusion protein consisting of the human oestrogen α D, E and F domains and recombinant oestrogen β receptors. The binding affinity of bisphenol-A to oestrogen receptors was seen to vary between the preparations, and was approximately 2-4 orders of magnitude lower than that for 17β-oestradiol. Bisphenol-A glucuronide did not competitively displace 17β-oestradiol in any of the oestrogen receptor preparations.

In vitro systems

Cell proliferation assays

The oestrogenic activity of bisphenol-A was assessed in four MCF-7 cell strains (Villalobos et al., 1995). MCF-7 cells strains BUS, BB, ATCC and BB104 were cultured in the presence of human serum that had been treated with charcoal-dextran to remove endogenous oestrogens and so inhibit cell proliferation. Substances with oestrogenic activity can overcome this inhibition. Bisphenol-A elicited a proliferative response in each cell type in this assay. On a molar basis, it

was calculated that the oestrogenic potency of bisphenol-A, as measured in this assay, was approximately 4-5 orders of magnitude lower than that of 17β-oestradiol.

In further studies using MCF-7 cells (strain not specified), and measuring the ability of putative oestrogen agonists to stimulate cell proliferation, it was calculated (on a molar basis) that the oestrogenic potency of bisphenol-A was approximately 3 (Brotons et al., 1995) and 4 (Olea et al., 1996) orders of magnitude lower than that of 17β-oestradiol. In the Olea et al. (1996) study, hydroxytamoxifen, an oestrogen antagonist known to act via the oestrogen receptor, was shown to inhibit the activity of bisphenol-A, demonstrating that the assay response was due to interaction with the oestrogen receptor.

In two very briefly reported studies, bisphenol-A stimulated MCF-7 cell proliferation (Dodge et al., 1996; Coldham et al., 1997). No further details are available for either of these studies.

The oestrogenic activity of bisphenol-A was assessed in MtT/E-2 cells, an oestrogen-responsive rat pituitary cell line (Maruyama et al., 1999). A statistically significant increase in cell proliferation was observed at concentrations from 10^{-6} M bisphenol-A upwards. No further details are available.

Receptor assays

MCF-7 cells (strain not stated) were used in assessing the effect of adult human serum on the ability of bisphenol-A to bind to oestrogen receptors (Nagel et al., 1997). The reference competitor in these assays was non-radioactive 17β-oestradiol. MCF-7 cells were cultured in several concentrations of bisphenol-A in the absence or presence of human serum in multiwell plates containing non-radioactive 17β-oestradiol and [^{3}H]oestradiol for 18 hours. Three independent tests were conducted, and the relative binding affinity of bisphenol-A to oestrogenic receptors in lysed MCF-7 cells was determined by scintillation counting or fluorometric measurement of DNA. The mean relative binding affinity of bisphenol-A was approximately 4 orders of magnitude lower than that of 17β-oestradiol in both serum and serum-free media. The relative binding affinity of bisphenol-A in serum was 1.7 fold higher than that measured in serum-free medium. Thus, the presence of adult human serum produced a negligible increase in the oestrogenic activity of bisphenol-A in this assay.

In a briefly reported study, the interaction of bisphenol-A and 17β-oestradiol with receptors for progesterone in MCF-7 cells was investigated (Olea et al., 1996). 17β-Oestradiol (1 nM) was reported to increase progesterone receptor levels nearly 15 fold over the control value. Bisphenol-A was reported to increase progesterone receptor levels with no change in oestrogen receptor levels. No further information was provided. The results of this study indicate that in vitro bisphenol-A can also stimulate an increase in progesterone receptor levels, although the extent to which this occurs was not quantified.

Bisphenol-A was one of several substances tested in a yeast assay looking at interactions with oestrogenic receptors (Sohoni and Sumpter, 1998). The assay used a recombinant strain of yeast (*S. cerevisiae*), which contains an oestrogen-inducible expression system. In the presence of oestrogens, a reporter gene (Lac-Z) encoding for the enzyme β-galactosidase is expressed, which can be monitored by measuring a colour change reaction in the culture medium. A vehicle-only control was included in the test. The oestrogenic activity of the test substances was expressed as a potency relative to 17β-oestradiol by determining the molar concentrations required to produce the same response. Bisphenol-A produced a positive response; the magnitude of the response was approximately 4 orders of magnitude lower than that of 17β-oestradiol. Hydroxytamoxifen, an oestrogen antagonist known to act via the oestrogen receptor, was shown to inhibit the

activity of 17β-oestradiol, demonstrating that the observed assay responses were due to interaction with the oestrogen receptor. As an additional part to this study, the interaction of bisphenol-A in a yeast strain (PGKhAR) containing an androgen-inducible expression system encoding for the enzyme β-galactosidase was also investigated. Bisphenol-A showed no androgenic activity. However, bisphenol-A did inhibit the action of dihydrotestosterone in this assay. It was approximately as potent as the anti-androgen flutamide over the concentration range in which oestrogenic activity of bisphenol-A was also observed. These results of this study in yeast indicate that bisphenol-A has both anti-androgenic and oestrogenic activity.

In a gene transcription assay, recombinant yeast (*S. cerevisiae*) strain BJ3505 was used to determine the oestrogenic activity of bisphenol-A (Gaido et al., 1997). In the presence of oestrogens a reporter gene encoding for the enzyme β-galactosidase is expressed. Again, bisphenol-A showed oestrogenic activity; the activity of bisphenol-A was found to be approximately 4 orders of magnitude lower than that of both 17β-oestradiol and diethylstilbesterol (DES). As an additional part to this study, the activity of bisphenol-A in a yeast strain (YPH500) containing a progesterone-inducible expression system was investigated. Bisphenol-A apparently tested negative in this assay, but the experimental data were not presented.

In a further recombinant yeast cell assay, the oestrogenic activity of a number of test substances was expressed as potency relative to that of 17β-oestradiol by determining the molar concentrations required to produce the same response (Coldham et al., 1997). Bisphenol-A produced a positive response that was approximately 4 orders of magnitude lower than that of 17β-oestradiol. This result further indicates that bisphenol-A exhibits oestrogenic activity in the recombinant yeast cell bioassay.

MtT/E-2 cells were transfected with an oestrogen inducible reporter gene encoding for the enzyme β-galactosidase, and the transcription activation of bisphenol-A investigated (Maruyama et al., 1999). Transfected cells were cultured with bisphenol-A for 24 hours. Bisphenol-A was observed to stimulate gene expression; a statistically significant increase in enzyme activity was seen at concentrations from 10^{-6} M upwards. No further information is available.

The ability of bisphenol-A to stimulate transcriptional activity of the oestrogen receptors α (ERα) and β (ERβ) was determined in human embryonal kidney cells transfected with a reporter gene and human oestrogen receptors α and β (Kuiper et al., 1998). Transfected embryonal kidney cells were cultured in bisphenol-A for 24 hours. Bisphenol-A was observed to stimulate reporter gene activity for ERα more than for ERβ. The transcriptional activity of 1,000 nM bisphenol-A at the α and β receptor was 50% and 41%, respectively, of that observed with 1,000 nM 17β-oestradiol.

In a study for which the results were briefly reported, Snyder et al. (2000) investigated the oestrogen binding activities of 17β-oestradiol, bisphenol-A and bisphenol-A glucuronide in HepG2 human hepatoma cells cotransfected with either a ERα or ERβ plasmid and an oestrogen responsive plasmid encoding for luciferase activity. For bisphenol-A, EC_{50} values for ERα and ERβ induction of luciferase activity were $6.4 \cdot 10^{-7}$ M and $8.9 \cdot 10^{-7}$ M, respectively (compared with $1.9 \cdot 10^{-9}$ and $1.0 \cdot 10^{-8}$ for 17β-oestradiol). Bisphenol-A glucuronide induced only minimal activity in ERα and ERβ activation at the highest concentration tested ($3 \cdot 10^{-5}$ M). The results of this study indicate that bisphenol-A can stimulate ERα and ERβ–mediated gene expression. However, no significant expression (α or β–mediated) is observed with bisphenol-A glucuronide.

In a study reported as an abstract only (Zacharewski and Matthews, 2000), MCF-7 cells were transfected with human (α) or mouse (β) oestrogen receptor and an oestrogen inducible gene

encoding for luciferase, and the transcription activity of bisphenol-A and bisphenol-A glucuronide investigated. Bisphenol-A was observed to stimulate oestrogen receptor α- and β–mediated gene expression. Again, no significant expression (α- or β-mediated) was reported with bisphenol-A glucuronide. Elsby et al. (2001) investigated the modulatory effects of human and rat liver microsomal metabolism on the oestrogenicity of bisphenol-A in a well reported series of experiments (see Section 4.1.2.1 for a summary of the metabolic studies conducted). The oestrogenic activity of oestradiol, bisphenol-A and 5-hydroxy bisphenol-A were determined in a gene transcription assay. 5-Hydroxy bisphenol-A was included in this assay as it was observed by Elsby et al. (2001) *in vitro* (and is postulated to be the hydroxylated metabolite of bisphenol-A identified by Knaap and Sullivan (1966) in rats *in vivo*) and its oestrogenic activity has not been previously determined. The assay used a recombinant strain of yeast (*S. cerevisae*) containing an oestrogen-inducible expression system (Lac-Z) encoding for the enzyme β–galactosidase. β-galactosidase expression was measured by a colour change reaction in the culture medium.

Oestradiol, bisphenol-A and 5-hydroxy bisphenol-A were all observed to be active in the yeast assay. Comparing the EC_{50} concentrations, it was observed that bisphenol-A was approximately 35 fold less potent than oestradiol and 10 fold more potent than 5-hydroxy bisphenol-A in the yeast oestrogenicity assay.

Elsby et al. (2001) next investigated the oestrogenic activity of bisphenol-A in a coupled microsomal metabolism-yeast oestrogenicity assay. Incubations containing 0.5 mg of human or immature rat microsomal protein were incubated with 0–4 mM bisphenol-A for 45 minutes. Reactions were initiated by the addition of UDPGA (for glucuronidation) or NADPH (for oxidation). Incubations were analysed by HPLC and also incorporated into a yeast oestrogenicity assay.

An approximately 2.5-fold decrease in oestrogenic activity was seen in the presence of UDPGA. For immature rat liver microsomes, oestrogenic activity was decreased approximately 6-fold in the presence of UDPGA. HPLC analysis of both rat and human incubations indicated the formation of bisphenol-A glucuronide. No significant effect on the activity of bisphenol-A in the yeast assay was seen following incubation of either human or rat liver microsomes in the presence of NADPH. HPLC analysis of these incubations indicated the formation of a minor metabolite: 5-hydroxy bisphenol-A. Thus, there was a reduction in the oestrogenic activity of bisphenol-A following glucuronidation by human and immature rat liver microsomes. However, *in vitro* oxidation of bisphenol-A is observed to have no significant effect on the oestrogenic activity of bisphenol-A.

There are two studies (Gould et al., 1998b; Nikula et al., 1999) that suggest that at a molecular level, the activities of bisphenol-A and oestradiol, in terms of receptor interaction and its consequences, are somewhat different.

Prolactin release assays

Xenoestrogens may have an affect on the neuroendocrine axis. Thus, the ability of bisphenol-A to stimulate prolactin release *in vitro* was investigated in a series of experiments by Steinmetz et al. (1997). Anterior pituitary cells from ovariectomised F344 rats were incubated with various concentrations of 17β-oestradiol or bisphenol-A for 72 hours. Both bisphenol-A and 17β–oestradiol increased prolactin release. Bisphenol-A activity in this assay was found to be approximately 3 orders of magnitude lower than that of 17β-oestradiol.

In further studies, GH_3 pituitary cells were incubated with 10 nM oestradiol or 1 μM bisphenol-A for 7 days. Both 17β-oestradiol and bisphenol-A increased prolactin release by 2- to 3 fold in a time-dependent manner. Cell numbers were also observed to increase by 50-60% within 3-5 days.

Steinmetz et al. (1997) also investigated the induction of prolactin gene expression by bisphenol-A. GH_3 cells transfected with a rat prolactin reporter gene and a luciferase encoding sequence were incubated with 1 pM 17β-oestradiol, 1 nM bisphenol-A or 1 nM TRH (a known inducer of the prolactin gene) for 24 hours. Luciferase activity was determined in cell lysate by luminometry. Luciferase activity was increased 1.5- to 2.5 fold with both 17β-oestradiol and bisphenol-A. Higher doses of either compound were stated not to increase prolactin gene expression (data not available). TRH produced a 6- to 8-fold increase in prolactin gene expression. It was next examined whether bisphenol-A regulates transcription through the oestrogen responsive element (ERE). Anterior and posterior pituitary cells from untreated ovariectomised F344 rats were transfected with ERE/luciferase plasmid expressing the luciferase gene. Cells were incubated with 10 nM 17β-oestradiol or 1 μM bisphenol-A for 24 hours. Like 17β-oestradiol, bisphenol-A stimulated ERE-dependent gene expression, suggesting its binding to oestrogen receptors in both tissues.

In a further study, Steinmetz et al. (1997) investigated the induction of prolactin regulating factor (PRF) by bisphenol-A. Posterior pituitary cells, which were removed from F344 and Sprague Dawley rats that had been subcutaneously exposed to about 0.25 mg/kg bisphenol-A or about 0.1 mg/kg 17β-oestradiol for 3 days, were co-cultured in the presence of GH_3 cells transfected with a rat prolactin reporter gene and a luciferase encoding sequence for 24 hours. Luciferase activity, designating induction of the prolactin promoter, was determined in cell lysate by luminometry. Posterior pituitary cells from controls of both strains of rat increased PRF activity 3 to 5 fold, indicating basal PRF activity. Cells harvested from 17β-oestradiol and bisphenol-A treated F344 rats increased PRF activity 15 to 17 fold. PRF activity in cells from Sprague Dawley rats treated with oestradiol or bisphenol-A was unchanged, indicating a marked strain difference (the author reports that results were observed with F344 rats only, as this strain is sensitive to exogenous oestrogens that induce hyperprolactinaemia). These results together with the results observed in F344 rats *in vivo* (see *in vivo* section) indicate that bisphenol-A can cause induction of PRF in the posterior pituitary leading to increased prolactin levels.

To summarise the *in vitro* oestrogenic data, bisphenol-A has oestrogenic activity in these systems and, overall, its activity is generally 3-5 orders of magnitude less than that of 17β–oestradiol. Bisphenol-A has also been shown to increase prolactin release, and there is limited evidence for anti-androgenic activity and stimulation of progesterone activity.

In vivo systems

The oestrogenic activity of bisphenol-A and its influence on prolactin release has been assessed *in vivo* in several studies generally using an assay based upon the uterotrophic response in the rat. These studies are presented below under sub-headings of the route of exposure used. Studies have also been grouped under sub-headings for the effect of bisphenol-A on the growth and development of the mammary gland and prolactin release.

Oral exposure

Rats

In an unpublished study for which the full test report is available (Central Toxicology Laboratory, 1999a), groups of 10 immature (21-22-day-old) female Alpk rats received daily doses of 0, 0.002, 0.02, 0.2, 1, 10, 100, 200 or 800 mg/kg bisphenol-A by gavage for three consecutive days. Animals were killed 24 hours after the final dose, the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels determined and the uterine wet and dry weight recorded together with a histopathological examination of the uteri. 17β–Oestradiol (0.4 mg/kg) administered by the same route and dosing regime served as a positive control.

Clinical signs of toxicity were observed only in animals receiving 800 mg/kg bisphenol-A; comprising hunched posture, subdued behaviour, salivation and piloerection. A statistically significant increase (21%) in serum ALT was seen at 800 mg/kg bisphenol-A compared to controls. No increase in AST levels was seen in treated animals. Compared to controls, a statistically significant increase in uterine wet and dry weights was observed at 200 (30% and 26%, respectively) and 800 mg/kg bisphenol-A (114% and 76%). At necropsy, endometrial hypertrophy/hyperplasia, lumenal epithelial apoptosis (800 mg/kg only), endometrial glandular epithelial apoptosis and increased stromal neutrophils were observed in the 200 and 800 mg/kg bisphenol-A dose groups. There were no treatment-related uterus changes at bisphenol-A doses of 100 mg/kg and below. For the positive control, statistically significant increases in the uterine wet (294%) and dry (203%) weight were observed, together with histopathological changes in the uteri consistent with those seen with bisphenol-A. Therefore, in this screening assay for oestrogenic activity using the oral route of exposure, changes to the uteri were observed in Alpk rats at 200 mg/kg bisphenol-A and above. No effects were observed at dose levels up to, and including, 100 mg/kg.

The activity of bisphenol-A was investigated in a rat uterotrophic study by Ashby and Tinwell (1998). Immature (21-22-day-old) female Alpk:AP rats (7-10 per dose level) received daily doses of 0, 400, 600 or 800 mg/kg bisphenol-A by gavage for 3 consecutive days. Animals were killed 24 hours after the final dose, the presence or absence of vaginal opening recorded, and the uterine wet and dry weight determined. DES (0.04 mg/kg), administered by the same route of administration and dosing regime, served as the positive control.

No clinical signs of toxicity or effects on body weight gain were observed with bisphenol-A. Compared to controls, uterine wet weights were increased by 31, 38 and 118%, and uterine dry weights by 40, 40 and 150%, following administration of 400, 600 and 800 mg/kg bisphenol-A, respectively. Premature vaginal opening was not observed in bisphenol-A treated groups. DES produced increases in uterine wet and dry weights of >250% and premature vaginal opening in 60% animals. Thus, an oestrogenic activity was observed with bisphenol-A in this immature rat uterotrophic assay following oral administration.

The effect of bisphenol-A on oestrus cyclicity was investigated in a uterotrophic assay by Rubin et al. (2001). Groups of 4-6 ovariectomised Sprague Dawley rats were administered 0, 0.2, 2.0 and 16.9 mg/kg bisphenol-A in the drinking water for 3 consecutive days. Vaginal cytology was conducted before and daily during treatment. Animals were sacrificed after treatment and uterine wet weights determined. Esterone (0.02 or 0.17 mg/kg) administered by the same route and dosing regime served as a positive control.

Bisphenol-A had no effect on uterine wet weight or vaginal cytology. Compared to controls, a statistically significant increase in uterine wet weight (317%) was observed with 0.17 mg/kg esterone, along with cornified vaginal smears on the day of sacrifice.

Laws et al. (2000) conducted a series of experiments investigating the oestrogenic activity of a number of substances, including bisphenol-A, using different biological endpoints.

In a well reported experiment, a uterotrophic assay was conducted in immature (21-day-old) and ovariectomised Long Evans rats. Groups of 6 immature and 6 ovariectomised rats received 0, 100, 200 or 400 mg/kg bisphenol-A daily by gavage for 3 consecutive days. Animals were sacrificed at 6 hours, and 24 hours for immature animals, after the last dose and the uteri removed and weighed. Compared to controls, a statistically significant increase in uterine wet weight of approximately 260 and 310% was seen in immature rats 6 hours after administration of 200 and 400 mg/kg bisphenol-A, respectively. However, by 24 hours post-dosing in both treatment groups, uterine weight had returned to control levels. Bisphenol-A had no effect on uterine weight in ovariectomised animals.

The next study by Laws et al. (2000) was conducted to investigate the effects of bisphenol-A on vaginal opening. Groups of 7 or 8 immature Long Evans rats received 0, 50, 100, 200 or 400 mg/kg day bisphenol-A by gavage from 21 to 35 days of age and the day of vaginal opening was recorded. Bisphenol-A had no effect on body weight gain or on the time of vaginal opening.

In a further study by Laws et al. (2000) in Long Evans rats, groups of 6 ovariectomised and 9-15 “intact” females received 0 or 100 mg/kg day bisphenol-A by gavage for 25 days. Vaginal smears were taken daily to assess the effect of bisphenol-A on oestrus cyclicity. The authors report that oestrogenic activity in ovariectomised animals would result in persistent oestrous status, which would be reflected by the appearance of cornified epithelial cells in vaginal smears. No effect on vaginal cytology was observed in ovariectomised animals administered bisphenol-A. In “intact” animals, extended oestrous was observed in bisphenol-A treated animals so that the mean number of 4-5 day oestrus cycles was seen to decrease from 5.2 in controls to 3.7 in treated animals.

In the final study, Laws et al. (2000) compared the oestrogenic activity of bisphenol-A following oral and s.c. administration. Groups of 6 ovariectomised rats received 0 or 200 mg/kg bisphenol-A daily by gavage or s.c. injection for 3 consecutive days. Animals were sacrificed 6 hours after the last dose, the uteri removed and weighed. Compared to controls, a statistically significant increase in uterine wet weight of approximately 130 and 270% was seen following oral and s.c administration, respectively.

Overall, the Laws et al. (2000) studies demonstrate that the immature rat model was more sensitive than ovariectomised adult rats in detecting oestrogenic activity on the basis of the uterotrophic response. However, the activity seen in immature rats 6 hours post-dosing was observed to be short term, as uterine weight returned to control level values by 24 hours. Greater changes in uterine weight were seen when bisphenol-A was administered by the s.c. route compared with those following oral exposure. Assays based on the time to vaginal opening and vaginal cytology did not detect oestrogenic activity, although an effect of bisphenol-A was observed on oestrous cyclicity.

Bisphenol-A was one of a number of chemicals tested in the peripubertal male rat assay, which is being developed for the detection of anti-androgens, oestrogens and metabolic modulators (Ashby and Lefevre, 2000). Groups of 10 immature male Alpk:APfSD rats (21-22 or 32-33-day-old) were given 20 consecutive daily oral (gavage) doses of 0, 100 or 150 mg/kg bisphenol-A. The endpoints studied were changes in the weights of testes, epididymides, seminal vesicles,

prostate, liver, kidney and body weight. The day of prepuce separation and the influence of initial body weight on final organ weight were also evaluated. The study also evaluated DES, which is often used as a positive control in oestrogenic activity assays. No effect on any parameter measured was observed with bisphenol-A. DES (40 μg) reduced the weights of all reproductive organs and produced marked delays in the day of prepuce separation. Therefore, bisphenol-A had no effect on any endpoint associated with potential oestrogenic activity in this immature male rat study.

In a briefly reported study (Dodge et al., 1996), ovariectomised Sprague Dawley rats received 0, 0.1, 1.0, 10 or 30 mg/kg bisphenol-A daily by oral gavage for 4 days or 5 weeks. Oestrogenic activity was measured by changes in uterine wet weight after 4 days and 5 weeks of dosing. In addition, the effect of bisphenol-A on serum cholesterol levels was determined in rats for both dosing regimes, along with bone mineral density in rats dosed for 5 weeks. Compared to controls, the authors report that the maximum, and statistically significant, increases in uterine wet weights were observed after 4 days of dosing; 29% and 37% in the 10 and 30 mg/kg dose groups, respectively. The authors also report that bisphenol-A lowered serum cholesterol levels after 4 days of dosing. However, no results were presented for the effect of bisphenol-A on uterine wet weights and serum cholesterol levels after 5 weeks of dosing. No effects were observed on bone mineral density with bisphenol-A. Thus, the limited details of this study suggest that bisphenol-A increases uterine wet weight in ovariectomised rats.

In a well reported study (Gould et al., 1998b), groups of 4-5 immature (21-day-old) female Sprague Dawley rats received 0, 5, 10, 25, 50, 100 or 150 mg/kg bisphenol-A daily by gavage for 3 consecutive days. Animals were sacrificed 20 hours after the last dose, the uteri removed, weighed and then assays conducted to determine uterine progesterone receptor levels and peroxidase activity, two oestrogen-responsive proteins. Oestradiol (0.5 μg), administered by the i.p. route and using the same dosing regime, served as a positive control. Bisphenol-A had no effect on uterine wet weight. Compared to controls, a statistically significant increase in uterine peroxidase activity was seen at 100 (50%) and 150 mg/kg bisphenol-A (108%). A statistically significant but not dose-related increase in progesterone receptor levels of 34-76% was observed in all bisphenol-A treatment groups. Compared to controls, the positive control oestradiol produced a statistically significant increase in uterine wet weight (375%), peroxidase activity (717%) and progesterone receptor levels (514%). The toxicological significance of these observed increases in oestrogen responsive proteins is unclear.

The effect of bisphenol-A on the growth of prostate and seminal vesicles in the rat was investigated in an androgen and anti-androgen assay (Michna, 2000). In the androgen assay, groups of 7-10 orchiectomised Wistar rats received 0, 3, 50, 200 or 500 mg/kg bisphenol-A by oral gavage daily for 12 days. A further group received 500 mg/kg bisphenol-A and the anti-androgen flutamid (3 mg/kg) by the same dosing regime. The assay also included a control group of "intact" males along with a positive control group receiving 1 mg/kg testosteronpropionat (TP) by s.c. injection. Animals were sacrificed 24 hours after the last dose and the kidney, liver, prostate and seminal vesicles removed, weighed and examined (no further details available). Blood was also taken from animals 5 minutes after the first and last dose, but the reasons for taking it are not reported.

A statistically significant decrease (22%) in body weight gain was observed with 500 mg/kg bisphenol-A and flutamid. No statistically significant increases or decreases were seen in absolute organ weights. However, after correction for body weight, a statistically significant increase in prostate weight was seen at 500 mg/kg in the absence (37%) and presence (16%) of flutamid compared to orchiectomised controls. No statistically significant effects were seen in seminal vesicle, liver or kidney weights. Comparing the "intact" and orchiectomised controls, a

statistically significant decrease in body weight (10%), prostate weight (89%) and seminal vesicle weight (89%) was seen in orchiectomised males. Compared to orchiectomised controls, TP produced a statistically significant increase in prostate (1313%), seminal vesicle (888%) and liver (12%) weight. The results of histopathological examination were not reported.

The anti-androgen assay used the same test protocol as the androgen assay but animals received 1 mg/kg TP (by s.c. injection) plus 0, 3, 50, 200 or 500 mg/kg bisphenol-A. A positive control group that received TP and flutamid was also included (but no "intact" control group).

A statistically significant decrease (10%) in body weight gain was seen at the top dose (500 mg/kg and 1 mg/kg TP). No statistically significant increases or decreases were seen in absolute organ weights. However, after correction for body weight, a statistically significant increase in prostate weight (15%) was seen at the top dose compared to controls. No statistically significant effects were seen on seminal vesicle, liver or kidney weight. A statistically significant decrease in prostate (55%) and seminal vesicle weight (63%) was seen with TP. Again, the results at histopathology were not reported.

The results of the Michna (2000) study show a stimulatory effect on prostate growth (37%) with 500 mg/kg bisphenol-A, which was antagonised by the anti-androgen flutamid. Bisphenol-A exhibited no activity in the anti-androgen assay. Thus, the data indicate androgen activity of bisphenol-A, albeit limited, at 500 mg/kg.

In a study cited in the BUA (1995) review (Bornmann and Loeser, 1959), oral administration of bisphenol-A in orchiectomised rats resulted in "uncertain and very faint" oestrogenic activity, even after "high" doses. A single s.c. injection of 2400 mg/kg bisphenol-A apparently triggered oestrus in ovariectomised animals. No further information is provided. Consequently, the limited details available for this study mean that no reliable conclusions can be drawn from the data.

Mice

In a well reported study (Tinwell et al., 2000), a uterotrophic assay was conducted in immature (19-20-day-old) AP mice. Administration of 5-bromodeoxyuridine (0.8 mg/ml) in deionized water to these animals during the acclimatisation and dosing period also allowed uterine hyperplasia to be determined.

Groups of 12 immature (19-20-day-old) female AP mice received 0, 0.5, 1, 5, 10, 50, 100, 200 or 300 mg/kg bisphenol-A daily by gavage for 3 consecutive days. Animals were sacrificed 24 hours after the last dose, the uteri removed and weighed. Uterine samples were then taken, stained for bromodeoxyuridine and labelling index determined by light microscopy. Uterine hypertrophy was determined by light microscopy. DES (10 μg/kg), administered by the same route of administration and dosing regime, served as a positive control. Bisphenol-A had no effect on body weight gain or uterine wet weight, nor did administration of bisphenol-A lead to uterine hypertrophy; no significant increase was seen in the height of the uterine epithelium or endometrium. However, compared to controls, a statistically significant increase in the number of cells in the uterine epithelium (181%) and endometrial stroma (166%) was seen at 200 mg/kg, and in the uterine epithelium (384%) and endometrial glands (54%) and stroma (172%) at 300 mg/kg. Compared to controls, DES produced a statistically significant increase in uterine wet weight (317%), height of the uterine epithelium (161%) and endometrium (79%); and in the number of cells in the uterine epithelium (618%), and endometrial glands (49%) and stroma (325%). Thus, the only effect observed on the uterus in this immature mouse uterotrophic assay was hyperplasia at 200 mg/kg and above. No effect on the uterus was seen at concentrations up to, and including, 100 mg/kg bisphenol-A.

Parenteral routes of exposure

Rats

A number of older studies are available which were designed to investigate the oestrogenic potential of bisphenol-A. These reports are generally brief and lack significant information on the test protocol and effects observed.

Bisphenol-A showed an oestrogenic effect (cornification of the vagina) after injection (by an unstated route) of 100 mg bisphenol-A in oily solution to ovariectomised rats (Dodds and Lawson, 1936; 1938). An oestrus response was also observed in ovariectomised rats injected with an unstated amount of bisphenol-A six times over three days (Campbell, 1940). Reid and Wilson (1944) also reported that bisphenol-A had shown oestrogenic activity in the rat. No further details are available for any of these studies.

In a recent unpublished study for which the full test report is available (Central Toxicology Laboratory, 1999b), groups of 10 immature (21-22-day-old) female Alpk rats received daily doses of 0, 0.002, 0.02, 0.2, 1, 10, 100, or 800 mg/kg bisphenol-A by s.c injection for three consecutive days. Animals were killed 24 hours after the final dose, the serum ALT and AST levels determined and the uterine wet and dry weight recorded together with a histopathological examination of the uteri. 17β-Oestradiol (0.4 mg/kg) administered by the same route and dosing regime served as a positive control.

Clinical signs of toxicity were observed only in the top dose (800 mg/kg) group; fur staining in several animals and a statistically significant decrease (10%) in body weight gain compared to controls. A statistically significant decrease in serum ALT levels was seen in animals at 800 mg/kg (16%). Serum AST levels in treated and control animals were similar. Compared to controls, a statistically significant increase in wet uteri weight (117%) was seen at 800 mg/kg bisphenol-A only. In bisphenol-A-treated animals, no increase in uterine dry weight achieved statistical significance when compared to controls. At necropsy, endometrial hypertrophy/hyperplasia, lumenal epithelial apoptosis, endometrial glandular epithelial apoptosis and increased stromal neutrophils were seen at 100 mg/kg bisphenol-A and above. Uterine lumenal epithelial apoptosis and endometrial glandular epithelial apoptosis were also seen in animals at 10 mg/kg. For the positive control, statistically significant increases in the uterine wet (218%) and dry (166%) weight were observed, together with histopathological changes in the uteri consistent with those seen with bisphenol-A. Therefore, in this screening assay for oestrogenic activity using the s.c route of exposure, bisphenol-A produced changes in the uteri in Alpk rats.

The activity of bisphenol-A was investigated in a recent rat uterotrophic study using the s.c route of administration (Ashby and Tinwell, 1998). Immature (21-22-day-old) female Alpk:AP rats (7-10 per dose level) received daily injections of 0, 400, 600 or 800 mg/kg bisphenol-A for 3 consecutive days. Animals were killed 24 hours after the final dose, the presence or absence of vaginal opening recorded, and the uterine wet and dry weight determined. DES (0.04 mg/kg), administered by the same route of administration and dosing regime, served as the positive control.

No clinical signs of toxicity or effects on body weight gain were observed with bisphenol-A. Compared to controls, uterine wet weights were increased by 50, 71 and 103%, and uterine dry weights by 50, 67 and 100%, following administration of 400, 600 and 800 mg/kg bisphenol-A, respectively. Premature vaginal opening was observed in 0, 0, 57 and 47% rats at 0, 400, 600 and 800 mg/kg bisphenol-A, respectively. DES produced increases in uterine wet and dry weights of

>200% and premature vaginal opening in 30% animals. Thus, an oestrogenic activity was observed with bisphenol-A in this immature rat uterotrophic assay following s.c. administration.

A further study investigating the activity of bisphenol-A in a rat uterotrophic assay following s.c. administration is available (Goloubkova et al., 2000). Groups of ovariectomised Wistar rats (6 per dose group) received daily injections of 0, 11, 78, 128 and 250 mg/kg for 7 days. The study also included a control group of sham surgery animals. Animals were killed after treatment (it is not reported how long after the final injection), serum prolactin levels and uterine wet weight determined, and prolactin-expressed cells were identified in pituitary glands by immunohistochemical staining. It is not reported whether a positive control was used in this study.

A statistically significant decrease in body weight gain (6%) was observed in animals receiving 250 mg/kg compared to controls. A statistically significant and dose-related increase in mean uterine wet weight was observed at 11 (approx 35%), 78 (50%), 128 (80%) and 250 mg/kg (155%) compared to controls. Even at 250 mg/kg bisphenol-A, the uterine wet weights were not restored to levels seen in the sham control animals. Compared to controls, statistically significant increases in mean anterior pituitary gland weights (approx >40%) and mean prolactin levels (approx >700%) were seen at 128 and 250 mg/kg bisphenol-A. A statistically significant increase in prolactin-immunopositive cells was also seen at 250 mg/kg (data not presented). The number of prolactin-immunopositive cells was seen to decrease in ovariectomised animals; 24% decrease in ovariectomised controls compared to sham control animals.

An immature rat uterotrophic assay using both the s.c. and oral route of administration is available (Yamasaki et al., 2000). Female CD rats (8 animals per dose group) were given daily s.c. injections of 0, 8, 40 or 160 mg/kg bisphenol-A in sesame oil, or daily oral (gavage) doses of 0, 40, 160 or 800 mg/kg bisphenol-A in sesame oil, on postnatal days 18-20. Animals were sacrificed 24 hours after the final dose and uteri removed and weighed. A repeat study using the same experimental protocol was conducted. The initial study also determined plasma concentrations of bisphenol-A in 4 females per dose group 1 hour after the last injection. These results have not been reported in this summary.

In both the initial and repeat study, no clinical signs of toxicity, effects on body weight gain or immature vaginal opening were observed for either the oral or s.c. route of administration. In the initial study, a statistically significant and dose-related increase in absolute wet and dry uterine weight (44%) was seen following oral administration of 800 mg/kg bisphenol-A, compared to controls. Statistically significant increases in the relative wet and dry uterine weights ($\geq$13%) were seen at 160 mg/kg and above. In the repeat study, statistically significant increases in absolute and relative wet and dry uterine weights ($\geq$14%) were seen at 160 mg/kg and above.

In the initial study using s.c. administration, a statistically significant and dose-related increase in absolute and relative wet and dry uterine weight ($\geq$14%) was seen at 8 mg/kg and above, compared to controls. In the repeat study, statistically significant increases in absolute wet and dry uterine weights ($\geq$47%) were seen at 40 mg/kg and above, compared to controls. Increases in relative wet and dry uterine weights ($\geq$14%) were statistically significant at 8 mg/kg and above.

Yamasaki et al. (2000) investigated time course changes in uterine weight after s.c. administration of bisphenol-A. This study employed the same dose levels and experimental procedure described above, with the addition that animals were sacrificed 6, 12, 18 and 24 hours after the last injection. Statistically significant increases were seen in uterine wet and dry weight for all sample times at 40 and 160 mg/kg, compared to controls. The increases at 6 hours were greater than those observed at 24 hours, but the coefficient of variation was lower at 24 hours

than at 6 hours. Thus, the authors suggest that autopsy at 24 hours after final administration of the test substance is more suitable, based on the coefficients of variation at low-dose levels.

A series of exploratory studies examining the growth, differentiation and gene expression in the female rat reproductive tract was conducted by Steinmetz et al. (1998).

In a briefly reported experiment, groups of ovariectomised F344 rats (number not reported) received a single i.p. injection of 0, 19, 37.5, 75, 150 or 200 mg/kg bisphenol-A. 17β-Oestradiol (10 μg/kg) administered by the same route, served as a positive control. Animals were injected i.p. with 5-bromodeoxyuridine 19 hours after administration of bisphenol-A or oestradiol, and killed 1 hour later. Uteri and vaginas were removed and cell proliferation determined in these tissues by bromodeoxyuridine immunostaining. A statistically significant increase in the number of labelled epithelium cells in both uteri and vagina was observed at 37.5 mg/kg bisphenol-A and above. Maximum labelling in the uterine epithelium was observed at 75 and 150 mg/kg. A secondary increase in vaginal epithelium labelling was observed at levels causing toxicity in some animals, 200 mg/kg, and at an additional dose level of 300 mg/kg bisphenol-A (no further data provided). The limited details prevent any conclusions to be drawn on the possible cause of this secondary increase. The authors report that labelled epithelial cells in both uteri and vagina were observed with the positive control. This study demonstrates that bisphenol-A increases cell proliferation in the uteri and vaginas of ovariectomised rats.

The next study was conducted to compare the ability of bisphenol-A and 17β-oestradiol to induce *c-fos* gene expression in the F344 rat uterus and vagina. Groups of ovariectomised rats (number not reported) received a single i.p injection of 0 or 50 mg/kg bisphenol-A. 17β-Oestradiol (10 μg/kg), administered by the same route, served as a positive control. Animals were killed 2, 6 and 24 hours later. Uteri and vaginas were removed and c-*fos* gene expression determined in each. Both bisphenol-A and 17β-oestradiol increased c-*fos* messenger RNA levels in the uterus 14- to 17-fold and 7- to 9-fold in the vagina above control values within 2 hours. In the uterus, c-*fos* expression returned to basal levels after 6 hours following both bisphenol-A and 17β-oestradiol treatment. In the vagina, bisphenol-A-induced c-*fos* expression remained elevated for up to 6 hours compared to transient increases observed with 17β-oestradiol. The results of this study show the potential of bisphenol-A to induce *c-fos* gene expression in the uterus and vagina of F344 rats.

In the final study, groups of ovariectomised F344 and Sprague Dawley rats (number not reported) were implanted subcutaneously with capsules containing crystalline bisphenol-A or 17β-oestradiol. Controls received empty capsules. Animals were killed after 3 days and uterine sections examined, the heights of the luminal epithelial cells being measured. The rate of release of bisphenol-A and 17β-oestradiol from the capsules was estimated to be approximately 0.3 and 0.006 mg/kg/day, respectively. In F344 rats a statistically significant increase of 2.5-fold and 3.5-fold were observed in epithelial cell height compared to controls following bisphenol-A and 17β-oestradiol treatment, respectively. Compared to controls, a statistically significant increase in uterine wet weight of nearly 2-fold and 3-3.5 fold was observed following treatment with bisphenol-A and 17β-oestradiol, respectively. Similar to 17β-oestradiol, bisphenol-A resulted in hypertrophy of the luminal epithelium and stimulated mucus secretion in the uterus, and hyperplasia and cornification of the vaginal epithelium. However, in Sprague Dawley rats uterine cell height and uterine wet weight were significantly altered only with 17β-oestradiol. The results of this study show a marked strain difference in the oestrogenic activity of bisphenol-A, but not of 17β-oestradiol. Based on the estimated release of bisphenol-A and oestradiol, the potency of bisphenol-A in this study is approximately 50-fold less than that of oestradiol.

Overall, the Steinmetz et al. (1998) studies demonstrate that the molecular and morphological alterations induced by bisphenol-A in the uterus and vagina are qualitatively similar to those induced by 17β-oestradiol. The studies also indicate that the reproductive tract of F344 rats appears to be more sensitive than that of Sprague Dawley rats to the effects of bisphenol-A, although there is no information to indicate which strain is more relevant to humans.

Long et al. (2000), investigated if the rat vagina (an oestrogen target tissue) responds to bisphenol-A in a strain-specific manner in F344 and Sprague Dawley rats. However, the data reported for F344 rats are from the Steinmetz et al. (1998) study. Only the results in Sprague Dawley rats are reported here.

Groups of 4 ovariectomised Sprague Dawley rats were administered a single i.p. injection of 0 or 0.2-150 mg/kg bisphenol-A and cell proliferation in vaginal epithelium determined 19 hours later. Animals were injected with bromodeoxyuridine 1 hour prior to sacrifice, vaginal tissue taken, stained for bromodeoxyuridine and labelling index determined by light microscopy. 17β–Oestradiol (0.02-2 μg/kg) administered by the same route, served as a positive control. No significant increase in the number of labelled epithelial cells was seen in Sprague Dawley rats treated with bisphenol-A (in comparison, in F344 rats, a statistically significant increase in labelled vaginal epithelial cells was seen at 37.5 mg/kg and above in the Steinmetz et al. (1998) study). The positive control produced a significant increase in labelled cells.

The Long et al. (2000) study also conducted further experiments investigating potential strain differences between F344 and Sprague Dawley rats; metabolic clearance of bisphenol-A, stimulation of c-*fos* gene expression by bisphenol-A and vaginal epithelium concentration of oestrogen receptors. However, since the study did not concurrently investigate the activity of bisphenol-A on vaginal epithelium in F344 rats, these experiments are considered to be of limited value. Consequently, they are not discussed in detail here, except to report that clearance of [^{3}H]-bisphenol-A from blood (administered by i.v. injection) followed the same time course in both rat strains, stimulation of c-*fos* gene expression by bisphenol-A showed no strain difference and analysis of [^{3}H]-bisphenol binding showed there were no significant differences in concentration or affinity for bisphenol-A of oestrogen receptor in vaginal tissue.

Overall, no effect on vaginal epithelium was seen in Sprague Dawley rats in this study. This suggests that there may be a significant difference in the oestrogenic activity of bisphenol-A between F344 and Sprague Dawley rats.

In a study in Sprague Dawley rats, which was reported in abstract form only, ovariectomised rats received a single i.p. injection of 0, 25, 50 or 100 mg/kg bisphenol-A (Bond et al., 1980). Oestrogenic activity was measured by changes in uterine water content; oestrogens can cause a fluid distension of the uterus. A statistically significant increase in % uterine water content was reported in the 50 and 100 mg/kg dose groups at 18 hours post-dosing. No further details are available.

In a study reported in abstract form only (Cummings, 1997), bisphenol-A was investigated in the delayed implanting model. In this study, female Holtzman rats identified as sperm positive (day 0) were hypophysectomised two days later. Removal of the pituitary prior to implantation permits the blastocysts to remain viable but unattached in the uterus. Oestrogenic activity is then detected as the ability to induce implantation. Animals received progesterone at 2 mg/rat on days 0-6 and 4 mg/rat on days 7-8. On day 7, rats were injected (s.c.) with 25-200 mg/kg bisphenol-A or 1 μg of the positive control, estrone. On day 9, dye was administered to animals by intra-hepatic infusion, under anaesthesia, and the implantation sites were observed as blue bands after 10 minutes. All treated rats had implantations following administration of bisphenol-A at

200 mg/kg, 78% at 100 mg/kg, 50% at 50 mg/kg and 30% at 25 mg/kg. Implantation was observed in all animals treated with the positive control. The results of this study indicate that bisphenol-A administered by the s.c. route can induce implantation of the blastocysts in hypophysectomised Holtzman rats, a reflection of oestrogenic activity.

In an early study (Bitman and Cecil, 1970), the ability of bisphenol-A to increase glycogen content in the uterus was investigated as a measure of its oestrogenic activity. Immature (21-23-day-old) Wistar rats were injected subcutaneously with unstated doses of bisphenol-A. The authors report that in this assay, the minimum effective dose was 0.25 mg/bisphenol-A/animal (using the default body weight value in Table 4.24, this corresponds to a dose of 1.4 mg/kg bisphenol-A). No further details are available.

In a well reported study, the oestrogenic activity of bisphenol-A was investigated *in vivo* in MtT/E-2 cells implanted in rats (Maruyama et al., 1999). The authors state that this cell type will develop into tumours in response to oestrogen. Groups of 5-6 ovariectomised F344 rats were inoculated at two sites in the subcutaneous fat pads with MtT/E-2 cells. Animals then received 0, 0.1, 1 or 10 mg bisphenol-A by the i.p. route (assuming a rat weighs 0.175 kg, this is equivalent to 0, 0.6, 5.7 and 57 mg/kg), three times a week, starting on the day the fat pads were injected. Oestrogen (0.5 mg), given by s.c. injection, served as the positive control. Test sites were examined twice a week and the diameters of any tumours measured. Animals were sacrificed when the tumours reached 1-2 cm in diameter. At necropsy, the pituitary and uterus were removed and weighed, and serum prolactin levels measured.

The tumour results were briefly reported. The time to appearance of "visible" tumours occurred in a dose-dependent manner in bisphenol-A-treated animals, on days 25, 32 and 36 at 57, 5.7 and 0.6 mg/kg respectively. Tumours were seen in control and oestrogen-treated animals at 41 and 22 days, respectively. Compared to controls, no significant increase in serum prolactin levels or pituitary and uterus weights were seen in bisphenol-A-treated animals. A statistically significant increase in prolactin (697%), and pituitary (102%) and uterus (545%) weights was observed with oestrogen. The results of this study suggest that bisphenol-A administered by the ip route expressed oestrogenic activity towards inoculated MtT/E-2 cells in F344 rats. However, the limited reporting of the results mean no reliable conclusions can be reached from this study. Furthermore, the toxicological significance of this experimental approach is unclear.

Mice

In a well reported uterotrophic assay in CFLP mice (Coldham et al., 1997), prepubertal 18-day-old females received 0, 0.05, 0.5 or 5 mg bisphenol-A daily by s.c. injection for 3 days. Mice were sacrificed 24 hours after the final injection and uterine wet weight determined. The oestrogenic activity of bisphenol-A was expressed as the potency relative to 17β-oestradiol, by determining the molar concentration required to produce similar increases in the ratio of uterine wet weight/body weight. The authors report that treatment with 5 mg bisphenol-A was ceased due to symptoms of acute toxicity (the nature of which was not specified). At 0.05 and 0.5 mg bisphenol-A, uterine wet weights were similar to controls. Compared to controls, a statistically significant dose-related increase in uterine wet weight (77-471%) was observed with 5-100 ng 17β-oestradiol. Therefore, bisphenol-A showed no oestrogenic activity in this uterotrophic assay in mice.

An exploratory assay examining the oestrogenic activity of xenoestrogens by determining their effect on uterine vascular permeability was conducted by Milligan et al. (1998). In this generally well reported study, 12 and 6 ovariectomised Swiss mice were administered 0 or 10^{-5}mol (equivalent to 23 mg) bisphenol-A by s.c. injection, respectively. (^{125}I)-Radiolabelled albumin

was then administered by i.v injection 3.5 hours later. A blood sample was taken 0.5 hours later and animals were immediately sacrificed. The uteri and a thigh muscle sample were removed, and the radioactivity in these samples and in the plasma was determined. The ratio of the (^{125}I) counts per minute per milligram of tissue to (^{125}I) counts per minute per microliter of plasma was used as an index of tissue vascular permeability. Uterine vascular permeability was observed to increase in bisphenol-A treated animals compared to controls. Various concentrations of 17β–oestradiol (10^{-12}, 10^{-10} and 10^{-9} mol) were also tested in this experiment. Compared to controls, uterine vascular permeability was seen to increase at 10^{-10} and 10^{-9} mol 17β-oestradiol. The muscle vascular permeability in both bisphenol-A and 17β-oestradiol treated animals was similar to controls. The results suggest that s.c. administration of 23 mg bisphenol-A produced an increase in uterine vascular permeability, which (using the default values in **Table 4.24**) corresponds to a dose of approximately 900 mg/kg. The toxicological significance to human health of increased uterine vascular permeability is unclear.

Prolactin release

A study was conducted by Steinmetz et al. (1997) to determine the influence of bisphenol-A on prolactin release, as the study authors reported that *in vivo*, oestrogens can affect the neuroendocrine axis and thus affect prolactin release, by acting directly on the pituitary lactotroph (an oestrogen responsive cell) or indirectly via hypothalamo-pituitary factors that regulate lactotrophs, such as prolactin regulating factor (PRF). Groups of 12 F344 and 8 Sprague Dawley ovariectomised rats were implanted subcutaneously with capsules containing crystalline bisphenol-A or 17β-oestradiol. Controls received empty capsules. The release of bisphenol-A and 17β-oestradiol was estimated from an *in vitro* experiment to be approximately 40-45 μg/day and 1.2-1.5 μg/day, respectively. Using default weight values (see **Table 4.24**) this corresponds to about 0.25 mg/kg bisphenol-A and 0.01 mg/kg oestradiol. Animals were killed after 3 days, serum analysed for prolactin and the anterior pituitaries removed and weighed. Compared to controls, bisphenol-A and 17β-oestradiol increased basal prolactin levels 7 to 8 fold and 10 fold in F344 rats, respectively. However, in Sprague Dawley rats only 17β-oestradiol produced an increase in prolactin levels (3-fold). Bisphenol-A did not alter anterior pituitary weights in either rat strain. 17β-Oestradiol doubled anterior pituitary weights in F344 rats but produced no significant increase in Sprague Dawley rats. The results indicate a marked strain difference in the influence of bisphenol-A on prolactin release in rats.

Effects on mammary gland

The influence of bisphenol-A on the normal growth and development of the mammary gland of rats was investigated by Colerangle and Roy (1997). An indication of changes in mitotic activity in the female mammary gland is considered to be a reflection of oestrogenic action. The authors stated that Noble rats were used in this study as they are particularly sensitive to oestrogenic activity; oestrogen treatment of female Noble rats for 11-12 months induced an 80-90% incidence of mammary tumours. Groups of rats (6 per group) were implanted subcutaneously with osmotic minipumps containing bisphenol-A administering a daily dose of 0, 0.1 or 54 mg/kg bisphenol-A. DES (0.1 mg/kg), administered by the same means served as a positive control. Animals were killed 11 days after implantation of the minipumps and mammary glands removed. Mammary gland growth was assessed by counting the number of mammary structures (terminal ducts (TDs), terminal end buds (TEBs) and lobules) and cells in 16 mm areas of the mammary gland.

In the mammary gland, the conversion of immature structures to mature structures was significantly increased with exposure to bisphenol-A. The average number of combined TDs and

TEBs was seen to decrease (controls 53%; low dose 38%; high dose 22%), and average number of lobules was seen to increase (47%, 62% and 78%) at both dose levels of bisphenol-A. The low- and high-dose bisphenol-A groups induced a 1.4- and 2.2-fold increase in cell numbers over controls, respectively. DES produced a 600% increase in cell numbers over controls.

However, there are concerns about the conduct of this study; Ashby and Odum (1998) draw attention to the fact that the same positive control (DES) data used in a 1996 study also appears in two other reports by Colerangle and Roy (1995 and 1997), and that the vehicle control data has also been duplicated. This raises uncertainties as to whether the control data were generated concurrently with the bisphenol-A data and raises questions about the validity of this study.

Summary of studies investigating endocrine modulating activity

Bisphenol-A has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with bisphenol-A glucuronide *in vitro.* The available data also indicate that there is a marked strain difference in the response to bisphenol-A in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories (Kanno et al., 2001). Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

4.1.2.9.2 Effects on fertility

One-generation studies

In a study that was well reported but not conducted to current regulatory guidelines (General Electric, 1976c), CD rats (10 per sex per group) were fed bisphenol-A in the diet for 10 weeks and then mated, 1 male to 1 female. The dietary levels used, 0, 1,000, 3,000 or 9,000 ppm corresponded to mean doses of approximately 0, 70, 200 and 650 mg/kg in males, and 0, 100, 300 and 950 mg/kg in females. Dosing was maintained throughout gestation and weaning of the F_1 generation. Following weaning, parental (F_0) animals were weighed, and then sacrificed and discarded.

At sacrifice, there was evidence of general toxicity in parental animals only at the top dose: a statistically significant reduction in body weight gain of 18% in males and 12% in females. Food consumption was not affected at any dose. No effects were observed on fertility index, litter size or post natal survival rate. At day 21 post partum, there was a statistically significant decrease in pup body weight gain relative to controls: 7% in the mid dose and 12% in the high dose (there was no effect at the low dose). No other parameters were investigated in the pups, which were used in a 90-day feeding study (see Effects on development section). The results of this one-generation study indicate that bisphenol-A did not produce any adverse effect on fertility up to dose levels at which parental toxicity was observed: 650 mg/kg in males and 950 mg/kg in females.

Using the same method, the study was repeated with 0, 100, 250, 500, 750 or 1,000 ppm bisphenol-A in the diet, which corresponded to mean dose levels of approximately 0, 5, 15, 30, 50 and 60 mg/kg in males, and 0, 10, 25, 50, 75 and 100 mg/kg in females, respectively (General Electric, 1978). Prior to mating, vaginal smears were taken for 21 consecutive days to determine the effect of bisphenol-A on the oestrous cycle.

At sacrifice, no effect was observed on body weight gain in the parental animals. No effects were observed on the oestrous cycle or on fertility index, litter size, post natal survival or pup body weight. Thus, in this additional one-generation study, in the absence of parental toxicity, no effect was observed on fertility or the offspring at the dose levels employed.

Two-generation studies

The effect of bisphenol-A on fertility was evaluated in an extensive oral two generation reproduction toxicity study in Crj;CD (SD) IGS rats (Chemical Compound Safety Research Institute, 2000). The F_0 generation consisted of groups of 25 rats per sex per group administered 0, 0.2, 2, 20 and 200 μg/kg/day bisphenol-A by gavage during a premating period of 10 weeks for males and 2 weeks for females and a 2-week mating period. Males and females from each group were randomly paired and co-habited for 2 weeks. Females were also administered the test material during gestation and lactation. F_0 males and females were sacrificed after the mating period and weaning of F_1 pups, respectively. Twenty-five male and female F_1 generation offspring from each group were retained after weaning for assessment of their reproductive capacity. F_1 animals were administered bisphenol-A for a 10-week premating period and a 3-week mating period (see below). Again, females received the test material during gestation and lactation, and male and female parental animals were sacrificed at the same times used for the F_0 generation. Twenty-five male and female F_2 generation offspring from each group were retained after weaning for assessment of sexual maturation. Males and females were administered the test material until they were sacrificed at the age of 7 and 14 weeks, respectively.

For all F_0 and all reared F_1 and F_2 animals, observations and weighings were performed regularly. In addition to determining reproductive capacity, various other parameters were assessed. Learning tests were conducted using a water filled multiple T-maze with 6 male and 6 female F_1 animals per dose group at 5-6 weeks of age. Several reflex assessments were determined in 1 rat per sex per litter until successfully completed. Sexual maturation (vaginal opening and preputial separation) was determined in F_1 and F_2 parent animals, along with anogenital distance (AGD). After sacrifice, all F_0 and F_1 parent animals were subjected to a thorough macroscopic and microscopic examination. In males, this included examination and weighing of the epididymis, prostate and seminal vesicles (including the coagulating gland). Serum testosterone, oestradiol, prolactin, LH, FSH, T3, T4 and TSH concentrations were also determined in 6 animals per sex per group from the F_0 and F_1 generations. Seminal vesicles and coagulating gland were weighed and subjected to histological examination. The motility and morphology of sperm in the epididymis was also determined in F_0 and F_1 males. All pups that were not selected for further assessment were sacrificed and also underwent histopathological examination.

In parental animals, no clinical signs of toxicity, nor any effects on body weight gain, food intake or treatment-related deaths were observed in any generation. No effect on behaviour (i.e. performance in learning tests) was observed in F_1 animals. Oestrus cycle, fertility index and the number of implantations in F_0 and F_1 females were not affected by treatment with bisphenol-A. (The mating period for F_1 animals was extended for a week, as at the end of the first week mating was confirmed in only 19/25 females administered 0.2 μg/kg/day, compared to 24/25,

22/25, 23/25 and 21/24 at 0, 2, 20 and 200 μg/kg/day respectively. At the end of the 3-week mating period no significant effect on the fertility index was observed between treated and control animals). No significant differences were observed between bisphenol-A and control animals for the time to preputial separation or vaginal opening. Compared to controls, a statistically significant decrease (≤5%) in AGD was seen in F_1 males at 0.2, 20 and 200 μg/kg/day, F_1 females at 20 and 200 μg/kg/day and F_2 males and females at 20 and 200 μg/kg/day. These decreases were not statistically significant when the ratio of the AGD to body weight was determined (the AGD is correlated with body weight). No changes in the motility and morphology of sperm were observed in F_0 and F_1 treated males. No treatment-related changes were observed in any of the serum hormone levels measured. Bisphenol-A had no effect on sexual maturation or the oestrus cycle in F_2 animals and F_2 females, respectively. At necropsy, no treatment-related macroscopic findings or organ weight changes were observed in F_0 and F_1 parental animals.

In the offspring (all live pups up to day 21), no clinical signs of toxicity or effects on body weight gain during lactation were observed in F_1 and F_2 pups. No treatment-related changes were seen in the litter size, survival, sex ratio, AGD and reflex ontogeny. At necropsy, no treatment-related macroscopic findings were observed in F_1 and F_2 pups. Compared to controls, a statistically significant decrease in the absolute (17%) and relative (20%) weight of seminal vesicles (including the coagulating gland) was observed in F_2 males only at 2 μg/kg/day. No other treatment-related changes in organ weight were observed in F_1 and F_2 pups.

The slight (≤5%) changes seen in AGD in F_1 and F_2 parental animals are not considered to be toxicologically significant as they are not statistically significant once correlated to body weight. The decrease in seminal vesicle weight at 2 μg/kg/day is also considered not to be toxicologically significant as no statistically significant decrease was observed at 20 or 200 μg/kg/day, histopathological examination revealed no morphological changes in the seminal vesicles and there were no weight changes in associated organs (prostate gland, testis and epididymis). Therefore, in this two-generation study, no parental toxicity or effect on fertility was observed at the low-dose levels employed.

Multigeneration studies

The effects of bisphenol-A on fertility and reproductive performance have been investigated in a comprehensive, good-quality multigeneration study (Tyl et al., 2000). The overall design of this study was based on the OECD two-generation reproduction toxicity study guideline, with additional dose groups and an extension to include the production of an F_3 generation. Groups of 30 male and 30 female Sprague Dawley rats were exposed to bisphenol-A in the diet at concentrations of 0, 0.15, 0.3, 4.5, 75, 750 or 7,500 ppm, which equated to approximately 0 (control), 0.001, 0.02, 0.3, 5, 50 or 500 mg/kg/day, respectively.

Exposure to bisphenol-A began for the F_0 generation at about 7 weeks of age and continued throughout a 10-week pre-breed exposure period, a 2-week mating period (when F_0 animals were mated [one male and one female] within each dose group) and gestation. F_0 males were sacrificed after the F_1 delivery period. Exposure of F_0 females to bisphenol-A continued throughout lactation until weaning (post-natal day 21) when F_0 animals were sacrificed. Selected F_1 animals were similarly mated to produce the F_2 generation and selected F_2 animals were mated to produce the F_3 generation. The same exposure regime was used for F_1 and F_2 animals with direct exposure to bisphenol-A in the diet commencing approximately at post-natal day 21. Selected F_3 animals were exposed to bisphenol-A only for a 10-week period from weaning as they were not mated. For the F_0 generation and retained F_1, F_2 and F_3 animals, clinical signs of

toxicity, body weights and food consumption were reported. Oestrous cycles were monitored in the last 3 weeks of the pre-breed exposure period and during the mating period for all generations. At the necropsy of adult animals, sperm samples were taken for analysis of epididymal sperm number, motility (using a computer assisted sperm motion analysis system) and morphology, testicular-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production, a number of organs were weighed and selected organs were examined histopathologically. Parameters assessed in the young offspring included litter size, body weights, survival, gross appearance, anogenital distance (AGD) (in F_2 and F_3 offspring only), sexual development and, for animals killed at weaning, gross appearance of organs at necropsy with attention given to reproductive organs.

There was evidence of general toxicity in adults of all generations at 500 mg/kg/day, seen as a statistically significant reduction in body weight gain ($\geq$13% in all generations). An increased incidence of renal tubular degeneration was also seen in F_0, F_1 and F_2 (but not F_3) females at 500 mg/kg. Chronic hepatic inflammation was also seen in both sexes and all generations. These aspects are described in greater detail in Section 4.1.2.6.1; the liver effects are not considered to be treatment-related.

Considering the reproduction-related parameters, there was no effect on mating. Compared to controls, a statistically significant decrease in the average number of live pups per litter was seen at 500 mg/kg/day in all generations on the day of birth (F_1: 11.5 compared to 14.3 for controls; F_2: 10.8 compared to 14.6; and F_3: 10.9 compared to 14.8). These decreases were observed with no effect on post implantation loss or the number of dead pups per litter. No further increase or decrease in the number of post-natal deaths between treated and control groups were seen on day 4. After standardisation of litter sizes on post-natal day 4 the number of pups per litter remained equivalent across all dose groups up to weaning. At necropsy, a statistically significant decrease in absolute (not relative) uterine weight in F_0, F_1 and F_2 parental animals (22-35%) was seen at 500 mg/kg/day. Statistically significant reductions in both absolute (16-34%) and relative (15-34%) paired ovarian weights were seen in F_0, F_1, F_2 and F_3 (absolute only) females at 500 mg/kg/day. A statistically significant increase in paired ovarian follicle count (43%) was seen in F_0 females only at 500 mg/kg/day. No treatment-related effects were observed in reproductive organs of adult animals. Compared to controls, the only changes in sperm endpoints were a statistically significant decrease in epididymal sperm count (18%) in the F_1 generation at 500 mg/kg/day and a statistically significant decrease in daily sperm production (19%) in the F_3 generation at 500 mg/kg/day (with no effect on efficiency of daily sperm production). The effects seen on ovarian follicle counts, epididymal sperm counts and sperm production at 500 mg/kg were not consistent (they were only observed in 1 generation) and are thus considered to be chance findings.

In the offspring, a statistically significant decrease in mean body weight per litter was seen at 500 mg/kg/day on post-natal days 7-21 in F_1, F_2 and F_3 males and females (12-27%) compared to controls. No treatment-related effect was seen on gestational indices, sex ratios, or nipple and/or areola retention in male pups. Compared to controls, statistically significant increases in AGD (which was only measured in F_2 and F_3 animals) were seen on the day of birth at 0.001 (by 3%), 0.02 (3%), 0.3 (3%) and 50 mg/kg/day (4%) in F_2 females only. At 500 mg/kg/day, a statistically significant delay in vaginal patency in females was observed in F_1 (day 33.0 compared to day 30.5 in controls), F_2 (day 34.5 compared to day 31.0) and F_3 (day 33.8 compared to day 31.3) animals. A statistically significant delay in preputial separation was seen in males in F_1 (day 45.8 compared to day 41.9 in controls), F_2 (day 47.9 compared to day 42.1) and F_3 (day 45.2 compared to day 42.1) animals. A statistically significant delay in preputial separation was also

seen at 50 mg/kg/day but only in F_1 males (day 43.6); given that this was seen in only one generation at this dose, it is considered not to be treatment-related, but to be a chance finding.

To summarise, an adverse effect on reproduction was observed in this study; a statistically significant decrease in the average number of live pups per litter at birth was seen in all generations at 500 mg/kg/day. Although this effect was only seen at a dose level at which there was also parental toxicity, it is not clear whether or not this finding could be a secondary consequence of this parental toxicity, or whether it represents a direct effect of bisphenol-A on fertility. A statistically significant decrease was also seen in adult ovarian weights and in mean pup body weights (males and females from postnatal day 7), along with a statistically significant delay in vaginal patency and preputial separation, in all generations at 500 mg/kg/day. However, acquisition of developmental landmarks is dependent on both age and weight (i.e. heavier animals acquire the landmark earlier and lighter animals later), and a statistically significant decrease in body weight gain was seen in males and females of all generations at 500 mg/kg. In addition, if these effects were related to the oestrogenic activity of bisphenol-A, a different pattern of results would have been expected. Thus, a chemical with oestrogenic activity would be expected to hasten the onset of vaginal patency in the offspring of exposed dams, whilst delaying preputial separation (Biegel et al., 1998). Thus, it is considered that the delays in vaginal patency and preputial separation are related to decreases in body weight, and not a direct developmental effect. In addition, all these effects on developmental landmarks were observed in the presence of parental systemic toxicity ($\geq$13% decrease in body weight gain in both sexes along with renal tubule degeneration in the kidneys of females) and are considered a secondary consequence of parental toxicity. The observed statistically significant increase in AGD was neither dose-related nor consistent across generations, being observed only in F_2 females at 0.001, 0.02, 0.3 and 50 mg/kg/day. The magnitude of the increases (0.03 to 0.04 mm) was minimal and no correlating effects of any kind were observed. Thus, the changes in AGD are not considered to be of toxicological significance.

Overall, this study showed 500 mg/kg bisphenol-A causes a reduction in the number of pups per litter. Although this finding was observed in the presence of some parental toxicity, it is not clear whether or not this finding could be a secondary consequence of this parental toxicity, or whether it represents a direct effect of bisphenol-A on fertility. The no effect level for parental toxicity is 50 mg/kg/day (see Section 4.1.2.6.1). Thus, the NOAEL for both parental and reproductive toxicity is 50 mg/kg/day in this well conducted multigeneration study.

Continuous breeding study

The effects of bisphenol-A on fertility and reproductive performance have been extensively studied in CD-1 mice using the test system known as the "Fertility Assessment by Continuous Breeding" (NTP, 1985b). This system involves four successive tasks. Task 1 is a preliminary 14-day toxicity study, conducted so that appropriate dose levels for the subsequent tasks can be selected. Task 2, the continuous breeding phase, involves a 14-week cohabiting phase during which reproductive performance is monitored. In Task 3, an optional "cross-over" mating trial is conducted; control males are mated with high-dose females and high-dose males are mated with control females. This is to determine whether any adverse effect seen in Task 2 is mediated through males or females. In Task 4, the reproductive offspring taken from the Task 2 final litters is assessed. The test substance is administered continuously through Tasks 2, 3 and 4 (except during the Task 3 mating phase).

Bisphenol-A was administered in the diet. Groups of twenty males and females (F_0 generation) were continuously exposed to the test substance at concentrations of 0, 0.25, 0.5 or 1.0% (using default values -see **Table 4.24**, Section 4.1.2.6.1- daily intakes of bisphenol-A are estimated to

have been 0, 300, 600 and 1,200 mg/kg in males, and 0, 325, 650 and 1,300 mg/kg in females) during a one-week premating period and a 14-week mating trial (Task 2). The dose levels were selected on the basis of a preliminary study (Task 1), in which statistically significant reductions in body weight gain (>10%) were seen at dietary bisphenol-A concentrations of 1.25% and above. The control group comprised of forty animals of each sex receiving the diet only. After the premating period, males and females from each group were randomly paired and allowed to cohabit for 14 weeks. During the cohabiting period the reproductive performance was monitored by counting the number of F_1 generation litters produced by each breeding pair and recording on the day of birth the litter size, proportion of live pups, litter size and sex ratio of the pups; all pups were then immediately removed and discarded. All litters produced after the cohabiting period remained with their mothers until weaning on day 21 post partum. The twenty F_0 males and twenty F_0 females from the top dose group (1.0% bisphenol-A) were then mated with twenty control females and twenty control males, respectively. Bisphenol-A was discontinued in the diet during this 7-day cohabitation period and then reinstated for 21 days upon separation of the breeding pairs. A control group of twenty untreated breeding pairs was also included (Task 3). The same reproductive assessment as described for the continuous breeding phase was conducted. Parental animals were sacrificed within 1 week of delivery. A maximum of twenty male and twenty female F_1 generation offspring (from the final litters of the control and high-dose groups in the continuous breeding phase) were retained after weaning for assessment of their reproductive capacity (Task 4). After rearing to sexual maturity, each F_1 female was paired with a F_1 male from the same dose group for 7 days. The resulting litters were evaluated and discarded on the day of birth as described for the litters produced during the F_0 generation cohabitation phase. For all control and high-dose F0 and all reared F_1 animals, liver, kidneys, adrenals and reproductive organs were weighed and subjected to histopathological examination. In males, sperm analysis (concentration, motility and morphology) was undertaken, and effects on the oestrous cycle assessed in females.

There were no clinical signs of toxicity among F_0 generation animals. In the continuous breeding phase, a statistically significant decrease in maternal body weight was observed after each litter (between 6 and 9%), at the top dose, on postnatal day 0 compared to controls. No effect was observed on maternal postnatal (day 0) body weight following the cross-over mating phase. However, at study termination, a small but statistically significant decrease in body weight (4%) was observed in treated females compared to controls. No adverse effects on body weight gain were observed in treated males. An adverse effect on fertility was observed in the continuous breeding experiment and cross-over mating experiment. In the continuous breeding phase, a statistically significant decrease compared to controls was observed in the number of litters produced per pair (4.5 and 4.7 compared to 5.0 for controls), litter size (6.5 and 9.8 compared to 12.2 for controls) and the number of live pups per litter (6.3 and 9.7 compared to 12.1 for controls) in the high and mid-dose group. The litter size reductions occurred across all matings and the magnitude of all these decreases were dose-related. No effects on fertility were observed in the low-dose group. A statistically significant decrease in litter size (controls: 11.4, treated males: 9.1, treated females: 5.9) and number of live pups per litter (controls: 11.3, treated males: 8.4, treated females: 5.5) were observed in the cross-over mating. In the continuous breeding phase, a statistically significant decrease in live pup weight (6%) on postnatal day 0 was observed in females at the top dose after adjustment for litter size, including live and still births. In the continuous breeding phase a small but statistically significant decrease in body weight gain (4%) was only observed in treated females at study termination. No effect was observed on the sex ratio in the F_1 generation.

In the F_1 litters used in the cross-over breeding experiment, post natal (day 0) pup weights were significantly increased in males (9-11%) and in females (8-10%) in the mid- and high-dose

groups compared to controls. These increases were no longer evident in either sex at 21 or 74 days of age. Deaths among F_1 generation were observed during lactation (day 0-21) and post weaning (day 21-74). At the top dose there were only 8 litters that had at least one male and one female for the mating phase, and therefore only 11 breeding pairs at the top dose compared to 19-20 breeding pairs in the control, low-dose and mid-dose groups. In those litters selected for mating deaths had been observed in 6%, 4%, 14% and 38% animals up to day 74 in the control, low-dose, mid-dose and high-dose groups, respectively. It is not known how many animals of this total died during lactation. However, it does raise the possibility that there may be potential effects on pups due to exposure to bisphenol-A via the milk. In the F_1 generation, bisphenol-A treatment had no effect on the fertility index, litter size, number of live pups per litter, sex ratio or mean pup weights at birth.

At necropsy of the F_0 generation (controls and top dose group only), treatment-related effects were seen at the highest dose level; for both sexes relative liver weight was increased about 28% and relative combined kidney/adrenal weight increased 10-16% compared to controls, and relative seminal vesicle weight and proportion of motile sperm were decreased 19% and 39% compared to controls, respectively. Histopathological changes were reported in the liver and kidney of Task 3 (F0) animals. Minimal multifocal necrosis of the liver was observed in 4/38 control males and 11/38 control females. Slight multifocal necrosis was observed in one control female. In treated animals, minimal multifocal necrosis was observed in 3/19 males and 3/19 females, slight necrosis in 9/19 males and 5/19 females and moderate necrosis in 3/19 males and 3/19 females. Multinucleated giant hepatocytes (slight to moderately severe/high) were observed only in treated animals: 11/19 males and 4/19 females. Centrilobular hepatomegaly in the liver was observed only in treated males; the severity was minimal, slight, moderate and moderately severe/high in 1/19, 7/19, 3/19 and 5/19 animals, respectively. Tubular cell 'nuclear variability' (slight to moderate) was observed in the kidney of treated animals only; 6/19 males and 12/19 females. Large microcalculi in the kidney were observed in females only; minimal in 1/38 controls and minimal, slight and moderate in 1/19, 4/19 and 2/19 treated animals, respectively. No histological change that was graded severe/high was seen in the liver or kidney of any animal. An amplification of spontaneous tubular and interstitial lesions normally observed in these mice was also observed in bisphenol-A treated animals. No histological changes were observed in male and female reproductive organs and no effect was observed on the oestrous cycle. Overall, the signs of general systemic toxicity were not marked in this study and therefore the effects on fertility are not considered to be a consequence of parental toxicity.

At necropsy of the F_1 generation, treatment-related effects of similar magnitude were generally observed in males and females; compared to controls, increased relative liver weights (6-29%) and kidney/adrenal weights (13-20%) were observed in all treated groups. In males, a statistically significant decrease in relative right epididymis weight (11%, 16% and 18%) was observed in all treated groups, compared to controls. Left testis/epididymis weights were significantly decreased by 10% at the mid dose and 9% at the high dose, and seminal vesicle weight was significantly decreased by 28% at the top dose. A statistically significant decrease in sperm motility in the mid-dose group only was not considered treatment-related, but a chance finding. Similar histopathological changes to those observed in the F_0 generation were also observed in males and females of the F_1 generation; the presence or increased incidence of multifocal necrosis and multinucleated giant hepatocytes in the liver and cortical tubular dilatation and tubular casts in the kidney at the low dose and above compared to controls. At the top dose, multifocal "mineralization" of hepatic cells was observed in females, along with microcalculi and "mineralization" of renal cells in both males and females. No histological changes were observed in the male and female reproductive organs.

In this study, adverse effects on fertility, namely a reduction in litter size and number of live pups per litter, were observed in each litter from the F_0 generation in the continuous breeding experiment at approximately 600 mg/kg and above, and at the only dose level tested in the crossover breeding experiment, approximately 1,200 mg/kg. A treatment-related decrease in the number of litters produced was also observed at 1,200 mg/kg during the continuous breeding phase. These effects were observed in the absence of significant parental toxicity. No effect on fertility was observed at 300 mg/kg, though no histopathology was conducted on these animals. In the F_1 generation at 300 mg/kg the only effect observed was a statistically significant decrease in epididymis weight of 11%. Histological examination was conducted on all F_1 animals, and the only effects observed were toxicity to the liver and kidney at all doses. No adverse effect on fertility was observed in the F_1 generation up to approximately 1,200 mg/kg, which might have been expected in view of the observed effects on fertility in the F_0 generation. Nevertheless, the absence of effects following the single F_1 mating does not detract from the reproducible results across the 4-5 litters produced by each F_0 generation pair. Therefore, overall, an adverse effect on fertility has been observed with bisphenol-A at approximately 600mg/kg and above. At 300 mg/kg no adverse effects on fertility were observed, though a decrease was seen in F_1 epididymis weight. This effect is considered treatment-related as the magnitude of the decrease was dose-related. Although this was the only effect observed on reproductive organs at 300 mg/kg, the health significance of this finding is not clear. Therefore, taking a cautious approach a no effect level could not be identified in this study due to the epididymis weight change observed at 300 mg/kg.

In a study which was briefly reported (Bolon et al., 1997), the results being presented in summary form only, the effect of bisphenol-A treatment on ovarian follicle count was determined in stored ovaries of the CD-1 mice from the NTP (1985b) continuous breeding study presented above. One ovary from each of 10 females per dose group was examined in F_0 mice from Task 3 and F_1 mice from Task 4 (see above). Each ovary was embedded in a paraffin block, sliced to produce approximately 400 sections and every 10^{th} section was stained and the number of "small", "growing" and "antral" follicles determined. No significant differences were observed in the mean number of small, growing or antral follicles in ovaries from F_0 females administered 1,300 mg/kg bisphenol-A (Task 3), and F_1 females administered 325, 650 and 1,300 mg/kg (Task 4), compared to controls. Thus, at dose levels up to 1,300 mg/kg bisphenol-A did not affect ovarian follicle count.

Continuous breeding study

The effects of bisphenol-A on fertility and reproductive performance in CD-1 mice when administered by subcutaneous implant were also investigated in the 'Fertility Assessment by Continuous Breeding' test system (NTP, 1984). The protocol for this 4-task study was identical to that described previously (see above).

Bisphenol-A was administered via subcutaneous implant. Groups of twenty males and females (F0 generation) were continuously exposed to the test substance at concentrations of 0, 25, 50 or 100 mg during a one-week premating period and 17-week mating trial (Task 2). Dose levels could not be varied with body weight and due to difficulties experienced in preparing accurate bisphenol-A dose levels and release from the implants, implant doses of 0, 25, 50 or 100 mg gave a total release of approximately 0, 10, 20 and 40 mg bisphenol-A over 18 weeks, respectively. The dose levels were selected on the basis of a preliminary study (Task 1), in which an increase in mean reproductive tract weight in females was observed from 6.25 mg (corresponding to a total release of 1.6 mg bisphenol-A over 2 weeks). The control group comprised of forty animals of each sex. Task 3 and Task 4 were not conducted as the study was

terminated on the completion of Task 2 due to the technical problems experienced with the subcutaneous implants. All control and high-dose animals were sacrificed at the end of the continuous breeding experiment and liver, brain, pituitary and reproductive organs were weighed. No detailed histopathological examination was conducted.

There were no clinical signs of toxicity or adverse effects on body weight gain in parental animals. However, several animals in each treatment group expelled their implants through cutaneous lesions that developed directly over the implants or at the site of incision. When this occurred, animals were re-implanted with the original dose; several animals received new implants on three different occasions. No effect was observed on the fertility index, number of live pups per litter, sex ratio and the group mean live pup weights. At necropsy, no effect was observed on parental organ weights.

Although technical problems with the method of administration were encountered, no effects were observed on fertility. However, no parental toxicity was observed and no histological examination was conducted, limiting the value of this study.

Other related studies

The effect of bisphenol-A on preimplantation was investigated in mouse embryos (Takai et al., 2001). Superovulation was induced in female B6C3F1 mice, which were then mated with males of the same strain. Two-cell embryos were obtained 40 hours after induction of superovulation by flushing the oviducts. Embryos were then cultured for 48 hours in 0, 1nM or 100 μM bisphenol-A. Seven embryos, now blastocytes, were transferred to each uterine horn of recipient ICR mice on day 3 of pseudopregnancy, which was induced by mating ovarian hyperstimulated females with vasectomised ICR males in a parallel procedure. The presence of a vaginal plug was used to identify day 1 of pseudopregnancy. Pups (randomly culled to six per litter where appropriate) were delivered and weaned by the recipient mother. Pups were weighed on postnatal day 21.

The rate of *in vitro* development of two-cell embryos to blastocytes was 62.1% (272/438) in control cultures after 48 hours. Compared to controls, a statistically significant increase in development was seen at 1 nM bisphenol-A (16%) with a decrease at 100 μM (46%). A statistically significant increase in degenerated embryos (not quantified) was also seen at 100 μM. Blastocytes that developed in the presence of bisphenol-A appeared to be morphologically normal, and no significant difference was seen in the number of cells per blastocyst. Compared to controls, no significant effect was seen on the number of pups per litter, sex ratio or pup body weight at birth, following pre-implantation treatment with bisphenol-A. A statistically significant increase in pup body weight was observed on post-natal day 21 at 1 nM (39%) and 100 μM (34%).

Overall, in view of the nature of the exposure in this study, which is not relevant to humans, it does not add significantly to the overall database and no conclusions can be drawn from it.

In a poorly reported sperm abnormality study, which was available in abstract form only, male C_3H/He mice received 5 daily intraperitoneal injections of 0 or 85 mg/kg bisphenol-A (Bond et al., 1980). It was reported that no morphological changes were detected between the sperm of treated and control animals. No further details are available.

4.1.2.9.3 Effects on development

Rats

Bisphenol-A has been tested for developmental toxicity in a NTP (1985c) study. In the dose-finding study, groups of 8 time-mated CD rats were gavaged with 0, 500, 1,000, 1,500, 2,000, 2,500 or 3,000 mg/kg bisphenol-A in corn oil on days 6 to 15 of gestation. Animals were sacrificed on day 20 of gestation and the foetuses were examined for gross morphological abnormalities only.

Deaths were observed in 1, 5, 3 and 5 rats at 1,500, 2,000, 2,500 and 3,000 mg/kg, respectively. Clinical signs of toxicity were observed at all doses and included diarrhoea, lacrimation, lethargy, rough coat, wheezing, wet urogenital area, arched back, dyspnea, bloody nose, disorientation, alopecia, piloerection and vaginal bleeding. Compared to controls, a statistically significant decrease in maternal body weight gain ($\geq$24%) was observed at 1,000 mg/kg and above during the gestation period, and during the treatment period a decrease in maternal body weight was observed at 1,500 mg/kg and above.

In this dose-finding study, bisphenol-A exhibited developmental toxicity (resorptions) only at doses that produced severe maternal toxicity. From the results of this study, dose levels in the range 0-1,280 mg/kg bisphenol-A were used in the main study.

In the main study, two tests were performed and the data from both tests combined (NTP, 1985c; Morrissey et al., 1987). Thus in total, groups of 27-29 time-mated CD rats were gavaged with 0, 160, 320, 640 or 1,280 mg/kg bisphenol-A in corn oil on days 6 to 15 of gestation. Animals were sacrificed on day 20 of gestation and the foetuses were subjected to routine external, visceral and skeletal examination.

No deaths were observed in the 0, 160, 320 and 640 mg/kg dose groups. At 1,280 mg/kg, deaths were observed in 7/27 females and because of this high mortality rate the top dose group was not included in statistical analysis. Outward signs of toxicity observed in bisphenol-A treated animals included rough coat, piloerection, alopecia, pica, excess salivation, wet urogenital area, dyspnea, chromodacryorrhea, tremors, red-stained coat, wheezing and vocalisation. Compared to controls, a statistically significant decrease in mean maternal body weight gain was observed in dams at all dose levels for the treatment period (35-54%) and the gestation period (11-14%). No effect was observed on gravid uterine weights. When maternal body weight gain was corrected for gravid uterine weight a statistically significant decrease was still apparent at all dose levels (26-34%).

At necropsy, no effect was observed on mean relative liver weight in dams. Pregnancy rates were not affected by treatment with bisphenol-A, nor was there any effect on the number of implantation sites per litter, % resorptions per litter, number of live foetuses per litter, sex ratio, mean fetal body weight per litter, % foetuses malformed per litter and % litters with malformed foetuses. In conclusion, this study provides no evidence of developmental toxicity in the rat at exposure levels which are toxic to the mother. A maternal no effect level could not be identified; instead a LOAEL of 160 mg/kg was identified for clinical signs of toxicity and a statistically significant decrease (26%) in body weight gain. No foetal effects were seen at the highest dose level evaluated, 640 mg/kg.

A developmental toxicity study using the i.p. route of administration is available (Hardin et al., 1981). Groups of 12, 4 and 12 mated female Sprague Dawley rats were given daily i.p. injections of 0, 85 or 125 mg/kg bisphenol-A in corn oil on days 1 to 15 of gestation. Dams were sacrificed on day 21 and the foetuses were subjected to routine external, visceral and skeletal examinations.

No reliable information on maternal toxicity was provided; only 3/12 females mated at 125 mg/kg were found to be pregnant but no further details are given to explain this result. In maternal tissues at necropsy, minor signs of toxicity to the lungs and peritonitis were observed at 125 mg/kg only. Compared to controls, a statistically significant decrease in the number of live foetuses per litter, foetal body weights and crown-rump lengths was observed at 85 and 125 mg/kg. Significant increases were observed in the number of litters with incomplete ossification, reduced sternebrae and enlarged cerebral ventricles at 85 and 125 mg/kg compared to controls. However, no conclusions can be drawn from this study on the developmental toxicity of bisphenol-A, as in addition to the limited detail available, there are design weaknesses in this study; the small number of litters available in the treated groups (4 at 85 mg/kg and 3 at 125 mg/kg) and the i.p. route of administration, which could result in unrealistically high exposure of the reproductive organs, is of questionable relevance to human exposure by the inhalation, dermal and oral routes.

In a poorly reported study available in abstract form only, pregnant female rats received 1 mg/l bisphenol-A in the drinking water throughout gestation and up to weaning of the F_1 offspring (Sharpe et al., 1996). Assuming that a female rat drinks 20 ml water a day and weighs 175 g, a dose of 1 mg/l corresponds to approximately 0.114 mg/kg. The focus of the study was to study the effects of oestrogenic substances on the development of the male reproductive organs; consequently observations were restricted mainly to the male reproductive system of the offspring. Exposure to bisphenol-A was reported to produce "a highly significant decrease in testis size (5-15%) and daily sperm production in males in adult life", although testicular morphology was normal, but no data were presented in support of these findings. There are also some design weaknesses in this study: the group sizes were not reported and the type of statistical analysis used is not known. This study was recently repeated (see below) to determine whether the results reported were reproducible.

In a well designed and reported study which was based on the protocol of the study described by Sharpe et al. (1996), groups of 28 female Han Wistar rats were administered 0, 0.01, 0.1, 1.0 or 10 ppm bisphenol-A in the drinking water, continuously for 10 weeks (Cagen et al., 1999a). The study contained two control groups to determine whether there was any significant variation in control values for the endpoints measured. Females were dosed throughout a 2-week pre-mating period. Females were then placed with untreated males. During a 2-week mating period, both sexes were exposed to bisphenol-A in the drinking water. Dosing of the females was maintained throughout gestation and lactation of the F_1 offspring. An additional dose group of 28 females served as a positive control, receiving 0.1 ppm of DES in the drinking water for the same period. Following weaning (22 days post partum), a maximum of 4 males per litter were maintained untreated until 90 days of age when they were sacrificed. F_0 females, F_1 females and surplus F_1 males were sacrificed 22 days post partum and subjected to a thorough macroscopic and microscopic examination. In 90-day-old F_1 males, as well as macroscopic and microscopic examination, the daily sperm production and epididymal sperm count were also determined.

Combining water consumption values in females during the premating, gestation and lactation periods, levels of 0, 0.01, 0.1, 1.0 or 10 ppm bisphenol-A in the water corresponded to mean doses of approximately 0, 0.002, 0.02, 0.2 and 1.8 mg/kg/day, respectively. No significant differences were observed between the two control groups, so they were pooled for analysis purposes. No treatment-related effects on body weight gain, food consumption, water consumption, or fertility were observed in F_0 females. In F_1 males, no treatment-related effects were observed on body weight gain, testes, prostate or preputial gland weight, testes histopathology, sperm count, or daily sperm production. In the positive control group, a slight decrease in water and food consumption was observed in F_0 females throughout most of the

exposure period. A statistically significant decrease in body weight gain during gestation, an increase in the duration of gestation, and a decrease in litter size on days 0, 1 and 4 of lactation were observed in the positive control group. A slight decrease in sperm production and cauda epididymal sperm concentration was also observed in F_1 males in the positive control group. In conclusion, the effects of bisphenol-A previously reported by Sharpe et al. (1996) at 1.0 ppm were not reproduced in this study, and no effects were observed at the additional dose levels of 0.01, 0.1 and 10 ppm.

Since the Sharpe et al. (1996) study, this group has reported a temporal downwards shift in the normal range of testes weights in control animals in their laboratory (Sharpe et al., 1998). Sharpe et al. (1998) state that although the decrease in testis weight in controls over time is unexplained it followed a permanent change in water supply to the animal facility. While Sharpe remains confident in the validity of the original study he does state that "we now consider that biological factors, of which we are unaware and for which we have not controlled, have the potential to exert developmental effects on testis weight which are at least as great as the maximum effect that can be induced by the addition of a potent oestrogen (DES) to the mother's drinking water during pregnancy and lactation." On this basis, the inability of Cagen et al. (1999a) to reproduce the Sharpe et al. (1996) result suggests that the effect seen on testis weight in Sharpe et al. (1996) may not have been due to an effect of bisphenol-A but to uncontrolled factors.

In a well reported study (Kwon et al., 2000), the effects of bisphenol-A on pubertal development and reproductive functions were investigated. Groups of 8 time-mated Sprague Dawley rats were administered 0, 3.2, 32 or 320 mg/kg bisphenol-A in corn oil by gavage from day 11 of gestation to postnatal day 20. An additional dose group of 8 animals served as a positive control and received 15 μg/kg DES for the same period. Dams were sacrificed when pups were weaned (post-natal day 21) and selected organ weights determined. F_1 males were killed on post-natal day 180 and reproductive organ weights were determined (testes, epididymides, ventral and dorsolateral lobes of the prostate and seminal vesicles). For F_1 females, 1-3 pups/litter were sacrificed on post-natal day 10, the brains removed and the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) determined. The volume of the SDN-POA was determined as it has been reported by Nagao et al. (1999) that sexual behaviour has been closely associated with the size of the SDN-POA; reductions in volume cause loss in sexual behaviour. Pubertal development (day of vaginal opening and first ovulation) was determined in remaining F_1 females, along with oestrous cyclicity from approximately 4 months of age. At approximately 6 months of age the lordosis (curvature of the lumbar and cervical spine) behaviour was evaluated in 1-2 F_1 females/litter. Remaining F_1 females were sacrificed on post-natal day 180 and ovaries and uteri examined by light microscopy along with ventral prostates in males.

In treated dams, no effect was observed on the number of live pups per litter or body weight during pregnancy, lactation or at termination compared to controls. In F_1 females, no effect was observed on body weight, the volume of the SDN-POA, pubertal development, oestrous cyclicity or lordosis behaviour. In F_1 males, no effect was seen on body or reproductive organ weights. No treatment-related effects were observed in F_1 animals of either sex on microscopic examination. Compared to controls, the only effects observed with DES were a statistically significant increase in liver weight (17%) in dams at termination, a statistically significant increase in SDN-POA volume in F_1 females (approximately 39%), and irregular oestrous cyclicity in F_1 females. Therefore, no adverse effects on pubertal development or reproductive function were observed in this study at dose levels of 3.2, 32 or 320 mg/kg bisphenol-A.

In a poorly reported study, which is available in abstract form only, pregnant Sprague Dawley rats were administered 0, 0.005, 0.05, 0.5, 5 or 50 mg/l bisphenol-A in the drinking water from day 2 of gestation until pups were 21 days old (Gould et al., 1998a; Liaw et al., 1998). Assuming

that a female rat drinks 20 ml water a day and weighs 0.175 kg, daily intakes of bisphenol-A are estimated to have been 0, 0.0006, 0.006, 0.06, 0.6 and 6 mg/kg. DES was administered as a positive control at 0.05 mg/l. Dam body weights, organ weights and uterine implant sites were examined post natally on day 21. Female offspring were examined for adverse effects on puberty, oestrous cycle pattern, and hypothalamo-hypophyseal regulation of luteinizing hormone. The only effect reported in dams was an increase in relative kidney weight of unstated magnitude in females at the top dose. No effect was observed on litter size or sex ratio. In female offspring, no effect was observed on age or body weight at vaginal opening, age of first ovulation or subsequent oestrous cyclicity. For DES a decrease in body weight gain, relative ovary weight, food consumption, weight gain during gestation, and serum luteinizing hormone concentrations were observed in dams. In the offspring, vaginal opening occurred significantly earlier and at a lighter body weight. No effect was observed on ovulation. No further details are available. In conclusion, bisphenol-A did not exhibit developmental toxicity in this low-dose study.

In a well reported dietary study (General Electric, 1976c), pairs of mating F_0 CD rats were fed bisphenol-A in the diet at 0, 1,000, 3,000 and 9,000 ppm throughout their mating period and up to day 21 post partum. The F_1 animals (15 per sex per dose) were then used in a 90-day feeding study and fed bisphenol-A in the diet at identical parental dose levels. Bisphenol-A in the diet at 0, 1,000, 3,000 and 9,000 ppm, corresponded to mean dose levels of 0, 68, 206 and 671 mg/kg in F_1 males and 0, 75, 228 and 699 mg/kg in F_1 females. Routine haematology, biochemical and urinalysis were performed monthly. Following necropsy, microscopic examination was performed on animals in the top dose group only. Prior to commencement of the 90-day study, day 21 post partum, statistically significant decreases in F_1 body weight gain of 7 and 12% were observed relative to controls, at 3,000 and 9,000 ppm, respectively. No effect on body weight gain was observed at 1,000 ppm. At the end of the 90-day study, statistically significant reductions in mean body weight of 11% and 24% in males, and 17% and 22% in females, were observed in the mid- and high-dose groups, respectively, relative to controls. Food consumption was reduced by 14% in males at the top dose and $\geq$12% in females from the mid-dose and high-dose in comparison to controls. No treatment-related changes were observed at necropsy or following microscopic examination, in males or females at the top dose. In conclusion, there were no effects at approximately 70 mg/kg in this study. At higher doses the only effect observed was an adverse effect in body weight gain, but it is not clear if this decrease is the result of palatability or a toxic effect.

Using the same protocol, the study was repeated with 0, 100, 250, 750 and 1,000 ppm bisphenol-A in the diet (General Electric, 1978). This resulted in mean doses of 0, 7, 17, 34, 51 and 70 mg/kg in males, and 0, 8, 20, 39, 60 and 82 mg/kg in females. No treatment-related effects were observed in body weight gain or food consumption. At necropsy, no treatment-related changes were observed. In conclusion, there were no adverse effects in animals at about 70 mg/kg.

In a drinking water study (Rubin et al., 2001), groups of 6 female Sprague Dawley dams received 0, 0.1 or 1.2 mg/kg bisphenol-A from day 6 of pregnancy to weaning. The offspring were sacrificed at various time points and “genital tracts” examined microscopically. The authors state that at all time points, offspring were selected from as many different litters as possible from each group. Male offspring were sacrificed at 3 (no. of pups = 12) and 5 months (no. of pups = 18) of age, and female offspring at 8 (no. of pups = 12) and 12-16 months (no. of pups = 34). Effects on anogenital distance were also determined in animals sacrificed during the neonatal period (12 males and 12 females). In female pups, vaginal cytology was also determined for 18 consecutive days at 4 and 6 months of age (in 23, 18 and 28 offspring from the

0, 0.1 and 1.2 mg/kg groups, respectively) and the day of vaginal opening recorded. In addition, 8 female offspring from each dose group were ovariectomised and killed 3 months later, at which time luteinizing hormone (LH) levels were determined.

A statistically significant increase in pup body weight gain was observed for combined male and female weights at 0.1 and 1.2 mg/kg on postnatal days 4, 7, 11 and 22, compared to controls. Animals were not weighed separately by sex on these days. On days 11 and 22, animals exposed to 0.1 mg/kg were heavier than those exposed to 1.2 mg/kg. Statistically significant increases in body weight gain were observed in females only, from day 28. On days 87 and 110, the increase in bodyweight gain in females at 0.1 mg/kg was statistically significant when compared to females at 0 and 1.2 mg/kg. These increases in bodyweight gain were not quantified.

Bisphenol-A did not affect the mean number of pups per litter, sex ratio, day of vaginal opening or anogenital distance. No macroscopic abnormalities were observed in genital tract tissues at any time during the study. Vaginal cytology demonstrated effects on the oestrus cycle in the offspring of animals exposed to 1.2 mg/kg; only 21% and 23% of these animals exhibited regular oestrus cycles at 4 and 6 months, respectively. The authors report that the pattern of non-regular oestrous cyclicity varied in individual females and was not easily defined. No effect on oestrus cycle was seen in the offspring of animals exposed to 0.1 mg/kg, compared with controls. In ovariectomised animals, a statistically significant decrease in LH levels (19%) compared to controls was observed only at 1.2 mg/kg.

In conclusion, bisphenol-A treatment produced an increase in pup body weight gain at 0.1 and 1.2 mg/kg. However, this increase was not dose-related and when sexes where examined separately, was statistically significant only in females. At 1.2 mg/kg, an adverse effect on the oestrus cycle was observed, although no distinct pattern in disruption was observed. A statistically significant decrease in LH levels in ovariectomised females was also seen at 1.2 mg/kg.

In a series of experiments investigating how sampling strategy can influence the outcome of studies investigating endocrine activity (Elswick et al., 2001a), time mated Sprague Dawley rats were administered bisphenol-A in the drinking water at 0, 0.005, 0.05, 0.5, 5 or 50 mg/l from day 2 of gestation to the end of weaning (post-natal day 21). The authors estimated daily intakes of bisphenol-A to have been approximately 0, 0.001, 0.01, 0.1, 1 or 10 mg/kg/day. This study was conducted in two blocks of animals separated by 4 months and resulted in 13-16 pregnant dams per dose group. Two males per litter from the initial block of animals, and 1 male per litter from the subsequent block of animals, were retained until 6 months of age. These males were then sacrificed and weights of organs of the reproductive tract were measured, including ventral prostate weight. A histopathological examination was also performed on ventral prostates.

In the initial block, no treatment-related effects were observed on ventral prostate weights. In the subsequent block, compared to concurrent controls, statistically significant increases of approximately 19, 15, and 8% at 0.01, 1 and 10 mg/kg/day bisphenol-A were observed. It is reported that these statistically significant increases remained when the data for both blocks were combined (data not shown). No treatment-related microscopic findings were observed in either block at necropsy. Although an increase in ventral prostate weight was observed in the second block, this was based on only 1 pup per litter and was not dose-related. Furthermore, the authors point out that the control mean prostate weight was considerably lower in the second block (0.387 g) compared to the first (0.517 g), and the standard error of the mean was approximately twice that of the first block (0.174 g compared to 0.092 g). These concerns over the different control mean values and large variability remained when the data for the two blocks were combined. The authors also point out that ventral prostate weight was not correlated with

terminal body weight. A historical control base for control ventral prostate weights was not available for the laboratory. No information was provided on other organ weights of the reproductive tract. The authors felt that the large intra-litter variability of ventral prostate weight affects the ability to interpret the results. Overall, this investigation of sampling strategy suggests that the number of pups sampled may influence the outcome of results. In view of this, and given the concerns raised by the authors in relation to the intra-litter variability of prostate weights, no reliable conclusions can be drawn from this study in relation to the effect of bisphenol-A.

In a dietary study, available as an abstract only (Fritz and Lamartiniere, 1999), the effect of bisphenol-A on the male reproductive tract was investigated. Male Sprague Dawley rats were exposed to 0, 2.2 and 23.1 μg/kg/day bisphenol-A from "conception" to day 70 post partum. The authors report that "lifetime" exposure to bisphenol-A resulted in significantly reduced body weights from days 1-70 postpartum. Compared to controls, no significant effect was seen on the sex ratio, anogenital distance (in both sexes), age of testes descent, dorsolateral and ventral prostates and testes weights in bisphenol-A-treated animals. It was reported that a dose-related increase in seminal vesicles and epididymis weight was seen in bisphenol-A-treated animals. A dose-related decrease in sperm density and motility was reported with bisphenol-A, though this decrease was not significant. A significant increase in androgen receptor protein levels was reported at 23.1 μg/kg/day bisphenol-A only. No further information is available. Due to the limited information provided no reliable conclusions can be drawn from this study.

In a study reported as an abstract only (Piersma et al., 1998), several chemicals including bisphenol-A were tested in a reproduction/developmental toxicity screening assay (OECD Guideline 421). In addition to the test guideline protocol, reproductive hormones were assessed and histological examinations performed on the reproductive organs of parents and pups. The authors report that all the tested chemicals showed one or several reproductive toxic effects; infertility, superovulation, preimplantation loss, resorptions, phenotypic feminization, reduced pup weight or testicular pathology. No further details are available. The limited details from this poorly reported study mean no reliable conclusions can be drawn from this data in relation to bisphenol-A.

A postnatal developmental study using the s.c route of administration is available (Nagao et al., 1999). Groups of 30-31 male and female Sprague Dawley pups were given s.c. injections of 0 or 300 mg/kg bisphenol-A in corn oil daily on postnatal days 1 to 5. On postnatal day 21, 5 male and 5 female control and treated pups were sacrificed and histopathological examination of the testes, epididymides, prostates, seminal vesicles, ovaries and uteri undertaken. At 12 weeks of age, males and females (number not reported) were mated with untreated animals to evaluate their reproductive function. Sperm-positive females were sacrificed on day 13 of gestation and the number of implants and live and dead embryos determined. Females that were not sperm-positive after 14 days cohabitation with males were sacrificed approximately 6 days later. Masculine sexual behaviour was evaluated in the treated males; animals were housed with an ovariectomised female (brought into sexual activity by treatment with estradiol benzoate) and the number of mounts, intromissions and ejaculations, and latency to first mount, intromission and ejaculation recorded over a one hour period. Five control, and five treated, males and females were then sacrificed and histopathological examination of the testes, epididymides, prostates, seminal vesicles, ovaries and uteri undertaken. A further five control males and five treated males were sacrificed, a histopathological examination of the brain conducted and the region of the SDN-POA identified and its volume calculated.

No clinical signs of toxicity were observed in the treated pups. All male and female rats treated with bisphenol-A showed normal reproductive function and the day of preputial separation and testicular descent in males, and day of vaginal opening in females, was comparable to controls.

There was no effect on masculine sexual behaviour, nor were histopathologic changes seen in the reproductive organs of treated animals. Bisphenol-A did not affect the volume of the SDN-POA. Therefore, no effects on postnatal development were observed with bisphenol-A in this study.

A study available as an abstract only, also examined the effect of bisphenol-A on the SDN-POA (Laessig et al., 1999). Pregnant Sprague Dawley rats (number not reported) were given a single i.p. injection of 0 or 5 mg/kg bisphenol-A on day 16 of gestation. Offspring were weighed and the anogenital distance measured on the day of birth and post natal day 6. Animals were sacrificed on day 6 and the volume of the SDN-POA determined. DES (5μg/kg) was also tested in this study. Bisphenol-A increased body weight in males and females and also anogenital distance in males on the day of birth and post natal day 6. Bisphenol-A did not affect the volume of the SDN-POA. The authors report that the positive control DES increased body weight in males and females, anogenital distance in males and had "oestrogenic-like" effects on the SDN-POA in females. No further details are available.

In a well reported postnatal study (Fisher et al., 1999), groups of 48 and 25 male Wistar rats were given daily s.c injections of 0 or approximately 37 mg/kg bisphenol-A respectively from 2 to 12 days old. Three to 7 test animals and 5-20 control animals were sacrificed at 10, 18, 25, 35 and 75 days of age and the testes and epididymides removed and dissected to leave the efferent ducts attached to the testes. The rete testis and efferent ducts were fixed for histological examination. Tissues from animals up to 25 days old were fixed in Bouin's fixative and perfusion fixation was used in tissues from 35- and 75-day-old animals. The authors report that Bouin's fixative causes negligible shrinkage and so data for the two fixative techniques can be compared directly. The shape and height of epithelial cells in the efferent duct was determined using image analysis. Immunoexpression of the water channel aquaporin-1 (AQP-1) was also determined in the efferent ducts using an antibody to AQP-1. The effects of 0.37, 0.037 and 0.0037 mg/kg DES, as a positive control were also investigated in this study, with animals receiving s.c injections on post natal days 2, 4, 6, 8, 10 and 12.

No effect on testes weight was observed with bisphenol-A on days 18 and 25 (results for day 10 are not reported). Although a slight but statistically significant decrease compared to controls was observed on day 35, no effect was seen on day 75. It is probable that the result at 35 days was a chance finding, not related to bisphenol-A treatment. Rete testis morphology was unaffected by bisphenol-A treatment. The level of AQP-1 immunostaining in the efferent ducts of bisphenol-A treated animals was comparable to controls from day 10-75. At days 18 and 25, a significant decrease in efferent duct epithelial cell height was observed with bisphenol-A. However, as no effect on cell height was observed after day 25 these decreases are not considered to be biologically significant. DES produced statistically significant reductions in testes weight at 0.0037 mg/kg and above, and produced effects in all parameters measured. Overall, no adverse effect on the testes or efferent duct was observed with bisphenol-A in this study.

A further postnatal developmental study is available (Saunders et al., 1997). In this well reported study, groups of 12 and 8 male Wistar rats were given s.c. injections of 0 or 0.5 mg bisphenol-A in corn oil on postnatal days 2, 4, 6, 8, 10 and 12. Animals were sacrificed on day 18 and the testes and pituitary removed. Immunohistochemistry was performed on the testes and pituitary to determine if there was any effect on oestrogen responsive gene expression; the FSHβ subunit was measured in pituitaries and the α subunit for inhibin was measured in testes. The diameter of seminiferous tubules was also determined using image analysis. Using the above test protocol, a positive control group of 12 animals was administered 10 μg DES in corn oil. The body weight of animals was determined on day 18, and using this data the dose of bisphenol-A was determined to be approximately 13 mg/kg.

No effect on body weight gain, testes weight or seminiferous tubule diameter was observed in bisphenol-A-treated animals. The authors report that in bisphenol-A-treated animals the intensity of immunopositive staining for FSHβ subunits in pituitaries and α subunit for inhibin in testes was indistinguishable from controls. Compared to controls, DES produced a statistically significant decrease in testis weight and seminiferous tubule diameter, and the intensity of the staining in the testes and pituitaries appeared to be reduced. Therefore, no effects on the testes, pituitaries or seminiferous tubule were observed with bisphenol-A in this postnatal study.

Mice

Bisphenol-A has been tested for developmental toxicity in a NTP study using CD-1 mice (NTP, 1985a). Two tests were performed and as the same signs of maternal toxicity were observed in both tests the data were combined. Overall, groups of 29-34 time-mated female mice were dosed orally by gavage with 0, 500, 750, 1,000 or 1,250 mg/kg bisphenol-A in corn oil on days 6 to 15 of gestation. Animals were sacrificed on day 17 of gestation and the foetuses were subjected to routine external, visceral and skeletal examinations. Data are also provided on the additional dose level of 250 mg/kg, which was used only in the first test.

Maternal deaths were observed in 0/29, 0/13, 2/28, 1/28, 2/32 and 6/33 females at 0, 250, 500, 750, 1,000 and 1,250 mg/kg, respectively. Rough coats were observed in animals at 0 and 250 mg/kg during the treatment period. In addition, piloerection, alopecia, dyspnea, arched back, vocalisation, lethargy, wheezing or vaginal bleeding were also observed in animals at 500, 750, 1,000 or 1,250 mg/kg. A decrease in pregnancy rates was observed; 90% (26/29), 92% (12/13), 88% (23/26), 78% (21/27), 77% (23/30) and 78% (21/27) at 0, 250, 500, 750, 1,000 and 1,250 mg/kg, respectively. As this reduction in pregnancy rates was not dose-related, it is considered not to be a treatment-related effect. Compared to controls, a decrease in maternal body weight gain of 4-10% and 32-43%, for both the treatment and gestation period, was observed at 1,000 and 1,250 mg/kg, respectively.

At necropsy, a dose-related statistically significant increase in mean relative liver weight (9-26%) was observed in dams in all bisphenol-A treatment groups compared to controls. At 1,250 mg/kg a statistically significant increase was observed in % resorptions per litter (40% compared to 14% in controls). A dose-related decrease in mean fetal body weight per litter was observed in the bisphenol-A treated groups that was statistically significant at 1,250 mg/kg when compared to the control value; 1%, 1%, 9% and 14% at 500, 750, 1,000 and 1,250 mg/kg, respectively. No statistically significant effect was observed on the number of implantation sites per dam, the number of live fetuses per litter and the sex ratio. Bisphenol-A administration had no significant effect on the % of fetuses malformed per litter or the % of litters with malformations. Overall, a significant increase in resorptions and decrease in fetal body weight was observed only at 1,250 mg/kg in the presence of severe maternal toxicity.

In the study reported by Nagel et al. (1997) and vom Saal et al. (1998), groups of 6, 7 and 7 pregnant CF-1 mice were given by gavage 0, 2 and 20 μg/kg/day of bisphenol-A in tocopherol-stripped corn oil, respectively, once daily on days 11-17 of gestation. An additional control group of 5 untreated pregnant females was included. Pups were weaned when 23 days old, and male litter mates were housed three per cage. One male per litter was individually housed to avoid possible dominance hierarchy influences on reproductive organs (which the authors believe can occur). No further details on this possible phenomenon were provided. Males were sacrificed when 6-month-old, the entire reproductive tract was removed and the testes, epididymides, preputial glands, seminal vesicles and prostate weights determined. The daily sperm production of 5 randomly chosen males per group was compared with 8 control males.

The right testis was removed, weighed then homogenised and the nuclei of step 14-16 spermatids (which survive this homogenisation process) counted to determine the daily sperm production per g of testis.

There were no significant differences between the untreated control group and vehicle control group for male offspring body weight gain or prostate weight, so these groups were pooled for analysis purposes. A statistically significant increase in prostate weight of 30% at 2 μg/kg and 35% at 20 μg/kg bisphenol-A was observed before and after adjusting for body weight. At 20 μg/kg a statistically significant decrease (19%) in sperm efficiency (daily sperm production per g testis) was also observed. This study was recently repeated to determine whether the reported results observed at these very low doses were reproducible (see below).

In a well designed and reported study which was based on the protocol of the study described by Nagel et al. (1997) and vom Saal et al. (1998), groups of 28 female CF-1 mice were administered by gavage 0, 0.2, 2.0, 20 or 200 μg/kg of bisphenol-A in tocopherol-stripped corn oil, once daily on days 11-17 of gestation (Cagen et al., 1999b). The study contained two control groups to determine whether there was any significant variation in control values for the endpoints measured. An additional dose group of 28 females was included and administered DES at 0.2 μg/kg in corn oil for the same period as vom Saal et al. (1997) had reported an increase in prostate size at 0.02-2.0 μg/kg DES in an earlier study. Pups were weaned when 23 days old, and male litter mates were housed to a maximum of four per cage. At 90 days of age, males were necropsied for evaluation of selected reproductive parameters.

A comparison of the two control groups showed no significant differences between any of the parameters measured and therefore the two groups were pooled for evaluation purposes. Body weight gain was unaffected in treated dams and their offspring. No effect was observed in water and feed consumption in treated dams. A statistically significant difference in mean litter size was observed at 200 μg/kg (9.60 pups/litter) compared to the control (12.37 pups/litter). However, the mean litter sizes at 0 and 200 μg/kg were both higher than values typically observed with this strain of mice (a mean of 8.75 pups/litter from a total of 7,400 litters). The number of live pups per litter was not affected by treatment. In males, decreases in mean cauda epididymis sperm concentration (up to 17%) and daily sperm production (up to 12%) were observed at 0.2 μg/kg and above compared to controls. However, the magnitude of these decreases was not dose-related and not statistically different from controls. No treatment-related effects were observed in prostate, preputial gland, seminal vesicle or epididymis weights. No effects were seen in the DES group. In conclusion, the effects reported by Nagel et al. (1997) and vom Saal et al. (1998) with 2 and 20 μg/kg bisphenol-A were not reproduced in a study that used larger group sizes (28 mice compared to 7). In addition, no effects were observed at the additional dose levels of 0.2 and 200 μg/kg bisphenol-A.

The protocol of the study reported by Nagel et al. (1997) and vom Saal et al. (1998) was repeated by Ashby et al. (1999). In addition, this study also repeated the protocol of the vom Saal et al. (1997) study, which reported an increase in prostate size with DES. The conditions of the studies were reproduced exactly, except that group sizes were 7-8 pregnant CF-1 mice instead of 6-7, and three male pups from each litter (instead of one as used by vom Saal) were individually housed. Additional parameters were evaluated in this study, and the data were also used to determine whether individual data should be corrected for the effect of animal body weight, litter (dam) or individual differences, housing conditions (isolated or group housed), and vehicle versus naive group effects.

Again, the effects reported by Nagel et al. (1997) and vom Saal et al. (1997; 1998) were not reproduced; no statistically significant increase in prostate weight or decrease in sperm efficiency was observed in the offspring of animals exposed to either 2 or 20 μg/kg bisphenol-A or 0.2 μg/kg DES. The litter was observed to be the more appropriate unit for statistical analysis. No changes considered to be treatment-related were seen with either bisphenol-A or DES in the additional parameters measured; testes/body weight ratio, epididymal/body weight ratio, daily sperm production and day of vaginal opening. Therefore, the results of this study are in agreement with those reported by Cagen et al. (1999b), that low doses of bisphenol-A do not increase prostate weight or reduce sperm efficiency in CF1 mice.

The effect of bisphenol-A on male reproductive development was investigated in CD-1 mice (Gupta, 2000). Fifteen pregnant mice were administered 0 or 50 μg/kg bisphenol-A on days 16-18 of gestation. Following delivery, litter sizes were adjusted to contain 8 pups ($\geq$ 3 males). Body weight and anogenital distances were determined on post-natal day 3, 21 and 60, for 45, 30 and 15 pups, respectively. On these days, 15 males per dose level (1 male from each litter) were sacrificed and prostate weight determined. Prostate cell preparations were also undertaken on days 3, 21 and 60 and androgen binding measured. Epididymis weight was determined on post-natal day 60. Additionally, on post-natal day 15, 4 males per group were sacrificed and prostate growth determined under light microscopy. DES (0.1 μg/kg) administered by the same route and dosing regime served as a positive control. (A dose level of 200 μg/kg DES was also included to compare the current findings with those reported by Gill et al. (1979). The results with 200 μg/kg DES are not reported in this summary). This study also determined the effects of bisphenol-A on prostate development *in vitro*. Fetal urogenital sinuses were obtained from mice on day 17 of gestation and cultured with bisphenol-A (0, 5 or 50 pg/ml) in the presence and absence of testosterone (to mimic *in vivo* conditions) for 6 days.

The author states that neither bisphenol-A nor DES induced fetal resorption or affected litter size (data not shown). Bisphenol-A had no effect on body weight gain of pups. Compared to controls, bisphenol-A produced a statistically significant increase in anogenital distance of 22, 25 and 33%, and in prostate weight of 56, 39 and 101%, on days 3, 21 and 60, respectively, following correction for body weight. The author reports that the uncorrected values also showed similar effects (data not shown). A statistically significant decrease in epididymis weight (35%) was observed on day 60. No effect was observed on testicular and vas deferens weight (data not shown). Bisphenol-A was also observed to increase the size of the prostate gland (on post-natal day 15), although this increase was not quantified. An increase (statistically significant) in prostate size was obtained *in vitro* in both the absence and presence of testosterone. Compared to controls, an increase of approximately 400-500% in androgen receptor binding activity was observed in prostate treated with bisphenol-A. However, this increase was only statistically significant on days 21 and 60.

For 0.1 μg/kg DES, statistically significant increases in anogenital distance (of 34, 10 and 27%) and prostate weight (of 38, 47 and 75%) were seen on days 3, 21 and 60 respectively. A statistically significant increase in androgen binding activity (of approximately 350%) was observed on days 21 and 60. Prostate size was increased both *in vivo* and *in vitro*.

Thus, in contrast to the findings of Ashby et al. (1999) and Cagen et al. (1999b) in CF-1 mice, an effect on epididymis and prostate weight was observed in CD-1 mice in this study at 50 μg/kg. A statistically significant increase in anogenital distance was also observed.

Recently Elswick et al. (2001b) raised concerns regarding the statistical analysis of the observations and resulting conclusions of the study by Gupta (2000). Gupta has responded to

these questions (Gupta, 2001) and has addressed all of the points raised. However, one key concern was the statistical analysis applied to the measurement of AGD in the offspring. Although clarification on this point was provided, there remain concerns that an inappropriate statistical analysis may have been applied to the data related to AGD.

In a study to investigate potential strain differences in the sensitivity of responses to oestrogens, Spearow et al. (1999) investigated the effects of exposure to 17β-oestradiol in various strains of mice. Although not directly involving an investigation of bisphenol-A, this study has been included because of its potential relevance to the interpretation of the results of studies with bisphenol-A in different mice strains. Groups of male CD-1, C57BL/6J, C17/J1, S15/J1, E/J1 and CN-/J1 mice (approx 16/dose) received siliastic implants containing 0, 2.5, 10, 20 or 40 μg 17β–oestradiol at 22-23 days of age. Animals were killed when 43 days old, testes removed and weighed, and light microscopy undertaken on the testes to determine spermatogenic index and the percentage of seminiferous tubules showing sperm maturation to the elongated spermatid stage of development.

Statistically significant differences in testes weight in control animals were seen between strains, heavier testes weights being observed in CD-1 and S15/J1 mice. Compared to controls, testes weight decreased in all strains of mice, though testes weight was affected by strain and dose. Strain accounted for more variation in testes weight than dose of 17β–oestradiol. C57BL/6J mice were the most sensitive (2.5 μg 17β-oestradiol produced a 60% decrease) and CD-1 mice the most resistant (40 μg produced a 30% decrease). Testicular histology also revealed strain differences. Low to moderate doses of 17β-oestradiol were reported to have "obliterated" spermatogenesis. In contrast, very little inhibition of spermatogenesis was seen in CD-1 mice with increasing doses of 17β-oestradiol. Compared to controls, statistically significant decreases were also seen in the % of seminiferous tubules with elongated spermatids. Again strain differences were evident. Gamete maturation to the elongated spermatid stage of development was completely eliminated in C57BL/6J mice at 10 μg. In contrast, an abundance of normally maturing spermatids was found in the testes of all CD-1 mice at 20 μg. The results of this study show marked genetic differences in sensitivity to the effects of 17β-oestradiol on the male reproductive tract.

4.1.2.9.4 Summary of toxicity to reproduction

No human data are available. Bisphenol-A has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with bisphenol-A glucuronide *in vitro*. The available data also indicate that there is a marked strain difference in the response to bisphenol-A in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories (Kanno et al., 2001). Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of bisphenol-A on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. Although no effect on fertility was seen in the rat two-generation study, low-dose levels were employed (0.2-200 μg/kg/day). In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of bisphenol-A. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse, it is prudent to assume that bisphenol-A may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg. The continuous breeding study in the mouse provides some evidence that bisphenol-A can cause adverse effects on fertility. In the F_0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F_1 generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F_1 generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F_0 generation), there was no effect on epididymal weight. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern. Although no effects were seen in the two-generation rat study, it is not considered suitable for use in the risk characterisation due to the low-dose levels employed (0.2-200 μg/kg/day). However, this data combined with that for the multigeneration study does provide a comprehensive dose-response range for effects on fertility in the rat. In addition, comparing the rat and mouse data it can be seen that similar toxicological profiles were observed for effects on fertility; effects were seen in both species at approximately the same dose level (i.e. reductions in litter size at 500 mg/kg/day in the rat and at 600 mg/kg/day in the mouse). Consequently, it is considered that the NOAEL of 50 mg/kg/day identified in the rat multigeneration study is also likely to produce no adverse effects in mice for which there is only a LOAEL of 300 mg/kg/day (for a small but statistically significant decrease in epididymal weight in F_1 males only). Therefore, the NOAEL of 50 mg/kg/day identified from the multigeneration study will be used for risk characterisation purposes, in relation to effects on fertility.

No evidence that bisphenol-A is a developmental toxicant was observed in standard development studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day, respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1,000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F_1-F_3). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg. However, additionally, some studies have investigated the potential of bisphenol-A to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at dose levels in the range 2–50 μg/kg.

However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. It is noted that in contrast to the studies showing effects on the male reproductive tract, the studies that did not find an effect of bisphenol-A also did not show any effects of DES. Furthermore, no functional changes in reproductive parameters or reproductive organ development were observed in a recent rat two-generation study using similar dose levels. The reasons for the differences in these results are unclear. Recent evidence from one study suggests that there are differences in the sensitivity of different mice strains to the effects of oestrogens, which may be related to the selection of strains for large litter size. This difference in sensitivity may in part explain some of the differences in the current database, although the relevance of these rodent strain differences in relation to human health remains unclear.

Overall, in standard developmental studies in rodents, there is no convincing evidence that bisphenol-A is a developmental toxicant. However, the available and apparently conflicting data from studies conducted using low doses (in the μg/kg range) do raise uncertainties. Overall, the majority of EU member states felt that the studies reporting effects at low doses could not be dismissed. However, the member states disagreed on how these studies should be used, if at all, in the risk characterisation for this endpoint. The disagreements were based on differing views about the uncertainties surrounding the reproducibility of the findings and their biological significance, if any, to human health.

This issue was referred to the Competent Authorities in June 2001. It was agreed unanimously by the Competent Authorities that further work was required to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. In addition, it was agreed that a provisional NOAEL of 50 mg/kg/day for developmental effects, derived from the rat multi-generation study, should be used in the risk characterisation in the interim, whilst awaiting the outcome of further testing, with the aim of identifying those scenarios which are clearly of concern irrespective of the outcome of the further testing.

4.1.3 Risk characterisation

The section below, titled "General aspects" provides an overview of the occupational use, exposure and toxicological profile of bisphenol-A, identifying the lead effects and where appropriate, identifying NOAELs and/or LOAELs.

4.1.3.1 General aspects

Occupational exposure to bisphenol-A occurs during the:

- manufacture of bisphenol-A,
- manufacture of PC,
- manufacture of articles from PC,
- manufacture of epoxy resins and moderated epoxy resins,
- manufacture of powder coatings, liquid epoxy paints and lacquers,
- use of epoxy resin-based powder coatings, paints and lacquers,
- use of bisphenol-A in PVC production,
- manufacture of thermal papers,
- manufacture of tin plating additive,
- manufacture of tetrabrominated flame retardants (TBBA).

Bisphenol-A usually occurs as white to light tan/cream flakes or powder. Vapour pressures of $4.1 \cdot 10^{-10}$ kPa and $5.3 \cdot 10^{-9}$ kPA at 25°C have been quoted so exposure to vapour is not considered to be a problem. Exposure to bisphenol-A will be in the form of inhalation/ ingestion of dust and by skin contact with the flakes or powder.

The bisphenol-A manufacturing process is largely an enclosed system with breaches for product sampling, product bagging and tanker/silo filling and some maintenance activities. The results ranged from none detected (nd) to 23.3 mg/m^3 8-hour TWA. Product bagging and tanker/silo filling were reported to be full-shift activities. The highest results were obtained where maintenance activities or cleaning was carried out during the sampling period. A reasonable worst-case scenario for 8-hour TWA for manufacturing activity would be 5 mg/m^3. A reasonable worst-case scenario for short-term exposures is 10 mg/m^3.

It was reported that there was little or no opportunity for exposure to bisphenol-A during the manufacture of polycarbonate, as the bisphenol-A entered the plant as a solution and was piped directly into a closed system. However, four respirable dust samples for PC dust had been collected in 1990-1991, although they were not analysed for bisphenol-A. Further dust sampling was undertaken from 1993 to 1996. These were for TIP and were not analysed for bisphenol-A. These results ranged from 0.1 to 1.1 mg/m^3. The 90th percentile for these figures was 1.0 mg/m^3. It was reported by industry that there is a maximum of 100 ppm residual bisphenol-A in the PC polymer. Taking this into account the reported results range from $7 \cdot 10^{-6}$ to $1.1 \cdot 10^{-4}$ mg/m^3, 8-hour TWA with a 90th percentile of $1 \cdot 10^{-4}$ mg/m^3, 8-hour TWA. In 2000, the same company took a personal sample to confirm that there was no exposure to bisphenol-A in the PC manufacturing plant. The sample was analysed for bisphenol-A. The result was less than $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA. EASE modelling resulted in a range of 0 to $1 \cdot 10^{-4}$ mg/m^3, 8-hour TWA. A reasonable worst-case scenario for this activity would be $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA. A reasonable worst-case scenario for short-term exposures is 0.5 mg/m^3. There is reported to be no opportunity for exposure to bisphenol-A during the manufacture of articles from polycarbonate, due to the stability of the polymer, and the retention of any residual bisphenol-A within the polymer matrix. As the manufacturing process does not use any higher temperatures

than those used for extrusion in the PC manufacturing industry, the same results have been used to represent exposure in the manufacture of articles from PC. The reasonable worst-case scenario is therefore $1 \cdot 10^{-3}$ mg/m^3, 8-hour TWA.

A number of responses from companies manufacturing epoxy resins and modified epoxy resins highlighted the charging of vessels with bisphenol-A prills or flakes as the main source of exposure in this industry. Short-term exposures during this activity ranged from 0.32 to 17.5 mg/m^3, with 8-hour TWAs of up 1.2 mg/m^3. A reasonable worst-case scenario would be an 8-hour TWA of 0.7 mg/m^3. A reasonable worst-case scenario for short-term exposure would be 11 mg/m^3.

Manufacture of liquid epoxy resin-based paints is not reported to be a source of significant exposure to bisphenol-A given the very low (10 ppm) quantity of residual bisphenol-A in the uncured epoxy resin, most of which would be retained within the resin matrix.

The residual amount of bisphenol-A in epoxy resins for powder paints is reported to be about 300 ppm. Calculations made using this figure and total inhalable particulate exposure measurements from the HSE's NEDB, gave an estimated exposure of up to 0.02 mg/m^3 8-hour TWA. Industry supplied data for personal exposure across all activities ranging from 0.3 to 10 mg/m^3, 8-hour TWA for total inhalable particulate. This is calculated to give a range of personal exposures to bisphenol-A of $9 \cdot 10^{-5}$ to $3 \cdot 10^{-3}$ mg/m^3. Given that the amount of residual bisphenol-A in powder paints is likely to be lower than that calculated, a reasonable worst-case scenario of 0.01 mg/m^3 8-hour TWA has been estimated. A short-term reasonable worst-case estimate of 0.3 mg/m^3 has been made based on data from SPI.

Exposure to total inhalable particulate during the use of powder paints has been calculated at up to 0.04 mg/m^3 8-hour TWA. Two actual measured exposure results of 0.003 and 1.063 mg/m^3 were reported in a NIOSH paper. A reasonable worst-case scenario for an 8-hour TWA is estimated to be 0.5 mg/m^3 for spraying coating powders and 0.005 mg/m^3 for dip-painting.

The use of bisphenol-A in PVC manufacture is being phased out. As handling of bisphenol-A is considered to be similar to industries such as thermal paper manufacturing, the EASE data for that scenario were used to generate data for PVC manufacturing. A reasonable worst-case scenario was estimated to be 0.1 mg/m^3 8-hour TWA. A short-term reasonable worst-case exposure is estimated to be 1 mg/m^3.

Thermal paper manufacturers reported only one exposure result for bisphenol-A, which was lower than the limit of detection for one-hour-long sample. An 8-hour TWA calculated from this result gave a figure of less than 0.25 mg/m^3. The estimated range predicted using EASE was 0 to 0.04 mg/m^3. A reasonable worst-case scenario for an 8-hour TWA for this industry is estimated to be 0.1 mg/m^3. A reasonable worst-case scenario for short-term exposure would be 4 mg/m^3.

Small quantities of BPA are used in the manufacture of tin plating additives. EASE predictions gave an exposure range of 0.02 to 0.05 mg/m^3 8-hour TWA, with the only source of exposure identified being the charging of the reactor vessel with bisphenol-A. A reasonable worst-case scenario would be an 8-hour TWA of 0.05 mg/m^3.

One company currently manufactures TBBA using bisphenol-A. No exposure data were available, but EASE was used to estimate exposure during the packaging process. This gave an estimated exposure range of $6 \cdot 10^{-6}$ to $1.5 \cdot 10^{-5}$ mg/m^3 8-hour TWA.

In summary, 8-hour TWAs rarely exceeded 5 mg/m^3 in bisphenol-A manufacturing facilities, and rarely exceeded 0.5 mg/m^3 in the other industries discussed. Short-term exposures could reach as high as 43.6 mg/m^3, but were more usually less than 10 mg/m^3.

Dermal exposure to BPA can occur during manufacturing and use of bisphenol-A. During manufacturing operators can come into contact during product sampling and during bag filling and other filling operations. Using the EASE model, dermal exposure during sampling was estimated to be in the range 0 to 0.1 mg/cm^2/day. Exposure is likely to be towards the lower end of the range as the activity takes less than five minutes to complete. The extent of the area of dermal contamination is estimated to be 420 cm^2.

Filling operations are full-shift activities, so the potential for dermal exposure is greater. The EASE estimation gave a range of 1-5 mg/cm^2/day. Again, the extent of the area of dermal contamination is estimated to be 420 cm^2.

The only potential for dermal exposure during PC manufacturing was during the bagging of PC granules. The EASE estimation gave a range of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 420 cm^2.

The same exposure range was used to estimate exposure during the manufacture of articles from PC, when loading PC granules from the big bags to the extruder i.e. $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 420 cm^2.

The main source of exposure identified during epoxy resin manufacturing was the charging of reactors. The EASE estimation gave a range of 0.1 to 1 mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 420 cm^2.

Estimations of dermal exposure during two maintenance activities were carried out using EASE as an illustration of the potential dermal exposures during general maintenance activities. The EASE prediction gave a range of 0.1 to 1 mg/cm^2/day for both activities. The extent of the area of dermal contamination for these activities is estimated to be 840 cm^2.

The range of dermal exposure predicted using EASE during epoxy resin-based powder coating manufacture was $3 \cdot 10^{-4}$ to $1.5 \cdot 10^{-3}$ mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 1,300 cm^2.

The range estimated using EASE for powder coating application was $6 \cdot 10^{-4}$ to $1.8 \cdot 10^{-3}$ mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 1,300 cm^2.

During the use of bisphenol-A in PVC manufacture, EASE predicted exposures in the range 0 to 0.1 mg/cm^2/day, for exposures during charging reactors. The extent of the area of dermal contamination is estimated to be 420 cm^2.

Charging reactors was the only activity identified by the thermal paper manufacturers and the tin plating additive manufacturers where the potential for dermal exposure arises. This activity takes about 5 to 10 minutes per shift. EASE was used to estimate a range of dermal exposure. The range predicted was 0 to 0.1 mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 420 cm^2.

Dermal exposure during bag filling of TBBA was estimated using EASE. The range predicted, taking into account the fact that there is only 3 ppm BPA in the final product is $3 \cdot 10^{-7}$ mg/cm^2/day to $3 \cdot 10^{-6}$ mg/cm^2/day. The extent of the area of dermal contamination is estimated to be 420 cm^2.

These values have been provided as a first approximation of this exposure and are based on the limited information obtained.

No information is available on the toxicokinetics of bisphenol-A in humans. In experimental animals, toxicokinetic data are available from three oral studies in a single species, the rat and from an *in vitro* dermal absorption study, using human skin. These studies provide the basis for a general understanding of the main features of the toxicokinetic profile. Following oral administration, absorption from the gastrointestinal tract is rapid and extensive, although it is not possible to reliably quantify the extent of absorption. Following dermal exposure, the available data suggest that there is limited absorption, in the region of about 10% of the applied dose.

Bisphenol-A was removed rapidly from the blood, and metabolism data indicate extensive first pass metabolism following absorption from the gastrointestinal tract. A clear sex difference was observed in the clearance of parent compound from the blood. In females parent compound was present in the blood at much later sampling times. There are no data available to explain why this sex difference was observed. In view of this first pass metabolism, the bioavailability of unconjugated bisphenol-A is probably limited following oral exposure, at no more than 10-20% of the administered dose. Limited data are available for the distribution of bisphenol-A following oral administration: an *in vivo* DNA adduct study shows that bisphenol-A reaches the liver, an *in vivo* micronucleus study suggests that bisphenol-A or a metabolite reaches the bone marrow, a limited toxicokinetic study suggests that bisphenol-A or a metabolite reaches the testes, and a repeated dose study in pregnant rats suggests that bisphenol-A reaches the liver of both the dam and fetus. However, because of first pass metabolism it is likely that the distribution and bioavailability of unconjugated bisphenol-A is limited following oral exposure. There is also evidence of enterohepatic circulation occurring.

The major metabolic pathway in rats involves glucuronide conjugation; limited sulphate conjugation may also occur. Approximately 10% and 20% of the administered dose was recovered in the urine as the glucuronide metabolite in males and females, respectively. There are no data available to explain why this sex difference was observed. In addition, data from cell free systems and *in vivo* studies on the interaction of bisphenol-A with DNA, supported by a chemical photodecomposition study, suggest that limited oxidation of bisphenol-A to bisphenol O-quinone by cytochrome P450 may occur.

The major route of excretion is via the faeces with the urinary route being of secondary importance: over 7 days post dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post dose. A sex difference was also observed in elimination, with females excreting approximately twice as much radioactivity in the urine (24-28%) than males (14-16%). Again, there are no data available to explain why this sex difference was observed. Limited data from studies of uncertain reliability also suggest that bisphenol-A can be excreted in the milk, though the data do not allow a quantitative determination to be made.

The first pass metabolism and extensive and rapid elimination of bisphenol-A suggest that the potential for transfer to the foetus and bioaccumulation may be limited. This is supported by data from toxicokinetic studies in pregnant rats that suggest limited distribution of bisphenol-A to the foetus, but no evidence for accumulation, and results from a repeated dose study in pregnant rats which show limited distribution to the fetal liver, with no evidence to indicate accumulation in the liver, the only organ tested.

There are no data on the toxicokinetics of bisphenol-A following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route. No useful information is available on the effects of single exposure to bisphenol-A in humans. Oral LD_{50} values beyond 2,000 mg/kg are indicated in the rat and mouse, and dermal LD_{50} values above 2,000 mg/kg are evident in the rabbit. Few details exist of the toxic signs observed or of target organs. For inhalation, a 6-hour exposure to 170 mg/m^3 (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed. These data indicate that bisphenol-A is of low acute toxicity by all routes of exposure relevant to human health.

Limited human anecdotal information of uncertain reliability is available from written industry correspondence suggesting that workers handling bisphenol-A have in the past experienced skin, eye and respiratory tract irritation. It cannot be determined whether the reported skin reactions were related to skin sensitisation or irritation. However, a recent well conducted animal study clearly shows that bisphenol-A is not a skin irritant. A recent well conducted animal study shows that bisphenol-A is an eye irritant; effects persisted until the end of the study (day 28 post-instillation) in 1 of 3 rabbits. Overall, taking into account the animal and human evidence, bisphenol-A has the potential to cause serious damage to the eyes.

Slight and transient nasal tract epithelial damage was observed in rats exposed to bisphenol-A dust at 170 mg/m^3 (the highest attainable concentration) for 6 hours. Slight local inflammatory effects in the upper respiratory tract were observed in rats exposed to 50 mg/m^3 and 150 mg/m^3 of bisphenol-A in 2 and 13 week repeat inhalation studies, but were not observed at 10 mg/m^3 in the same studies. Increased exposure did not increase the severity of the response at 50 and 150 mg/m^3. Taken together with anecdotal human evidence, these data suggest bisphenol-A appears to have a limited respiratory irritation potential.

With respect to skin sensitisation in humans, there are several reports of patients with dermatitis responding to bisphenol-A in patch tests. However, it is unclear whether bisphenol-A or related epoxy resins were the underlying cause of the hypersensitive state. Anecdotal information indicates skin inflammation in workers handling bisphenol-A, although given the uncertain reliability of this information no conclusions can be drawn from it. In animals, a skin sensitisation test performed to current regulatory standards is not available. The available studies are negative, but the test reports lack detail and no reliable justifications were given for the choice of concentrations used. In the study using the highest challenge concentration, 50% in a guinea pig closed-patch test, a sensitisation rate of 12.5% was obtained. It is possible that the concentrations used in all the available studies were not maximised and a greater response might have been obtained with higher induction and challenge concentrations. Based on the findings from the most robust study, bisphenol-A may possess a skin sensitisation potential, albeit a limited one. Bisphenol-A in the presence of UV light can also elicit skin responses in humans, and reproducible positive results for photosensitisation have been obtained in mouse ear swelling tests. Mechanistic studies in mice have suggested this is an immune-mediated process. Therefore, examination of the available human and experimental animal studies leaves the picture somewhat unclear as to whether one or more of the following are properties of bisphenol-A; (1) orthodox skin sensitisation (2) photosensitisation (3) bisphenol-A eliciting a response in people previously skin sensitised to another substance (e.g. epoxy resins). Thus, the precise nature of the hazardous properties of bisphenol-A on the skin is unclear, but clearly skin

reactions can be a potential consequence of repeated skin exposure in humans. Overall, taking all of the data available into account, bisphenol-A is considered capable of producing skin sensitisation responses in humans. There are no data from which to evaluate the potential of bisphenol-A to be a respiratory sensitiser.

No useful information is available on the effects of repeated exposure in humans. In animals there are no data relating to repeated dermal exposure. Repeat inhalation studies are available in the rat. The principal effect was the same as that observed following a single exposure-slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed following exposure to 50 and 150 mg/m^3 (6 hours/day, 5 days/week for 2 or 13 weeks; 150 mg/m^3 is close to the highest attainable concentration), and a NOAEL of 10 mg/m^3 was identified in rats in this 13 week study.

Dietary studies in rats produced a decrease in body weight gain and minor changes in organ weight at 100 mg/kg/day and above in 90-day studies. These effects are difficult to interpret in terms of their toxicological significance in the absence of other findings (e.g. histopathological changes). An inconsistent finding of caecal enlargement was seen in some 90-day studies. The caecal enlargement was observed at 25 mg/kg/day and above and was without any associated histological abnormalities. In addition, it was not observed in a 2-year study at doses up to about 140 mg/kg/day or a multigeneration study at doses up to 500 mg/kg/day. Consequently, this is not regarded as a toxicologically significant observation of relevance to humans. Overall, a NOAEL of 74 mg/kg/day has been established for rats from a 2-year study.

Dietary studies in mice consistently indicated that the liver is a target organ in this species with changes being observed in the size and nucleation state of hepatocytes in 2-year and 90-day studies. The incidence and severity of these treatment-related multinuclear giant hepatocytes was markedly greater in males than in females. It was not possible to identify a no effect level for males, the effect being observed at all dose levels used from the lowest dose tested of 120 mg/kg/day (2-year study). Even at this lowest dose level a large proportion (84%) of the animals examined showed signs of this effect. In females, a no-effect level of 650 mg/kg/day was identified for these cellular changes in the 2-year study. The mechanism by which changes arise and their significance for human health is not clear but cannot be dismissed as being of no significance. The only other findings in mice were significant reductions in body weight gain at dose levels of 650 mg/kg/day and above. Thus, a LOAEL of 120 mg/kg/day in males for multinuclear giant hepatocytes and 650 mg/kg/day in females for a reduction in body weight gain of unknown magnitude, were identified in a 2-year study.

In a 90-day dietary study in dogs, a no effect level of approximately 80 mg/kg/day was identified, with increases in relative liver weight being the only finding observed at approximately 270 mg/kg/day. In the absence of changes in histopathology, this finding is of doubtful toxicological significance.

No human data regarding mutagenicity are available. However, bisphenol-A appears to have demonstrated aneugenic potential *in vitro,* positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems, there is information that shows bisphenol-A disrupts microtubule formation. Bisphenol-A has been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. However, some deficiencies in the conduct of these studies have been noted and the negative results cannot be taken as entirely conclusive. Bisphenol-A does not appear to be aneugenic *in vivo*, since a

recently conducted, standard mouse bone marrow micronucleus test has given a negative result. Bisphenol-A was negative in a briefly reported dominant lethal study in rats but, given the limited details provided, this is not regarded as an adequate negative result. The only other data in somatic cells *in vivo* are from a ^{32}P-postlabelling assay, which showed that bisphenol-A is capable of producing DNA adduct spots in rat liver following oral administration. These adduct spots were not characterised fully.

Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies (see below), it does not appear that bisphenol-A has significant mutagenic potential *in vivo*. Any aneugenic potential of bisphenol-A seems to be limited to *in vitro* test systems and is not of concern. The relevance of the finding that bisphenol-A can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health.

There are no human data contributing to the assessment of whether or not bisphenol-A is carcinogenic. In animals, a dietary carcinogenicity study in two species, F344 rats and B6C3F$_1$ mice, is available. A small increased incidence of leukaemias was seen in male and female F344 rats along with increases in the frequency of mammary gland fibroadenomas in male rats. These increases were not statistically significant, were slight and in a strain prone to these tumours. An increased incidence in benign Leydig cell tumours seen in male rats was within historical control limits. In mice, a small increased incidence in lymphomas was observed in males, but was not statistically significant and there was no dose-related trend. No increased incidence in any tumour type was observed in female mice. Overall, all of these tumour findings in rats and mice are not considered toxicologically significant. Consequently, it is concluded that bisphenol-A was not carcinogenic in this study in both species. No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, bisphenol-A did not exhibit properties that raise concern for potential carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m^3 in a 13-week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m^3. Taking into account all of the animal data available the evidence suggests that bisphenol-A does not have carcinogenic potential.

Bisphenol-A has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. The available data also indicate that there is a marked strain difference in the response to bisphenol-A in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories (Kanno et al., 2001). Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of bisphenol-A on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although

this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of bisphenol-A. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse, it is prudent to assume that bisphenol-A may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg. The continuous breeding study in the mouse provides some evidence that bisphenol-A can cause adverse effects on fertility. In the F_0 generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F_1 generation. A small but statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F_1 generation, but the significance of this finding is uncertain because a comparable effect was not seen in F_0 mice. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern. For risk characterisation purposes, although no effects were seen in the two-generation rat study it is not considered suitable due to the low-dose levels employed (0.2-200 μg/kg/day). However, this data combined with that for the multigeneration study does provide a comprehensive dose-response range for effects on fertility in the rat. In addition, comparing the rat and mouse data it can be seen that similar toxicological profiles were observed for effects on fertility; effects were seen in both species at approximately the same dose level (i.e. reductions in litter size at 500 mg/kg/day in the rat and at 600 mg/kg/day in the mouse). Consequently, it is considered that the NOAEL of 50 mg/kg/day identified in the rat multigeneration study is also likely to produce no adverse effects in mice for which there is only a LOAEL of 300 mg/kg/day (for a small but statistically significant decrease in epididymal weight in F_1 males only). Therefore, the NOAEL of 50 mg/kg/day identified from the multigeneration study will be used for risk characterisation purposes with respect to effects on fertility.

No evidence that bisphenol-A is a developmental toxicant was observed in standard development studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1,000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F_1-F_3). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg. However, additionally, some studies have investigated the potential of bisphenol-A to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at dose levels in the range 2– 0 μg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. Furthermore, no functional changes in reproductive parameters or reproductive organ development were observed in a recent rat two-generation study using similar dose levels. The reasons for the differences in these results are unclear. Recent evidence from one study suggests that there are differences in the sensitivity of different mice strains to the effects of

oestrogens, which may be related to the selection of strains for large litter size. This difference in sensitivity may in part explain some of the differences in the current database, although the relevance of these rodent strain differences in relation to human health remains unclear.

Overall, in standard developmental studies in rodents, there is no convincing evidence that bisphenol-A is a developmental toxicant. However, the available and apparently conflicting data from studies conducted using low doses (in the μg/kg range) do raise uncertainties. Overall, the majority of EU member states felt that the studies reporting effects at low doses could not be dismissed. However, the member states disagreed on how these studies should be used, if at all, in the risk characterisation for this endpoint. The disagreements were based on differing views about the uncertainties surrounding the reproducibility of the findings and their biological significance, if any, to human health.

This issue was referred to the Competent Authorities in June 2001. It was agreed unanimously by the Competent Authorities that further work was required to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. In addition, it was agreed that a provisional NOAEL of 50 mg/kg/day for developmental effects, derived from the rat multi-generation study, should be used in the risk characterisation in the interim, whilst awaiting the outcome of further testing, with the aim of identifying those scenarios which are clearly of concern irrespective of the outcome of the further testing.

Overall, the hazardous properties of bisphenol-A have been evaluated in animals to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of eye irritation, respiratory irritation, skin sensitisation, repeated dose toxicity to the respiratory tract, effects on the liver and reproductive toxicity have been identified. No dose-response information is available on eye irritation. A NOAEL of 10 mg/m^3 has been identified for repeated dose toxicity to the respiratory tract. A LOAEL of 120 mg/kg has been identified for liver effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg/day has been established in a multigeneration study for effects on fertility and as a provisional NOAEL for effects on development; this value is used in the risk characterisation.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

To conduct the risk characterisation for workers and consumers, it is necessary to compare human exposure for the inhalation/dermal route with oral N(L)OAELs from repeated dose animal studies, because of the absence of significant inhalation/dermal toxicity data. A direct comparison between exposure and effects must take account of first pass liver metabolism, which is likely to limit systemic bioavailability by the oral route. To compensate for this limited oral bioavailability (assumed to be 10% of administered dose), the animal N(L)OAELs for reproductive toxicity have been reduced by a factor of 10 for the comparison of inhalation or dermal exposure and effects. Thus, the "systemic" value used for comparison in the risk characterisation for reproductive toxicity is a NOAEL of 5 mg/kg/day. For liver effects, no adjustment is made for first pass metabolism as it is assumed that the effects seen are mediated prior to metabolism in this organ.

4.1.3.2 Workers

The health effects of concern for bisphenol-A are eye irritation, respiratory irritation, skin sensitisation, repeated dose toxicity to the respiratory tract, effects on the liver and reproductive

toxicity. Of the other toxicity endpoints, the acute studies indicate bisphenol-A is of low acute toxicity via the oral, dermal and inhalation routes. The data indicate that bisphenol-A is not a skin irritant. Under normal occupational conditions, workers are not likely to be exposed to concentrations which would lead to adverse health effects from a single exposure. There are no concerns for mutagenicity and carcinogenicity.

A risk characterisation for each health effect of concern for bisphenol-A is presented below. In order to carry out the risk characterisation for workers of some of these health effects the following assumptions have been made; the body weight of the average worker is 70 kg and the worker inhales 10 m^3 air per working day.

4.1.3.2.1 Eye and respiratory tract irritation

Anecdotal human evidence suggests that respiratory irritation has been reported in workers, though there are no quantitative details. In an acute inhalation study, slight and transient nasal tract epithelium damage was observed in rats following exposure to 170 mg/m^3 (the only concentration tested and the highest attainable concentration) for 6 hours. A NOAEL for these effects was not identified in this study. However, a NOAEL of 10 mg/m^3 was identified in rats from a 13-week repeat exposure study. Overall, it is considered that bisphenol-A may have limited respiratory tract irritation potential that should be considered for risk characterisation.

No clinical signs of eye irritation were reported in an acute inhalation study in rats following exposure to 170 mg/m^3 bisphenol-A for 6 hours. However, anecdotal human evidence, albeit limited, suggests that eye irritation can occur following occupational exposure to bisphenol-A. A recent well conducted animal study showed that irritation was observed following instillation of bisphenol-A into rabbit eyes. Since bisphenol-A has the potential to cause eye and respiratory irritation short-term peak exposures need to be considered.

Manufacture of bisphenol-A

During the manufacture of bisphenol-A a reasonable worst-case scenario for short-term exposures is 10 mg/m^3. Although eye irritation is unlikely to be expressed providing good occupational hygiene practices are in operation, it is possible that eye irritation could occur at these concentration levels and therefore concerns are raised for human health and **conclusion (iii)** is reached.

There are insufficient experimental animal data to make a quantitative assessment of whether respiratory irritation would be observed at this exposure level, though the workplace human anecdotal evidence suggests it may occur. Therefore, there are concerns for human health arising from these exposures to bisphenol-A and **conclusion (iii)** is reached.

Manufacture of polycarbonate

During the manufacture of polycarbonate a reasonable worst-case scenario for short-term exposure is 0.5 mg/m^3. Therefore, eye and respiratory irritation are unlikely to be expressed during normal use because exposure is low, providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Manufacture of articles from polycarbonate

No short-term occupational exposure data are available. However, the eye and respiratory irritation of the substance are unlikely to be expressed during normal use because exposure is negligible (8-hour TWA of $1 \cdot 10^{-3}$ mg/m^3), providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Manufacture of epoxy resins and moderated epoxy resins

A task specific (sampling times 15 to 132 mins) peak mean concentration of up to 3.96 mg/m^3 bisphenol-A (range, none detected to 43.6 mg/m^3) was reported during the manufacture of epoxy resins, in process operators, for a 16 minute sampling period. It is possible that eye irritation will occur over these concentration ranges. Since concerns are raised for human health, **conclusion (iii)** is reached.

There are insufficient experimental animal data to make a quantitative assessment of whether respiratory irritation would be observed over these exposure ranges, though the human anecdotal data suggests it may occur. Therefore, there are concerns for human health arising from these exposures to bisphenol-A and **conclusion (iii)** is reached.

Powder coatings manufacturing

During the manufacture of powder coatings a reasonable worst-case scenario for short-term exposures is 0.3 mg/m^3. Therefore, eye and respiratory irritation are unlikely to be expressed during normal use because exposure is low, providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Powder coatings use

No short-term occupational exposure data are available. However, the eye and respiratory irritation of the substance are unlikely to be expressed during normal use because exposure is low (8-hour TWA of up to 0.5 mg/m^3), providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Use of bisphenol-A in PVC manufacture

On the basis of modelled data, short-term exposures of up to 1 mg/m^3 are predicted. Therefore, eye and respiratory irritation are unlikely to be expressed during normal use because exposure is low, providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Thermal paper manufacturing

One result for a one-hour sample taken during and following the charging of bisphenol-A pellets into a metal container was reported. As the task was reported to take 10 minutes, a short-term exposure (15 min) was calculated using the worst-case scenario for the charging of bisphenol-A pellets from a bulk bag into a metal container, and gave a result of less than 4 mg/m^3. Therefore, eye and respiratory irritation are unlikely to be expressed during normal use because exposure is low, providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Manufacture of tin plating additives

No short-term occupational exposure data are available. However, the eye and respiratory irritation of the substance are unlikely to be expressed during normal use because exposure is low (8-hour TWA of 0.05 mg/m^3), providing good occupational hygiene practices are in operation. Overall, **conclusion (ii)** is reached.

Manufacture of TBBA

Eye and respiratory tract irritation of the substance are unlikely to be expressed during normal use because exposure is negligible (estimated exposure range of $6 \cdot 10^{-6}$ to $1.5 \cdot 10^{-5}$ mg/m^3). Overall, **conclusion (ii)** is reached.

4.1.3.2.2 Skin sensitisation

Bisphenol-A has been associated with skin sensitisation responses in humans. The balance of evidence suggests that bisphenol-A is at least capable of inducing skin responses in hypersensitive individuals. Consequently, repeated skin contact with bisphenol-A may result in dermatitic responses. In order to avoid this, skin exposure should be controlled for all exposure scenarios. **Conclusion (iii)** is reached.

4.1.3.2.3 Repeated dose toxicity to the respiratory tract

The principal effect following repeated exposure to bisphenol-A was slight local inflammatory effects in the upper respiratory tract. A NOAEL of 10 mg/m^3 and a LOAEL of 50 mg/m^3 can be identified in rats for respiratory irritation from 2 and 13-week repeat exposure studies. It is particularly noted that the effects seen at 50 mg/m^3 were slight and that an increase in the exposure concentration to 150 mg/m^3 produced only a slightly greater response, indicating a shallow dose-response curve. Furthermore, extending the duration of exposure from 2 weeks to 13 weeks at these exposure concentrations had only marginal effects. The margins of safety (MOS) for the NOAEL and LOAEL for each occupational exposure scenario are shown in **Table 4.25**.

Overall, bisphenol-A is considered to have limited effects on the respiratory tract as only slight local inflammatory effects were observed at both 50 and 150 mg/m^3 in a 13-week repeated dose study. The smallest margin of safety observed was 2 for the NOAEL in the manufacture of bisphenol-A. However, this is considered sufficient for this health effect for the following reasons:

- only minor effects were observed at the LOAEL and there is a shallow dose-response curve; increased exposure (150 mg/m^3) had only marginal effects on the severity of the response,
- there are no data to suggest that humans are more sensitive than the rat for local effects on the respiratory tract and,
- rats are obligate nasal breathers, therefore the amount deposited in nasal turbinates in humans would be less than in rats for comparable exposures.

Table 4.25 Calculated margins of safety for each exposure scenario

Process	Inhalation exposure ($mg \cdot m^{-3} \cdot day$)	MOS for repeated dose effects on respiratory tract		Conclusion
		NOAEL ($10\ mg \cdot m^{-3}$)	LOAEL ($50\ mg \cdot m^{-3}$)	
Manufacture of bisphenol-A	5	2	10	ii
Manufacture of PC	0.001	10,000	50,000	ii
Manufacture of articles from PC	0.001	10,000	50,000	ii
Manufacture of epoxy resin	0.7	15	70	ii
Powder coating manufacture	0.01	1,000	5,000	ii
Powder coating use	0.5	20	100	ii
PVC manufacture	0.1	100	500	ii
Thermal paper manufacture	0.1	100	500	ii
Tin plating manufacture	0.05	200	1,000	ii
Manufacture of TBBA	$1.5 \cdot 10^{-5}$	>650,000	>3, 000, 000	ii

The lack of a lifetime inhalation study is not considered a concern, as only slight effects were observed at 50 mg/m^3 and the dose-response curve for this effect was shallow up to 150 mg/m^3. Since the minimum MOS is considered sufficient, all the other MOS for the various occupational exposure scenarios are also considered sufficient and thus **conclusion (ii)** applies.

4.1.3.2.4 Effects on the liver and toxicity to reproduction

Effects on the liver (multinucleated giant hepatocytes) were observed in mice. There are no data to indicate the relevance of the effects for humans but they cannot be discounted and so must be taken into account. A NOAEL could not be identified for the liver effects. A LOAEL of 120 mg/kg/day was identified in male mice from a 2-year study; females were appreciatively less sensitive. There is also limited data to ascertain the dose-response curve for the incidence of these liver effects. In the 2-year study giant multinucleated hepatocytes were observed in 41/49 and 41/50 mice at 120 and 600 mg/kg/day, respectively. In addition, the lack of a NOAEL is also compounded by the fact that their consequence for the functioning of the liver, the underlying mechanism involved and its relevance to human health are all unclear.

A NOAEL of 50 mg/kg has been identified for reproductive effects (effects on fertility and provisionally for effects on development) from a multigeneration study in the rat. Effects on fertility and on development were seen at 500 mg/kg in the same study. In order to compensate for first pass metabolism, these values have been adjusted by a factor of 10 for comparison with inhalation and dermal exposure estimates. The estimated body burdens arising from inhalation and dermal exposure are given in **Table 4.26**.

Table 4.26 Calculations of the contribution of inhalation and dermal exposure to total body burden

Process	Inhalation exposure		Dermal exposure		Combined exposure
	Inhalation exposure 8-hour TWA ($mg \cdot m^{-3}$)	Estimated body burden * ($mg \cdot kg^{-1} \cdot day^{-1}$)	Dermal exposure # ($mg \cdot day^{-1}$)	Estimated body burden + ($mg \cdot kg^{-1} \cdot day^{-1}$)	Combined body burden ($mg \cdot kg^{-1} \cdot day^{-1}$)
Manufacture of bisphenol-A	5	0.71	420	0.6	1.3
Manufacture of PC	$1 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	0.04	$6 \cdot 10^{-5}$	$1.6 \cdot 10^{-4}$
Manufacture of articles from PC	$1 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	0.04	$6 \cdot 10^{-5}$	$1.6 \cdot 10^{-4}$
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3
Powder coating manufacture	0.01	$1 \cdot 10^{-3}$	2	$3 \cdot 10^{-3}$	$4 \cdot 10^{-3}$
Powder coating use	0.5	0.07	2.3	$3 \cdot 10^{-3}$	0.07
Use of BPA in PVC manufacture	0.1	0.01	42	0.06	0.07
Thermal paper manufacture	0.1	0.01	42	0.06	0.07
Manufacture of tin plating additive	0.05	$7 \cdot 10^{-3}$	42	0.06	0.07
Manufacture of TBBA	$1.5 \cdot 10^{-5}$	$2 \cdot 10^{-6}$	$1.3 \cdot 10^{-3}$	$1.9 \cdot 10^{-6}$	$3.9 \cdot 10^{-6}$

* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m^3 air inhaled per working day.
\# Taking into account area exposed, as indicated in 4.1.1.2.1
\+ Assuming 10% absorption by the dermal route

These body burdens are heavily based on model calculations for dermal exposure, particularly as worst-case scenarios have been used that assume PPE is not being worn and there is maximum exposure to a substance that can cause skin reactions as a consequence of repeated skin exposure. However, refinements have been made on the basis of the estimated skin surface area that is likely to be exposed. For each of the exposure scenarios, using the estimated body burdens derived in the previous table, MOSs have been calculated and are presented in **Table 4.27**.

Table 4.27 Calculated margins of safety for each exposure scenario

Process	Liver toxicity MOS based on LOAEL	Conclusion	Reproductive toxicity (effects on fertility and development) MOS based on NOAEL*	Conclusion for effects on fertility	Conclusion for effects on development
Manufacture of bisphenol-A	90	**iii**	4	**iii**	**iii**
Manufacture of PC	$7.5 \cdot 10^5$	ii	$3 \cdot 10^4$	ii	i
Manufacture of articles from PC	$7.5 \cdot 10^5$	ii	$3 \cdot 10^4$	ii	i
Manufacture of epoxy resins	90	**iii**	4	**iii**	**iii**
Powder coating manufacture	$3 \cdot 10^4$	ii	1,250	ii	i
Powder coating use	1,700	ii	70	ii	i
Use of BPA in PVC manufacture	1,700	ii	70	ii	i
Thermal paper manufacture	1,700	ii	70	ii	i
Manufacture of tin plating additive	1,700	ii	70	ii	i
Manufacture of TBBA	$3 \cdot 10^6$	ii	$1 \cdot 10^6$	ii	i

Liver LOAEL = 120 mg/kg/day
Reproductive toxicity NOAEL = 50 mg/kg/day, for effects on fertility and on development
* Note that in determining the MOS for reproductive toxicity, the NOAEL has been reduced by a factor of 10 to 5 mg/kg/day to account for first pass metabolism.

Liver effects

Based on these calculations the manufacture of bisphenol-A and the manufacture of epoxy resins give a cause for concern for human health. However, these values need to be interpreted with great care because of the uncertainties regarding exposure, the relevance of these effects for humans and as the limited dose-response data does not allow for an estimation of where the NOAEL lies, as the response was found in most animals. Where the MOSs have been calculated to be very large >1,000, then even allowing for the many uncertainties, there would appear to be little cause for concern. However, for MOSs of 90, there remains some concern because the incidence of response in mice at the LOAEL of 120 mg/kg/day was high and the NOAEL may lie at a level some distance below this (i.e. a MOS based on a NOAEL could be much smaller). Thus, **conclusion (iii)** is reached for the manufacture of bisphenol-A and the manufacture of epoxy resins. **Conclusion (ii)** is reached for all other occupational scenarios.

Toxicity to reproduction

In relation to effects on fertility, based on these calculations, the manufacture of bisphenol-A and the manufacture of epoxy resins would give a cause for concern for human health and **conclusion (iii)** is reached. In all other cases the MOSs are larger (70 or greater) and give less cause for concern. This is because unlike for the liver, a NOAEL is used and comparisons made with the LOAEL for this endpoint would give MOSs an order of magnitude larger. In addition, the calculations of body burdens are based on modelled estimates of dermal exposure, which are very much worst-case predictions. Given these considerations, the MOS of 70 is concluded to be sufficiently large, even allowing for variations in toxicokinetics and toxicodynamics within and

between species. For these other occupational scenarios, **conclusion (ii)** is reached in relation to effects on fertility.

In relation to developmental effects, based on a provisional NOAEL of 50 mg/kg, the MOS of 4 for the manufacture of bisphenol-A and for the manufacture of epoxy resins is insufficient to provide reassurance and therefore **conclusion (iii)** is reached. For all other scenarios, the MOS is at least 70. In the light of the uncertainties surrounding the conflicting findings in studies investigating the developmental effects of bisphenol-A at low doses, it has been agreed by the member state Competent Authorities that further information should be obtained to resolve these uncertainties. Thus, **conclusion (i)** is reached for all other scenarios. Further information is required in relation to the potential for bisphenol-A to cause effects on development, particularly in the low-dose range (levels in the μg/kg region).

4.1.3.2.5 Conclusion for workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

The risk characterisation for workers leads to concerns for eye and respiratory tract irritation, effects on liver and toxicity for reproduction (effects on fertility and on development) during the manufacture of bisphenol-A and the manufacture of epoxy resins. In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact.

Conclusion (i) There is need for further information and/or testing.

In relation to developmental toxicity, in view of the uncertainties surrounding the potential for bisphenol-A to produce adverse effects at low doses (in the μg/kg range), the EU Competent Authorities have agreed that further work is required to resolve these uncertainties. Thus, conclusion (i) is reached for the manufacture of PC, manufacture of articles from PC, powder coating manufacture and use, and in the manufacture of PVC, thermal paper, tin plating additives and TBBA.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion is reached in relation to eye and respiratory tract irritation, effects on liver following repeated exposure and effects on fertility for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, manufacture of PVC, thermal paper manufacture, manufacture of tin plating additive and manufacture of TBBA. This conclusion is also reached in relation to repeated dose toxicity to the respiratory tract for all scenarios.

4.1.3.3 Consumers

The key health effects of concern for bisphenol-A are eye and respiratory tract irritation, skin sensitisation, repeated dose toxicity to the respiratory tract, effects on the liver following

repeated exposure and reproductive toxicity. The acute toxicity studies indicate that bisphenol-A is of low toxicity via the oral, dermal and inhalation routes and it is not a skin irritant. There are no concerns for mutagenicity and carcinogenicity.

The relevant routes of exposure for the consumer are inhalation, oral and dermal.

4.1.3.3.1 Eye and respiratory tract irritation

The potential for eye and respiratory tract irritation could arise as a result of consumer use of paints and varnishes containing bisphenol-A. In these applications, the concentration of bisphenol-A in the product is $\leq$ 0.0004%. At these concentrations, there is no concern that the irritating properties of bisphenol-A will be expressed and therefore there are no concerns for this endpoint and **conclusion (ii)** is reached.

4.1.3.3.2 Skin sensitisation

Dermal exposure to bisphenol-A, leading to potential concerns for skin sensitisation, can result from the consumer use of paints, varnishes, wood fillers and adhesives which contain bisphenol-A. In these applications, the concentration of bisphenol-A in the product is $\leq$ 0.0004%. At these concentrations, there is no concern that the sensitising properties of bisphenol-A will be expressed and therefore there are no concerns for this endpoint and **conclusion (ii)** is reached.

4.1.3.3.3 Repeated dose toxicity and effects on reproduction

Oral exposure

Potential concerns for repeated dose toxicity and for reproductive effects arise from those consumer exposure scenarios which involve repeated exposure to bisphenol-A. Scenarios for which exposures are single, relatively rare events (application of paints and varnish, use of wood fillers, exposures immediately following dental treatment) are not relevant in relation to concerns for these endpoints, and thus will not be considered further.

The sources of consumer exposure which could result in repeated exposure to bisphenol-A are food contact applications (infant feeding bottles; polycarbonate tableware; wine from epoxy-resin lined vats; canned food). In addition, the use of adhesives will be considered in relation to these endpoints, since although it is generally unlikely to be a daily event, some consumers may have relatively frequent use. With the exception of adhesives use, each of these scenarios results in oral exposure only; use of adhesives results in dermal exposure only.

Some of these sources will result in exposure to adult and/or infant or child consumers. **Table 4.28** gives calculations of body burdens for adult and/or infant and child consumers from sources involving oral exposure and MOSs for repeated dose toxicity and reproductive effects. Body burdens have been calculated using the following assumptions: oral absorption is 100%; an adult consumer weighs 70 kg; a young child (1.5-4.5 years) weighs 14.5 kg; a 1-2 month baby weighs 4.5 kg; a 4-6 month baby weighs 7 kg, an infant (6-12 months) weighs 8.7 kg. The estimates of body burdens arising for child consumers are derived using values that could represent a realistic worst-case scenario of the highest food and drink intake relative to bodyweight (2 kg intake, 14.5 kg bodyweight). Bodyweight values for adults and young children are based on UK data (HMSO, 1990; 1992; 1995).

Table 4.28 Calculated body burdens and MOSs for repeated dose toxicity and reproductive effects (oral exposure)

Source of exposure	Daily ingestion of bisphenol-A (mg)	Estimated body burden (mg/kg/day)	MOS		Conclusions		
			Liver toxicity [1]	Reproductive toxicity (effects on fertility and on development) [2]	Liver toxicity	Effects on fertility	Effects on develop-ment
Infant feeding bottles (1-2 month baby)	0.035	0.008	15,000	6,250	ii	ii	i
Infant feeding bottles (4-6 month baby)	0.050	0.007	17,000	7,100	ii	ii	i
Canned food (infant 6-12 months)	0.04	$5 \cdot 10^{-3}$	26,000	11,000	ii	ii	i
Polycarbonate tableware (young child, 1.5-4.5 years)	0.010	$7 \cdot 10^{-4}$	171,000	71,500	ii	ii	i
Canned food (young child 1.5-4.5 years)	0.200	0.014	8,500	3,600	ii	ii	i
Canned food (adult)	0.10	0.0014	84,000	35,000	ii	ii	i
Wine (adult)	0.500	0.007	17,000	7,000	ii	ii	i
Canned food + wine (adult)	0.600	0.009	14,000	6,000	ii	ii	i

1) Based on LOAEL of 120 mg/kg
2) Based on NOAEL of 50 mg/kg

The margins between exposures and the LOAELs are at least three orders of magnitude for liver toxicity and for reproductive toxicity, for all the exposure scenarios in **Table 4.28**.

These margins are considered not to give rise to concern for liver toxicity or for effects on fertility, for a number of reasons. The estimates of intake of bisphenol-A for scenarios involving polycarbonate tableware, canned food (for infants and young children) and wine, are based on worst-case estimates, in which all food and drink, including all the wine, is taken in from sources giving rise to potential bisphenol-A exposure; these are likely to overestimate actual intake. For infant feeding bottles, the assumption that all intake is from bottled sources is more realistic; for adults, the estimates of intake of canned food are also more realistic. With respect to liver effects, there are uncertainties in relation to the relevance of these effects for humans and the limited dose-response data does not allow for an estimation of where the NOAEL lies. However, MOS values of the magnitudes calculated (7,000 and above) are considered sufficient to allow for these uncertainties and the variation in toxicokinetics and toxicodynamics within and between species. In relation to effects on fertility, these margins are also considered sufficient to allow for the variation in toxicokinetics and toxicodynamics within and between species. **Conclusion (ii).**

However, in relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, **conclusion (i)** is reached for all scenarios, for developmental toxicity. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

Dermal exposure

In relation to potential exposure arising from the use of adhesives, exposure occurs only as a result of dermal contact. Based on the available information for dermal absorption, the contribution to total body burden arising from dermal exposure is calculated on the basis of 10 % uptake. In order to compensate for first pass metabolism, the NOAEL for reproductive toxicity has been adjusted by a factor of 10 for comparison with the dermal exposure estimates. The estimated exposures arising from the use of adhesives for an adult consumer and the resultant MOSs are shown in **Table 4.29**.

Table 4.29 Calculated body burdens and MOSs for repeated dose toxicity and reproductive effects as a result of use of adhesives (dermal exposure)

Exposure to bisphenol-A per event (mg)	Estimated body burden (mg/kg/day)	MOS		Conclusions		
		Liver toxicity 1)	Reproductive toxicity (effects on fertility and on development) 2)	Liver toxicity	Effects on fertility	Effects on development
0.014	$2 \cdot 10^{-5}$	$6 \cdot 10^{6}$	$2.5 \cdot 10^{5}$	ii	ii	i

1) LOAEL = 120 mg/kg/day
2) NOAEL = 5 mg/kg/day (allowing for first pass metabolism)

In relation to liver toxicity and effects on fertility, these MOSs are considered sufficient to allow for variation in toxicokinetics and toxicodynamics within and between species, particularly in view of the fact that adhesives use is likely to be an infrequent event. **Conclusion (ii)**.

In relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, **conclusion (i)** is reached. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

4.1.3.3.4 Conclusion for consumers

Conclusion (i) There is need for further information and/or testing.

Further information is required in relation to the potential for bisphenol-A to produce adverse effects on development.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

There are no concerns for all other endpoints, given that consumer exposure is very low.

4.1.3.4 Humans exposed via the environment

The key health effects are reproductive toxicity (effects on fertility and on development) and liver effects following repeated exposure. Irritation and sensitisation are of low concern where exposure is dissipated throughout the environment.

4.1.3.4.1 Regional exposure

In **Table 4.21**, the total daily human exposure to bisphenol-A via the environment is estimated to be $1.78 \cdot 10^{-5}$ mg/kg/day for regional sources. Comparisons of this intake estimate with the NOAELs and LOAELs for reproductive toxicity and liver effects respectively to derive MOSs are shown in the table below. Given the low levels of exposure for the regional scenario, these exposures are considered not to be of concern in relation to repeated exposure toxicity to the liver and effects on fertility and **conclusion (ii)** is reached.

In relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, **conclusion (i)** is reached for developmental toxicity. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Table 4.30 Risk characterisation for systemic effects following exposure via the environment (regional sources)

Exposure		Effect (systemic)					
		Reproductive effects NOAEL 50 mg/kg/day				Liver LOAEL 120 mg/kg/day	
Source	Value (mg/kg/day)	Effects on fertility		Effects on development			
		MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
Regional	$1.78.10^{-5}$	2.10^{6}	ii	2.10^{6}	i	6.10^{6}	ii

4.1.3.4.2 Local exposure

The human health systemic effects of concern include reproductive toxicity and effects to the liver. The highest local exposure is in the locality of plants producing PVC. Exposure is estimated to be 0.059 mg/kg/day. Comparisons of this intake estimate with the NOAELs and LOAELs for reproductive toxicity and liver effects respectively to derive MOSs are shown in **Table 4.31**.

Table 4.31 Risk characterisation for systemic effects following exposure via the environment (local sources)

Exposure		Effect (systemic)					
		Reproductive effects NOAEL 50 mg/kg/day				Liver LOAEL 120 mg/kg/day	
Source	Value (mg/kg/day)	Effects on fertility		Effects on development			
		MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
Local	0.059	800	ii	800	i	2,000	ii

The lowest MOSs are for reproductive toxicity. In relation to effects on fertility, this MOS is considered to be acceptable, given that the exposure estimate is the highest value obtained for all local scenarios, and it is based on modelled rather than measured data, which will overestimate actual exposures. Similarly, the MOS of 2,000 for repeated exposure toxicity to the liver is considered to be acceptable. Overall, **conclusion (ii)** is reached for effects on fertility and for liver toxicity in this scenario.

In relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, **conclusion (i)** is reached for developmental toxicity. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

4.1.3.4.3 Conclusion for humans exposed via the environment

Conclusion (i) There is need for further information and/or testing.

This conclusion is reached in relation to developmental toxicity. Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion applies for all other endpoints in relation to local and regional exposure.

4.1.3.5 Combined exposure

The worst-case combined exposure would be for someone who is exposed via the regional/local environment near to a PVC production plant, and who is also exposed via food contact materials as described in Section 4.1.1.3.

The exposures for these component parts are presented in **Table 4.32**. In this table, comparisons are made with the LOAEL of 120 mg/kg for liver effects and with the NOAEL of 50 mg/kg for

reproductive effects. Exposures are primarily via the oral route and therefore are compared directly with the oral NOAELs.

Table 4.32 Conclusions for reproductive effects and liver toxicity, for combined exposure scenarios

Exposure		Effect (systemic)					
		Reproductive effects NOAEL 50 mg/kg/day				Liver LOAEL 120 mg/kg/day	
Source	Value	Effects on fertility		Effects on development			
	(mg/kg/day)	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
Regional	$9 \cdot 10^{-3}$	5,500	ii	5,500	i	$1 \cdot 10^{4}$	ii
Local	0.069	725	ii	725	i	1,700	ii

Conclusion (i) There is need for further information and/or testing.

In relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, **conclusion (i)** is reached for developmental toxicity. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

The MOSs for effects on fertility and for liver effects are considered to be sufficient to provide reassurance that adverse health effects would not occur.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

The physico-chemical properties of bisphenol-A are available from the literature although the exact values for end points such as vapour pressure can be difficult to verify. There will be slight variation in values quoted by manufacturers according to the nature of the material they produce. Given the low vapour pressure at normal temperatures, lack of flammability and the general stability, the risks arising from the physico-chemical properties are small. In common with many organic materials, the finely powdered material is a significant dust explosion hazard (Grossel, 1988). However, this appears to be well known within the manufacturing industry and it is considered that there are adequate controls for this risk in place. Given the controls used during manufacture and use the risk from this is small. Overall, the risk from physico-chemical properties is low.

5 RESULTS

5.1 INTRODUCTION

There are four companies that manufacture bisphenol-A in four countries within the EU: Germany, The Netherlands, Belgium and Spain. Total production within the EU is estimated to be about 700,000 tonnes per annum (based on data up to 1999). Allowing for imports and exports, a representative EU consumption of bisphenol-A is estimated to be approximately, 690,000 tonnes/year.

It is primarily used in the production of polycarbonate and epoxy resins, and there are a number of minor uses in the thermal paper and PVC industries. Polycarbonates are used in a range of applications including optical media, glazing, food containers and as polycarbonate blends in the electronics industry. Epoxy resins are used as protective coatings, structural composites, electrical laminates, electrical applications and adhesives.

5.2 ENVIRONMENT

Bisphenol-A is a solid of low vapour pressure ($5.3 \cdot 10^{-9}$ kPa) with a water solubility of ~300 mg/l at 20°C and a log octanol-water partition coefficient (log K_{OW}) of 3.4. Hydrolysis and photolysis in water are negligible but it is considered readily biodegradable, possibly with a short period of adaptation. The log K_{OW} value implies a low to moderate bioaccumulation potential in aquatic species and moderate adsorption to soils and sediment. The substance chiefly partitions to water and it may be relatively mobile in the environment. The main route of environmental exposure is from its use in the thermal paper and PVC industries.

Aquatic toxicity data are reported for freshwater and marine fish, invertebrates and algae. The available data cover 'conventional' endpoints (such as reproduction and mortality), and non-conventional ones, such as endocrine disruption effects. The available data suggest that endocrine disruption may be the most sensitive endpoint.

The lowest values from acute studies with freshwater species are: 96-hour LC_{50} of 4.6 mg/l for fish (fathead minnow *Pimephales promelas*) (results for saltwater species are similar); 48-hour EC_{50} of10.2 mg/l for *Daphnia magna* (based on measured concentrations – a lower value of 3.9 mg/l is reported based on nominal concentrations, and a 96-hour LC_{50} of 1.1 mg/l is reported for the saltwater mysid *Mysidopsis bahia*); 96-hour EC_{50} (based on cell count) of 2.73 mg/l for algae (*Selenastrum capricornutum*) (a 96-hour EC_{50} (based on cell count) of 1.1 mg/l is reported for marine algae (*Skeletonema costatum*)).

Chronic studies are also reported for fish, invertebrates and algae. The lowest NOEC value for a "conventional" endpoint from chronic studies is that for egg hatchability in *Pimephales promelas* from a full life cycle test, at 16 µg/l. The lowest values from chronic studies for invertebrates and algae are a 21-day NOEC >3.146 mg/l for *Daphnia magna* and a 96-hour $EC_{10}$of 0.40 mg/l for *Skeletonema costatum*. No effects on larval growth, development or sexual differentiation were reported for the African clawed frog (*Xenopus laevis*) at nominal concentrations up to 0.5 mg/l in a 90-day flow-through study. Based upon the endpoint for fish a PNEC of 1.6 µg/l is derived using an assessment factor of 10. A $PNEC_{sediment}$ of 26 µg/kg wet weight (60 µg/kg dry weight) can be derived from this using the equilibrium partitioning method.

Other effects at lower concentrations have been reported for fish (LOEC of 1 µg/l for effects on *P. promelas* spermatogenesis) and aquatic snails (effects on egg production). Both of these areas

require further work in order to clarify the levels at which effects may occur, and to consider the significance of these effects. As a preliminary approach, an assessment factor of 10 is applied to the LOEC of 1 µg/l derived for effects on spermatogenesis in fathead minnows to give a "conservative" PNEC of 0.1 µg/l.

A $PNEC_{microorganisms}$ of 320 mg/l has been derived from a NOEC based on cell growth of ≥320 mg/l for *Pseudomonas putida*.

Toxicity data for soil-dwelling organisms are not available, but a $PNEC_{soil}$ of 23 µg/kg wet weight can be derived from the 'conventional' aquatic PNEC using the equilibrium partitioning method for screening risk assessment purposes.

There are no known biotic or abiotic effects of bisphenol-A in the atmosphere, and in particular effects on plants due to atmospheric exposure are unknown. Based on structural considerations, it is unlikely to be an ozone depleter or greenhouse gas, nor is it thought to contribute to low-level ozone formation. It is therefore not possible to derive a PNEC.

A PNECoral of 33 mg/kg food has been derived for the secondary poisoning assessment from a NOAEL of 50 mg/kg body weight (based on a reduction in litter size) from a three-generation multi-dose level feeding study in rats.

Results

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the following scenarios for the water and sediment compartments:

Thermal paper recycling
Use as an inhibitor in PVC production
Preparation of additive packages for PVC processing
Use as an anti-oxidant in the production of plasticisers for use in PVC processing.

For these uses further refining the PNEC for water will not change the outcome of the assessment. Although these scenarios are referred to as generic in the exposure section, the PEC estimates are based on data from the industry and use areas and are considered representative. It appears unlikely that the provision of further information would alter the conclusions. The use of bisphenol-A in the manufacture of PVC resin is due to be phased out in Europe by the end of 2001 under a voluntary agreement by industry.

Conclusion (i) There is need for further information and/or testing.

This conclusion applies to the following scenarios for the water and sediment compartments:

Bisphenol-A production [7]
Epoxy resin production
Thermal paper production
Phenoplast cast resin processing

[7] Four uses only take place on sites where bisphenol-A is produced. Emissions from these processes are included in the site-specific emissions for bisphenol-A production and so are not separately identified. These are:
Polyol/polyurethane production
Brake fluid manufacture
Polyamide production
Polycarbonate production

Use as a anti-oxidant in PVC processing
Use as a plasticiser in PVC processing
Regional concentration

These scenarios do not give rise to a risk when the PNEC based on the standard endpoint of egg hatchability is used. However, if a "conservative" PNEC based on research studies indicating effects on snails and sperm development in fish is used, all scenarios and the regional concentration give rise to a risk. There is considerable uncertainty over the validity of the lower PNEC. Recent research studies on snails have raised the possibility of effects at still lower concentrations. If these studies were to be used as the basis for a PNEC derivation, the much lower value would have implications for possible risk reduction measures. It is therefore considered that further studies on the toxicity of bisphenol-A to snails are needed, to provide a more robust basis for the derivation of a PNEC. The re-investigation of the effects on sperm development in fish is also required. The apparently elevated levels measured in sediment will also be considered when the aquatic assessment is refined.

Conclusion (i) also applies to the following uses of bisphenol-A for the terrestrial compartment:

Epoxy resin production
Phenoplast cast resin processing
Thermal paper recycling
Use as an inhibitor in PVC production
Preparation of additive packages for PVC processing
Use as an anti-oxidant in the production of plasticisers for use in PVC processing
Use as an anti-oxidant in PVC processing
Use as a plasticiser in PVC processing
Regional concentration

The equilibrium partitioning method has been used, so testing on terrestrial organisms could revise the PNEC. It is currently not clear what testing would be appropriate, as the most sensitive effects in aquatic organisms appear to be related to endocrine disruption. It is proposed to await the outcome of the further work on aquatic organisms before deciding on testing for the terrestrial compartment. In addition, the UK Department of Environment, Food & Rural Affairs is conducting research into endocrine disruption in the earthworm *Eisenia andrei* and bisphenol-A is one of the test compounds. The project aim is to develop molecular markers of exposure to, and population level effects of, endocrine disruption for use in field and laboratory studies. It is expected that this work will provide relevant information, to a timescale compatible with that of the aquatic tests.

A revision of the PNECoral value will also be considered if additional information on the interpretation of mammalian developmental data becomes available as a result of further studies being conducted for the human health assessment.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

This conclusion applies to microorganisms in wastewater treatment plants and to the air compartment for all scenarios. It also applies to the terrestrial compartment for the following:

Bisphenol-A production
Thermal paper manufacture

This conclusion also applies to the water, sediment and terrestrial compartments for the following uses:

Unsaturated polyester production
Can coating production
Tyre manufacture
Alkoxylated bisphenol-A production
Tetrabromobisphenol-A production and use
Phenoplast cast resin production

For these six scenarios, emissions are negligible and PECs have not been calculated in this assessment (these processes are either completely dry, or any aqueous effluent produced is disposed of through incineration).

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

The key health effects of exposure to bisphenol-A are eye irritation, respiratory tract irritation, skin sensitisation, repeated dose toxicity to the respiratory tract, effects on the liver and reproductive toxicity (effects on fertility and on development). No dose-response information is available on eye irritation. A NOAEL of 10 mg/m^3 has been identified for repeated dose toxicity to the respiratory tract. A LOAEL of 120 mg/kg has been identified for liver effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg/day has been established in a multigeneration study for effects on fertility and on development.

5.3.1.1 Workers

For the effects of eye irritation and local effects to the respiratory tract from repeated inhalation exposure, risk reduction measures are required for the manufacture of bisphenol-A and for the manufacture of epoxy resin.

For skin sensitisation, there are concerns for all exposure scenarios where there is the potential for skin contact.

In relation to effects on the liver following repeated exposure, effects on fertility and effects on development, the risk characterisation has identified concerns for workers exposed during the manufacture of bisphenol-A and the manufacture of epoxy resins.

In relation to developmental toxicity, in view of the uncertainties surrounding the potential for bisphenol-A to produce adverse effects at low doses (in the μg/kg range), the EU Competent Authorities have agreed that further work is required to resolve these uncertainties. Thus, conclusion (i) is reached for the manufacture of PC, manufacture of articles from PC, powder coating manufacture and use, and in the manufacture of PVC, thermal paper, tin plating additives and TBBA.

Results

Conclusion (iii) There is a need for limiting the risks; risk reduction measure which are already being applied shall be taken into account

This conclusion applies to the manufacture of bisphenol-A and the manufacture of epoxy resins, in relation to concerns for eye and respiratory tract irritation, effects on liver and toxicity for reproduction (effects on fertility and on development). In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact.

Conclusion (i) There is need for further information and/or testing.

This applies to the manufacture of PC, manufacture of articles from PC, powder coating manufacture and use and the manufacture of PVC, thermal paper, tin plating additives and TBBA.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This conclusion is reached in relation to eye and respiratory tract irritation, effects on liver following repeated exposure and effects on fertility for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, manufacture of PVC, thermal paper manufacture, manufacture of tin plating additive and manufacture of TBBA. This conclusion is also reached in relation to repeated dose toxicity to the respiratory tract for all scenarios.

5.3.1.2 Consumers

For eye and respiratory tract irritation and for skin sensitisation, exposure is very low and it is concluded that there is no concern for these endpoints. For repeated dose toxicity to the liver and for effects on fertility, conclusion (ii) is reached for all exposure scenarios.

In relation to effects on development, in view of the uncertainties surrounding the potential for bisphenol-A to produce adverse effects at low doses (in the μg/kg range), the EU Competent Authorities have agreed that further work is required to resolve these uncertainties. Thus, conclusion (i) is reached for this endpoint, for all exposure scenarios.

Results

Conclusion (i) There is need for further information and/or testing.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This conclusion applies to all consumer exposure scenarios in relation to eye and respiratory tract irritation, skin sensitisation, liver effects following repeated exposure and effects on fertility.

5.3.1.3 Humans exposed via the environment

The key health effects are reproductive toxicity (effects on fertility and on development) and liver effects following repeated exposure. Irritation and sensitisation are of low concern where exposure is dissipated throughout the environment.

Given the low levels of exposure for both the regional and local exposure scenarios, there are no concerns for repeated exposure toxicity to the liver or for effects on fertility and conclusion (ii) is reached.

In relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, conclusion (i) is reached for developmental toxicity. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

Results

Conclusion (i) There is need for further information and/or testing.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This applies to both regional and local exposure scenarios in relation to liver effects following repeated exposure and effects on fertility.

5.3.1.4 Combined exposure

The worst-case combined exposure would be for someone who is exposed via the regional/local environment near to a PVC production plant, and who is also exposed via food contact materials.

The MOSs for effects on fertility and for liver effects are considered to be sufficient to provide reassurance that adverse health effects would not occur.

In relation to effects on development, in light of the uncertainties surrounding the potential for bisphenol-A to produce effects at low doses, in the μg/kg range, the EU Competent Authorities have agreed that further information is required to resolve these uncertainties. Thus, conclusion (i) is reached for developmental toxicity. More information on the potential for bisphenol-A to cause developmental effects, particularly in the μg/kg range, is required.

Results

Conclusion (i) There is need for further information and/or testing.

Further research is needed to resolve the uncertainties surrounding the potential for bisphenol-A to produce adverse effects on development at low doses. At the 29th Technical Meeting (18-21st September 2001), it was decided to set up a Steering Committee to further develop the details of the requirement for further research.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

This conclusion is reached in relation to liver effects following repeated exposure and effects on fertility.

5.3.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

There are no significant risks from physico-chemical properties.

6 REFERENCES

Aardema MJ, Albertini S, Arni P, Henderson LM, Kirsch-Volders M, Mackay JM, Sariff AM, Stringer SA and Taalman RDF (1998). Aneuploidy: A Report of an ECETOC Task Force. Mutat Res. **410**, 3-79.

Alexander HC, Bartlett EA, Boggs GU (1985a). Bisphenol-A: Flow-Through Acute Toxicity to the Fathead Minnow. Dow Company Report: ES-764.

Alexander HC and Batchelder TL (1975). The Pollutional Evaluation of Compounds. Dow Chemical Company. Laboratory Report Code ES-6.

Alexander HC, Dill DC, Milazzo DP, Boggs GU (1985b). Bisphenol-A: Algal Toxicity Test. Dow Company Report: ES-762. 1985.

Alexander HC, Dill DC, Smith LW, Guiney PD and Dorn P (1988). Bisphenol-A: Acute aquatic toxicity. Environmental Toxicology and Chemistry **7**, 19-26.

Alexander HC, Milazzo DP and Boggs GU (1985c). Bisphenol-A: Daphnid Static Acute Toxicity Test. Dow Company Report. ES-763.

Allen H and Kaidbey K (1979). Persistent photosensitivity following occupational exposure to epoxy resins. Arch. Dermatol. **115**, 1307-1310.

Andersen HK, Wollenberger L, Halling-Sorensen B and Kusk KO (2001). Development of copepod nauplii to copepodites – A parameter for chronic toxicity including endocrine disruption. Environmental Toxicology and Chemistry **20(12)**, 2821-2829.

Angelini G, Rigano L, Foti C, Grandolfo M, Vena GA, Bonamonte D, Soleo L and Scorpiniti AA (1996). Occupational sensitisation to epoxy resin and reactive diluents in marble workers. Contact Dermatitis **35**, 11-16.

Arenholt-Bindslev, Breinholt V, Preiss A and Schmaltz G (1999). Time-related bisphenol-A content and estrogenic activity in saliva samples collected in relation to placement of fissure sealants. Clin. Oral Invest. **3**, 120-125.

Ashby J and Odum J (1998). The importance of protocol design and data reporting to research on endocrine disruption. Environ. Health Perspect. **106**, 315-316.

Ashby J and Tinwell H (1998). Uterotrophic activity of bisphenol-A in the immature rat. Environ Health Perspect. **106**, 719-720.

Ashby J, Tinwell H and Haseman J (1999). Lack of effects for low dose levels of bisphenol-A and diethylstilbestrol on the prostate gland of CF1 mice exposed *in utero*. Reg. Toxicol. and Pharmacol. **30**, 156-166.

Ashby J and Lefevre PA (2000). The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators. J. Appl. Toxicol. **20**, 35-47.

Atkinson A and Roy D (1995a). *In vivo* DNA adduct formation by bisphenol-A. Environ. Mol. Mutagen. **26**, 60-66.

Atkinson A and Roy D (1995b). *In vitro* conversion of environmental estrogenic chemical bisphenol-A to DNA binding metabolite(s). Biochem. Biophys. Res. Comm. **210**, 424-433.

Bayer AG (1988). Manufacturers Safety Data Sheet.

Bayer AG (1989). Manufacturers Safety Data Sheet.

Bayer AG (1993). Manufacturers Safety Data Sheet.

Bayer AG (1996). Studies on the ecological behaviour of bisphenol-A. Study Number 600 A/96.

Bayer AG (1999a). Fish, prolonged toxicity test (*Brachydanio rerio*) 14 day study of Bisphenol-A. Study Number 707 A/98 Fl.

Bayer AG (1999b). Fish, juvenile growth test (*Oncorhynchus mykiss*) of Bisphenol-A. Study Number: 707 A/98FF.

Bayer AG (1999c). Bisphenol-A monomer in milk from returnable polycarbonate bottles. Unpublished test data.

Belfroid AC, Murk AJ, Voogt P de, Schäfer AJ, Rijs GBJ and Vethaak AD (1999). Hormoonontregelaars in water. Orienterende studie naar de aanwezigheid van oestrogeen-actieve stoffen in watersystemen en afvalwater in Nederland. Institute for Inland Water Management and Waste Water (RIZA), Vol. RIKZ-rapport 99.007/99.024, Lelystadt, The Netherlands.

Berg C, Halldin K, Brunström B (2000). Effects of bisphenol-A and tetrabromobisphenol-A on sex organ development in quail and chicken embryos. Department of Environmental Toxicology, Evolutionary Biology Centre, Uppsala University, Sweden.

Biegel LB, Flaws JA, Hirshfield AN, O'Connor JC, Elliott GS, Ladics GS, Silbergeld EK, Van Pelt CS, Hurtt ME, Cook JC and Frame SR (1998). 90-Day feeding and one-generation reproduction study in Crl:CD BR rats with 17β-estradiol. Toxicol. Sci. **44**, 116-142.

Biles JE, McNeal TP, Begley TH and Hollifield HC (1997). Determination of bisphenol-A in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. J.Agric. Food Chem. **45**, 3541-3544.

Billard R, Breton B, Richard M (1981). On the inhibitory effect of some steroids on spermatogenesis in adult rainbow trout (*Salmo gairdneri*) Can. J. Zool. **59**, 1479-1487.

Bitman J and Cecil HC (1970). Estrogenic activity of DDT analogs and polychlorinated biphenyls. J. Agr. Food Chem. **18**, 1108-1112.

Bolon B, Bucci TJ, Warbitton AR, Chen JJ, Mattison DR and Heindel JJ (1997). Differential follicle counts as a screen for chemically induced ovarian toxicity in mice: results from continuous breeding bioassays. Fund. Appl. Toxicol. **39**, 1-10.

Bond GP, McGinnis PM, Cheever KL, Harris SJ, Plotnick HB and Niemeier RW (1980). Reproductive effects of bisphenol-A. Society of Toxicology 19th Annual Meeting, March 9-13, Washington D.C., Abstracts of papers: A23.

Bornmann G and Loeser A (1959). Monomer-Polymer Studie uber Kunststoffe aus ε - Caprolactam oder Bisphenol. Arzneim. Forsch. **9**, 9-13.

Boutrup S et al. (1998). Environmental hazardous substances in the County of Aarhus. Environmental Division Hojbjerg, Aarhus County, Denmark, p. 16.

Bowmer T and Gimeno S (2001). The Effects of Bisphenol-A on the Development of the Male Carp Reproductive Tract When Exposed During Sexual Differentiation – Implications for Test Design and Risk Assessment. Unpublished TNO Report.

Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V and Olea N (1995). Xenoestrogens released from lacquer coatings in food cans. Environ. Health Perspect. **103**(6), 608-612.

Bruze M and Zimerson E (1985). Contact allergy to dihydroxydiphenyl methanes (bisphenol-F). Dermatosen Beruf Umwelt **33**, 216-220.

BUA (1995). BUA Report 203. Bisphenol-A (2,2-Bis-(4-hydroxy-phenyl)propane). German Chemical Society.

Cagen SZ, Waechter JM, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE and Harris LR (1999a). Normal reproductive organ development in Wistar rats exposed to bisphenol-A in the drinking water. Reg. Tox. Pharmacol. **30**, 130-139.

Cagen SZ, Waechter JM, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE and Harris LR (1999b). Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol-A. Toxicol. Sci. **50**, 36-44.

Campbell NR (1940). Molecular structure in relation to oestrogenic activity: derivatives of 4:4'-dihydroxydiphenylmethane. Proc. Roy. Soc., Ser B **129**, 528-538.

Carreon RE (1982). Bisphenol-A Polycarbonate: Acute Toxicological Properties and Industrial Handling Hazards. Dow Chemical Company Unpublished Report.

Caspers N (1998). No estrogenic effects of bisphenol-A in Daphnia magna STRAUS. Bull. Environ. Contam. Toxicol. **61**, 143-148.

CEFAS (2002). Sampling for Priority Chemicals in the Environment. Confidential Report for UK Department for Environment, Food and Rural Affairs (DEFRA). Report No C1337.

Central Toxicology Laboratory (1999a). Bisphenol-A: Uterotrophic Assay in Immature Rats (Oral Dosing). CTL Unpublished Report No: CTL/P/6029.

Central Toxicology Laboratory (1999b). Bisphenol-A: Uterotrophic Assay in Immature Rats (Subcutaneous Dosing). CTL Unpublished Report No: CTL/P/5943.

Chahoud I, Werner W and Faqi IS (1999). Determination and kinetics of bisphenol-A in dams and fetuses after multiple exposure to pregnant rats. Nann. Schm. Arch. Pharmacol. 359, 663, Abstract of papers: 661.

Chemical Compound Safety Research Institute (2000). Two-Generation Reproduction Study of Bisphenol-A in Rats. Chemical Compound Safety Research Institute Unpublished Report - Study No: SR-98101.

Clark LB, Rosen RT, Hartman TG, Alaimo LH, Louis JB, Hertz C, Ho CT, Rosen JD (1991b). Determination of nonregulated pollutants in three New Jersey publicly owned treatment works (POTWs). Research Journal WPCF **63**(2), 104-113.

Clark LB, Rosen RT, Hartman TG, Louis JB and Rosen JD (1991a). Application of particle beam LC/MS for the analysis of water from publicly owned treatment works. Inter. J. Environ. Anal. Chem. **45**, 169-178.

Coldham NG, Dave M, Sivapathasundaram S, McDonnel DP, Connor C and Sauer MJ (1997). Evaluation of a recombinant yeast cell estrogen screening assay. Environ. Health Perspect. **105**, 734-742.

Colerangle JB and Roy D (1995). Perturation of cell cycle kinetics in the mammary gland by stilbene estrogen, diethylstilbestrol (DES). Cancer Lett. **94**, 55-63.

Colerangle JB and Roy D (1997). Profound effects of the weak environmental estrogen-like chemical bisphenol-A on the growth of the mammary gland of Noble rats. J. Steroid Biochem. Molec. Biol. **60**(1-2), 153-160.

CRC (1995). Handbook of Chemistry and Physics. 75th Edition.

Cummings A (1997). Estrogenicity of bisphenol-A and octylphenol measured via the delayed implanting rat model. Biology of Reproduction, **56**(Suppl 1), 146 (Abstract 256).

Dean BJ and Brooks TM (1978). Toxicity Tests with Diphenylol Propane (DPP): *In vitro* Mutation Studies. Shell Research Report TLGR.0111.78.

del Olmo M, González-Casado A, Navas NA and Vilches JL (1997). Determination of bisphenol A (BPA) in water by gas chromatography-mass spectrometry. Analytica Chimica Acta 346, 87-92.

DIPPR (1994). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis.

Dodds EC and Lawson W (1936). Synthetic oestrogenic agents without the phenanthrene nucleus. Nature **137**, 996.

Dodds EC and Lawson W (1938). Molecular structure in relation to oestrogenic activity. Compounds without a phenanthrene nucleus. Proc. Roy. Soc. (London) Series B**125**, 222-232.

Dodge JA, Glasebrook AL, Magee DE, Philips DL, Sato M, Short LL and Bryant HU (1996). Environmental estrogens: Effects on cholesterol lowering and bone in the ovariectomised rat. J. Steroid Biochem. Molec. Biol. **59**, 155-161.

Dorn PB, Chou CS and Gentempo JJ (1987). Degradation of bisphenol-A in natural waters. Chemosphere **16**(7), 1501-1507.

Dow Chemical Company (1957). Results of the Range Finding Toxicological Tests on Bisphenol-A - Regular Grade and Bisphenol-A - E.R. Grade. Unpublished Report.

Dow Chemical Company (1985). Purity Determination of Bisphenol-A. Unpublished Report.

Dow (1984). Bisphenol-A Biodegradability Data. Internal Company Report.

Dow (1988). Bisphenol-A: Determination of Acute Bacterial Toxicity. Internal Company Report.

Du Pont (1962). Summary of toxicological tests on bisphenol-A. Letter from Rowe VK., Dow Chemical Company to Clayton JW, Du Pont dated 2/05/1962. EPA/OTS Document #878214650; Order No.206607 (NTIS), 1-3.

Eadsforth CV (1983). Diphenylol Propane: Determination of the n-Octanol/Water Partition Coefficient Using a Reverse-Phase HPLC Method. Shell Company Report: SBGR.83.104.

Earls AO, Clay CA and Braybrook JH (2000). Preliminary Investigation into the Migration of Bisphenol-A from Commercially-Available Polycarbonate Baby Feeding Bottles. LGC Technical Report LGC/DTI/2000/005.

EC (1996). Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk assessment for new notified substances and Commission Regulation (EC) 1488/94 on Risk assessment for existing substances. Parts 1-4. European Commission (EC), Office for Official Publications of the EC, Luxembourg.

EC (2002). Draft Environmental Risk Assessment Report for Sodium Hypochlorite, May 2002 version. Rapporteur Country: Italy. European Commission, European Chemicals Bureau, http://ecb.jrc.it/.

Elsby R, Maggs JL, Ashby J and Park KB (2001). Comparison of the modulatory effects of human and rat liver microsomal metabolism on the estrogenicity of bisphenol-A: implications for extrapolations to humans. J. Pharmacol. Exp. Ther. **297**, 103-113.

Elswick BA, Welsch F and Janszen DB (2001a). Effect of different sampling designs on outcome of endocrine disrupter studies. Reproductive Toxicology **14**, 359-367.

Elswick BA, Miller FJ and Welsch F (2001b). Comments to the editor concerning the paper entitiled "Reproductive malformation of the male offspring following maternal exposure to oestrogenic chemicals" by C. Gupta. Exp. Biol. Med. **226**, 74-75.

Emmitte JA (1978). The Acute Toxicity of Parabis A and Bisphenol-A to the Sheepshead Minnow, *Cyprinodon variegatus*. Dow Company Report, TWC 102.

Estlander T, Jolanki R, Henriks-Eckerman M and Kanerva L (1999). Occupational contact allergy to bisphenol-A. Contact Dermatitis **40**, 52-53.

Eyre and Spottiswood (1965). London.

Feldman D and Krishnan A (1995). Estrogens in unexpected places: Possible implications for researchers and consumers. Environ. Health Perspect. **103**, 129-133.

Fennell TR, Snyder RW, Maness SC, Gaido KW, Sumner SCJ and Welsch F (2000). Metabolism and disposition of bisphenol-A in female rats. The Toxicologist **54**, 382, Abstracts of papers: 1740.

Fenske M, Maack G, Schäfers C and Segner H (2001). Assessment of estrogenic effects on reproduction and sexual differentiation in zebrafish (*Danio rerio*): Alterations in vitellogenesis and reproductive physiology. Poster presentation.

Ferracane JL and Condon JR (1990). Rate of elution of leachable components from composite. Dent. Mater. **6**, 282-287.

Fisher JS, Turner KJ, Brown D and Sharpe RM (1999). Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. Environ. Health Perspect. **107**, 397-405.

Flammarion P, Brion F, Babut M, Garric J, Migeon B, Noury P, Thyband E, Tyler CR, Palazzi X (2000). Induction of fish vitellogenin and alterations in testicular structure: preliminary results of estrogenic effects in chub (*Leuciscus cephalus*). Ecotoxicology **9**, 127-135.

Foureman P, Mason JM, Valencia R and Zimmering S (1994). Chemical mutagenesis testing in *Drosophila. X.* Results of 70 coded chemicals tested for the National Toxicology Program. Environ. Mol. Mutagen. **23**, 208-227.

Freeman K and Warin AP (1984). Contact dermatitis due to bisphenol-A in semi-synthetic waxes. Contact Dermatitis **11**, 259-260.

Fregert S and Rorsman H (1960). Hypersensitivity to diethylstilbesterol. Acta. Derm.-Venereol. **40**, 206-209.

Fregert S and Rorsman H (1961). Allergy to a carbon-functional organic silicon compound, dimethyldi-(4-hydroxyphenyl)-silane. Nature **192**, 989-990.

Fregert S and Rorsman H (1962). Hypersensitivity to epoxy resins with reference to the role played by bisphenol-A. J. Invest. Dermatol. **39**, 471-472.

Fritz WA and Lamartiniere CA (1999). The influence of lifetime exposure to dietary bisphenol-A on the male rat reproductive tract. The Toxicologist **48**, 46, Abstracts of papers: 217.

Fromme H, Otto T, Pilz K, Lahrz T and Führling D (1998). Expositionsmonitoring endokrin wirksamer Substanzen in verschiedenen Umweltkompartimenten. Umweltbundesamt, Institut für Umweltanalytik und Humantoxikologie: Umweltforschungsplan, UFOPLAN 216 02 001/12. Berlin, p. 79.

FSA (2001). Survey of bisphenols in canned foods. Food Standards Agency UK (FSA), Food Surveillance Information Sheet Number 13/01. http://www.foodstandards.gov.uk/fsainfsheet/2001/no13/13bisp.htm#summary.

Furun L, Boquing L, Zheyong S, Zheng L, Yayan Y and Yurong X (1990). Treatment of wastewater containing bisphenol-A. Water Treatment **5,** 105-124.

Fung EYK, Ewoldsen NO, St Germain HA et al. (2000). Pharmacokinetics of bisphenol-A released from a dental sealant. JADA **131**, 51-58.

Furukawa F, Nishikawa A, Mitsui M, Sato M, Suzuki J, Imazawa T and Takahashi M (1994). A 13-week subchronic toxicity study of bisphenol-A in B6C3F1 mice. Eisei Shikensho Hokoku **112**, 89-96.

Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ and McDonnell DP (1997). Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol. Applied Pharmacol. **143**, 205-212.

Gage JC (1970). The subacute inhalation toxicity of 109 industrial chemicals. Brit. J. Industr. Med. **27**, 1-18.

Gandrass J (1999). Bisphenol A (BPA) in der Wasserphase der Elbe - Elbe-Längsprofil im September 1998. GKSS-Forschungszentrum Geesthacht GmbH, Institut für Physikalische und Chemische Analytik, Organische Spurenanalytik, personal communication on 31 March 1999, Geesthacht.

Garrod ANI, Guiver R and Rimmer DA (2000). Potential exposure of amateurs (Consumers) through painting wood preservative and antifoulant preparations. Ann. Occup.Hyg. **44**, 421-426.

Gary MA and Metcalfe CD. (1997) Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to p-nonylphenol. Environ. Tox. Chem. **16**, 1082-1086.

Gaul JC (1960). Sensitivity to bisphenol-A. Arch. Dermatol. **82**, 1003.

General Electric (1976a). Bisphenol-A: Fourteen Day Range Finding Study in Rats. Unpublished Report of General Electric (IRDC study 313-076).

General Electric (1976b). Bisphenol-A: Nineteen Day Oral Toxicity Study in Dogs. Unpublished Report of General Electric (IRDC study 313-079).

General Electric (1976c). Reproductive and Ninety Day Oral Toxicity Study in Rats. Unpublished Report of General Electric (IRDC study 313-078).

General Electric (1978). Reproductive and Ninety Day Oral Toxicity Study in Rats. Unpublished Report of General Electric (IRDC study 313-112).

Gerberick GF and Ryan CA (1990). A predictive mouse-ear swelling model for investigating topical photoallergy. Food. Chem. Toxicol. **28**, 361-368.

Geurtsen W, Spahl W and Leyhausen G (1999). Variability of cytotoxicity and leaching of substances from four light-curing pit and fissure sealants. J. Biomed Mater. Res. **44**, 73-77.

Gill WH, Schumacher FB, Bibbo M, Straus FH, Schoenberg HW (1979). Association of diethylstilbesterol exposure *in utero* with cryptorchidism, testicular hyperplasia, and semen abnormalities. J. Urol. **122**, 36-39.

Gold LS, Sawyer CB, Magaw R, Backman GM, De Veciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, Pike MC and Ames BN (1984). A carcinogen potency database of the standardised results of animal bioassays. Environ. Health Perspect. **58**, 9-319.

Goloubkova T, Flavia MM, Rodrigues RLP, Cecconello AL and Spritzer PM (2000). Effects of xenooestrogen bisphenol-A on uterine and pituitary weight, serum prolactin levels and immunoreactive prolactin cells in ovariectomised Wistar rats. Arch. Toxicol. **74**, 92-98.

Gould JC, Liaw JJ, Elswick BA, Stedman DB, Turner M and Welsch F (1998a). Maternal effects and secretion into milk of low doses of bisphenol-A given to rats via drinking water. Toxicologist **42**, 175, Abstracts of papers: 866.

Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP and Gaido KW (1998b). Bisphenol-A interacts with the estrogen receptor alpha in a distinct manner from estradiol. Molec. Cell Endocrinol. **142**, 203-214.

Grimalt F and Romaguera C (1981). Contact dermatitis caused by polyamide trousers pockets. Dermatosen **29**, 35-39.

Grossel SS (1988). Safety considerations in conveying of bulk solids and powders. J. Loss Prev. Process Ind. 1988, **1**, April.

Gupta C (2000). Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proc. Soc. Exp. Biol. Med. **224**, 61-68.

Gupta C (2001). Response to the letter by B. Elswick et al. from the Chemical Industry Institute of Toxicology. Exp. Biol. Med. **226**, 76-77.

Halldin K, Berg C, Brunström B (2000). Distribution of bisphenol-A and tetrabromobisphenol-A in quail embryos and effects on reproduction variable in adults following *in ovo* exposure. Department of Environmental Toxicology, Evolutionary Biology Centre, Uppsala University, Sweden.

Hamid A and Hume WR (1997a). A study of component release from resin pit and fissure sealants *in vitro*. Dent. Mater. **13**, 98-102.

Hamid A and Hume WR (1997b). The effect of dentine thickness on diffusion of resin monomers *in vitro*. J. Oral Rehabilitation **24**, 20-25.

Hanai Y (1997). Bisphenol-A Eluted from Nursing Bottles. Unpublished Data. Environmental Science Research Center, Yokohama National University.

Hansch C and Leo A (1979). Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley & Sons Inc., Appendix II, 286.

Hardin BD, Bond GP, Sikov MR, Andrew FD, Beliles RP and Niemeier RW (1981). Testing of selected workplace chemicals for teratogenic potential. Scand. J. Work. Environ. Health. **7**, 66-75.

Hartley WR, Thiyagarajah A, Anderson MB, Broxson MW, Major SE, Zell SI (1998). Gonadal development in Japanese Medaka (*Oryzias latipes*) exposed to 17ß oestradiol. Marine Environment Research **46**, 145-148.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. **1**, 3-142.

Hayakawa R, Matsumaga K, Takeuchi Y, Tatsumi H, Masamoto Y (1985). Occupational contact dermatitis from bisphenol-F. Skin Res. **27**, 494-500.

Hazleton Laboratories (1985). Bisphenol-A: Acute Oral Toxicity Study in the Rat. Hazleton Laboratories Europe Limited. Dow Chemical Company Unpublished Report.

Heinonen J, Honkanen J, Kukkonen JVK and Holopainen IJ (2002). Bisphenol A accumulation in the frehswater clam *Pisidium amnicum* at low temperatures. Arch. Environ. Contam. Toxicol. **43**, 50-55.

Hendricks AJ, Maas-Diepeveen JL, Noordsij A, Van der Gaag MA (1994). Monitoring response of XAD concentrated water in the Rhine delta: A major part of the toxic compounds remains unidentified. Water Research **28**(3), 581-598.

HMSO (1990). The Adults Survey: Dietary and nutritional survey of British adults. HMSO.

HMSO (1992). Food and nutritional intakes of British infants aged 6-12 months. Mills A and Tyler H HMSO.

HMSO (1995). The Toddlers Survey. Gregory JR, Collins DL, Davies PSW, Hughes JM and Clarke PC. National Diet and Nutrition Survey; Children Aged 1½ - 4½ years. **1**: Report of the Diet and Nutrition Survey. HMSO.

Holness DL and Nethercott JR (1989). Occupational contact dermatitis due to epoxy resin on fibreglass binder. J. Occup. Med. **31**, 87-89.

Holness DL and Nethercott JR (1993). The performance of specialised collections of Bisphenol-A epoxy resin system components in the evaluation of workers in an occupational health clinic population. Contact Dermatitis **28**, 216-219.

Honma M, Hayashi M, Shimada H, Tanaka N, Wakuri S, Awogi T, Yamamoto KI, Kodani NU, Nishi Y, Nakadata M and Sofuni T (1999). Evaluation of the mouse lymphoma tk assay (microwell method) as an alternative to the *in vitro* chromosomal aberration test. Mutagenesis **14**(1), 5-22.

Howard PH, Boethling RS, Jarvis WF, Meylan WM and Michalenko EM (1990). Handbook of Environmental Fate and Exposure Data for Organic Chemicals. **1**. Large Production and Priority Pollutants. Lewis Publishers Inc, Michigan, USA.

Howe SR and Borodinsky L (1998). Potential exposure to bisphenol-A from food-contact use of polycarbonate resins. Food Additives and Contaminants **15**, 370-373.

Howe SR, Borodinsky L and Lyon RS (1998). Potential exposure to bisphenol-A from food-contact use of epoxy coated cans. J. Coatings Technology **70**(877), 69-74.

Hubbard NW et al. (1948). J. Am. Chem. Soc.**70**, 3259-3261.

In Vitro Technologies (2001). Assessment of Skin Penetration of Bisphenol-A. *In Vitro* Technologies, Unpublished Draft Report No IVT M2000-46, Baltimore, Maryland, USA.

IPCS (1993). Bispenol-A; International Chemical Safety Cards, 8th Series prepared by IPCS. International Programme on Chemical Safety (IPCS).

Ivett JL, Brown BM, Rodgers C, Anderson BE, Ressnick MA and Zeiger E (1989). Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. IV. Results with 15 chemicals. Environ. Mol. Mutagen. **14**, 165-187.

Japanese Environment Agency (1996). Chemical Substances and Environment.

Japanese Ministry of Construction (1998). The Report on Monitoring of Endocrine Distrupters in the Environment (The first half of the survey).

JETOC (1996). Mutagenicity test data of existing chemicals. Japan Chemical Industry Ecology-Toxicology and Information Centre. 223-224.

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkyphenolic chemicals. Environmental Toxicology and Chemistry **15**, 194-202.

Jolanki R, Estlander T and Kanerva L (1987). Occupational contact dermatitis and contact urticaria caused by epoxy resins. Acta. Derm. Venereol. Suppl. **134**, 90-94.

Jolanki R, Kanerva L, Estlander T, Tarvainen K, Keskinen H and Henriks-Eckerman MJ (1990). Occupational dermatoses from epoxy resin compounds. Contact Dermatitis **23**, 172-183.

Jolanki R, Kanerva L and Estlander T (1995). Occupational allergic contact dermatitis caused by epoxy diacrylate in ultraviolet-light-cured paint, and bisphenol-A in dental composite resin. Contact Dermatitis **33**, 94-99.

Jones WH (1968). Toxicity and Health Hazard Summary. Eastman Kodak Company Unpublished Report.

Jones CA, Huberman E, Callaham MF, Tu A, Halloween W, Pallota S, Sivak A, Lubet RA, Avery MD, Kouri RE, Spalding J and Tennant RW (1988). An interlaboratory evaluation of the Syrian hamster embryo cell transformation assay using eighteen coded chemicals. Toxicol. *In vitro* **2**, 103-116.

Kanerva L, Jolanki R and Estlander T (1991). Allergic contact dermatitis from non-diglycidyl-ether-of-bisphenol-A epoxy resins. Contact Dermatitis **24**, 293-300.

Kanno J, Onyon L, Haseman J, Fenner-Crisp P, Ashby J and Owens W (2001). The OECD program to validate the rat uterotrophic bioassay to screen compounds for in vivo estrogenic responses: phase 1. Environ. Health Perspect. **109**, 785-794.

Kawamura Y, Koyama Y, Takeda Y and Yamada T (1998). Migration of bisphenol-A from polycarbonate products. J. Food Hyg. **99**.

Kawamura Y, Sano H and Yamada T (1999). Migration of bisphenol-A from can coatings to drinks. J. Food Hygienic Soc. Japan. **40**, 158-165.

Kedderis GL and Held SD (1996). Prediction of furan pharmacokinetics from hepatocyte studies: comparison of bioactivation and hepatic dosimetry in rats, mice and humans. Toxicol. Appl. Pharmacol. **140**, 124-130.

Kirk-Othmer (1994). Encyclopedia of Chemical Technology Fourth Edition Volume 9 Wiley Interscience. Epoxy resins. 730-755.

Kirk-Othmer (1996). Encyclopedia of Chemical Technology Fourth Edition Volume 18 Wiley Interscience. Phenolic resins. 603-644.

Kirk-Othmer (1996). Encyclopedia of Chemical Technology Fourth Edition Volume 19 Wiley Interscience. Polycarbonates. 584-608.

Klečka GM, Gonsior SJ, West RJ, Goodwin PA and Markham DA (2000). Biodegradation of Bisphenol-A in Aquatic Environments – River Die-Away. Dow Chemical Company Report.

Kloas W, Lutz I, Einspanier R (1999). Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. Sci. Total Environ. **225**, 59-68.

Kloas W, Schrag B, Ehnes C, Segner H (2000). Binding of xenobiotics to hepatic estrogen receptor and plasma sex steroid binding protein in the teleost fish, the common carp (*Cyprinus carpio*). Gen. Comp. Endocrinol. **119**, 287-299.

Kluwer (1991). Chemical Safety Sheets: Working Safely with Hazardous Chemicals.

Knaap JB and Sullivan LJ (1966). Metabolism of bisphenol-A in the rat. Toxicol. Appl. Pharmacol. **8**, 175-184.

Korenman and Gorokhov (1973). Russian Journal of Physical Chemistry **47**(8).

Körner W, Boz U, Süßmuth W, Hiller G, Harf V and Hagenmaier H (1998). Input/output balance of estrogenic active compounds in a major municipal sewage plant in Germany. Organolhalogen Compounds **37**, 269-272.

Krajewska D and Rudzki E (1976). Sensitivity to epoxy resins and triethylenetetramine. Contact Dermatitis **2**, 135-138.

Krishnan AV, Stathis P, Permuth SF, Tokes L and Feldman D (1993). Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology **132**, 2279-2286.

Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B and Gustafsson J (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology **139**(10), 4252-4263.

Kuribayashi S, Nishiyama H, Ishibe T, Takada H, Iguchi T, Irie H and Mori C (1999). Determination of nonylphenol and bisphenol-A using GC-MS in human umbilical cord. Abstract from International symposium on Environmental Endocrine Disrupters, Kobe, Japan, 9th December 1999.

Kusk KO, Wollenberger L (1999). Fully defined saltwater medium for cultivation of and toxicity testing with marine copepod *Acartia tonsa*. Environmental Toxicology and Chemistry **18**(7), 1564-1567.

Kwak HI, Bae MO, Lee MH, Lee YS, Lee BJ, Kang KS, Chae CH, Sung HJ, Shin JS, Kim JH, Mar WC, Sheen YY, Cho MH (2001) Effects of nonylphenol, bisphenol-A, and their mixture on the viviparous swordtail fish (*Xiphophorus helleri*). Environmental Toxicology and Chemistry **20**(4), 787-795.

Kwon S, Stedman DB, Elswick, BA, Cattley RC and Welsch W (2000). Pubertal development and reproductive functions of Crl:CD BR Sprague Dawley rats exposed to bisphenol-A during prenatal and postnatal development. Toxicol. Sci. **55**, 399-406.

Laessig SA, McCarthy MM and Silbergeld EK (1999). Prenatal exposure to estrogenic endocrine disrupting chemicals (EDCs) affects sexual differentiation in the rat brain. The Toxicologist **48**, Abstract No 220, 46.

Larroque M, Brun S and Blaise A (1989). Migration of constitutive monomers from epoxy resins used as coating material for wine vats. Sciences des Aliments **9**, 517-531.

Larson DF, Mayal B and Anderson RM (1977). Hemolysis due to chemical contamination of clinical perfusion apparatus. Ann. Thorac. Surg. **23**, 374-375.

Laws SC, Carey SA, Ferrell JM, Bodman GJ and Cooper RL (2000). Estrogenic activity of octylphenol, nonylphenol, bisphenol-A and methoxychlor in rats. Toxicol. Sci. **54**, 154-167.

Leuschner J (2000a). Acute Skin Irritation Test (Patch Test) of Bisphenol-A in Rabbits. Laboratory of Pharmacology and Toxicology KG, Unpublished Test Report No. 12664/99.

Leuschner J (2000b). Acute Eye Irritation Study of Bisphenol-A by Instillation into the Conjunctival Sac of Rabbits. Laboratory of Pharmacology and Toxicology KG, Unpublished Test Report No. 12665/99.

Lewis JB, Rueggeberg FA, Lapp CA, Ergle JW and Schuster GS (1999). Identification and characterisation of estrogen-like components in commercial resin-based dental restorative materials. Clin. Oral Invest. **3**, 107-113.

Liaw JJ, Stedman D, Gould JC, Elswick B and Welsch F (1998). Reproductive development in female rats prenatally and lactationally exposed to bisphenol-A. Toxicologist **42**, 176, Abstracts of papers: 867.

Ligon WV, Maxam J, Grade H (1998). Major Organic Volatiles Evolved on Moulding from the Polymers: Lexan EM3110-754, Lexan 920-80189, Lexan 103-112, Noryl PX1005X-701 and Noryl GTX 910-71653. GE Research and Development Center Report 98CRD026.

Ligon WV, Silvi N, Caraher J, Grade H (1997). Volatiles Produced on Extrusion from Lexan EM3110, Lexan 103 and Lexan 920. GE Research and Development Center Report 97CRD165.

Lindholst C, Pedersen KL, Pedersen SN (2000). Estrogenic response of bisphenol-A in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology **48**, 87-94.

Lindholst C, Pedersen SN and Bjerregaard P (2001). Uptake, metabolism and excretion of bisphenol-A in the rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology **55**, 75-84.

Lobos JH, Leib TK and Su TM (1992). Biodegradation of bisphenol-A and other bisphenols by a gram-negative aerobic bacterium. Applied and Environmental Microbiology **58**(6), 1823-1831.

Lockwood DD (1984a). Bisphenol-A - Polycarbonate Grade: Eye Irritation Potential. Dow Chemical Company Unpublished Report.

Lockwood DD (1984b). Bisphenol-A - E.R Grade: Eye Irritation Potential. Dow Chemical Company Unpublished Report.

Long X, Steinmetz R, Ben-Jonathon N, Caperell-Grant A, Young PCM, Nephew KP and Bigsby RM (2000). Strain diferences in vaginal responses to the xenoestrogen bisphenol-A. Environ. Health Perspect. **108**(3), 243-247.

Lysak A and Marcinek J (1972). Multiple toxic effects of simultaneous action of some chemical substances on fish. Roczniki nauk rolniczych **94**(3), 53-63.

MAFF (1998). Joint Food Safety and Standards Group. Food Surveillance Information Sheet – Number 167, November 1998.

Maguire HC (1988). Experimental photoallergic contact dermatitis to bisphenol-A. Acta. Derm. Venereol. **68**, 408-412.

Maruyama S, Fujimoto N, Yin H and Ito A (1999). Growth stimulation of a rat pituitary cell line MtT/E-2 by environmental oestrogens *in vitro* and *in vivo*. Endocrine J. **46**, 513-520.

Matsui S, Okawa Y and Ota R (1988). Experience of 16 years operation and maintenance of the Fukashiba industrial wastewater treatment plant of the Kashima petrochemical complex -II Biodegradability of 37 organic substances and 28 process waters. Water Science Technology **20**(10), 201-210.

Matsumoto G and Hanya T (1980). Organic constituents in atmospheric fallout in the Tokyo area. Atmospheric Environment **14**, 1409-1419.

Matsumoto G (1982). Comparative study on organic constituents in polluted and unpolluted inland aquatic environments, III: Phenols and aromatic acids in polluted and unpolluted waters. Water Res. **16**, 551-557.

Matsumoto G, Ishiwatari R and Hanya T (1977). Gas chromatographic-Mass spectrometric identification of phenols and aromatic acids in river waters. Water Research **11**, 693-698.

Mellon Institute of Industrial Research (1946). Range Finding Tests on Diphenylol Propane. Study No. 9-12. Union Carbide Corporation Unpublished Report.

Mellon Institute of Industrial Research (1948). The Acute and Subacute Toxicity of Diphenylol Propane. Study No. 11-13. Union Carbide Corporation Unpublished Report.

Mellon Institute of Industrial Research (1965). Range Finding Tests on Bisphenol-A. Study No. 28-49. Union Carbide Corporation Unpublished Report.

Merck Index (1989). The Merck Index. an Encyclopedia of Chemicals Drugs and Biologicals. 11th Edition. Budavari S et al. (eds), Rahway, N.J. Merck & Co.

Michna H (2000). Report of the Effects of Bisphenol-A on the Growth of Prostate, Seminal Vesicle, Kidney and Liver in the Rat: (Anti-)Androgen Test. Institute of Morphology and Tumour Research, DSHS Cologne.

Miles-Richardson SR, Kramer VJ, Fitzgerald SD, Render JA, Yamini B, Barbee SJ, Giesy JP (1999). Effects of waterborne exposure to 17ß estradiol on secondary sex characteristics and gonads of fathead minnow (*Pimephales promelas*). Aquatic Toxicology **47**, 129-145.

Milligan SR, Balasubramanian AV and Kalita JC (1998). Relative potency of xenobiotic estrogens in an acute *in vivo* mammalian asay. Environ. Health Perspect. **106**, 23-26.

MITI (1977). Results of bisphenol-A bioaccumulation test.

Miyakoda H, Tabata M, Onodera S and Takeda K (1999). Passage of bisphenol-A into the fetus of the pregnant rat. J. Health Sci. **46**, 318-323.

Miyakoda H, Tabata M, Onodera S and Takeda K (2000). Comparison of conjugative activity, conversion of bisphenol-A to bisphenol-A glucuronide, in fetal and mature male rat. J. Health Sci. **46**, 269-274.

Mobil Oil Corporation (1993). Aerobic biodegradation study of XXXXX. Study Number 65163.

Moon HJ, Lee YK, Lim BS and KIM CW (2000). Component elution from dental pit and fissure sealants. J. Dent. Res. **79**, 191.

Moore MM, Collard DD and Harrington-Brock K (1999a). Failure to adequately use positive control data leads to poor quality mouse lymphoma assessments. Mutagenesis **14**, 261-263.

Moore MM, Harrington-Brock K and Cole J (1999b). Issues for conducting the microtiter version of the mouse lymphoma thymidine kinase (tk) assay and a critical review of data generated in a collaborative trial using the microtiter method. Mutagenesis **14**, 271-281.

Morrissey RE, George JD, Price CJ, Tyl RW, Marr, MC and Kimmel CA (1987). The developmental toxicity of bisphenol-A in rats and mice. Fundam. Appl. Toxicol. **8**, 571-582.

Mountfort KA, Kelly J, Jickells SM and Castle L (1997). Investigations into the potential degradation of polycarbonate baby bottles during sterilization with consequent release of bisphenol-A. Food Additives and Contaminants **14**, 737-740.

Mountfort KA (1997). Investigations into the Potential Degradation of Polycarbonate Baby Bottles During Sterilization with Consequent Release of Bisphenol-A. Ministry of Agriculture, Fisheries and Food, Report FD 97/08.

Moura C, Margarida D and Vale T (1994). Contact dermatitis in painters, polishers and varnishers. Contact Dermatitis **31**, 51-53.

Myhr BC and Caspary WJ (1991). Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded chemicals in the National Toxicology Program. Environ. Mol. Mutagen. **18**, 51-83.

Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M and Welshons WV (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol-A and octylphenol. Environ. Health Perspect. **105**, 70-76.

Nagao T, Saito Y, Usumi K, Kuwagata M and Imai K (1999). Reproductive function in rats exposed neonatally to bisphenol-A and estradiol benzoate. Repro. Toxicol. **13**, 303-311.

Nathanson D, Lertpitayakun P, Lamkin MS, Edalatpour M and Chou LL (1997). *In vitro* elution of leachable components from dental sealants. JADA **128**, 1517-1523.

Nathanson D, Lertpitayakun P, Lamkin MS, Edalatpour M and Chou LL (1998). *In vitro* evaluation of leachable components from dental sealants. J. Dent. Res. **77**, 241.

Nehring Institute (1998). Statistical Evaluation on Extractable Bisphenol-A in Can Coatings. Data from Unpublished Reports (dated between January and November 1998) from Institut Nehring.

Nikula H, Talonpoika T, Kaleva M and Toppari J (1999). Inhibition of hCG-stimulated steroidogenesis in cultured mouse leydig cells by bisphenol-A and octylphenols. Toxicol. Appl. Pharmacol. **157**, 166-173.

NIOSH (1979). Health Hazard Evaluation Determination Report - Greenheck Fan Corporation 1979.

NIOSH (1984). Health Hazard Evaluation Report - Dale Electronics 1984. NIOSH.

Nitschke KD, Quast JF and Wolfe EL (1985a). Bisphenol-A: Acute Aerosol Toxicity Study with Fischer 344 Rats. Dow Chemical Company Unpublished Report.

Nitschke KD, Quast JF, Schuetz DJ and Wolfe EL (1985b). Bisphenol-A: 2 Week Aerosol Toxicity Study with Fischer 344 rats. Dow Chemical Company Unpublished Report.

Nitschke KD, Lomax LG, Schuetz DJ, Hopkins PJ and Weiss S.W (1988). Bisphenol-A: 13 Week Aerosol Toxicity Study with Fischer 344 Rats. Dow Chemical Company Unpublished Report.

Noda M, Komatsu H and Sano H (1999). HPLC analysis of dental resin composites components. J. Biomed. Mater. Res. **47**, 374-378.

NTP (1982). Carcinogenesis Bioassay of Bisphenol-A (CAS No. 80-05-7) in F344 Rats B6C3F1 Mice (Feed Study). National Toxicology Program. Technical Report No. 215, Order No. PB82-184060 (NTIS), 1-116.

NTP (1984). Bisphenol-A: Reproductive and Fertility Assessment in CD-1 Mice when Administered Via Subcutaneous Silastic Implants. National Toxicology Program. Report NTP-84-015; Order No. PB84-155308 (NTIS) 1-192.

NTP (1985a). Teratologic Evaluation of Bisphenol-A (CAS No. 80-05-7) Administered to CD-1 Mice on Gestational Days 6 Through 15. National Toxicology Program. Report NTP-85-088; Order No. PB85-205102 (NTIS).

NTP (1985b). Bisphenol-A: Reproductive and Fertility Assessment in CD-1 Mice when Administered in the Feed. National Toxicology Program. Report NTP-85-192; Order No. PB86-103207 (NTIS) 1-346.

NTP (1985c). Teratologic Evaluation of Bisphenol-A (CAS No. 80-05-7) Administered to CD(R) Rats on Gestation Days 6 Through 15. National Toxicology Program. Report NTP-85-089; Order No. PB85-205112 (NTIS).

Ochi T (1999). Induction of multiple microtubule-organizing centers, multipolar spindles and multipolar division in cultured V79 cells exposed to diethylstilbesterol, estradiol-17β and bisphenol-A. Mutat. Res. **431**, 105-121.

Oehlmann J, Schulte-Oehlmann U, Tillmann M and Markert B (2000). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol-A and Octylphenol as xeno-estrogens. Ecotoxicology **9**, 383-397.

Oehlmann J, Schulte-Oehlmann U, Duft M and Tillmann M (2001). Effect of Environmental Hormones in Prosobranch Molluscs. Presentation at 2nd Status Seminar on Endocrine Disrupters held in Berlin, April 2001.

Ohsako S, Sakaue M, Ishimura R, Kurosawa S, Yomnemoto J and Tohyama C (1999). Effects of Low-Dose Bisphenol-A on Rat Spermatogenesis. Abstract from International symposium on environmental endocrine disrupters, Kobe, Japan, 9th December 1999.

Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, Pedraza V, Soto AM and Sonnenschein C (1996). Estrogenicity of resin-based composites and sealants used in dentistry. Environ. Health Perspect. **104**, 298-305.

Panter GH, Thompson RS, Sumpter JP (1998). Adverse reproductive effects in male fathead minnows (*Pimephales promelas*) exposed to environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. Aquatic Toxicology **42**, 243-253.

Patussi V, Kokelj F and Buttazzi P (1995). Occupational airborne allergic contact dermatitis due to 3-amino-methyl-3,5,5-trimethylcyclohexylamine. Contact Dermatitis **32**, 239.

Pawlowski S, Islinger M, Völkl A and Braunbeck T (2000). Temperature-dependent vitellogenin-mRNA expression in primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes at 14°C and 18°C. Toxicology *in Vitro* **14**, 531-540.

Peltonen K, Pfaffli P, Itkonen A and Kalliokosi P (1986a). Determination of the presence of bisphenol-A and the absence of diglycidyl ether of bisphenol-A in the thermal degradation products of epoxy powder paint. Am. Indust. Hyg. Assoc. Journal **47**, 399-403.

Peltonen K, Zitting A, Koskinen H and Itkonen A (1986b). Free radicals from photodecomposition of bisphenol-A. Photochem. Photobiol. **43**, 481-484.

Pfeiffer E, Rosenburg B and Metzler M (1996). Bisphenol-A disturbs microtubule assembly and induces micronuclei *in vitro*, in: Hormonal Carcinogenesis II, JJ Li, JA Gustafsson, S Nandi and LI Sekely (eds.) Springer Verlag, New York, 450-453.

Pfeiffer E, Rosenburg B, Deuschel S and Metzler M (1997). Interference with microtubules and induction of micronuclei *in vitro* by various bisphenols. Mutation Research **390**, 21-31.

Pickford DB, Caunter JE, Hetheridge MJ, Hutchinson TH (2000) Bisphenol-A: Determination of effects on larval growth, development and sexual differentiation of the African clawed frog (*Xenopus laevis*). Brixham Environmental Laboratory.

Pienta RJ (1996). Transformation of Syrian hamster embryo cells by diverse chemicals and correlation with their reported carcinogenic and mutagenic activities. de Serres FJ and Hollender A (eds), Chemical Mutagens, principles and methods for their detection **6**, 175-202, Plenum, New York.

Piersma AH, Verhoef A, Elvers LH and Wester P (1998). Toxicity of compounds with endocrine activity in the OECD 421 reproductive toxicity screening test. Teratology **57**, Abstract No P82, 243-244.

Pohanish RP and Greene SA (1996). Hazardous Materials Handbook. New York. London. Van Nostrand Rheinhold.

Polycarbonate Resin Manufacturers Group of Japan (1999). Q&A Concerning polycarbonate and bisphenol-A.

Pottenger LH, Domoradzki JY, Markham DA and Hansen SC (1997a). Bioavailability of ^{14}C-Bisphenol-A in Fischer Rats Following Oral, Subcutaneous or Intraperitoneal Administration (Part A). Dow Chemical Company Unpublished Report K-001304-12A.

Pottenger LH, Domoradzki JY, Markham DA and Hansen SC (1997b). Bioavailability of ^{14}C-Bisphenol-A in Fischer Rats Following Oral, Subcutaneous or Intraperitoneal Administration (Part B). Dow Chemical Company Unpublished Report K-001304-12B.

Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ and Waechter JM, Jr (2000). The relative bioavailability and metabolism of bisphenol-A in rats is dependent upon the route of administration. Toxicological Sciences **54**, 3-18.

Prens EP, de Jong G and van Joost T (1986). Sensitisation to epichlorohydrin and epoxy system components. Contact Dermatitis **15**, 85-90.

Procter & Gamble Company (1969). Guinea Pig Closed Patch Test. Unpublished Data. NTIS/OTSO206621, Doc. I.D. 878214688/9.

Pulgar R, Olea-Serrano MF, Novillo-Fertrell A, Rivas A, Pazos P, Pedraza V, Navajas JM and Olea N (2000). Determination of bisphenol-A and related aromatic compounds released from bis-GMA-based composites and sealants by High Performance Liquid Chromatography. Environ. Health Perspect. **108**, 21-27.

Reiff B (1979). The Acute Toxicity of Disphenylolpropane to Rainbow Trout (Salmo Gairdneri). Shell Company report. GRR-TLGR.79.146.

Reid EE and Wilson E (1944). The relation of oestrogenic activity to structure in some 4,4'-dihydroxydiphenylmethanes. J. Am. Chem. Soc. **66**, 967-969.

Richardson GM (1997). An assessment of adult exposure and risks from components and degradation products of composite resin dental materials. Human and Ecological Risk Assessment **3**, 683-697.

Romaguera C, Grimalt F and Lecha M (1981). Occupational purpuric textile dermatitis from formaldehyde resins. Contact Dermatitis **7**, 152-153.

Romaguera C, Grimalt F and Vilaplana J (1986). Occupational dermatitis from epoxy resins. Contact Dermatitis **174**, 187.

Rubin BS, Murray MK, Damassa DA, King JC and Soto AM (2001). Perinatal exposure to low doses of bisphenol-A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect. **109**, 675-680.

Rudel AR, Melly SJ, Geno PW, Sun G and Brody JG (1998). Identification of alkylphenols and other estrogenic phenolic compounds in wastewater, septage and groundwater on Cape Cod, Massachusetts. Environmental Science and Technology **32**(7), 861-869.

Sattelberger A, Scharf S, Lorbeer G (1999). Hormonell wirksame Substanzen in Fliessgewässern - Datenbericht. Umweltbundesamt Wien, Bericht BE-150, Wien, p. 30.

Saunders PTK, Majdic G, Parte P, Millar MR, Fischer JS, Turner KJ and Sharpe RM (1997). Fetal and perinatal influence of xenoestrogens on testis gene expression. The fate of the male germ cell, Ivell and Holstein (eds), Plenum Press, New York.

Sax NI and Lewis RJ (1996). Sax's Dangerous Properties of Industrial Materials, 9th Edition. New York. London. Van Nostrand Reinhold.

Schäfers C, Wenzel A, Schmitz A, Böhmer W (2001). Effects of Xenoestrogens on the life cycle of fish. Poster Presentation.

Schaefer WR, Hermann T, Meinhold-Heerlein I, Deppert WR and Zahradnik HP (2000). Exposure of human endometrium to environmental estrogens, antiandrogens and organochlorine compounds. Fertility and Sterility **74**, 558-563.

Scharf S, Sattelberger R and Lorbeer G (1998). Potentiell hormonell aktive Substanzen in der aquatischen Umwelt - erste Untersuchungsergebnisse aus Österreich. Wiener Mitteilingen, Wasser - Abwasser – Gewässer **153**, 45-58.

Schmalz G, Preiss A and Arenholt-Bindslev D (1999). Bisphenol-A content of resin monomers and related degradation products. Clin. Oral Invest. **3**, 114-119.

Schulte-Oehlmann U, Tillmann M, Markert B, Oehlmann J, Watermann B and Scherf S (2000). Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part II: Triphenyltin as a xeno-androgen. Ecotoxicology **9**(6), 399-412.

Schweikl H, Schmalz G and Rackebrandt K (1998). The mutagenic activity of unpolymerized resin monomers in *Salmonella typhimurium* and V79 cells. Mutat. Res. **415**, 119-130.

Sharpe RM, Majdic G, Fischer J, Parte P, Millar MR and Saunders PTK (1996). Effects on testicular development and function. 10th International Congress of Endocrinology, June 12-15, San Francisco. S23-4.

Sharpe RM, Turner KJ and Sumpter JP (1998). Endocrine disrupters and testis function. Environ. Health Perspect. **106**, A220-221.

Shell Oil Company (1984). BPA dust exposure assessment. EPA/OTS Document #40-8486025; Order No.509966 (NTIS), 1-3.

Shell Oil Company (1999). Mammalian Erythrocyte Micronucleus Test. Unpublished Test Report BPA 99-01.

Shell (1999). Bisphenol-A: An Assessment of Anaerobic 'Primary' Biodegradability. Company Report CT.98.47148.

Shioda T and Wakabayashi M (2000). Effect of certain chemicals on the reproduction of medaka (*Oryzias latipes*). Chemosphere **40**, 239-243.

Simoneau C, Roeder G and Anklam E (2000). Migration of bisphenol-A from baby bottles: effect of experimental conditions and European survey. 2nd International Symposium on Food Packaging: Ensuring the Safety and Quality of Foods (ILSI conference), Vienna, Austria, 8-10 November 2000.

Sipes IG (2001). The *in vitro* Metabolism of Bisphenol-A. Unpublished Report - Dept of Pharmacology and Toxicology, University of Arizona.

Smeets JMW, van Holsteijn I, Giesy JP, Seinen W, and van der Berg M (1999). Estrogenic potencies of several environmental pollutants, as determined by vitellogenin induction in carp hepatocyte assay. Toxicological Sciences **50**, 206-213.

Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SCJ and Fennell TR (2000). Metabolism and disposition of bisphenol-A in female rats. Toxicol. Appl. Pharmacol. **168**, 225-234.

Sohoni P and Sumpter JP (1998). Several environmental oestrogens are also anti-androgens. J. Endocrinol. **158**, 327-339.

Spearow JL, Doemeny P, Sera R, Leffler R and Barkley M (1999). Genetic variation in susceptibility to endocrine disruption by estrogen in mice. Science **285**, 1259-1261.

Springborn Bionomics Inc. (1985a). 96 hour Flow-Through Acute Toxicity Test with the Atlantic Silverside. Report Number BW-85-6-1802. Commissioned by the Society of the Plastics Industry.

Springborn Bionomics Inc. (1985b). Acute Toxicity of Bisphenol-A to the Mysid (*Mysidopsis bahia*) Under Flow Through Conditions. Report Number BW-85-8-1825. Commissioned by the Society of the Plastics Industry.

Springborn Bionomics Inc. (1985c). Acute Toxicity of Bisphenol-A to the Marine Alga *Skeletonema costatum*. Report Number BW-85-8-1829. Commissioned by the Society of the Plastics Industry.

Srinivas CR, Devadiga R and Aroor AR (1989). Footwear dermatitis due to bisphenol-A. Contact Dermatitis **20**, 150-151.

Stachel B (1999). Concentrations of phenols in the River Elbe in July 1998. Arbeitsgemeinschaft für die Reinhaltung der Elbe (ARGE Elbe), personal communication on 31 March 1999, Hamburg.

Staples CA, Dorn PB, Klecka GM, O'Block ST, Branson DR and Harris LR (2000). Bisphenol-A concentration in receiving waters near US manufacturing and processing facilities. Chemosphere **40**, 521-525.

Stasenkova KP, Schumskaja NL and Grinberg AE (1973). Regularities governing the biological action of bisphenol-A derivatives as a function of their chemical structure (In Russian). Gig Tr. Prof. Zabol 30-33.

Steinhagen WH, Harrington WW and Wong KL (1987). Irritation response to bisphenol-A (BPA) aerosol in mice and rats. The Toxicologist **7**, Abstract 781.

Steinmetz R, Brown NG, Allen DL, Bigsby RM and Ben-Jonathan N (1997). The environmental estrogen bisphenol-A stimulates prolactin release *in vitro* and *in vivo*. Endocrinology **138**, 1780-1786.

Steinmetz R, Mitchner NA, Grant A, Allen DL, Bigsby RM and Ben-Jonathan N (1998). The xenoestrogen bisphenol-A induces growth, differentiation, and c-*fos* gene expression in the female reproductive tract. Endocrinology **139**, 2741-2747.

Stephenson RR (1983). Diphenylol Propane: Acute Toxicity to Daphnia Magna and Selenastrum Capricornutum. Shell Group Research Report SBGR.83.216.

Stone CM and Watkinson RJ (1983). Diphenylol Propane: An Assessment of Ready Biodegradability. Shell Group Research Report SBGR.83.425.

Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW and DeLuca JG (1996). Revalidation of the *in vitro* alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat. Res. **368**, 59-101.

Sumpter JP, Tyler CR, Sherazi A (2001). Bisphenol-A: Multigeneration study with the fathead minnow (*Pimephales promelas*). Brunel University.

Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M and Magara Y (2001). Estrogenic influences of estradiol-17ß, p-nonylphenol and bisphenol-A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. Water Science and Technology **43**(2), 109-116.

Takada H, Isobe T, Nakada N, Nishiyama H, Iguchi T, Irie H and Mori C (1999). Bisphenol-A and Nonylphenols in Human Umbilical Cords. International Conference at Verita, Ascona; Switzerland on March 7-12: Environmental Endocrine Disrupting Chemicals - Conference Report, 49.

Takahashi O and Oishi S (2000). Disposition of orally administered 2,2-bis (4-hydroxyphenyl) propane (bisphenol-A) in pregnant rats and the placental transfer to fetuses. Environ. Health Perspect. **108**, 931-935.

Takahashi O and Oishi S (2001). Testicular toxicity of dietary 2,2-bis (4-hydroxyphenyl) propane (bisphenol-A) in F344 rats. Arch. Toxicol. **75**, 42-51.

Takahashi T (1998). Environmental Hormone Increases by PC Containers with Boiling Water – elution study by Professors of Niigata University. Exerpted from Mainichi Shinbun, dated May 29,1998.

Takahata J, Tamakawa K, Takahashi Y, Seki T, Tsunoda A, Nohmi T and Sofuni T (1990). Mutagenicity of environmental chemicals II, bisphenol-A. Sendai-shi Eisei Kenkyushoho, (Pub. 1991) **20**, 245-247 (Japan).

Takai Y, Tsutsumi O, Ikezuki Y, Kamei Y, Osuga Y, Yano T and Taketan Y (2001). Preimplantation exposure to bisphenol-A advances postnatal development. Reproductive Toxicology **15**, 71-74.

Takao T, Nanamiya W, Nagano I, Asaba K, Kawabata K and Hashimoto K (1999a). Exposure with the environmental estrogen bisphenol-A disrupts the male reproductive tract in young mice. Life Sciences **65**, 2351-2357.

Taylor JA, Judy BM, Rottinghaus BA, Blackwell KJ, Rottinghaus GE, Alworth LC, vom Saal FS and Welshons WV (1999). Bisphenol-A bioaccumulates in the serum of pregnant mice. Environmental Hormones, Past, Present, Future, Tulane University, October 18-20.

Tennant RW, Stasiewicz S and Spalding JW (1986). Comparison of multiple parameters of rodent carcinogenicity and *in vitro* genetic toxicity. Environmental Mutagenesis **8**, 205-207.

Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B and Minor R (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science **236**, 933-941.

The Society of the Plastics Industry (1995). Report on the Potential Exposure to Bisphenol-A from Epoxy Can Coatings. The Society of the Plastics Industry Inc., Washington DC, USA, September 1995.

The Society of the Plastics Industry (1997). Bisphenol-A (A safety and handling guide). The Society of the Plastics Industry Inc., Washington DC, USA, April, 1997.

Thorgeirsson A and Fregert S (1977). Allergenicity of epoxy resins in the guinea pig. Acta Derm. Venereol. **57**, 253-256.

Til HP, Roverts WG and Beems RB (1978). Sub-Chronic (90 day) Oral Toxicity Study with Diphenylolpropane (DPP) in Rats. Unpublished Report (No. R 6229) of TNO, The Netherlands.

Tinwell H, Joiner R, Pate I, Soames A, Foster J and Ashby J (2000). Uterotrophic activity of bisphenol-A in the immature mouse. Reg. Toxicol. Pharmacol. **32**, 118-126.

TNO (2000). Environmental Risk Assessment of Bisphenol-A Release from Thermal Paper Recycling. Jongbloed RH, Hanstveit AO, Organisation for Applied Scientific Research (TNO), TNO Report V99.1148, The Netherlands.

Tsutsui T, Tamura Y, Yagi E, Hasegawa K, Takahashi M, Maizumi N, Yamaguchi F and Barrett C (1998). Bisphenol-A induces cellular transformation, aneuploidy and DNA adduct formation in cultured Syrian hamster embryo cells. Int. J. Cancer. **75**, 290-294.

Tsutsui T, Tamura Y, Suzuki A, Hirose Y, Kobayashi M, Nishmura H, Metzler M and Barrett CJ (2000). Mammalian cell transformation and aneuploidy induced by five bisphenols. Int. J. Cancer **86**, 151-154.

Turner SJ, and Watkinson RJ (1986). Diphenylol Propane: An Assessment of Inherent Biodegradability. Shell Company Report.

Tyl RW, Myers CB and Narr MC (2000). Three Generation Reproduction Toxicity Evaluation of Bisphenol-A Administered in the Feed to CD (Sprague Dawley) Rats. Unpublished Report- Research Triangle Institute RTI Project No 65C-07036-000.

UCD (1998). Use Category Document on Plastics Additives. Modified Version (June 1998) produced for the UK Environment Agency and circulated through the OECD Task Group on Environmental Exposure Assessment. Based on original report by Jolly et al. (1994).

Ullmann's Encyclopedia of Industrial Chemistry (1991). Encyclopedia of Industrial Chemistry. 5th completely revised edition. Gerhartz W (ed), Weinheim. VCH.

Union Carbide Corporation (1947). Unpublished Data in: American Industry Hygiene Association, Hygienic Guide Series, May-June, 1967.

Union Carbide Corporation (1962). The metabolism of bisphenol-A in the rat. Carnegie Mellon Institute for Industrial Research, EPA/OTS Document # 878214659; Order No. 206612 (NTIS), 1-9.

US EPA (1985). Bisphenol-A: Proposed test rule. US Environmental Protection Agency (EPA), Fed. Reg. **50**, 20691-20703.

Upmeier A, Degen GH, Diel P, Michna H and Bolt HM (2000). Toxicokinetics of bisphenol-A in female DA/Han rats after a single i.v. and oral administration. Arch. Toxicol. **74**, 431-436.

van Joost T, van Ulsen J and van Loon LA (1988). Contact allergy to denture materials in the burning mouth syndrome. Contact Dermatitis **18**, 97-99.

van Joost T (1988). Occupational sensitisation to epichlorohydrin and epoxy resin. Contact Dermatitis **19**, 278-280.

van Joost T, Roseyanto ID and Satyawan I (1990). Occupational sensitisation to epichlorohydrin (ECH) and bisphenol-A during the manufacture of epoxy resins. Contact Dermatitis **22**, 125-126.

Villalobos M, Olea N, Brotons JA, Olea-errano MF, Ruiz de Almodovar JM and Pedraza V (1995). The E-screen assay: a comparison of different MCF7 cell stocks. Environ. Health Perspect. **103**, 844-850.

Vinggaard AM, Körner W, Lund KH, Bolz U and Petersen JH (2000). Identification and quantification of estrogenic compounds in recycled and virgin paper for household use as determined by an *in vitro* yeast estrogen screen and chemical analysis. Chem. Res. Toxicol. **13**, 1214-1222.

vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer K, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S and Welshons WV (1997). Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbesterol and opposite effects at high doses. Proc. Natl. Acad. Sci. **94**, 2056-2061.

vom Saal FS, Cooke P, Buchanan DL, Palanza P, Thayer KA, Nagel SC, Parmigiani S and Welshons WV (1998). A physiologically based approach to the study of bisphenol-A and other oestrogenic chemicals on the size of reproductive organs, daily sperm production, and behaviour. Toxicol. Indust. Health **14**, 239 - 260.

Von Braun (1925). ANN, **472**, 65.

Voordeckers JW, Fennell DE, Jones K and Häggblom MM (2002). Anaerobic biotransformation of tetrabromobisphenol A, tetrachlorobisphenol A and bisphenol A in estuarine sediments. Environ. Sci. Technol. **36**, 696-701.

Wataha JC, Rueggeberg FA, Lapp CA, Lewis JB, Lockwood PE, Ergle JW and Mettenburg DJ (1999). *In vitro* cytotoxicity of resin-containing restorative materials after aging in artificial saliva. Clin. Oral Invest. **3**, 144-149.

Watts MM, Pascoe D, Carroll K (2001). Survival and precopulatory behaviour of *Gammarus pulex* (L.) exposed to two xenoestrogens. Water Research **35**(10), 2347-2352.

Wenzel A, Küchler T, Henschel KP, Schnaack W, Diedrich M and Müller J (1998)**.** Konzentrationen östrogen wirkender Substanzen in Umweltmedien. Umweltbundesamt, Fraunhofer-Institut für Umweltchemie und Ökotoxikologie Schmallenberg und Bergholz-Rehbrücke, Umweltforschungsplan UFOPLAN 216 01 011/11. Berlin, p. 88.

West RJ, Goodwin PA (1997a). Evaluation of Ready Biodegradability of Bisphenol-A Using the OECD 301F: Manometric Respirometry Test. Dow Company Report. Study ID 971108A.

West RJ, Goodwin PA (1997b). Evaluation of Ready Biodegradability of Bisphenol-A Using the OECD 301F: Manometric Respirometry Test. Dow Company Report. Study ID 971108B.

Whale GF, Warden JR and Bumpus RN (1999). Bisphenol-A: Toxicity in Marine Sediments to *Corophium volutator*. Shell Research Report No CT.99.47028.

Yamamoto T and Yasuhara A (1999). Quantities of bisphenol-A leached from plastic waste samples. Chemosphere **38**(11), 2569-2576.

Yamasaki K, Sawnki M and Takatsuki M (2000). Immature rat uterotrophic assay of bisphenol-A. Environ. Health Perspect. **108**, 1147-1150.

Yasuhara A, Shiraishi H, Nishikawa M, Yamamoto T, Uehiro T, Nakasugi O, Okumura T, Kenmotsu K, Fukui H, Nagase M, Ono Y, Kawagoshi Y, Baba K and Noma Y (1997). Determination of organic components in leachates from hazardous waste disposal sites in Japan by gas chromatography-mass spectrometry. J Chromatogr. A **774**, 321-332.

Zacharewski TR and Matthews JB (2000). Comparison of the interactions of bisphenol-A and its metabolite bisphenol-A glucuronide with oestrogen receptors α and β. Toxicologist **54**, Abstracts of papers: 1795.

Zincke and Greuters (1905). ANN, **343**, 85.

ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives

JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic

PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations

UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix 1 Euses modelling

The EUSES printout for bisphenol-A can been can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

In the running the EUSES program site-specific data and generic scenarios have been assigned to USE PATTERNS as follows:

Use Pattern	Site-specific data/Generic Scenario
1 Production	Bisphenol-A production – composite from site-specific data
2 Formulation	Epoxy resin production – site-specific data
2 Processing	Phenolplast cast resin processing
3 Formulation	PVC production use as an inhibitor – generic scenario
3 Processing	PVC processing use as an anti-oxidant – generic scenario
3 Private use	Preparation of additive package for use in PVC processing – generic scenario
4 Production	Use of additive package in PVC processing – generic scenario
4 Formulation	Plasticiser use – generic scenario
5 Production	Thermal paper production – site-specific data
5 Formulation	Thermal paper recycling – generic scenario

Appendix 2 Effect of a hypothetical phase out of bisphenol-A in thermal paper and PVC production, processing and use on the regional PEC_{water}

The current regional PEC_{water} of 0.12 µg/l is based on releases from all known lifecycle stages. At this concentration the use of bisphenol-A in PVC production, processing and use and thermal production and recycling presents a risk to the environment (using a PNEC of 1.6 µg/l), and risk reduction measures are recommended. However, there is considerable uncertainty over the PNEC derivation and it may be more appropriate to adopt a lower PNEC value for aquatic species. With respect to this a tentative PNEC of 0.1 µg/l is considered in the risk assessment report. When this PNEC is compared to the predicted background concentration (PECregional) a ratio greater than 1 is obtained, i.e. risk reduction measures are required for all uses.

This appendix looks at the effect of removing the emissions associated with use in the thermal paper industry and PVC industry from the regional PEC_{water} calculation and the likely implications for further work. Calculations have been performed using EUSES. It is not intended to pre-judge any risk reduction strategy, but is aimed at informing such a strategy.

Scenario 1: Effect of immediate phasing out of bisphenol-A from thermal paper and PVC production

Emissions included	Emissions excluded
BPA production	Thermal paper production and recycling
Polycarbonate bottle washing	PVC production
Epoxy resin production	
Phenoplast case resin processing	
PVC processing	
Losses from PVC articles in use [a)]	

a) Losses from PVC articles in use are included because it is assumed that the service life of articles is thirty years and therefore any effect through phasing out use in the production of PVC will not be noticed for ten years.

Based upon this scenario the regional PEC_{water} becomes 0.033 µg/l. At this level the concern would be removed for the background scenario and risk reduction measures would only be required where local concentrations exceeded the PNEC value.

Scenario 2: Effect of immediate phasing out of bisphenol-A from thermal paper and PVC industries

Emissions included	Emissions excluded
BPA production	Thermal paper production and recycling
Polycarbonate bottle washing	PVC production
Epoxy resin production	PVC processing
Phenoplast case resin processing	
Losses from PVC articles in use [a)]	

a) Losses from PVC articles in use are included because it is assumed that the service life of articles is thirty years and therefore any effect through phasing out BPA use in PVC will not be noticed for ten years.

Based upon this scenario the regional PEC_{water} becomes 0.032 µg/l.

Scenario 3: Effect of phasing out of bisphenol-A from thermal paper and PVC industries after 30 years

Process considered	Process not considered
BPA production	Thermal paper production and recycling
Polycarbonate bottle washing	PVC production
Epoxy resin production	PVC processing
Phenoplast case resin processing	PVC articles in use

Based upon this scenario the regional PEC_{water} becomes 0.0055 µg/l (5.5 ng/l)

These three scenarios suggest that phasing out bisphenol-A in two areas, thermal paper and PVC, could lead to a relatively large reduction in the background concentration of bisphenol-A. Of the remaining uses bisphenol-A production, epoxy resin production and (in Scenarios 1 and 2) losses from PVC articles in use are the major contributors to the resultant background concentration. Bisphenol-A production and epoxy resin production are both point source releases and measured data from the existing plants suggests that it should be possible to control the emissions from these plants to achieve virtually zero emissions. Losses from PVC articles in use are harder to quantify and the approach taken here is likely to have overestimated the actual level of releases. Their contribution to the regional PEC should therefore be treated with caution. It is likely that the release of bisphenol-A from PVC articles in use will decrease over the thirty-year period rather than remain constant, as articles are taken out of use and the bisphenol-A present in the article as an antioxidant reduces due to chemical reaction.

Input into EUSES for the scenarios above

Existing: Regional PEC_{water} 0.115 µg/l

Compartment	Regional (kg/day)	Continental (kg/day)
Air	5.8	39.6
WWTP	112.7	1,012.2
Surface water	14.7	121.2
Industrial soil	6.2	56

Scenario 1: Regional PEC_{water} 0.0334 µg/l

(minus thermal paper production and recycling, and PVC production)

Compartment	Regional (kg/day)	Continental (kg/day)
Air	5.8	39.6
WWTP	0.87	5.96
Surface water	7.89	59.8
Industrial soil	6.2	56

Scenario 2: Regional PEC_{water} 0.0315 µg/l

(As Scenario 1 minus PVC processing)

Compartment	Regional (kg/day)	Continental (kg/day)
Air	5.8	39.6
WWTP	0.01	0.11
Surface water	7.52	57.17
Industrial soil	6.2	56

Scenario 3: Regional PEC_{water} 0.0055 µg/l

(As Scenario 2 minus losses from PVC articles in use)

Compartment	Regional (kg/day)	Continental (kg/day)
Air	1.58	1.12
WWTP	0.01	0.11
Surface water	1.35	1.45
Industrial soil	0	0

European Commission

EUR 20843 EN **European Union Risk Assessment Report**
4,4'-isopropylidenediphenol (bisphenol-A), Volume 37

Editors: S.J. Munn, R. Allanou, K. Aschberger, F. Berthault, J. de Bruijn, C. Musset, S. O'Connor, S. Pakalin, G. Pellegrini, S. Scheer, S. Vegro.

Luxembourg: Office for Official Publications of the European Communities

2003 –X pp., 290 pp. – 17.0 x 24.0 cm

Environment and quality of life series

The report provides the comprehensive risk assessment of the substance 4,4'-isopropylidenediphenol (bisphenol-A). It has been prepared by the UK in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The environmental risk assessment for bisphenol-A concludes that there is concern for the water and sediment compartments arising from the use of the substance in thermal paper recycling and in production and processing of PVC. In addition there is a need for further information on risks to the water and sediment compartments for the other scenarios and to the terrestrial compartment. There is no concern for microorganisms in the sewage treatment plant and for the atmosphere.

The human health risk assessment for bisphenol-A concludes that there is concern for workers in relation to eye and respiratory tract irritation, effects on liver and toxicity for reproduction arising from exposure in the manufacture of bisphenol-A and of epoxy resins and also in relation to skin sensitisation in all scenarios. In addition there is a need for further information to adequately characterise the risks to workers, consumers and humans exposed via the environment in relation to effects on development at low doses.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commissions committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, private or national.

European Commission – Joint Research Centre
Institute for Health and Consumer Protection
European Chemicals Bureau (ECB)

European Union Risk Assessment Report

4,4'-isopropylidenediphenol (bisphenol-A)

CAS No: 80-05-7 EINECS No: 201-245-8

Series: 3rd Priority List Volume: 37

Publications Office
Publications.eu.int