European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

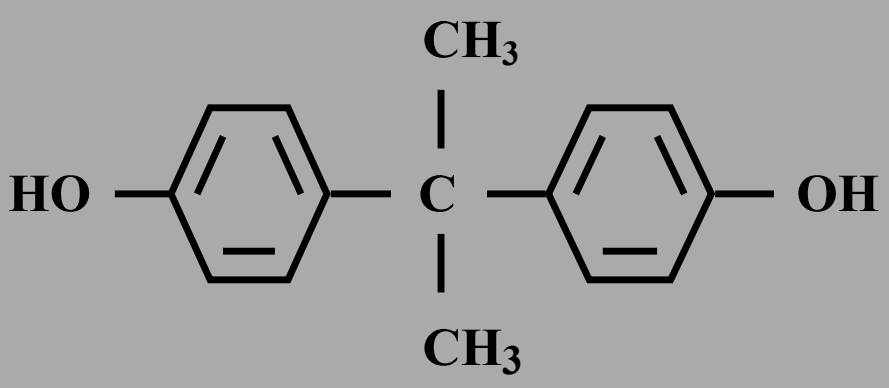
Existing Substances

European Union Risk Assessment Report

CAS No: 80-05-7

EINECS No: 201-245-8

4,4'-isopropylidenediphenol (bisphenol-A)



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4,4'-ISOPROPYLIDENEDIPHENOL

(BISPHENOL-A)

CAS No: 80-05-7

EINECS No: 201-245-8

RISK ASSESSMENT

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4,4'-ISOPROPYLIDENEDIPHENOL

(BISPHENOL-A)

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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.

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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

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[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.

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Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it.

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1 GENERAL SUBSTANCE INFORMATION

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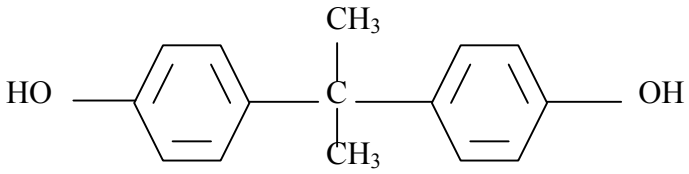
CAS-No: 80-05-7
EINECS-No: 201-245-8
IUPAC name: 2,2-bis(4-hydroxyphenyl)propane
Molecular weight: 228.29
Molecular formula: $C_{15}H_{16}O_2$
Structural formula:



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 $2.59 \cdot 10^{6}$ m^3/day
BPA 6	10 mg/Nm^3 (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

Nm^3: volume in m^3 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.

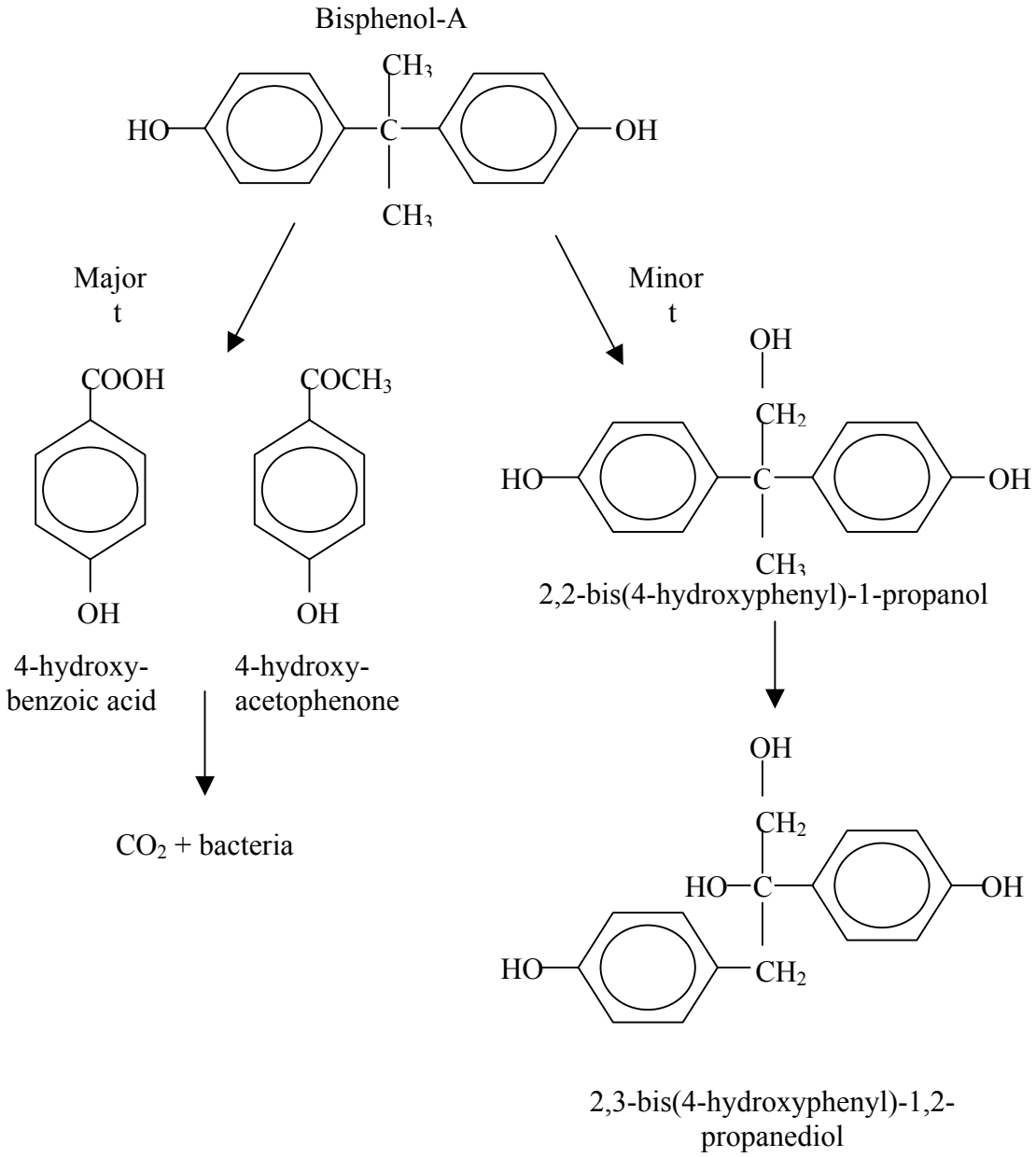


Figure 3.1 Possible degradation pathways of bisphenol-A

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:

$$Clocal_{inf} = \frac{Elocal_{water} \cdot 10^6}{EFFLUENT_{stp}} \qquad \text{(17 TGD)}$$

$$Clocal_{eff} = Clocal_{inf} \cdot Fstp_{water} \qquad \text{(18 TGD)}$$

$$EFFLUENT_{stp} = CAPACITY_{stp} \cdot WASTEWinhab = 2{,}000{,}000 \text{ l/d} \qquad \text{(19 TGD)}$$

$$Clocal_{water} = \frac{Clocal_{eff}}{(1 + Kp_{susp} \cdot SUSP_{water} \cdot 10^{-6}) \cdot DILUTION} \qquad \text{(30 TGD)}$$

$$DILUTION = \frac{EFFLUENT_{stp} + FLOW}{EFFLUENT_{stp}} = 10 \text{ [Default]} \qquad \text{(31 TGD)}$$

$$Clocal_{water,ann} = Clocal_{water} \cdot \frac{Temission}{365} \qquad \text{(32 TGD)}$$

$$PEClocal_{water} = Clocal_{water} + PECregional_{water} \qquad \text{(33 TGD)}$$

$$PEClocal_{water,ann} = Clocal_{water,ann} + PECregional_{water} \qquad \text{(34 TGD)}$$

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 10th 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 90th 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 90th 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 90th 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 90th 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 90th 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/m³)	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.

<u>Fish studies</u>

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 ≥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
$K_{soil-water}$	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 90th percentile. This has been calculated where there is sufficient data. Where insufficient data has been submitted, professional judgement has been used to estimate the 90th 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/m³)	Arithmetic mean 8-h TWA (mg/m³)	90th percent. 8-h TWA (mg/m³)	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/m³)	Arithmetic mean (mg/m³)
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/m³)	Mean 8-h TWA (mg/m³)	90th percentile 8-h TWA (mg/m³)
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/m³)	Mean result (mg/m³)	90th percentile (mg/m³)
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

<u>Epoxy paints and lacquers</u>

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°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/m³)	Mean (mg/m³)	RWS exposure (mg/m³)	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 $3 \cdot 10\text{-}^{7}$ 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/cm²/day)	RWS for dermal exposure (mg/cm²/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 ($mg/cm^2/day$)	RWS for dermal exposure ($mg/cm^2/day$)
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 97.5th 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 · 10-5 ± 2.5 · 10-5 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 (≥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.

<u>Dermal exposure</u>

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 (≥24%) and females (≥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 ≤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°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

<u>Inhalation exposure</u>

There are no data available.

<u>Oral exposure</u>

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 (≥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 (≥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 (≥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 (≥47%) were seen at 40 mg/kg and above, compared to controls. Increases in relative wet and dry uterine weights (≥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 (≥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 10th 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 (≥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 (≥ 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.

<u>Manufacture of bisphenol-A</u>

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.

<u>Manufacture of polycarbonate</u>

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 ≤ 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 ≤ 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	Effects on fertility		Effects on development			
	(mg/kg/day)	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	Effects on fertility		Effects on development			
	(mg/kg/day)	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.

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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.

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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.

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