THIS DOCUMENT HAS BEEN PREPARED ACCORDING TO THE PROVISIONS OF ARTICLE 136(3) “TRANSITIONAL MEASURES REGARDING EXISTING SUBSTANCES” OF REACH (REGULATION (EC) 1907/2006). IT IS NOT A PROPOSAL FOR A RESTRICTION ALTHOUGH THE FORMAT IS THE SAME

ANNEX XV RESTRICTION REPORT

SUBMITTED BY: United Kingdom
DATE: 30 November 2008

SUBSTANCE NAME: Bisphenol-A
IUPAC NAME: 4-[2-(4-hydroxyphenyl)propan-2-yl]phenol
EC NUMBER: 201-245-8
CAS NUMBER: 80-05-7

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A Proposal

A.1 Proposed Restriction

No restrictions on the manufacture or use of Bisphenol-A are proposed.

A.1.1 The identity of the substance(s)

Chemical Name:	Bisphenol-A
EC Number:	201-245-8
CAS Number:	80-05-7
IUPAC Name:	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol

A.1.2 Proposed risk management measures for occupational uses

- Establish an Indicative Occupational Exposure Limit (IOELV) – likely to be adopted in early 2009;
- Implementation of risk management measures (RMMs) following registration of Bisphenol-A under REACH, and
- Industry to voluntarily update the 'Safety and Handling Guide' to take account of the information within this Annex XV report.

A.2 Background to the transitional dossier

A.2.1 Human Health

The hazards and risks associated with Bisphenol-A (BPA) have been evaluated and agreed (2003 and 2008) under the Existing Substances Regulations (ESR) (793/93/EEC). The original human health risk assessment report (RAR) was agreed by the Technical Committee for New and Existing Substances (TCNES) in 2003 (EC, 2003). However, further information (new toxicology and exposure data) was submitted in October 2007 which was included as an addendum to the RAR. The addendum to the RAR was agreed by TCNES in 2008 (EC, 2008).

Whenever a conclusion (iii) was assigned under the ESR a risk reduction strategy was developed. A conclusion (iii) denotes that further risk management measures (RMMs) are required to control the risk. As ESR has been repealed by REACH (Registration, Evaluation and Authorisation of Chemicals) an Annex XV Restriction document has to be developed for this transitional substance. This Annex XV report **only** examines those human health scenarios that were assigned a conclusion (iii) following the update to the RAR. This Annex XV report **will not** revisit any other conclusions made in the RAR.

The RAR concluded that:

1. Workers

There is a need for reducing the risks (conclusion iii) from exposure to BPA because of the following human health effects:

- Repeated dose toxicity (local effects on the respiratory tract, effects of bodyweight gain and kidney, effects on the liver, toxic for reproduction) for manufacture of BPA and manufacture of epoxy resins.
- skin sensitisation at high concentrations of BPA (the maximum concentration investigated was 30% and some uncertainty remains as to whether high concentrations (>30%) of BPA can exert skin sensitising activity) for all occupational exposure scenarios:
    - Manufacture of BPA
    - Manufacture of polycarbonate (PC)
    - Manufacture of articles from PC
    - Manufacture of epoxy resins and moderated epoxy resins
    - Use of BPA in poly vinyl chloride (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.

2. Consumers

There were no human health effects which lead to a conclusion (iii) in the RAR for consumers. Therefore, no further risk management activity under REACH is required.

3. Man via the environment

There were no human health effects which lead to a conclusion (iii) in the RAR for man via the environment. Therefore, no further risk management activity under REACH is required.

4. Combined exposure

There were no human health concerns that lead to a conclusion (iii) in the RAR for combined exposure. Therefore, no further risk management activity under REACH is required.

This Annex XV report contains information from the 2003 and updated (2008) RAR (EC, 2003 & 2008). However, for full details of the information in the RAR and its update, reference should be made to the European Chemicals Bureau (ECB) website. The 2003 and 2008 RAR can be found at http://ecb.jrc.it/Documents/Existing-Chemicals/RISK_ASSESSMENT/SUMMARY/bisphenolasum325.pdf and http://ecb.jrc.it/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/bisphenola_add_325.pdf, respectively.

The original RAR and its addendum are collectively termed RAR within this Annex XV report.

A.2.2 Environment

Two environmental risk assessment reports for BPA were published under ESR (EC, 2003 & 2008). The conclusion of the last report for the freshwater compartment (and by implication the marine compartment) was that further investigations of toxicity to aquatic snails should be performed, due to continued uncertainties about the role of seasonal cycles on snail sensitivity. UK Government (the Environment Agency and the Department of the Environment, Food and Rural Affairs) has sponsored further research in this area, and this document presents the results of this study to bring the ESR work to an end.

In addition, two further aspects were raised by stakeholders during consultation for this report – i.e. possible sediment risks indicated by monitoring data and a specific fish toxicity test result – and these are also addressed for transparency and completeness.

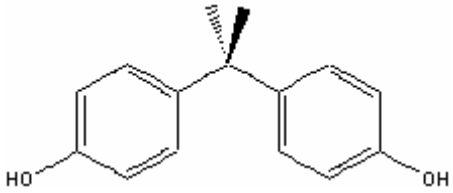
B. INFORMATION ON HAZARD AND RISK

B.1 Identity of the substance(s) and physical and chemical properties

B.1.1 Name and other identifiers of the substance(s)

Chemical Name:	Bisphenol-A
EC Number:	201-245-8
CAS Number:	80-05-7
IUPAC Name:	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol

B.1.2 Composition of the substance(s)

Chemical Name:	Bisphenol-A
EC Number:	201-245-8
CAS Number:	80-05-7
IUPAC Name:	4-[2-(4-hydroxyphenyl)propan-2-yl]phenol
Molecular Formula:	$C_{15}H_{16}O_2$
Structural Formula:	
Molecular Weight:	228.29
Typical concentration (% w/w)	99 – 99.8*

*Impurities typically include phenol (<0.06%), ortho and para isomers of bisphenol-A (<0.2%) and water (<0.2%).

B.1.3 Physico-chemical properties

The information outlined in Table 1.1 is from the RAR. For those physico-chemical properties where a value (as required by this Annex XV report) is not detailed in the RAR then this is indicated in Table 1.1 as 'not available'.

Table 1.1 Physico-chemical properties of Bisphenol-A

REACH ref Annex, §	Property	IUCLID section	Value	Comment
VII, 7.1	Physical state at 20 C and 101.3 KPa	3.1	White solid flakes or powder	Depends on manufacturing process
VII, 7.2	Melting / freezing point	3.2	155-157°C	Depends on manufacturing process
VII, 7.3	Boiling point	3.3	360°C at 101.3 kPa	Decomposition is also likely
VII, 7.4	Relative density	3.4 density	circa 1.1-1.2 kg/m^3 at 25°C	
VII, 7.5	Vapour pressure	3.6	4.1×10^{-10} kPa and 5.3×10^{-9} kPa at 25°C	
VII, 7.6	Surface tension	3.10	Not available	
VII, 7.7	Water solubility	3.8	300 mg/l	
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	Log Kow circa 3.3-3.5	
VII, 7.9	Flash point	3.11	circa 207°C	
VII, 7.10	Flammability	3.13	circa 532°C	
VII, 7.11	Explosive properties	3.14	Minimum explosive concentration 0.012 g/l with oxygen > 5%	
VII, 7.12	Self-ignition temperature		510°C	
VII, 7.13	Oxidising properties	3.15;	Not an oxidising agent	
VII, 7.14	Granulometry	3.5	Not available	
IX, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	Not available	
IX, 7.16	Dissociation constant	3.21	Not available	
IX, 7.17	Viscosity	3.22	Not applicable	
	Auto flammability	3.12	Circa 532°C	Decomposition is likely before these temperatures are reached.
	Reactivity towards container material	3.18	Not available	
	Thermal stability	3.19	Not available	

B.2 Manufacture and uses

B.2.1 Manufacture and import of a substance

The following information is a summary of that provided in the RAR:

Four companies within the EU manufacture BPA. 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 1,150,000 tonnes/year (taken from 2005/06 data). From the data submitted by the EU manufacturers net exports are in the region of 65,000 tonnes/year for 2005/06. However, other manufacturers exist who are not members of Cefic and so have not supplied information, so these tonnage figures may be an underestimate.

B.2.2 Uses

The occupational uses of BPA identified in Table 2.1 are those that were assigned a conclusion (iii) following the update to the RAR.

Table 2.1 Bisphenol-A use pattern data (2005/06)

Use	**Tonnes/year**	**Percentage of EU consumption**
Manufacture of polycarbonate (PC)	865,000	71.1
Manufacture of articles from polycarbonate	400*	0.05*
Manufacture of epoxy resins and moderated epoxy resins	191,520	25
Manufacture of polyvinylchloride (PVC)	1,800	0.3
Manufacture of liquid epoxy paints, lacquers and powder coatings	8,800 (Phenoplast) 3,600 (unsaturated)	1.4 0.5
Use of epoxy resin-based powder coatings, paints and lacquers	Not available	Not available
Manufacture of thermal papers	1,900	0.2
Manufacture of tin-plating additive	2,460**	0.4

*BPA Environmental Risk Reduction Strategy (RRS) (Defra, 2003)
** BPA RAR (EC, 2003)

B.2.3 Uses advised against by the registrants

No uses have been advised against by the registrants as BPA is a transitional substance.

B.3 Classification and labelling

B.3.1 Classification in Annex I of Directive 67/548/EEC

Classification: Repr. Cat. 3; R62
Xi; R37-41, R43

Labelling: Xn
R37-41-43-62

S: (2-) 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

B.3.2 Classification in classification and labelling inventory/Industry's self classification(s) and labelling

As this is a transitional substance no industry classification and labelling has been carried out.

B.4 Environmental fate properties

This Annex XV report is only concerned with those conclusion (iiis) for human health and the conclusion (i) for environment related to aquatic snail toxicity. Environmental fate data are therefore not relevant.

B.5 Human health hazard assessment

Full details of the human health hazard assessment can be found in section 4.1.2 of the RAR and its addendum (EC, 2003 & 2008).

B.5.1 Derivation of DNEL(s)/DMEL(s) or other quantitative or qualitative measure for dose response

The purpose of this transitional dossier is to develop risk reduction strategies for exposure situations for which conclusion (iii) was reached in the EU RAR. Therefore, derived no effect levels (DNELs) have only been calculated for the health endpoints and routes of exposure that are relevant to the exposure situations of concern identified in the RAR. The UK notes that appendix R.8-13 of the Chemical Safety Assessment (CSA) guidance states that EU Indicative Occupational Exposure Limit Values (IOELVs) may take the place of the short-term inhalation worker-DNEL and long-term inhalation worker-DNEL where the current scientific information supports the IOELV (CSA guidance, chapter R8, Appendix R8-13, p142, 2008).

In May 2004, the European Commission's Scientific Committee on Occupational Exposure Limits (SCOEL) published a recommendation for an IOELV for BPA of 10 mg/m^3 (8-hr TWA (time-weighted average)) and this recommendation has been included in the draft 3rd IOELV Directive which is due to be adopted early in 2009 . In making its recommendation, SCOEL took account of evidence for site of contact inflammation in the respiratory tract of rats inhaling BPA and evidence for systemic toxicity in oral dosing studies (SCOEL, 2004). Although new information has become available since the SCOEL recommendation was finalised it does not change the No-observed adverse effect level (NOAEL) on which the IOELV proposal is based. In accordance with the CSA guidance the UK proposes to adopt 10 mg/m^3 as the long-term inhalation worker-DNEL.

B.5.1.1 Overview of dose descriptors

The human health endpoints for which concerns have been identified in the RAR are:

- respiratory tract irritation
- skin sensitisation
- repeat dose toxicity
- reproductive toxicity

The dose descriptors that were identified in the RAR for these endpoints are summarised in Table 5.1 below.

With regards to skin sensitisation, a DNEL/DMEL cannot be calculated from the available information. In a guinea pig maximisation study, a sensitisation rate of 12.5% was obtained with the highest challenge concentration of 50%. This finding of skin sensitising potential was not confirmed in a recent local lymph node assay (LLNA). However, the maximum concentration investigated

in the LLNA was 30% and some uncertainty remains as to whether high concentrations (>30%) of BPA can still exert skin sensitising activity. Since no sensitising activity was seen in the LLNA, it is not possible to derive an EC3 (effect concentration 3) value; therefore, it is not possible to identify a suitable starting point from which to calculate a DNEL or DMEL for skin sensitisation. It is possible to make a qualitative judgement about the skin sensitising potency of BPA for the purposes of identifying suitable risk management measures (RMMs). The CSA guidance indicates that skin sensitisers for which the EC3 value is greater than 2% in a LLNA should be regarded as moderate sensitisers (CSA guidance, chapter 8, appendix 8-10, page 127). In the case of BPA, no sensitising activity was observed with a concentration of 30% and therefore, if an EC3 value were to be obtained it would be greater than 30%. On this basis, it can be concluded that BPA should be regarded as a moderate skin sensitiser.

Table 5.1 Dose descriptors identified in the RAR for endpoints of concern

Endpoint	**Quantitative dose descriptor or other information on potency**		**Associated relevant effect**	**Remarks on the study**
	Local effect	**Systemic effect**		
Irritation/corrosivity				
Respiratory tract	NOAEC 10 mg/m^3		Inflammatory effects in the upper respiratory tract	2 and 13 weeks repeat inhalation studies in the rat.
Repeated dose toxicity (sub-acute/sub-chronic/chronic)				
Oral	NOAEL 50 mg/kg/day		Reduction in bodyweight gain, kidney damage and liver toxicity	2-generation dietary study in mice.
Inhalation	NOAEC 10 mg/m^3		Slight upper respiratory tract epithelium inflammation and hyperplasia of the olfactory epithelium	13 weeks bioassay in the rat, exposure 6 hours per day, 5 days per week
Reproductive toxicity				
Oral	NOAEL 50 mg/kg/d		Increased duration of gestation, reduced pup bodyweight, seminiferous tubules hypoplasia, increased incidence of undescended testes and delayed acquisition of preputial separation	2-generation dietary study in the mouse
Skin sensitisation				
Skin sensitisation	Not possible to derive an EC3 value			

B.5.1.2 Exposure situations for which risk reduction strategies are required

In the RAR, conclusion (iii) was only identified for the occupational exposure scenarios.

The exposure scenarios identified are as follows:

- Manufacture of BPA
- Manufacture of epoxy resins
- There is a concern for skin sensitisation in all scenarios with a potential for skin contact with high concentrations (>30%) of BPA

The pattern of exposure for these uses includes short-term peak exposure by the inhalation and dermal routes and long-term repeated exposure by the inhalation and dermal routes. As no specific health concerns have been identified relating to short-term inhalation of BPA it is not necessary to derive a worker-DNEL short-term inhalation. The UK intends to refer to the 8-hr TWA IOELV of 10 mg/m^3 recommended by SCOEL in lieu of calculating a worker-DNEL long-term inhalation. In the case of short-term peak dermal exposure, there are no measured or modelled data from which to characterise this type of exposure therefore all dermal exposure will be assessed by comparison to the long-term dermal DNEL.

The following worker DNEL has been calculated:

Worker-DNEL long-term for dermal route

B.5.1.3 Worker-DNEL long-term dermal route

BPA has the potential to be absorbed across the skin and there is the potential for adverse systemic effects to arise as a result of skin exposure. No studies have been undertaken by the dermal route to characterise the dose-response relationship for systemic effects. Therefore, it will be necessary to obtain the worker DNEL long-term dermal by extrapolation. A NOAEL of 50 mg/kg/d was identified in oral studies in rodents for the effects of BPA on reproduction, bodyweight, kidney and liver.

Toxicokinetics studies indicate that following oral absorption, BPA undergoes extensive first pass metabolism resulting in decreased systemic bioavailability of free BPA. Although absorption following oral administration is estimated to be 100%, only around 5-10% reaches the systemic circulation. Since the RAR concluded that free BPA is the toxic entity responsible for the systemic effects of BPA it is necessary to take account of first pass metabolism when conducting the oral to dermal extrapolation for the reproductive, kidney and bodyweight effects of BPA. These organs may potentially be exposed to all of the material absorbed across the skin but only 10% of the material absorbed across the gastrointestinal (GI) tract. It is not necessary to take account of first pass metabolism when conducting the oral to dermal extrapolation for the effects of BPA in the liver, since this organ potentially receives all of the orally

dosed material. Given this variation, it is considered necessary to calculate a separate endpoint specific DNEL for the liver.

The RAR also identifies major differences between humans and rodents in the systemic availability of free unconjugated BPA after oral absorption. In both species, BPA is rapidly metabolised in the gut wall and the liver to BPA-glucuronide; however, in humans the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion resulting in low blood concentration of free BPA. In rats, BPA glucuronide is eliminated in the bile and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid in the intestinal tract. The enterohepatic recirculation results in slower excretion and increased systemic availability of free unconjugated BPA in rodents suggesting that rodents may be more sensitive to the effects of BPA than humans.

B.5.1.3.1 *Dermal DNEL derived for repeated dose effects on the liver*

A NOAEL of 50 mg/kg/d has been identified from a 2-generation study in the mouse for the liver effects. In the RAR, dermal absorption in both humans and animals is assumed to be 10%; whereas, the absorption following oral administration is estimated at 100%. In order to conduct a route-to-route extrapolation, there is a need to adjust for the differences in absorption between routes; therefore, the oral NOAEL of 50 mg/kg/d is multiplied by 10 to give the equivalent dermal NOAEL. As indicated above it is not necessary to take account of first pass effects when conducting the route-to-route extrapolation for the liver.

The corrected starting point is therefore 500 mg/kg/d.

Assessment factors (AF) and calculation for worker DNEL long-term dermal liver effects based on the animal NOAEL		
Uncertainties	**AF**	**Justification**
Interspecies differences	17.5 (2.5x7)	The dose descriptor is obtained from an oral study in the mouse. To use a value extrapolated from a mouse oral study to assess dermal exposure in humans it is necessary to apply an allometric scaling factor of 7 to take account of differences in basal metabolic rates between mice and human. For effects on the liver, the rodent-humans differences in the systemic availability of free unconjugated BPA are unimportant. On this basis a default factor of 2.5 to account for other species differences will be applied giving an overall AF of 17.5.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to BPA in the human population. The default factor of 5 for workers will therefore be used to take account of intraspecies differences.
Differences in duration of exposure	1	Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. A NOAEL of 50 mg/kg bw/day was identified in parental generations in subchronic reproductive toxicity studies in the mouse. The LOAEL was 600 mg/kg bw/day. In a chronic study with mice, some liver effects were observed at a dose level of 120 mg/kg bw/day but without an increase in severity at 650 mg/kg bw/day. Therefore, it is judged that an additional factor to extrapolate the subchronic NOAEL to chronic exposure is not necessary.
Dose response and endpoint specific/severity issues	1	There is more than one order of magnitude between the LOAEL (600 mg/kg/d) and the NOAEL (50 mg/kg/d) and only minor effects were observed at the LOAEL. It is, therefore, not necessary to apply an additional factor.
Quality of database	1	Several robust repeated exposure studies and a series of good quality 2- and multi-generation reproductive toxicity studies are available from which to characterise the effects of BPA in the liver. The quality of the database is therefore not considered to contribute uncertainty to this assessment and hence it is not necessary to apply an additional factor.
Overall assessment factor: 87.5		
Endpoint specific DNEL: 500/87.5 = 6 mg/kg/day		

B.5.1.3.2 *Dermal DNEL derived for repeated dose effects on bodyweight and kidney*

A NOAEL of 50 mg/kg/d has been identified in a 2-generation study in the mouse for effects on bodyweight and kidney. Oral absorption is estimated at 100% in the RAR, but to take account of the effect of first pass metabolism on the bioavailability of BPA following oral absorption, there is a need to adjust the NOAEL by a factor of 10 to give an internal oral NAEL (no adverse effect level) of 5 mg/kg/d. The RAR concludes that dermal absorption is 10% of the applied dose in both humans and animals; therefore the internal NAEL of 5 mg/kg/d is multiplied by 10 to give the equivalent dermal NOAEL.

The corrected starting point is therefore 50 mg/kg/day.

Assessment factors and calculation for worker DNEL long-term dermal repeated dose effects on bodyweight weight and kidney based on the animal NOAEL		
Uncertainties	**AF**	**Justification**
Interspecies differences	7	The dose descriptor is obtained from an oral study in the mouse. To use a value extrapolated from a mouse oral study to assess dermal exposure in humans it is necessary to apply an allometric scaling factor of 7 to take account of differences in basal metabolic rates between mice and humans. In view of the evidence that much lower levels of free BPA reach the systemic circulation in humans compared to the levels observed in rats and mice indicating that humans are likely to be less sensitive to the effects of BPA it is not considered necessary to apply an additional factor to take account of other species differences.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to BPA in the human population. The default factor of 5 for workers will therefore be used to take account of intraspecies differences.
Differences in duration of exposure	1	Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. In a mouse 2-year study a LOAEL of 120 mg/kg/day was identified for minor effects on bodyweight gain. Therefore it is judged that an additional factor to extrapolate the subchronic NOAEL to chronic exposure is not necessary.
Dose response and endpoint specific/severity issues	1	There is more than one order of magnitude between the LOAEL (600 mg/kg/d) and the NOAEL (50 mg/kg/d) and only minor effects were observed at the LOAEL. It is therefore not necessary to apply an additional factor.
Quality of database	1	Several robust repeated exposure studies and a series of good quality 2- and multi-generation reproductive toxicity studies are available from which to characterise the effects of BPA in the liver. The quality of the database is therefore not considered to contribute uncertainty to this assessment and hence it is not necessary to apply an additional factor.
Overall assessment factor: 35		
Endpoint specific DNEL: 50/35 = 1.4 mg/kg/day		

B.5.1.3.3 *Dermal DNEL derived for reproductive toxicity*

A NOAEL of 50 mg/kg/d has been identified in a 2-generation study in the mouse and in rat multigeneration study. Oral absorption is estimated at 100%

in the RAR but, to take account of the effect of first pass metabolism on the bioavailability of BPA following oral absorption, there is a need to adjust the NOAEL by a factor of 10 to give an internal oral NAEL of 5 mg/kg/d. The RAR concludes that dermal absorption is 10% of the applied dose in both humans and animals; therefore the internal NAEL of 5 mg/kg/d is multiplied by 10 to give the equivalent dermal NOAEL.

The corrected starting point is therefore 50 mg/kg/day.

Assessment factors and DNEL calculation for worker DNEL long-term dermal systemic effects based on the animal NOAEL		
Uncertainties	**AF**	**Justification**
Interspecies differences	4	The same NOAEL of 50 mg/kg/d has been identified from mouse and rat data; however, since the available information (see section 4.1.3.1 of RAR) shows that the rat is a better model for humans than the mouse the allometric scaling factor for the rat (4) is considered more appropriate than that for the mouse. In view of the evidence that much lower levels of free BPA reach the systemic circulation in humans compared to the levels observed in rats and mice indicating that humans are likely to be less sensitive to the effects of BPA it is not considered necessary to apply an additional factor to take account of other species differences.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to BPA in the human population. The default factor of 5 for workers will therefore be used to take account of intraspecies differences.
Differences in duration of exposure	1	The dose descriptor was obtained from multigenerational studies. It is therefore not necessary to apply a factor to take account of differences in duration of exposure.
Dose response and endpoint specific/severity issues	1	There is more than one order of magnitude between the LOAEL (600 mg/kg/d) and the NOAEL (50 mg/kg/d); it is therefore not necessary to apply an additional factor.
Quality of database	1	Several robust repeated exposure studies and a series of good quality 2- and multi-generation reproductive toxicity studies are available from which to characterise the systemic and reproductive effects of BPA. The quality of the database is therefore not considered to contribute uncertainty to this assessment and hence it is not necessary to apply an additional factor.
Overall assessment factor: 20		
Endpoint specific DNEL: 50/20 = 2.5 mg/kg/day		

B.5.1.3.4 Selection of worker-DNEL long-term dermal

BPA undergoes extensive first pass metabolism following oral absorption which results in decreased systemic bioavailability of free BPA - the toxic entity responsible for its systemic effects and, owing to the major species differences between rodents and humans in systemic availability of unconjugated BPA after oral absorption, endpoint specific DNELs have been calculated using animal data. It is therefore necessary to identify which of these DNELs is the critical DNEL for assessing long-term dermal exposure of workers.

Endpoint specific DNELs have been derived separately for reproductive toxicity (2.5 mg/kg/d) and the repeated dose effects in the liver (6 mg/kg/d) and on bodyweight/kidney (1.4 mg/kg/d). The lowest DNEL is derived for the repeated dose effects on bodyweight and kidney and it is, therefore, chosen as the critical DNEL for long-term dermal exposure in workers.

The worker DNEL long-term dermal route for systemic effects is therefore 1.4 mg/kg/day.

BPA is considered to be a moderate skin sensitiser. This DNEL does not address the skin sensitising potential of BPA and may not be adequate to protect against skin sensitisation in situations where there is a potential for direct skin contact with BPA.

B.5.2 Summary of critical DNELs

	Worker
DNEL long-term inhalation for local and systemic effects	10 mg/m^3 (8h-Time-weighted average (TWA))
DNEL long-term dermal for systemic effects	1.4 mg/kg/day
Skin sensitisation	BPA is considered to be a moderate skin sensitiser. As outlined in Section 5.1.1 the data available for this endpoint are not sufficient to allow a DNEL to be calculated for this endpoint.

B.6 Human health hazard assessment of physico-chemical properties

A conclusion (ii) for the human health assessment of physico-chemical properties was assigned in the RAR indicating the risks are adequately controlled.

B.7 Environmental hazard assessment

B.7.1 Aquatic compartment (including sediment)

B.7.1.1 Aquatic snail toxicity

A summary of the previously reported research on bisphenol-A toxicity to aquatic snails is provided below to place the new findings in context. However, EC (2008) should be consulted for full details and discussion. No further literature searches have been performed for this report.

Summary of previous findings

One study has been performed with the dogwhelk *Nucella lapillus* (a marine prosobranch gastropod):

1. Wild-caught adults were exposed for three months in the laboratory to nominal concentrations of 1, 25 and 100 µg/l, with renewal every 24 hours (Oehlmann *et al*., 2000). Enlarged pallial sex glands and an enhancement of oocyte production was observed. No oviduct malformations were found. A lower percentage of exposed specimens had ripe sperm stored in their vesicula seminalis and males exhibited a reduced length of penis and prostate gland when compared to the control. Statistically significant effects were observed at all the test concentrations (which were not confirmed analytically).

   The lack of measured exposure concentrations means that the results cannot be used directly for risk assessment. In addition, the use of wild specimens is a problem because they might be parasitized and their exposure history is unknown. The test methodology and statistical analysis has not been subject to any detailed scrutiny, but the comments on the main studies of this research group (reported below) may be relevant.

Two studies have been performed with *Potamopyrgus antipodarum* (a temperate species of freshwater prosobranch gastropod, common in Europe).

2. Jobling *et al*. (2003; corrected version published 2004) exposed laboratory-cultured snails to nominal bisphenol-A concentrations in water of 1, 5, 25 and 100 µg/l over 90 days in a semi-static system with 50% of the dosed water being replaced every four days. Temperature, light regime and physico-chemical parameters are not reported in the paper. No effects were seen on survival at any concentration of bisphenol-A. Exposure to 5 µg/l appeared to enhance growth (a significant increase in the mean shell height occurred between 6 and 9 weeks exposure), though this effect was not observed at higher concentrations. After three weeks, the absolute number of new embryos was significantly greater than the control at 1, 5 and 25 µg/l, but not 100 µg/l (it appears that less than one new embryo had been produced in the control – value read from a graph).

At 63 days, the 5 and 25 µg/l exposures had significantly more new embryos than the controls; numbers were higher than controls at 1 µg/l but lower at 100 µg/l, although the differences were not statistically significant. The highest number of new embryos (11) was observed in the 5 µg/l group at 63 days; the control only produced one new embryo at this time point (values read from a graph).

3. Duft *et al*. (2003) exposed *Po. antipodarum* to artificial sediments spiked with bisphenol-A. Stimulation of embryo production occurred at all nominal test concentrations (1, 10, 30, 100 and 300 µg/kg dry weight) after eight weeks' exposure at 15±1°C, i.e., the NOEC was below 1 µg/kg nominal dry weight. However, no analysis of the sediment was undertaken because of the reported short half-life of bisphenol-A in sediment. The concentrations of parent substance and any metabolites that the snails might have been exposed to over the course of the study are therefore unknown.

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

4. Oehlmann and co-workers have performed three series of experiments. (Oehlmann *et al*. (2000), Schulte-Oehlmann *et al*. (2001), Oehlmann *et al*. (2001) and Oehlmann *et al*. (2006)). In the first experiment, adult *M. 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, at 22ºC. A complex number of effects occurred, including 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.

   A second experiment using nominal bisphenol-A concentrations of 0.05-1.0 µg/l and the same semi-static exposure system over 180 days again detected the "superfeminisation" phenomenon in all of the treated groups (with the exception of the 0.05 µg/l (nominal) group), though at a lower incidence than in the high concentration experiment. Egg production was also stimulated as before, although the results over the whole 180-day exposure period showed a significant increase only at the two highest concentrations. The exposure period in this

second experiment included the season of the year (October to February) when spawning activity in this population of *M. cornuarietis* increases naturally. It was therefore considered that the effect of bisphenol-A might be masked to some degree by the natural increase. (The first experiment took place completely outside this active season.) 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 measured concentrations).

A third experiment with *M. cornuarietis* was performed using semi-static exposure with medium renewal every one or two days. Two replicates, each of 30 sexually mature snails, were exposed to five concentrations of bisphenol-A (0, 0.25, 0.5, 1, and 5 µg/l). Exposure was for five months (February-July, which is outside the main spawning period for this population of snails) at two different temperatures (20°C or 27°C) with analysis of all surviving animals at the end of the study. Survival, numbers of eggs and clutches, and numbers of eggs per clutch were recorded daily. Results were expressed in terms of median measured concentrations, which were between 39.0% and 48.3% of nominal levels. Snails exposed to bisphenol-A at 20°C produced significantly more clutches and eggs compared to controls. A NOEC could not be calculated because there were significant effects (compared to the control) at the lowest test concentration of 106 ng/l. EC_{10} values were estimated to be 14.8 ng/l (95% confidence interval 6.07 – 36.2 ng/l) and 18.0 ng/l (95% confidence interval 6.2-52.5 ng/l) for egg and clutch production, respectively. At 27°C, none of the treatment groups produced significantly more clutches, or eggs on a per female basis, than the control. A significant increase in egg production could only be detected if measured in terms of cumulative egg number, for the nominal 1 and 5 µg/l exposure groups. Based on measured concentrations, the NOEC for egg production was 205 ng/l (EC_{10} = 998 ng/l; 95% confidence interval 161-6,200 ng/l) and the NOEC for clutch production rose to >1,990 ng/l (EC_{10} = 2,090 ng/l; 95% confidence interval 796-5,460 ng/l). The temperature-related differences in NOECs are a direct consequence of the lower egg production in controls observed at 20°C (~500 eggs/female over the 5-month period). Females with oviduct malformations were only found at 20°C, with an incidence of 4.8%, 8.0%, 14.8% and 11.5% in the groups receiving 0.25, 0.5, 1, and 5 µg/l bisphenol-A respectively. Increased mortality was observed in those groups experiencing oviduct malformation (numbers are not cited, but from Figure 2E in the paper, around 10 deaths were observed in each treatment group at 20°C, compared to 3 in the control – like egg production, there was no clear dose-response). Some anti-androgenic effects (e.g. a significant concentration-dependent decrease in penis length of males at 20°C) were also observed (neither the magnitude of this change nor a NOEC/EC_{10} for the effect are indicated in the paper).

5. A separate series of studies with *M. cornuarietis* was performed in response to Commission Regulation EC No. 642/2005[1]. The main toxicity study (Warbritton *et al*. (2007a and 2007b) and Forbes *et al*. (2008)) was based on the methodology and results of an extensive series of earlier experiments on husbandry conditions, inter- and intra-laboratory variability, and a pilot toxicity study (Aufderheide *et al*. (2006), Selck *et al*. (2006) and Forbes *et al*. (2007a and 2007b)). The main finding of these initial studies was that the primary source of variability in egg production was at the level of the breeding pairs. The fecundity of adult snails over a 12-month period showed a decrease over the first few months and a plateau thereafter (with no evidence of seasonal variation).

   For the adult fecundity trial, replicate exposure aquaria were divided into ten equal size chambers using perforated glass partitioning. Each chamber randomly received a breeding pair of snails. Six replicate exposure aquaria were used for each bisphenol-A exposure concentration (0.1, 1, 25 and 640 µg/l (nominal)), with twelve replicate aquaria for the control. Exposure was for six months using an intermittent flow-through dosing system, and the test temperature was 25°C. The number of egg masses and the number of eggs per egg mass were counted. An egg hatchability trial was also conducted. Five females in each of three of the replicate exposure aquaria (and in six of the control aquaria) were randomly selected, and five consecutive egg masses were collected starting at two months after the beginning of the fecundity trial. The egg masses were placed individually in the appropriate test solutions and the percent hatch, the time to first hatch and the time to 50% hatch were recorded. Finally, a juvenile growth trial was also conducted: one egg clutch was selected randomly from each of five females in each replicate vessel (three replicates per bisphenol-A treatment and six from the controls) and exposed as in the hatchability trial; at 32 days post-hatch five juvenile snails were selected from each female's offspring, giving 25 young per replicate, and were placed in aquaria (one aquarium for each of the three replicates at each concentration). These snails were individually marked, and were weighed weekly over the three-month exposures. Animal gender was determined for each individual based upon internal examination of the gonads at the termination of the exposure.

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

   The results are expressed in terms of the nominal concentrations since the mean measured concentrations were 74-135% of the nominal concentrations. The overall NOEC from the study was

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

concluded to be 25 µg/l, related to the growth of juvenile female snails. It should be noted that this is a conservative value since the LOEC is significantly higher (i.e. the true NOEC lies somewhere between 25 and 640 µg/l). In addition, there were no significant differences between the fecundity (as eggs/female/month) of the controls and snails exposed to 25 µg/l at 22°C and 25°C.

6. Schirling *et al*. (2006) reported the development of a test method using eggs of *M. cornuarietis* to assess the effects of potential developmental and endocrine disruptors. Eggs were exposed to bisphenol-A in Petri dishes, and monitored endpoints were hatching weight, mortality, formation of eyes, formation of tentacles, hatching (all as percentages of the exposed organisms) and heart rate. The only significant effects were a reduction in the heart rate in the 100 µg/l exposure at nine days, and newly hatched snail weight at 100 µg/l. The study was intended as a development of the method and not for the determination of dose-responses, but a NOEC of 50 µg/l could be tentatively drawn.

The interpretation of these studies is discussed extensively in EC (2008). With the clear exception of the industry-sponsored study, the overall findings suggest that bisphenol-A can stimulate reproduction in aquatic snails at concentrations around or below 1 µg/l. However, several of these studies suffer from significant methodological limitations. In particular, changing snail densities, high individual variability in egg production (and the consequent need for a high level of replication), rapid loss of bisphenol-A from the test system, and questions over the validity of the statistical techniques used to interpret the data, mean that less reliance can be put on the findings of Oehlmann and co-workers than the industry-funded study. However, a recalculated EC_{10} of 2.1 µg/l using data from the Oehlmann *et al*. (2006) study was ultimately used in the species sensitivity distribution (SSD) to derive a PNEC for surface water of 1.5 µg/l.

The findings of mortality and morphological changes by Oehlmann and co-workers cannot easily be discounted as artefacts, especially as these have been observed in more than one species. There are certainly strain differences in the snail stocks used in the different laboratories, and it is possible that the role of seasonality was not sufficiently investigated in the industry-sponsored study (since those snails did not exhibit any seasonality in their breeding cycle). Differences in exposure regimes might also have an influence if metabolites are more potent than the parent substance. These uncertainties led the UK Government to commission further studies with native European gastropod mollusc species, and these are reported below.

<u>New studies</u>

Benstead *et al*. (2008) report the effects of bisphenol-A on the fecundity of three species of adult European gastropod snails during simulated spring and autumn conditions. The studies for each species are summarised separately.

The test facility was a windowless laboratory of the Environment Agency used for a variety of routine ecotoxicity tests.

1) *Planorbarius corneus*

This is a native European pulmonate species, with a simultaneous hermaphroditic reproductive strategy (an individual's sexual role is determined on each mating occasion by behavioural competition, but individuals can lay eggs at all times, having the option of self-fertilization). The aim of the study was to expose adult *P. corneus* to bisphenol-A using an experimental design that incorporates the natural autumnal decline in productivity. Under these conditions, the egg production rate of the control group would gradually decline to an expected minimum level, and the relative performance of the exposed groups could be observed. A 'positive' control substance (such as ethinyl oestradiol) was not used because of timing and budget constraints, and the fact that it is not clear whether any putative effect (an expected enhancement of egg production) is actually oestrogenic as such.

All animals were purchased from a commercial dealer in the UK in September 2007, and were originally collected from the wild. The experimental work took place during October to December 2007. The snails were initially held in a 100-litre glass holding tank; individuals were then drawn out, weighed and measured in the longest axis, and randomly allocated to the plate glass exposure tanks until each tank contained nine snails (40 tanks in all). No animal less than 18 mm long was used in the experiment, to ensure that only sexually mature snails were tested. The tanks were filled with Artificial Pond Water (APW) consisting of 588 mg/l $CaCl_2.2H_2O$, 123.25 mg/l $MgSO_4.7H_2O$, 64.75 mg/l $NaHCO_3$ and 5.75 mg/l KCl, with a working volume of nine litres per tank. All water used to make the media was filtered through activated carbon and a reverse osmosis filter[2] and allowed to equilibrate to the test temperature for 24 hours prior to the addition of the salts.

The snails were subsequently found to be laying eggs at a lower rate than anticipated from previous work in the same laboratory with this species (at least seven egg masses per adult in each fortnightly exposure cycle was expected before the experiment could begin, to ensure that a significant decline could be detected). The temperature of the air-conditioned rooms was therefore raised from 15°C to 19°C, and the photoperiod extended to 16 hours light–8 hours dark[3], to cause an increase in reproduction and to determine variability between the groups over 28 days. This was termed the 'pre-exposure' period.

[2] This was done in an effort to remove any bisphenol-A from the influent to the laboratory. Additional precautions included the use of glass or steel vessels for storage and transfer of media. The use of plastics (e.g. siphons for cleaning, gloves for handling snails) that had the potential to leach bisphenol-A or other possible oestrogenic substances was kept to a minimum.

[3] The tanks were lit by strip fluorescence light bulbs emitting cool white light with a measured lux value of 4000 at the height of the water surface.

At the end of this 28-day period, the individual tanks were divided into four groups of ten so that the mean and standard deviation of the reproductive output in each of these groups in the pre-exposure period was similar. These four groups were then randomly assigned to one of four treatments (nominal bisphenol-A exposure concentrations of 0.2, 2 or 20 µg/l[4], plus a control) and exposed for an 8-week period. The test media were renewed on a semi-static basis every 48 hours, when two thirds of the media in the tanks were removed to leave the animals in approximately 3 litres (to avoid shocking them). Faeces and excess food were then carefully removed from the tanks by vacuum siphon, before 6 litres of fresh media were returned. Algal and bacterial films slowly built up on the plate glass, so every two weeks the snails were completely removed from the tanks so that the tanks could be cleaned with chlorinated tap water and a clean cloth before refilling with fresh media. The snails were kept in correspondingly numbered vessels containing approximately 1 litre of water from the respective tank during this time. The snails' shells were gently cleaned with fingertips if necessary prior to their return so that the fresh media was not seeded with an appreciable amount of algae and bacteria.

During the first four weeks of the exposure phase, the temperature was reduced gradually from 19°C to 15°C (at a rate of approximately 1°C week), and the photoperiod was also changed to 12 hours light–12 hours dark (by allowing 30 minutes less light per week), to simulate the onset of autumn. The temperature and photoperiod then remained constant for the final four weeks, to allow the snails to continue declining in productivity without ceasing activity altogether. The snails were fed fish flakes at a rate of 0.25 g/snail at each partial media change during the 'pre-exposure' period at 19°C, reducing to 0.1 g/snail for the duration of the exposure to minimise water quality problems as the snails became less active. The total weight of food was adjusted for snail deaths as they occurred.

Test media samples were taken for chemical analysis weekly, either by sampling a 100 ml aliquot from each exposure tank 24 hours after a partial media change (i.e. the midpoint between partial media changes) or by sampling a 50 ml aliquot immediately prior to and after the partial media change, depending on the day of the week on which the samples were taken in the fortnightly cycle. Due to budget constraints, all aliquots within each concentration were pooled for analysis except on two occasions, when 500 ml samples were taken from each tank for individual analysis to ensure that spatial variability was acceptable. The samples were preserved by the addition of 1 ml of a 250 g/l copper sulphate solution and 1 ml of concentrated hydrochloric acid to sterilise the media and halt biological degradation, before being shipped to another Environment Agency laboratory for analysis. Aqueous samples were spiked with deuterated bisphenol-A before undergoing a simple solvent extraction using dichloromethane. Extracts were then concentrated by drying before derivatisation using trifluoroacetic

[4] The test concentrations were made by dilution of a stock solution of 12 mg/l of bisphenol-A (purity 97% w/w), prepared by sonication for approximately 2 hours followed by stirring for 24 hours. A new stock solution was prepared each week.

anhydride. An aliquot was then injected into a gas chromatograph with EI mass spectrometer detection (operated in SIM mode). Quantification was achieved using an internal standard. The analytical laboratory is not accredited for this method. The limit of detection was typically 0.04 µg/l, but rose to 0.08 µg/l for the samples collected on two occasions to check spatial variability (due to differences in sample volumes).

Prior to and after each complete or partial water change, a 100-200 ml sample was taken from each control tank for pH, dissolved oxygen and conductivity measurements (samples were not taken from any of the dosed tanks to avoid potential cross-contamination of other laboratory applications due to the shared use of probes). The samples were then pooled before the hardness was measured. The water temperature in each test tank was measured every 48 hours prior to the media being changed. Replacement media was stored in the same constant temperature facility for 24 hours prior to use.

Total reproductive output (as number of eggs per surviving snail) was monitored at each 48-hour partial water change by careful removal of all the egg masses laid using a cut-throat razor. The number of egg masses, number of eggs (and any abnormalities) within each mass (counted using a microscope), and egg mass dry weight were also measured. At the end of the exposure, all the surviving snails were placed into a 5% solution of magnesium chloride for at least two hours to relax the tissues, before re-weighing and measurement in the longest axis. The shells were then cracked and removed, and the soft parts weighed. The reproductive tract was then dissected away from the muscular parts in one piece wherever possible, to include the ovo-testis, the hermaphroditic ducts, the albumin gland, the prostate, sperm ducts and penile complex and the oothecal gland, oviducts and vagina. The remaining tissues were then re-weighed to give an indication of condition factor (all tissues were either preserved or frozen for any later investigations). Only the effect on “total reproductive output” was formally tested for significance (as this was the focus of the experiment).

Results

i) Physico-chemical data

The mean pH was 7.4. The average dissolved oxygen level was 85.1% of the air saturation value (the lowest measured value was 76%). Conductivity remained low over the experiment, with a mean value of 952 µS/cm, and the mean hardness was 453 mg/l $CaCO_3$ equivalents. These are all within expected ranges, although it should be noted that no measurements were made for exposure tanks.

Figure 7.1 describes the mean temperature of the exposure tanks prior to each media change, along with the temperature of the coolest tank (minimum) and the warmest tank (maximum) on each occasion. The temperature did not differ by more than 2°C at any time across the tanks. The temperature was held at a mean value of 18.7°C for the duration of the 4-week pre-exposure

period and following the simulation of the autumnal decline, the mean temperature was 14.9°C.

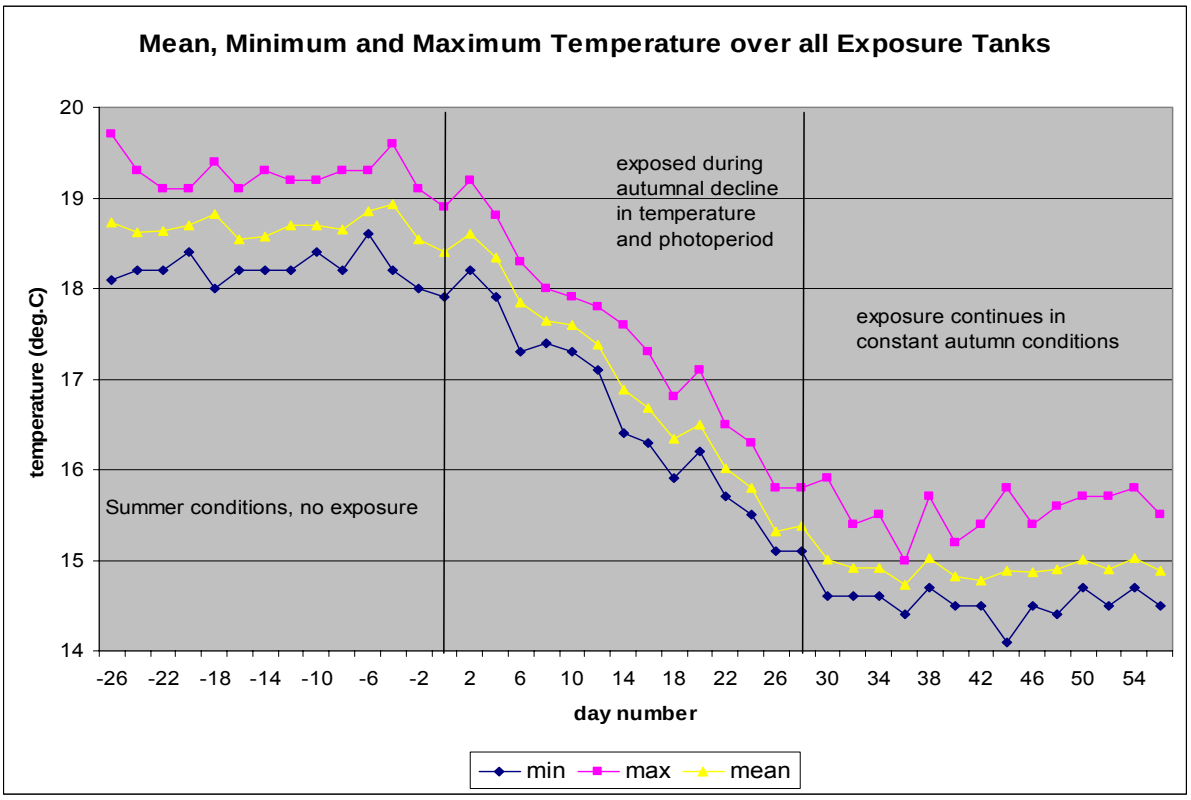


Figure 7.1 Mean, minimum and maximum temperatures of the exposure tanks

A small number of sample identification errors occurred at the analytical laboratory. Because the original identity of the samples could not be ascertained in every case, all results that are cast into doubt in this manner were excluded from the overall calculation of mean measured concentrations. In addition, there was a dosing error during the second week which gave an unacceptably high concentration in one of the controls in the second and third weeks, and one of the highest dose concentrations was abnormally low (6.45 µg/l) in the third week as well. Reproductive output data were therefore ignored from this particular control tank, and the analytical result from the pooled sample in week 2 was also excluded from the calculation of the mean exposure concentrations for the control and top dose. This left 21 of the 26 potential data points to contribute to the mean exposure level for the control, nominal 0.2 µg/l and 20 µg/l groups, and 24 for the nominal 2 µg/l group. The mean measured concentrations for the three exposure groups were 0.182±0.036, 1.43±0.26 and 13.1±3.4 µg/l, respectively (representing 90.4%, 71.5% and 66.0% of the nominal concentration in each case). Bisphenol-A was also measured in most of the control samples at concentrations generally close to the limit of detection - the mean control concentration of 0.052±0.03 µg/l is based on a calculation that assumes that a result below the detection limit is equivalent to 0.02 µg/l (this only concerns four samples but clearly adds some additional uncertainty). It would therefore appear that the measures taken to eliminate bisphenol-A from the control tanks were not

consistently successful (the highest concentration recorded for a control tank used in the study was 0.152 µg/l).

ii) Biological data

The snails began to lay eggs at the required rate during the 4-week pre-exposure period (all groups achieving the minimum quality criterion for egg productivity of 0.5 egg masses/snail per 48 hours). The average number of eggs produced per tank over this period was 417 per surviving adult, which is broadly similar to other studies with this species in the same laboratory under similar conditions. These values suggest that although the organisms had a lower than anticipated egg productivity on establishment in the laboratory, their reproductive tissues had not yet begun to regress. The co-efficient of variation for all the tanks over the pre-exposure period was 19.8%; following allocation to treatment groups, this was broken down into 18.3% for the control group, and 19.3%, 22.6% and 16.1% for the groups allocated to the 0.2, 2 and 20 µg/l nominal concentrations, respectively. This part of the experiment was designed to demonstrate that all groups were performing to a minimal acceptable standard and to generate an accumulated productivity rate in 'optimal' conditions that could be used as a comparison for the reduced egg production rate that was expected with the onset of the 'simulated autumn'. The data were also used to assess the variability between the groups, and to optimise the allocation of tanks to minimise the variation between concentrations by selecting the lowest between-group variation following six random allocations. It was expected that these measures would provide a dataset with sufficient power to make early detection of the reduced egg productivity of the control group in 'simulated autumn' conditions.

The egg production results are presented in Table 7.1 and graphically in Figure 7.2. Snails that are high producers in the pre-exposure period probably have a relatively high egg production capacity, and are therefore expected to have a relatively high egg production rate during the exposure period as well. On the other hand, the egg production might decrease more quickly than that of a snail group with a low pre-exposure reproduction. To compensate for these dependencies, the egg production in the treatment period and the rate of change in the egg production were adjusted for the egg production in the pre-exposure period in the same test vessel with an Analysis of Covariance (ANCOVA).

Table 7. 1 Mean egg production per exposure period

Treat-ment	Mean egg production per vessel				Mean egg production per vessel adjusted for reproduction in the pre-exposure period [5]					
	Pre-treatment period	First treatment period, day 2-28	Second treatment period, day 30-56	Total treatment period	first treatment period, day 2-28		second treatment period, day 30-56		Total treatment period	
					N	% of control	N	% of control	N	% of control
Control	410	188	57.6	246	191	-	58.0	-	249	-
0.2 μg/l	400	135	28.7	164	144	75	29.6	51	173	70
2 μg/l	397	121	37.4	158	131	69	38.5	66	170	68
20 μg/l	455	167	32.4	199	145	76	30.1	52	175	70
All exposed	417	141	32.8	174	140	75[a]	32.8	57[a]	173	69[a]

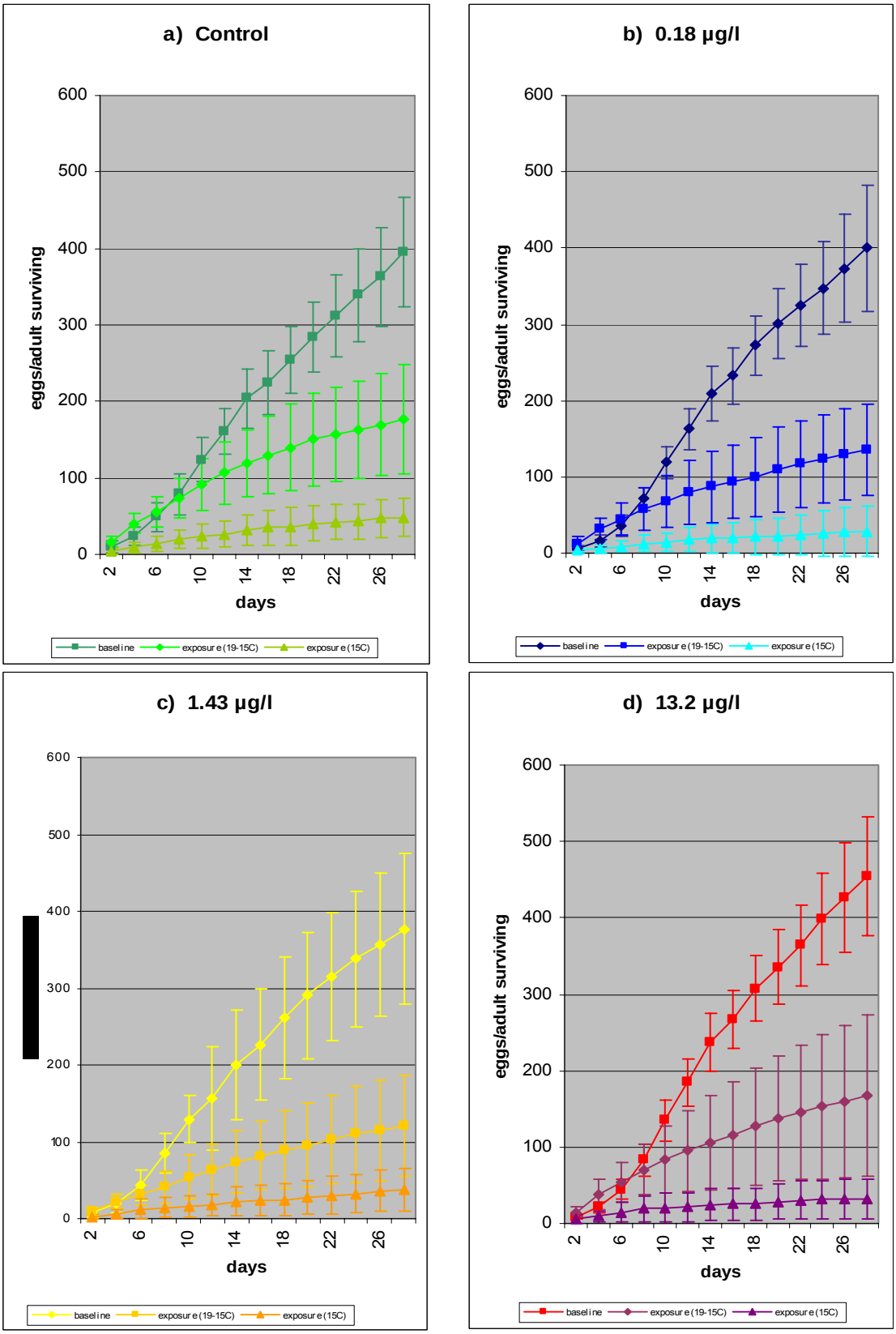
Note: a - % difference from control for the unadjusted egg production.

The difference between the average adjusted egg production in the control and the three treatment levels was tested with the two-sided Dunnett's test, for both exposure periods separately (days 2 to 28 and days 30 to 56) and for the combination of both exposure periods. The total significance level of 5% was assigned to this test. An additional question was whether the rate of change in egg production due to the simulated onset of autumn was significantly different in exposed snails than in the controls. Since no significance was left, the statistical testing of this question was only to see whether it merits further research.

As expected, the rate of egg production in the control group declined steadily with the decreasing temperature and photoperiod that the organisms experienced in the first 28 days of the exposure phase, from a mean value of 410 eggs per surviving adult over the pre-exposure period to 188 eggs per surviving adult (a decrease of 54.1%). This decline continued in the second 28-day period when the temperature and photoperiod were constant, reducing the productivity to 57.6 eggs per surviving adult. The organisms are not expected to cease to lay eggs entirely unless the temperature falls below 10°C and food is withheld (as would occur in winter when the snails retreat into the sediment).

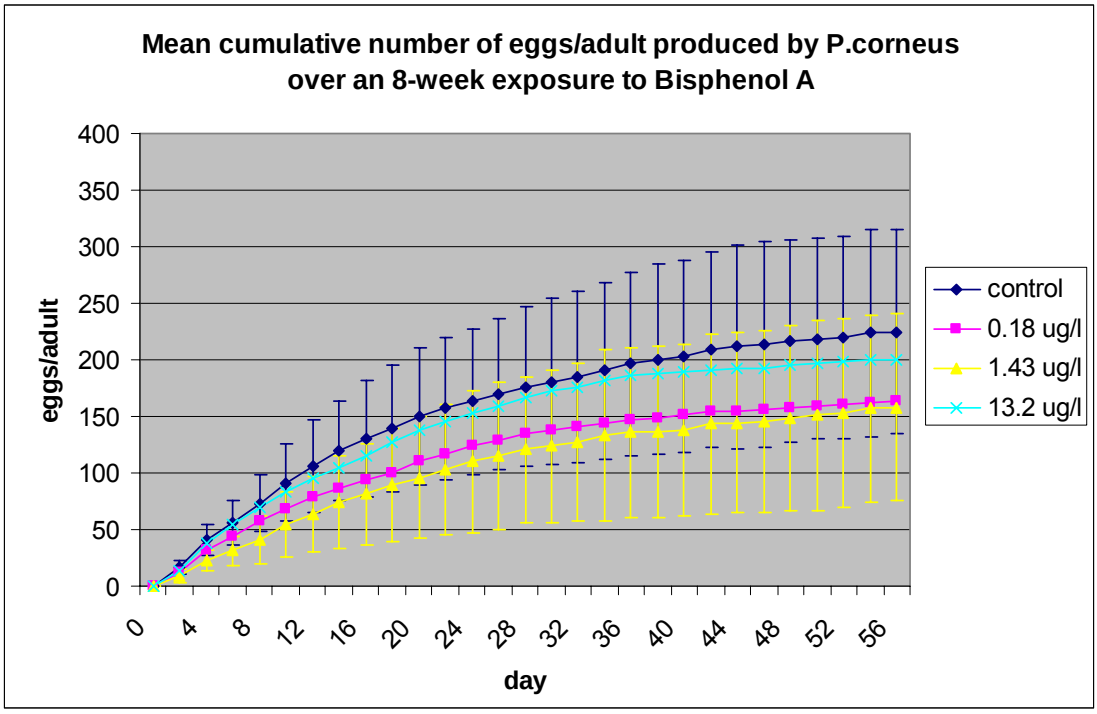
[5] The efficiency of correcting for the pre-treatment reproduction varied from 4.6 for the second period to 6.9 for the first one. For the comparison of the control with the combination of the three treatment levels, it is not efficient to adjust the reproduction for the reproduction in the pre-treatment period. The efficiency for this adjustment varied between 0.010 for the second period to 0.016 for the first period.

Figure 7.2 **Mean cumulative egg productivity per surviving adult in the pre-exposure period, the first 28 days of the exposure when the temperature/photoperiod were reduced and the second 28-day period of exposure at constant temperature/photoperiod. Error bars show standard deviation from the mean. (b – d: mean measured bisphenol-A exposure concentrations)**



It can be seen that fecundity in all of the groups exposed to bisphenol-A declined in a similar manner to the controls, but that the decrease in egg numbers was more pronounced. This is better illustrated in Figure 7.3, which compares the mean cumulative numbers of eggs produced by the control and exposed groups over the whole 56-day exposure period (only the standard deviation of the controls and the group with the lowest response are shown, for reasons of clarity). The lowest and intermediate exposure groups produced approximately 50 eggs less per adult over the duration of the exposure than either the control or the highest exposure group.

Figure 7.3 The mean cumulative number of eggs/surviving adult over the whole exposure. Error bars show standard deviation from the mean.



Although all of the exposure groups consistently produced around 30% less eggs than the control group over the whole experiment (when adjusted for the egg output during the pre-exposure phase), the variability in the data was such that these differences are not statistically significant at the 95% level (two-sided Dunnett's test, $p>0.1$). Nevertheless, this reduction in egg production *is* significant at the 87-90% confidence level. The additional statistical tests performed on other reproductive end points (the adjusted egg production per individual and the decline in reproduction rate, over both days 1-28 and days 29-56) found no statistically significant difference between the control and any treatment.

Twenty-one of the 360 animals (5.8%) died during the pre-exposure period and the overall mortality rate continued to rise as the temperature and photoperiod was reduced. The mean control mortality of 20% (including the deaths occurring in the pre-exposure period) was higher than expected from previous studies with this species in this laboratory and on the bounds of acceptability for invertebrate toxicity testing. Mortality was also relatively high

in the exposed groups, reaching 27.7% for the highest exposure group (also including deaths occurring in the pre-exposure period), and in some vessels only four of the original nine individuals survived to the end of the test. However, although the lowest survivorship occurred in the highest concentration during the exposure (76.8%), there does not appear to be any dose-dependency in survival during the exposure period and there is no statistically significant relationship present. (A Jonckheere-Terpstra test for the hypothesis that the survival does not decrease with increasing concentration gives a p-value of 0.23. Since the observed p-value is not below 0.05, this experiment does not indicate that bisphenol-A might have a negative effect on survival at the concentrations used in the experiment.)

Treatment groups were based on reproductive output rather than morphometry and this inadvertently led to snails in the 2 µg/l nominal group being slightly smaller initially than those in the other groups. Whilst this difference was statistically significant, it is unlikely that the results for other endpoints would have been seriously affected.

The relatively high level of mortality confounded the analysis of growth to some extent, since death of larger individuals can cause the mean group size to be smaller after the experiment than before. In addition, accurate weight measurement of dead snails is confounded by rapid water uptake and tissue breakdown. Therefore, at the end of the exposure, the growth of the organisms was calculated in terms of percentage increase in shell length. All of the groups grew between 3 and 4% with no significant differences, which was a similar increase to that seen in previous experiments.

At the end of the experiment, there was no significant difference in the mean condition factor between the exposure groups, this being a ratio of total weight to flesh weight, giving an impression of the body condition of the animal inside the shell (ANOVA, $p>0.05$). The pattern of response for the gonado-somatic index was a very slight increase in the weight of the reproductive organs with increasing test concentration, except in the case of the highest dose where a slight reduction was observed compared to the lower two doses (though still marginally higher than the controls); these changes were not significantly different.

Discussion

Whilst noting that this was a research project, breaking new ground in toxicity testing with aquatic snails, a number of drawbacks were identified by a project steering group of independent peer reviewers and UK government officials:

- Although bought from a dealer, the animals were wild-caught, so their age, exposure history and levels of parasitism are all unknown. These might all affect the snails' responsiveness as well as their ability to lay eggs (this species is known to be parasitized by trematodes, which can prevent an individual from breeding completely).

- Despite steps taken to remove bisphenol-A from the influent water, the control tanks appeared to contain the substance at levels close to the analytical limit of detection, and around four times lower than the first exposure group. Therefore it would seem that there were no unexposed snails in the experiment.
- The semi-static exposure regime resulted in a significant drop in bisphenol-A concentration over the duration of the test, especially in the highest exposure group. It is possible that metabolites might have been present in variable amounts, which could be a complicating factor (there is now some evidence that some metabolites might be more potent oestrogen mimics than the parent substance. For example, Ishibashi *et al*. (2005) report that 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene, a metabolite of bisphenol-A, has an oestrogenic activity around 250 times greater than that of bisphenol-A in male medaka (*Oryzias latipes*)).
- Tank temperatures varied over a range of up to 2°C at any one time (the vessels were heated indirectly by the ambient room temperature). Although this is consistent with standard test guidelines, the fact that temperature was used as a variable to influence snail output means that it should preferably have been better controlled.
- The level of mortality was fairly high (20% of controls if the pre-exposure phase is included). This suggests that the snails could have been stressed, which might be related to the artificial temperature rise and fall that was used at the beginning of the experiment.
- The intention to investigate reproduction at a time of natural decline inevitably meant that the feeding regime had to be adjusted, which adds a further complicating variable.
- The earlier work with the sexually reproducing species *M. cornuarietis* showed that individual females are highly variable in their egg output. The egg output of the hermaphrodite *P. corneus* is also highly variable. The authors took account of this in the design of the test (by maximising the number of organisms per replicate and the number of replicates per concentration, within the constraints of the laboratory, and by grouping snails of similar egg output into treatments). Nevertheless, this inherent variability remains an important consideration in any subsequent analysis, especially as the variability increased with time.

The test was designed with a particular type of statistical analysis in mind, and although only the effect on total reproductive output per snail was tested formally (to maximise the power of the statistical test for the end-point), there were no significant differences between bisphenol-A exposed snails and control snails at the 95% confidence level for any of the parameters measured (aside from the initial differences in length and weight of the snails at the outset of the exposure mentioned above). Nevertheless, it should be noted that this lack of significance is related to the high variability – in fact there *was* a consistent difference in response between the three exposed groups and

the ‘control’ (a reduction in egg output of around 30%), which is statistically significant if the confidence limit is lowered slightly from 95% to 90%. It remains unknown whether a more marked effect might have been detected if a true control group of unexposed animals had been used. In addition, independent statistical advice suggests that the methods of statistical analysis used for this study might not have been entirely appropriate.

The authors suggest that the response might be simply ‘on’ or ‘off’ rather than a concentration-related effect. To analyse the results further, the authors pooled the data of all three exposed tanks based on the assumption that all the treatment groups were responding to the same degree[6]. When this is done, the ‘bisphenol-A exposed group’ can be shown to be significantly different to the control at the 95% significance level (two-sided Student t-test, p=0.049) for the entire exposure period, although the effect of pooling the data is more pronounced in the second period (days 30-56) of the exposure (p=0.036) than in the first. This may cautiously be interpreted as a warning that some significant effect on reproduction might have occurred. However, this statistical test is conditional on that fact that a visual observation of the data suggested that the three exposure levels deviated in a similar way from the control. A new test would need to be performed to confirm whether this effect is genuine. This could require a high degree of replication given the variability involved.

2) *Bithynia tentaculata*

This is an egg-laying species of freshwater prosobranch snail, native to Europe, with a gonochoristic breeding strategy (i.e. individuals are male or female). An experiment was set up to investigate the effect of bisphenol-A on reproduction during simulated spring conditions, as water temperature, light intensity and photoperiod increase.

Snails were collected from the Lewes Brooks, Lewes, West Sussex in February and March 2008 and allowed to intermix in 100-litre holding tanks before individuals were drawn out, weighed and measured in the longest axis, and randomly allocated to beakers using a random number table until each vessel contained groups of 40 in a working volume of two litres of Artificial Pond Water, prepared as for the *Planorbarius* experiment. All snails were at least 7 mm long (i.e. sexually mature).

The numbered vessels were allocated to either the control, 0.2, 2 or 20 µg/l bisphenol-A (nominal) exposure groups and dosed semi-statically in a similar way as for the *Planorbarius* experiment: at 48-hour intervals, the test medium was removed and replaced, with the animals shutting their opercula for the brief period out of water (less than 1 minute). Test media samples were taken for chemical analysis weekly, and always from the pooled media taken from the vessels prior to a media change and 48 hours after the bisphenol-A was

[6] The difference between the reproductive output in the control and the combination of the three exposure levels was tested with a t-test. For this test the reproductive output was not corrected for the pre-treatment reproduction because this correction was not efficient.

added[7]. Samples were not collected between media changes because the aliquot would have removed an appreciable proportion of the vessel volume (200 ml per vessel, i.e. 10%) in which the stocking density of snails was maximal (1 snail per 50 ml). The samples were preserved and sent for chemical analysis at another Environment Agency laboratory in the same way as for the *Planorbarius* experiment.

For the first four weeks of bisphenol-A exposure, the light-dark cycle was 16:8 hours and the temperature was 16°C. The temperature was then raised by 1 degree per week over the next 4 weeks to end at 20°C. The initial measured lux value was 750 at the height of the water surface, but this was increased to 1000 in the second 4-week period by the addition of an extra light bulb. The snails were fed a concentrated monoculture of the alga *Chlorella vulgaris* constituting 0.15 mg of total organic carbon (TOC) per snail per day for the first 4-week period, increasing each week by 0.0375 mg TOC per snail per day over the second 4-week period, to culminate in double the original feed rate (0.3 mg TOC per snail per day).

At each 48-hour partial water change all the egg masses laid in the vessels were counted (by eye, using a bright light) and recorded. At the end of the exposure, all the snails were then re-weighed and measured in the longest axis. The shells were then cracked and removed, and the sex of the animal plus the presence of any parasites were noted.

Results

All measured media physico-chemical measurements taken were within the ranges described for prosobranch molluscs by Duft *et al*. (2003). During the first 4-week exposure at 16°C, the temperature did not vary by more than 1°C. However, when the temperature was increased during the last four weeks, the vessels experienced higher temperatures than intended (particularly in the last two weeks, when the temperature range in the tanks was 19.5 – 23.5°C) because the constant temperature room controls malfunctioned.

Delays in analytical laboratory turnaround time (approximately 6 weeks) meant that very low and decreasing measured concentrations of bisphenol-A were picked up too late to rectify. The measured concentration of the sample from the highest dose group (20 µg/l nominal) was only 2.18 µg/l in the first week, falling to 0.682 µg/l in the second week, and was not recorded again until the end of the 8-week exposure. This is believed to be due to microbial degradation associated with the build up of a biofilm on the sides of the test vessels that were not utilised for feeding by the prosobranchs in the same active manner as that of the pulmonates. Although measurements of bisphenol-A concentration are reported for all weeks of the study, these problems mean that the results are in the main meaningless. However in the last week of the study, when a thorough biofilm removal was undertaken, the

[7] The test media samples were combined with those from the *Potamopyrgus antipodarum* study prior to bisphenol-A analysis during the period when both studies were running concurrently. See the description of that study in the main text for more details.

analysis results were better (57 – 64.5% of nominal). This at least indicated that the bisphenol-A was being successfully spiked, although not successfully sustained. As for the *Planorbarius* experiment, the controls also had detectable levels of bisphenol-A; the highest detected concentration was 0.192 µg/l, although given the problems mentioned above, the reliability of these measurements is unclear. All other test media physico-chemical measurements conformed with those described for prosobranchs in Duft *et al*. (2007).

As expected, the rate of egg production in the control group increased slowly in the first 4 weeks of the exposure when the temperature and photoperiod were stable, and they continued to increase steadily with the increasing temperature and photoperiod that the organisms experienced in the second 4-week period. Mortality was extremely low across the treatments, at between 3.5% (controls) and 8% (highest dose). The sex ratio was determined to be 52:48 in favour of females over the test population at the end of the test. No visible new growth was laid down on the shell over the duration of the exposure. The number of eggs laid in each vessel was adjusted for the proportion of overtly parasitized[8] females present at the end of the test. However, no statistical analysis was made of these data because the variances between treatments were found to be unequal and there were insufficient replicates for a non-parametric analysis.

Discussion

The analytical problems unfortunately mean that most of the data collected in this experiment are unusable. The detection of bisphenol-A in the control vessels also suggests that there were no unexposed organisms. Parasitism is also a serious confounding factor for this species, since as well as visible parasites, it is possible that other parasites were present that were not seen. The test is therefore considered to be invalid for use in risk assessment, although it can be noted that the number of eggs laid per female was greater in all test groups when compared to the control.

3) *Potamopyrgus antipodarum*

This is another freshwater prosobranch species. Although native to New Zealand, it is widely naturalised in Europe, where the population is made up entirely of parthogenetically reproducing females. This species is very small, and begins reproducing when it achieves a length of approximately 3 mm. It does not lay eggs, but rather embryos develop within the body of the parent.

This experiment was performed during February and March 2008, and ran partly in parallel with the *B. tentaculata* study reported above, but it began 4

[8] The rate of visible parasitism by trematodes was not particularly high compared with previous experiments in this laboratory: 10.35% of the surviving females were parasitized (41/396). However, the rate per surviving female in each vessel did vary enormously, from a zero rate of parasitism to 57% (12/21 of the surviving females). These parasites seriously compromise reproduction (causing the snails to stop laying eggs), and so the number of laying females was reduced to account for the presence of these visibly parasitized females.

weeks earlier and therefore consisted of an 8-week exposure period under constant conditions (the light-dark cycle was 16:8 hours and the temperature was 16°C). This was done to ensure that test conditions were consistent as much as possible with the recommendations of Duft *et al*. (2007). The organisms used in the test were laboratory-reared and were fed with small quantities of alder leaves to limit reproduction in culture. For the experiment, groups of 80 adults were housed in 800 ml beakers (3 beakers per treatment) and snails were fed ground Hikari algal tablets at a rate of 0.2 mg/snail per day.

The reproductive success of 20 snails per replicate beaker[9] was examined after 14, 28, 56 and 84 days of exposure to the test substance for each treatment group. The sampled snails were narcotised for 45 to 90 minutes in a solution of 2.5% magnesium chloride hexahydrate in deionised or distilled water. After removal of the shell, the brood pouch was opened carefully with a dissecting needle and the embryos removed and counted using a microscope. The number of embryos per sampled female were calculated for each vessel, and then averaged across each of the 3 replicates per exposure group. Survival of the remaining snails was determined at the end of the test. After checking normality and equality of variance, ANOVA and two-tailed t-tests were performed on the data.

The test media samples were combined with those from the *B. tentaculata* study prior to bisphenol-A analysis during the period when both studies were running concurrently.

Results

All measured media physico-chemical measurements taken were within the ranges described for prosobranch molluscs by Duft *et al*. (2003). The temperature did not vary by more than ±1°C for the duration of the experiment (range 15.5 – 17°C). Hardness was slightly higher than expected (441 – 452 mg/l $CaCO_3$ equivalents, rather than 394 mg/l $CaCO_3$), but this was not thought to be detrimental to the snails.

The mean measured concentrations in the first 4 weeks of the exposure were 0.036 µg/l (control), 0.168 µg/l (0.2 µg/l nominal), 1.08 µg/l (2 µg/l nominal) and 10.3 µg/l (20 µg/l nominal). There were several problems with the chemical analyses (involving broken sample vials and incorrect dilution levels) which meant that there were no analytical data for 4 out of 8 samples from the first two weeks. The measured concentration for the highest dose group (nominal 20 µg/l) in week 1 was 6.86 µg/l, which is only 34.3% of nominal. The analytical issues described for the *B. tentaculata* study apply to this one as well, so that any data collected after the first four weeks of exposure are not reliable (since the concentrations of bisphenol-A that the snails were exposed to is unknown). Contamination of controls also suggests that no snails were unexposed (the highest recorded measurement was 0.068 µg/l in

[9] Removal at this rate will affect snail density in the test medium – the influence of this on the secretion of growth factors, aggressive interactions, etc., is unknown.

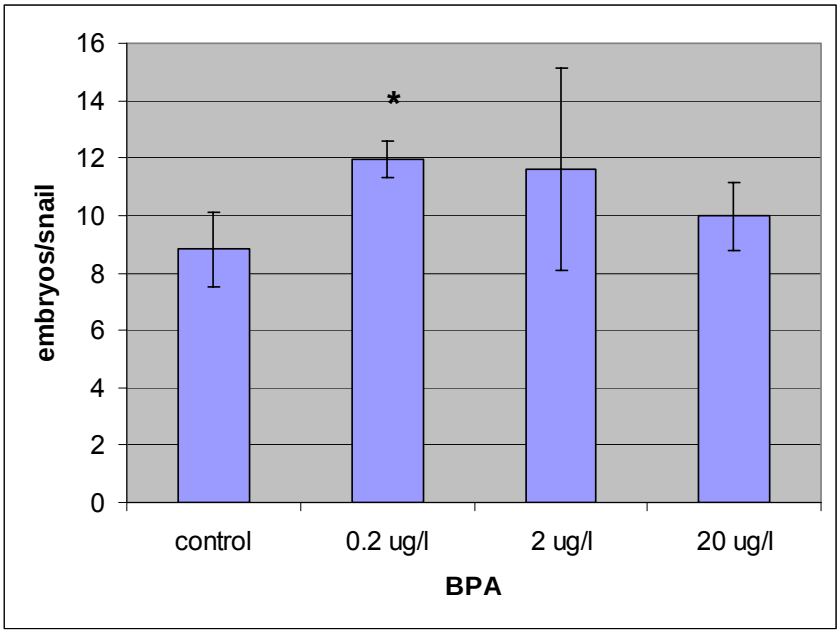
the first 4 weeks; two samples were below the detection limit and the fourth was not measured), and the highest dose group had variable exposure (as low as 34% nominal during the first four weeks).

Mortality was low across the treatments at between 2.9 and 5.4%. As expected, no visible new growth was laid down on the shell over the duration of the exposures and no other morphological features were recorded due to the small size of the organisms. After 28 days, the snails exposed to each of the test concentrations produced a greater number of embryos than the controls did (see Figure 7.4) – the difference between the control and lowest dose group was significantly different at the 95% confidence level using a t-test ($p = 0.03$), with about 30% more embryos produced on exposure to bisphenol-A (i.e. 12 rather than 9). The other two test concentrations also gave higher embryo numbers than the controls, but due to the high variability involved, the differences were not statistically significant at the 95% confidence level.

Discussion

The analytical problems unfortunately mean that most of the data collected in this experiment are unusable. The exposure concentrations are believed to have been reasonably well maintained during the first four weeks of the study, although the detection of bisphenol-A in the control vessels suggests that there were no unexposed organisms. Nevertheless, the formal statistical test suggests that there is a significant effect on reproductive output between treatments over 28 days, at least at a nominal concentration of 0.2 µg/l (0.168 µg/l measured). It is interesting that this 'effect' occurs at a mean measured bisphenol-A concentration that is less than five times higher than that in the control.

Figure 7.4 Mean number of embryos in the brood pouch of *Po. antipodarum* exposed to bisphenol-A (nominal concentrations indicated) for 28 days. Asterisk = difference from the control at $p<0.05$ (t-test).



The environmental implication of such a change, if it is a real effect rather than an artefact, is uncertain. Embryos can only be counted by killing the adult snail, so it cannot be determined if any snails are naturally 'high' producers in advance of the experiment.

As described earlier in this report, a previous study with this species (Jobling *et al*., 2003; corrected version published 2004) found a significant increase in new embryo production compared to controls at a nominal bisphenol-A concentration of 5 µg/l after 63 days (but not at a nominal 1 µg/l exposure, although this appears to have caused a significant effect after 3 weeks). The test conditions are not described in the paper, so it is difficult to make comparisons with the new study, although as a semi-static exposure it is likely that actual concentrations were lower than nominal. This makes the relevance of the findings difficult to establish. In addition, the embryo output of the control population in the earlier study appears very small in comparison to the current one, which may be due to seasonal differences (the Jobling et al. study was performed in the late summer when embryo numbers are very low; in early spring, the mean embryo number is expected to be above 15 (Oehlmann, 2008)).

Regardless of how the productivity data are interpreted, the major problems with the chemical analysis mean that the results should not be used for risk assessment.

Overall conclusion

Whilst the expected effect of bisphenol-A exposure from earlier studies might be an enhancement of reproductive output during the natural period of autumnal decline, the *P. corneus* results might indicate a possible reduction in fecundity compared to the controls, whilst there was possibly a slight enhancement of embryo production in *Po. antipodarum* during constant temperature conditions. However, these were preliminary research experiments, and the problems experienced with the test concentrations as well as numerous other drawbacks (as summarised above, including the unknown consequences of using wild-caught animals and the influence of changing snail densities over the course of the *P. corneus* experiment) prevent the results from being used formally for risk assessment. It remains unknown what effect, if any, might have been detected if the controls had not contained any bisphenol-A.

The results of this new research are not robust enough to warrant a revision of the current freshwater PNEC of 1.5 µg/l. Nevertheless, the observations hint at an effect. Whilst they cannot be used to either support or refute the hypothesis that low concentrations of bisphenol-A affect reproductive output in aquatic snail species, they do suggest that further work is needed.

Given the apparent high variability of reproductive output in snail species, and the resources already spent without arriving at a truly unequivocal result, a more closely controlled and statistically robust partial life cycle reproduction test method, using sustainable parasite-free laboratory cultures, is needed

before a request for any further testing is justified. Variability in control snail fecundity should be minimised as much as possible, and consideration should also be given to the best time of year to look for an effect for seasonally breeding species. The OECD test guidelines programme provides a vehicle for such efforts, although it is unlikely that a suitable ring-tested method will become available within the next few years. Nevertheless, it may be possible for industry and/or academia to perform further studies based on the standard operating procedures that have already been established. *Po. antipodarum* appears to be a suitable candidate species for further investigations with bisphenol-A.

The presence of low levels of bisphenol-A and other oestrogenic substances in laboratory water supplies is a generic issue that also needs to be taken into account. Effort should be spent on developing analytical methods that can reliably detect bisphenol-A (and perhaps its metabolites) at low concentrations.

B.7.1.2 Additional endpoints

1) Sediment monitoring data

A large amount of European sediment monitoring data were summarized in EC (2008), but were overlooked in the risk characterization. This oversight is addressed here.

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

It is therefore apparent that monitoring programmes have detected some sediment concentrations that are higher than predicted. For example, sediment concentrations above the sediment PNEC (24 µg/kg wwt) were measured in seven German rivers (the Elbe and its tributaries Mulde, Saale and Schwarze Elster, and the Weser and its tributaries Werra and Fulda) (see Table 7.2). Taking a wider view of the whole data set, the sediment PNEC is also exceeded by the highest 95th percentile measured sediment concentration from Germany of 311 µg/kg dwt (120 µg/kg wwt) and 95th percentile value from the whole data set of 256 µg/kg dwt (98.5 µg/kg wwt).

These findings suggest a potential sediment risk (with risk characterisation ratios up to 6) for a number of locations, but a number of factors need to be considered in this interpretation:

Table 7.2 Sediment concentrations above the $PNEC_{sediment}$ of 24 µg/kg wwt measured in Germany

River	Measured concentration µg/kg wwt[1]	Sampling date	Possible sources	Remark	Reference
Elbe	25.4 – 131.9	June 1998	Czech chemical plant Spolchemie (formerly Spolona), a manufacturer and user of bisphenol-A at the Bilina tributary.	Freshly deposited sediment (collected over a period of about 4 weeks) Apart from two sites (Grauerort and Schnakenburg[2]), constantly decreasing concentration from Schmilka (30 km from Bilina) to Blankenese (near estuary).	Heemken *et al*. 2002
	3.8 – 145.8	July 2000		Freshly deposited sediment (collected over a period of about 4 weeks) Constantly decreasing concentration from Schmilka (30 km from Bilina) to Cuxhaven (estuary).	Stachel *et al*. 2003
Mulde	29.2	June 1998	?	Freshly deposited sediment (collected over a period of about 4 weeks).	Heemken *et al*. 2002
	33.8	July 2000		Freshly deposited sediment (collected over a period of about 4 weeks).	Stachel *et al*. 2003
Saale	46.5	June 1998	Chemical factory producing antioxidants and stabilizing agents for plastics at the river Weisse Elster.	Freshly deposited sediment (collected over a period of about 4 weeks).	Heemken *et al*. 2002
	11.2	July 2000			Stachel *et al*. 2003
Schwarze Elster	50.8	June 1998	?	-	Heemken *et al*. 2002
	19.6	July 2000			Stachel *et al*. 2003
Weser	< 0.8 – 113.5	1999	?	Suspended particulate matter (SPM), 3 sampling sites.	Wesergutebericht 1999, in Stachel *et al*. 2003
Werra	< 0.8 – 62.3	1999	?	Suspended particulate matter (SPM), 1 sampling site.	Wesergutebericht 1999, in Stachel *et al*. 2003
Fulda	< 0.8 - 80	1999	?	Suspended particulate matter (SPM), 1 sampling site.	Wesergutebericht 1999, in Stachel *et al*. 2003
Berlin / Brandenburg	< 0.8 – 73.1	1996	?	Sediment, 12 sampling sites, surface waters.	Bherm *et al*. 1999, in Stachel *et al*. 2003

Note: 1) Calculated from dry weight values using a factor of 2.6 for comparison. 2) At two sampling sites additional sources must have been responsible for an increase of concentration: Grauerort - 36.9 compared to 25.4 µg/kg further upstream; and Schnackenburg - 88.5 compared to 74.6 µg/kg further upstream. Possible sources: Grauerort: bisphenol-A producing factory nearby. However the increased concentrations were not observed in July 2000 (Stachel *et al*. 2003).

- There has been insufficient time to analyze the monitoring database in more depth, but it should be noted that the usual approach is to select the mean, median or 90th percentile values, depending on the distribution function, whilst taking account of the number of data available from each site and whether they represent a local or regional scenario.

- These monitoring data have been taken at face value, in the absence of any information on data quality. If different laboratories are involved, then there could be differences in sampling, storage and analytical protocols. It is also not known whether any inter-laboratory calibration was performed, or whether the laboratories were accredited for bisphenol-A analysis. Therefore the measured concentrations may or may not be a reliable reflection of actual exposures.

- The sources of this sediment contamination may include accidental releases, historical pollution from sites that no longer exist, routine releases from industrial facilities near the sampling locations or further upstream, or some combination of all these possibilities. Without this information there is no way to identify which industrial scenario (if any) needs risk management. The German Umweltbundesamt (UBA) and industry representatives were contacted to seek some further information on the possible sources in Germany, and the following details were provided (UBA, 2008):

  "*Detailed information about possible bisphenol-A sources is only available for the River Elbe. The highest measured sediment concentration of 343 µg/kg dm and 379 µg/kg dm were found at Schmilka in the German part of the river near the German-Czech border. According to Stachel et al. (2003 & 2005) and Heemken et al. (2001), the bisphenol-A probably originates from the Czech factory "Spolana" in Usti nad Labem, which manufactures and uses this substance. The wastewater is treated inadequately in the sewage plant and is released into the Elbe via a small tributary, the Bilina. As the sediment concentration decreases further downstream, this source seems to be the most important effluent to the Elbe*."

  This production site was not considered in the original ESR report or its addendum. This exposure scenario will therefore need to be addressed by the Chemical Safety Assessment of this supplier under REACH. The situation concerning the other rivers remains unknown, but in one case at least emissions from a plant producing plastic antioxidants and stabilizing agents seem to be involved.

- Finally, the sediment PNEC is based on the freshwater PNEC and equilibrium partitioning theory. It could therefore in principle be revised with sediment organism toxicity data, although in general equilibrium partitioning is assumed to be a valid approach for this

type of substance. The freshwater data set is large, and so may provide some additional element of protection compared to the usual three-species approach to sediment PNEC derivation (e.g. it is influenced by fish toxicity, although only invertebrates are usually considered in sediments). At the same time, the study by Duft *et al*. (2003) mentioned in Section B.7.1.1 appears to show that at least one snail species (*Po. antipodarum*) might be sensitive to bisphenol-A when exposed in a sediment test system. The continuing uncertainties surrounding effects on molluscs (especially considering the significant and poorly understood variability in fecundity) means that the sediment PNEC should be re-evaluated if any further testing with snails is performed, even if standard sediment organism test data become available in the interim.

In addition, it would be worth reviewing the monitoring dataset in more depth, to establish which values might represent a regional background situation, and which might be influenced by point sources. This analysis could then be used to guide a more targeted monitoring campaign to trace the sources of the exposure. This task will require co-ordination between industry and the data providers.

2) Fish toxicity data

A paper selected as the critical aquatic toxicity study for bisphenol-A in a recent report by Environment Canada & Health Canada (2008) was not reviewed in depth for EC (2008). This is a study of the effects of bisphenol-A on maturation and quality of semen and eggs in the brown trout (Lahnsteiner *et al*., 2005). For completeness, this paper is briefly summarised below.

Wild-caught male and female brown trout (*Salmo trutta f. fario*) were exposed to three concentrations of bisphenol-A via a flow-through test system during the late pre-spawning and spawning period and the effect on maturation, quantity and quality of semen and eggs was investigated.

The fish were approximately 3 years old (length 15-20 cm), and were divided into four experimental groups consisting of 10 males and 6 females. The fish density (approximately 4 kg/m^3) was “approximately similar” in the four tanks. One tank acted as the control and the remaining ones were exposed to bisphenol-A $_{test}$ concentrations of 1.75, 2.40, 5.00 µg/l (estimated from the stock concentration based on water flow and test chemical injection rates). Dimethylsulfoxide was used as a co-solvent, at a concentration of 100-550 µg/l, depending on the test vessel. The fish were fed twice per week with small cyprinids and had a natural photoperiod.

Starting on 5 October fish were examined at weekly intervals for semen and egg production. Semen of all the males was stripped in the beginning (10 October), middle (28 October) and end (17 November) of spawning to examine semen quality using a variety of techniques. When females gave eggs they were completely stripped out, the time point of egg collection was recorded and then eggs were processed for quality determination.

Similar numbers of males (6-8) provided semen in the control group and at the two lowest test concentrations, while only one male from the highest concentration group provided semen. In males exposed to estimated bisphenol-A concentrations of 1.75 and 2.40 µg/l, semen quality was lower than control fish at the beginning of spawning (as measured by reduced sperm density, motility rate, and swimming velocity) and in the middle of spawning (as measured by reduced swimming velocity; also reduced sperm motility rate at 2.40 µg/l). Production of high quality semen was restricted to the end of the spawning season and delayed for approximately 4 weeks in comparison to the control. Semen of low quality (reduced semen mass, motility rate, and swimming velocity) was also given by the single male sampled at an exposure concentration of 5.00 µg/l.

The percentage of ovulated females was similar for the control group and the groups exposed to estimated bisphenol-A concentrations of 1.75 and 2.40 µg/l; however, females from the highest exposure group did not ovulate over the 103-day investigation period. While fish in the control group ovulated between 28 October and 12 November, ovulation occurred approximately 2 weeks later at an estimated bisphenol-A concentration of 1.75 µg/l, and approximately 3 weeks later at 2.40 µg/l. No effect was observed on the quality of eggs (egg mass, percentile mass increase during hardening, egg fertility).

A number of problems can be identified with this paper:

a) Concentrations: There is no indication that concentrations were confirmed by chemical analysis, either for the stock solution or the actual treatment solutions. The estimated concentrations in the paper are based on the relative flow rates of the stock solution (at 570 µg/l) and the dilution water. In view of the instability of bisphenol-A concentrations demonstrated in other studies, this means that the actual exposure levels have some uncertainty (although the flow rates were such that replacement of the water in the tanks would have occurred every three hours or so, hence degradation in the tanks may not have been as much of an issue as in other studies). Presumably a large number of stock solutions had to be made up, as the indicated addition rates would require over 6,000 litres of stock solution. [Note: the relative flows for the lowest exposure level appear to give an estimated concentration of 1.0 µg/l, rather than the 1.5 or 1.75 µg/l mentioned in the text.]

b) Statistics: Whilst the effects on female fish at the highest concentration are very obvious, only one tank was used for each exposure concentration. This means that the experimental unit is the tank, rather than the individual fish (i.e. the treatment is applied to the tank, not to the fish), and hence there is no replication. The same situation arose for other studies on bisphenol-A, and statisticians have commented that in these cases multiple comparison techniques are not appropriate. Hence the experimental design is not suitable for deriving

NOEC values. It is not possible to separate vessel effects (an example might be availability of food) and the effect of fish influencing each other from any effects related to the treatment (i.e. level of bisphenol-A).

The sperm swimming pattern data are expressed as percentages, adding up to 100%. A similar situation applied to the sperm cell types for fathead minnow (*Pimephales promelas*) in a previous study, and statistical advice at the time was that it is not appropriate to compare these values in the same way as other data because the values are not independent of each other. It is not clear whether the significance level of 0.05 was applied for each individual comparison or divided between the tests. The probability of false positive results increases with the number of tests if all use the 0.05 significance level. In the absence of the raw data from the experiment, independent statistical analysis is not possible.

c) Endpoints: The endpoints used in the species sensitivity distribution (SSD) for bisphenol-A are based on mortality, growth and reproduction, which are generally accepted to be population-relevant. Most of the endpoints in this study (e.g. sperm density and motility) cannot be related directly to these types of endpoints. The exceptions are:

   i) Sperm fertility, which was only tested at the end of the exposure period. This was not significantly different from the controls at the two lowest concentrations. (Only one out of eight males gave sperm at the highest dose: the semen masses were small and the fertility was low in comparison to the control. However, the representivity of the data are unclear.)
   ii) Egg fertility, which again was only tested at the end of the exposure period. These were not significantly different from the controls at the two lowest concentrations.
   iii) Absence of ovulation at the highest test concentration – it is possible that ovulation was delayed beyond the end of the experiment rather than stopped completely. Such an impact on egg production is surprising, since it does not appear to have been observed in the low microgram per litre concentration range for any other fish species exposed to bisphenol-A (i.e. for those experiments that included egg laying). It is notable that sperm was only collected from a single male at this exposure concentration. Whilst this may be due to bisphenol-A exposure, it is feasible that something else might have affected this tank (e.g. causing stress to the fish). The absence of replication means this cannot be determined.

   The effects seen on sperm *quality* are temporary effects, in that there appears to be a delay in the production of high quality sperm in comparison to the controls by about four weeks. Similarly the time point of ovulation is delayed at the two lowest test concentrations, but the eventual level of ovulation is similar to the controls. There is some

lack of clarity with regard to what is the “natural” spawning period – Section 2.2 of the paper indicates the second half of November, while Section 4.2 indicates that 28 October - 12 November is within the natural spawning period. Hence a comparison with the natural period is not clear. As described in the paper, a delay in spawning may have an effect on larval development if food is scare at the time of first feeding. It is not, however, possible to determine what would constitute a significant delay comparable to the “traditional” endpoints used in the SSD.

d) The use of wild individuals adds some uncertainty – for example, the paper notes that the fish were probably not fully adapted to hatchery conditions.

Given such concerns over the experimental design, it is considered inappropriate to use the results directly for the PNEC derivation. However, since the exposures at 5 µg/l do not show much evidence of the exposed fish having “caught up” by the end of the experiment, this could be taken as an effect level, with 2.4 µg/l as a no effect concentration (NOEC). Whilst this NOEC is not a comparable endpoint to those used in the SSD, it is still above the PNEC of 1.5 µg/l. This therefore provides some reassurance that the PNEC is protective of this sort of effect.

At the same time, questions about effects of bisphenol-A on spermatogenesis have been raised before for other fish species (notably *Pi. promelas*). Ideally, the study should therefore be repeated using a more statistically robust design, with analytical confirmation of test concentrations and a larger number of exposure groups, to establish a reliable reproductive NOEC for this species.

B.7.2 Terrestrial compartment

Not relevant for this dossier.

B.7.3 Atmospheric compartment

Not relevant for this dossier.

B.7.4 Microbiological activity in sewage treatment systems

Not relevant for this dossier.

B.7.5 Non compartment specific effects relevant for the food chain (secondary poisoning)

Not relevant for this dossier.

B.8 PBT and vPvB assessment

This information is not relevant for this Annex XV dossier.

B.9 Exposure assessment

B.9.1 General discussion on releases and exposure

The following is a discussion of the occupational exposures that resulted in a conclusion (iii) from the RAR.

B.9.1.1 Summary of existing legal requirements

The following discussion of existing legal requirements only details those related to occupational situations as it was only those that had a conclusion (iii) in the RAR.

B.9.1.1.1 Regulation 1907/2006 (REACH)

REACH (Registration, Evaluation, Authorisation (and Restriction) of Chemicals) will require those companies that manufacture and/or import chemicals in to EU to register them with the European Chemicals Agency (ECHA) in Helsinki. REACH will require these registrations to be supported by data on the substance. The amount and type of data that will be required increases with increasing tonnage.

Registration requires manufacturers and importers to submit:

- a technical dossier, for substances in quantities of 1 tonne or more, and
- a chemical safety report, for substances in quantities of 10 tonnes or more.

The **technical dossier** should contain information on the properties, uses and on the classification of a substance as well as guidance on safe use.

The chemical safety report (CSR) for substances manufactured or imported in quantities starting at 10 tonnes should document the hazards and classification of a substance and the assessment as to whether the substance is PBT or very vPvB. When the substance is classified as dangerous or as a PBT or VPvB then the CSR should also describe exposure scenarios. Exposure scenarios are sets of conditions that describe how substances are manufactured or used during their life-cycle and how the manufacturer or importer controls, or recommends to the user how to control exposures to humans and the environment. The exposure scenarios must include the appropriate risk management measures (RMMs) and operational conditions (OCs) that, when properly implemented, should ensure that the risks from the use of the substance are adequately controlled. Exposure scenarios should be developed to cover all “identified uses” which are the manufacturers’ or importers’ own uses, and uses that are made known to the manufacturer or importer by his downstream users and which the manufacturer or importer includes in his assessment. Relevant information from the exposure scenarios will need to be annexed to the safety data sheets (SDS) that will be supplied to downstream users and distributors.

As all those who manufacture BPA in the EU do so in quantities of at least 10 tpa, a CSR will need to be provided by the manufacturer or importer. In addition, as BPA is classified as a dangerous substance, exposure scenarios demonstrating that exposures are below the DNEL will need to be submitted. When a DNEL cannot be derived, (as outlined in Section 5.1) substances should be assigned to a hazard category for a more qualitative assessment.

The progressive implementation of REACH will have implications for the management of workplace exposure in the EU. Suppliers of substances that fall within the remit of REACH will have to demonstrate that exposures associated with identified uses are less than the DNEL (i.e. that the substance is adequately controlled), and will have to provide information on the measures that should be in place to control exposure (detailed in the CSR and passed onto the supply chain in the SDS).

B.9.1.1.2 Workplace Legislation

The key pieces of EU legislation that govern workplace health and safety are the Framework Directive (89/391/EEC) and its daughter directives including the Chemical Agents Directive (98/24/EC) (CAD). The Framework Directive outlines general principles for the management of workplace health and safety for all workplace hazards. CAD describes specific measures to be taken in relation to the control of chemical hazards. It requires employers to assess the risks to worker health and safety posed by chemical agents in the workplace and to take the necessary preventative measures to minimise those risks by:

- substitution of a hazardous process or substance with a process or substance which presents no or lower hazards to workers;
- designing work processes and engineering controls to minimise the release of a hazardous chemical agent;
- applying collective protection measures at the source of the risk e.g. adequate ventilation and appropriate organisational measures;
- where exposure cannot be prevented by other means, application of individual protection measures including personal protective equipment.

Employers should always, by preference, try to prevent exposure. Where it is not possible to do this, they must control exposure adequately by all routes. The Directive outlines a priority order (as above) in which risk management measures should be applied.

B.9.1.1.3 Occupational Exposure Limit (OEL) Values

The European Union (EU) has developed a programme whose objectives are to:

- prevent or limit the exposure of workers to dangerous substances at workplaces; and,
- to protect the workers that are likely to be exposed to these substances.

Setting occupational exposure limits is an essential part of this programme, which is endorsed under the following directives:

- Council Framework Directive 89/391/EEC on the introduction of measures to encourage improvements in the safety and health of workers at work;
- Council Directive 98/24/EC on the protection of the health and safety of the workers from the risks relating to chemical agents at work (the 'Chemical Agents Directive');
- Commission Directive 2000/39/EC establishing a first list of Indicative Occupational Exposure Limit Values (IOELVs) (for 63 agents);
- Commission Directive 2006/15/EC establishing a second list of IOELVs (for 33 agents);
- Council Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (the Carcinogens and Mutagens Directive).

The major task of the SCOEL is to give advice on the setting of OELs based on scientific data and where appropriate propose values. SCOEL may recommend OELs, which can be supplemented by further notations and information such as routes of absorption, as:

- Eight-hour time-weighted average (8hr-TWA);
- short-term exposure limits (STEL); and/or
- biological limit values (BLVs).

SCOEL aims to give health-based OELs that can be recommended when the available scientific data suggest that a clear threshold value can be identified for the adverse effects of the substance in question. For some adverse effects (in particular respiratory sensitisation and genotoxicity i.e. damage to genes), it is currently impossible to identify such limits. In these cases, SCOEL can recommend a pragmatic OEL, which is established on the basis of data on dose and risk.

The European Commission uses the scientific advice from SCOEL to make proposals for IOELVs. Limits based solely on scientific considerations are considered as adaptations to technical progress, and are incorporated in proposals for Commission directives within the framework of the CAD and are indicative. Limits that take account also of socio-economic and technical feasibility factors are included in proposals for Council directives under either the CAD or the Carcinogens and Mutagens Directive and are binding.

B.9.1.1.4 Classification and Labelling

Harmonised rules for classification and labelling are outlined in Council Directive 67/548/EEC (Dangerous Substances Directive) and 1999/45/EC (Dangerous Preparations Directive). These Directives will continue alongside the EC Classification, Packaging and Labelling (CLP) Regulations through the transitional period up to 1 June 2015. The CLP Regulation is expected to come into force in January 2009.

The main objective of these Directives is to communicate intrinsic hazardous properties of substances through classification and labelling. The Directives outline the classes of substances or preparations that are considered to be dangerous e.g. sensitisers. The Directives also outline the hazard symbols, risk and safety phrases and labelling and packaging requirements that should be adhered to when a substance is considered to be dangerous.

B.9.1.2 Summary of effectiveness of the implemented risk management measures

B.9.1.2.1 REACH (1907/2006)

As REACH is a European Regulation, it should be an effective legal instrument to aid BPA risk reduction. REACH requires manufacturers and importers to assess the risks that arise from the manufacture and/or use of their substances and to pass this information down the supply chain. The information supplied to downstream users will include improved information on the hazards plus improved information on risk management in the exposure scenario if one is required. As BPA is classified as dangerous (see section B.3.1), and is manufactured in quantities ≥10 tpa (see section B.2.1.1.1), such information will become available to the user.

This improved information will filter through to the end user after the substance has been registered. As BPA is manufactured by companies in >1000 tonnes per annum the information should be available from December 2010. Thus the information should be available to downstream users via extended safety data sheets in just under 2 years time.

B.9.1.2.2 Chemical Agents Directive (98/24/EC)

If industry applies the principles of 'good practice', as outlined by CAD, then this should ensure an effective reduction in exposure of humans to chemical substances in the workplace.

As much of the work, when manufacturing BPA or epoxy resins, is currently being carried out through closed systems it is clear that industry has used the principles of CAD to reduce exposure.

B.9.1.2.3 Indicative Occupational Exposure Limit Values

OELs are a useful means to establish EU minimum objectives of control for industry to achieve. An OEL is not a prevention measure in itself but must be seen as a complementary tool in the course of chemical risk management. The successful implementation of an OEL as a measure for risk management is dependent on the ability of industry sectors to be able to achieve exposure reduction in order to at least meet the OEL.

An OEL is an important tool in exposure control in the workplace. An OEL provides a 'benchmark' against which employers can assess the effectiveness

of the measures in place to control exposure. In the absence of air monitoring, employers can have no confidence that exposures have been controlled to appropriately low levels and, should employees become ill, they would have no evidence to demonstrate an adequate control regime. Although workplace monitoring can be undertaken in the absence of an OEL, the significance of the concentrations measured/found is often unclear.

SCOEL has considered BPA in the past (see section 5.1). In May 2004, SCOEL concluded that repeated inhalation exposure to 10 mg/m^3 BPA (as inhalable dust) would pose no concern for local or systemic toxicity and therefore recommended an 8hr-TWA IOELV at this level. In humans inhaling 10 m^3 of air, if it is assumed that all of the inhaled BPA would be retained and absorbed (a worst-case assumption), this would result in a body burden of just a little over 1 mg/kg/day. SCOEL stated that there is no toxicological basis for recommending an additional specific short-term IOELV; nor are "Sk" (skin) or "Sen" (sensitising) notations appropriate (SCOEL, 2004). BPA is listed in the Annex to the draft 3rd IOELV Directive, due to be adopted in early 2009, subject to the agreement of Member States.

As discussed above, for an IOELV to be effective industry sectors must be able to achieve reduction in exposures to at least meet the IOELV. Information from the RAR and in Tables 9.1 and 9.2 indicate that industry sectors are already achieving exposures which are below the IOELV. Therefore, the introduction of the IOELV is likely to be effective in maintaining long-term inhalation exposures below 10 mg/m^3.

B.9.1.2.4 Classification and Labelling

When substances and preparations are properly classified and labelled the potential hazards are identified and appropriate risk management measures are communicated on labels and in safety data sheets to those handling the substance or preparation. As the classification and labelling of BPA has been agreed and are listed in Annex I to Directive 67/548/EEC (as outlined in section 3.1) then effective communication of the hazards, risks and risk reduction measures should occur.

B.9.2 Manufacture of BPA

Introduction

The RAR states that BPA 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 BPA produced is usually of a very high purity.

Exposure values

The 8-hr TWA exposures for inhalation (presented in Table 9.1) given in the RAR came from data submitted by the 4 companies manufacturing BPA in the EU. The reasonable worst case (RWC) 8-hr TWA exposure is based on 123 samples collected between 2000 and 2007.

The RAR used the EASE model predictions to determine dermal exposure as no sampling data were provided by industry. However, industry provided information on control measures used to allow the mitigating effect of PPE to be taken into account when determining the RWC dermal exposure for BPA manufacture. The TGD (Technical Guidance Document on Risk Assessment) states that the use of PPE can be taken into account if the following two conditions are met:

- PPE is used regularly by the great majority (90%) of workers in the majority (90%) of facilities making or using the chemical and;
- the PPE used is appropriate and fit for purpose.

Information on PPE as well as on training and supervision of workers was provided by all companies manufacturing BPA. In general a set of measures are in place at the six BPA manufacturing plants in Europe:

- every employee is informed and trained with respect to the work and the associated hazards in both spoken and written forms;
- there is detailed written information on the following aspects of production available to every trained employee on;
    - general safety instructions for the plant,
    - instructions on the use of PPE
    - permit to work used for modifications and maintenance,
    - handling of dangerous chemicals,
    - substance and task specific information and instructions,
    - action plans in the event of accidents/spillages
    - rules for the disposal of chemicals.

The standard PPE is:

- safety footwear;
- protective clothing including a long-sleeved jacket and long-leg trousers;
- safety helmet;
- safety goggles and
- protective gloves – type (nitrile butadiene rubber coated cotton, latex or leather) varies depending on the company and the task.

The RAR stated that all of the information taken together gave confidence that the mitigating effects of PPE could be taken into account when determining the RWC dermal exposures for BPA manufacture. Therefore following the guidance given in the TGD exposures in the RAR were reduced by 90% to result in the long-term dermal exposures presented in Table 9.1.

Table 9.1 Reasonable worst case (RWC) long-term inhalation and dermal exposures from manufacture of BPA

Exposure	RWC exposures (mg/m^3)	RWC exposures (mg/kg/d*)
Long-term inhalation (8-hr TWA)	3	-
Long-term dermal	-	0.6

*Based on a 70kg adult

B.9.3. Uses of BPA

B.9.3.1 Manufacture of Epoxy resins

Introduction

Epoxy resin production is the second largest use of BPA in the EU. The RAR stated that in 2005/06 191,520 tonnes/year of BPA was used in the production of epoxy resins. There are 11 companies manufacturing BPA across 15 sites within the EU.

There are a number of different epoxy resins, which vary depending upon the starting constituents. However, diglycidyl ethers of BPA derived from BPA 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 BPA in the presence of an alkaline catalyst. Initially the dichlorohydrin of BPA is produced. The intermediate product then undergoes dehydrohalogenation with an alkali. In the preparation of commercial pure diglycidyl ether of BPA (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, BPA 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 fusion method, liquid epoxy resin is chain extended with BPA 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 BPA derived epoxy resins are most frequently cured with anhydrides, aliphatic amines, or polyamides, depending on the desired end properties. These include 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 protective coatings, structural composites, electrical laminates, electrical applications and adhesives.

Exposure values

Information from the RAR indicated that the RWC 8-hr TWA for inhalation exposure in the manufacture of epoxy resins was 0.7 mg/m^3. The exposure was derived from 53 samples within the UK's National Exposure Database (NEDB), 1 published study from the USA (NIOSH, 1979) which included 28 samples and exposure was also modelled using EASE (EASE parameters were non-dispersive use, dry manipulation, without local exhaust ventilation (LEV)).

As no dermal data were available for inclusion in the RAR, EASE was used to model exposure. The parameters used were wide-dispersive use, direct handling with extensive contact. The RWC exposure was calculated as 12 mg/kg/d (based on a 70 kg adult).

Information within the RAR indicated that charging of reactors with BPA was considered to be the main source of exposure with possible exposures during maintenance activities. Information from 5 of the 15 sites manufacturing epoxy resins indicated that dermal exposures are negligible because either BPA is manufactured on site and charged via a closed system to BPA or unloading is done under a nitrogen blanket. Charging at some sites is additionally done from hoppers within a closed system. The RAR stated that because only 5 of the 15 sites presented information it was not known whether these processes were representative (as some sites were thought to charge from bags). Therefore, no refinement of the dermal exposure figure was carried out in the update to the RAR. In addition, information was provided on the PPE (e.g. latex-neoprene blend gloves) worn during these activities. However, the use of PPE was not taken into account when looking at exposure as it was not known whether the information from companies was representative of the whole industry.

During the development of this transitional dossier further information was requested from the epoxy resin manufacturers. Information provided indicated that there are still 11 companies manufacturing epoxy resins containing BPA across the EU. These 11 companies operate from 17 different sites (1 in the Netherlands, 7 in Germany, 2 in Spain, 1 in the UK, 1

in Czech Republic, 3 in Italy, 1 in Poland and 1 in Hungary) (Pers. comm., 2008a).

As can be seen from the exposures in the RAR (discussed above) the majority of the exposure from the manufacture of epoxy resins occurs via the dermal route. Therefore, further information was requested from companies on how they handle BPA when manufacturing the resins. Information was provided from 13 of the 17 sites. Of these 13 sites, 10 use the 'Bisphenol A: A Safety and Handling Guide' when handling the substance. The Guide, which is available to download online (http://www.bisphenol-a.org/human/handguide.html), indicates that when handling the substance workers should wear 'gloves resistant to bisphenol-A e.g. PVC gauntlets, to protect the hands and lower arms'. Although PVC Gauntlets are an adequate material for protecting the skin from BPA no information is provided within the Guide on whether other gloves (such as seamless nitrile or butyl rubber) are appropriate for handling BPA, how often workers should change their PPE, how their PPE is maintained and whether there is an adequate PPE management programme.

In the other 3 sites a different industry standard (BG Chemie, 2006) is followed. The guide provides information on dealing with irritant and corrosive chemicals (BPA is classified as R37: Irritant to the respiratory tract). It details the measures to take to protect health including reference to protecting the skin (e.g. preventing hands becoming cracked and dry etc) and indicates that gloves made of resistant rubber or plastic should be used. BPA is not an irritant substance by the dermal route but is sensitising. Therefore, this guide may not be appropriate reference point for choosing appropriate PPE to protect the skin from a potential skin sensitiser. Again, no information is provided within the industry standard on a PPE management programme.

In order to take PPE into account when examining exposures it must meet the following two conditions:

- PPE is used regularly by the great majority (90%) of workers in the majority (90%) of facilities making or using the chemical and;
- the PPE used is appropriate and fit for purpose.

As information has only been provided from 79 % of sites manufacturing epoxy resins across the EU and no detail is provided within the Industry Guide on an effective PPE management programme a reduction in exposures because workers wear adequate PPE (gloves) cannot take place. Therefore the exposures outlined (see Table 9.2) from the RAR are taken forward to the risk characterisation.

Table 9.2 Reasonable worst case (RWC) long-term inhalation and dermal exposures from manufacture of epoxy resins

Exposure	RWC exposures (mg/m^3)	RWC exposures (mg/kg/d*)
Long-term inhalation (8-hr TWA)	0.7	-
Long-term dermal	-	12

*Based on a 70kg adult

B.9.3.2 All Occupational uses of BPA

Introduction

No information was provided on whether any of the industries outlined below use concentrations of BPA <30 %. Therefore the following occupational exposure scenarios are still considered relevant for the potential to sensitise the skin with high concentrations (>30%) of BPA:

- Manufacture of BPA
- Manufacture of PC
- Manufacture of articles from PC
- Manufacture of epoxy resins and moderated epoxy resins
- Use of BPA in PVC manufacture
- Manufacture of liquid epoxy paints, lacquers and powder coatings
- Use of epoxy resin-based powder coatings, paints and lacquers
- Manufacture of thermal papers
- Manufacture of tin-plating additive

New information from industry in June 2008 indicates that BPA is no longer used for the manufacture of tin-plating additives as BPA is thermally unstable (Pers comm., 2008b). This process will not be considered further within this transitional dossier.

Information received from industry in November 2008 indicates that there is no exposure to relevant concentrations (>30 %) of BPA in the manufacture of articles from polycarbonate and there is no handling of free BPA. The concentration of residual BPA monomer in polycarbonate is, at its highest, a few 10s of ppm (Pers. comm., 2008c).

Information from industry in November 2008 indicated that there is no exposure to high concentrations of BPA in the manufacture of liquid epoxy paints, lacquers and powder coatings and the use of epoxy resin-based powder coatings, paints and lacquers. As outlined in section 4.1.1.1.1 of the RAR (EC, 2008) the BPA concentration in liquid epoxy resins is less than 10 ppm and the residual amount of BPA in epoxy resins for powder coatings is about 300 ppm (Pers comm., 2008c).

Industry indicates that the potential for skin sensitisation in the three occupational scenarios (outlined above) is highly unlikely. Due to the information only being submitted in November 2008 the UK has not been able to verify the information. Therefore, it is recommended that information on the amount and concentration of BPA used in each occupational setting is submitted within the REACH registration dossiers.

Exposure values

From the available toxicology data BPA has been classified as a moderate sensitiser. Although, the dermal exposures derived in the RAR could be used to examine the potential of BPA to cause skin sensitisation no specific DNEL/DMEL could be derived for this hazard (see section 5.1). As no DNEL/DMEL can be derived then no RCR can be calculated (see B.10.1.3). Therefore, the dermal exposures for each occupational exposure scenario have not been included within this section.

B.10 Risk characterisation

According to REACH, if, exposure is less than the relevant DNEL (i.e. the risk characterisation ratio (RCR) <1) then the risk is adequately controlled. If exposure is greater than the relevant DNEL (i.e. RCR >1) then the risk is NOT controlled. The RCR for combined exposure is calculated by adding the relevant inhalation and dermal RCRs together and if they are <1 then the risk is adequately controlled.

B.10.1 Human health

B.10.1.1 Manufacture of BPA

The RCRs for the manufacture of BPA are presented in Table 10.1.

Table 10.1 Risk characterisation ratios for inhalation, dermal and combined exposures during manufacture of BPA

REASONABLE WORST CASE EXPOSURE SCENARIO	**RCR (RWC exposure / 8h TWA DNEL (mg/m^3))**
RCR for inhalation	3 / 10 = 0.3
RCR for dermal	0.6 / 1.4 = 0.43
RCR for combined exposure	0.3 + 0.43 = 0.73

Conclusion

As the RCRs for inhalation, dermal and combined exposures are less than 1 then the risks from manufacture of BPA are adequately controlled. Therefore,

no further RMMs for the manufacture of BPA are considered necessary, above those already in place.

B.10.1.2 Manufacture of epoxy resins

The RCRs for the manufacture of epoxy resins are presented in Table 10.2.

Table 10.2 Risk characterisation ratios for inhalation, dermal and combined exposures during manufacture of epoxy resins

REASONABLE WORST CASE EXPOSURE SCENARIO	**RCR (RWC exposure / 8h TWA DNEL (mg/m^3))**
RCR for inhalation	0.7 / 10 = 0.07
RCR for dermal	12 / 1.4 = **8.57**
RCR for combined exposure	0.07 + 8.57 = **8.64**

Conclusion

The RCR for inhalation during manufacture of epoxy resins indicates that exposures are adequately controlled (RCR less than 1). However, the RCRs for dermal and combined exposures are greater than 1 and therefore exposures are not considered to be adequately controlled. It is clear from the RCRs in Table 10.2 that the majority of risk for combined exposure comes via the dermal route. Therefore, if exposures via the dermal route are adequately controlled (overall) then the combined risk could also be adequately controlled.

If all 17 sites (to include the 4 who did not respond to the request for further information) manufacturing epoxy resins ensured staff wore appropriate PPE when in contact with BPA during manufacture of epoxy resins then potential dermal exposures could be reduced by 90 % of that outlined in Table 9.2. If dermal exposures were reduced by 90 % then the RCRs would be as outlined in Table 10.3.

Table 10.3 Consideration of the effect of PPE on the risk characterisation ratios for inhalation, dermal and combined exposures during manufacture of epoxy resins

REASONABLE WORST CASE EXPOSURE SCENARIO	RCR (RWC exposure / 8h TWA DNEL (mg/m^3))
RCR for inhalation	0.7 / 10 = 0.07
RCR for dermal	1.2 / 1.4 = 0.86
RCR for combined exposure	0.07 + 0.86 = 0.93

To ensure that dermal exposures are as low as possible across the epoxy resin industry, adequate PPE must be worn. Industry should ensure that all sites are visited to ensure that appropriate PPE is worn when handling BPA.

B.10.1.3 All Occupational uses of BPA

Conclusion

As no DNEL/DMEL can be derived for the sensitising potential of BPA then no risk characterisation can take place. As outlined in the REACH 'Guidance on Information requirements and Chemical Safety Assessment' when no risk characterisation can take place RMMs should be considered. The RMMs that should be considered when dealing with a moderate sensitiser are outlined in Table E.3-1 in the Guidance on Chemical Safety Assessment. The guide indicates that appropriate gloves should be worn, which will address the dermal and sensitising risks associated with BPA.

B.10.2 Environment

Not relevant for this Annex XV dossier.

B.11 Summary on hazard and risk

Manufacture of BPA

The risk characterisation indicates that the risk from manufacture of BPA in relation to concerns for repeated dose systemic effects and reproductive toxicity are low taking into account the current RMMs outlined in the RAR. Therefore, no further RMMs on the manufacture of BPA for repeated dose systemic effects and reproductive toxicity are proposed within this transitional dossier.

Manufacture of epoxy resins

When PPE is not worn the risk characterisation indicates that the risk from manufacture of epoxy resins in relation to concerns for repeated dose systemic effects and reproductive toxicity are high taking into account the RMMs outlined in the RAR. The majority of the exposure occurs from the dermal route. However, when it can be demonstrated that workers at all sites manufacturing epoxy resins wear appropriate PPE when handling the substance the risks are likely to be low (RCR less than 1).

Therefore, where BPA is handled all companies should follow the updated industry standard (see below) for handling BPA (i.e. wearing appropriate gloves when in contact with the substance) to ensure risks to workers are low. Therefore, no further RMMs for repeated dose systemic effects and reproductive toxicity on the manufacture of BPA are proposed within this transitional dossier.

All Occupational uses of BPA

As no DNEL can be determined for the sensitising potential of BPA then RMMs have to be considered. For all occupational scenarios (except use in tin plating) it is recommended that information on the amount and concentration of BPA used in each occupational setting be submitted within the REACH registration dossiers.

The REACH 'Guidance on Information requirements and Chemical Safety Assessment' outlines the general information that should be considered by industry and authorities when dealing with a moderate sensitiser. The information (outlined in Table E.3-1 of the Risk Characterisation part of the Guidance) that should be considered when dealing with a moderate sensitiser includes the following:

- Containment as appropriate;
- Minimise number of staff exposed;
- Segregation of the emitting process;
- Effective contaminant extraction;
- Good standard of general ventilation;
- Minimisation of manual phases;
- Avoidance of contact with contaminated tools and objects;
- Regular cleaning of equipment and work area;
- Management/supervision in place to check that the RMMs in place are being used correctly and operational conditions (OCs) followed;
- Training for staff on good practice;
- Good standard of personal hygiene.

For PPE the guidance states that the following should be considered:

- Substance/task appropriate gloves;

- Skin coverage with appropriate barrier material based on potential for contact with the chemicals;
- Substance/task appropriate respirator;
- Optional face shield;
- Eye protection.

As discussed previously within this report the BPA industry have an Industry 'Safety and Handling Guide' already available. Industry is in the process of revising the guide. They indicate that they will take account of the recommendations within this Annex XV report in the new version (Pers. comm., 2008c). Therefore the updated industry guidance document should include (as a minimum) the information outlined above (from Table E.3-1 of the Guidance on Information requirements and Chemical Safety Assessment') and the following for each occupational setting where BPA is used:

- the appropriateness and the suitability of PPE (including the most appropriate gloves to be worn when using BPA in each occupational situation);
- maintenance and replacement of PPE;
- information, instruction and training in the use of PPE and cleaning before and after eating;
- Management systems to examine general cleaning and maintenance procedures.

The REACH registration dossier (which includes the Chemical Safety Report, exposure scenarios and safety data sheets) should reflect the above information.

C. AVAILABLE INFORMATION ON ALTERNATIVES

Information and analysis of alternatives for human health have not been provided as the concerns for repeated dose systemic effects and reproductive toxicity appear to be adequately controlled using current control measures and appropriate PPE. The concerns related to the sensitisation potential should be adequately reduced by a voluntary agreement to update the safety and handling guide which outlines the information detailed in B.11.

D. JUSTIFICATION FOR ACTION ON A COMMUNITY-WIDE BASIS

Not applicable as no action on a community-wide basis is proposed.

E. JUSTIFICATION WHY A RESTRICTION IS THE MOST APPROPRIATE COMMUNITY-WIDE MEASURE

Not applicable as no restriction is proposed.

F. SOCIO-ECONOMIC ASSESSMENT OF PROPOSED RESTRICTION(S)

Not applicable as no restriction is proposed.

G. Stakeholder consultation

All the following stakeholders were consulted in 2008 by e-mail, fax, letter, telephone or via a meeting on the environmental part of this document during the preparation of this Annex XV dossier.

The section of this report concerning snail toxicity was circulated for comment in October 2008 to government officials and independent experts in the UK, Bisphenol-A producer representatives, and other expert stakeholders who were involved in the snail steering group discussions under the earlier ESR programme (particularly the German Umweltbundesamt (UBA)).

The discussion of sediment and fish data was sent to Bisphenol-A producer representatives and the German UBA for comment in November 2008.

Bayer Materials Science
Brunel University
Centre for Environment, Fisheries and Aquaculture Science (CEFAS)
Department for Business, Enterprise & Regulatory Reform (BERR)
Department for Environment, Food and Rural Affairs (DEFRA)
Department of Health, UK
Dow Chemical Company
Environment Agency
Health & Safety Executive – Northern Ireland
Health and Safety Laboratory (HSL)
Hexion Specialty Chemicals
Local Authorities Coordinators of Regulatory Services (LACORS)
National Assembly of Wales
Plastics Europe
Sabic Innovative Plastics
Scottish Environment Protection Agency
Scottish Executive
Swedish Chemicals Agency (Kemi)

H. OTHER INFORMATION

Not applicable.

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GLOSSARY

AF	Assessment Factor
BLV	Biological Limit Values
BPA	Bisphenol-A
CAD	Chemical Agents Directive (98/24/EC)
CAS	Chemical Abstracts Service
CEFIC	European Chemical Industry Council
CLP	EU Classification, Labelling and Packaging of Substances and Mixtures Regulation – to be implemented under the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS).
CSA	Chemical Safety Assessment
DGEBPA	Diglycidyl Ether of Bisphenol-A
DMEL	Derived Minimal Effect Level
DNEL	Derived No Effect Level
EASE	Estimation and Assessment of Substance Exposure
EC	European Community
ECB	European Chemicals Bureau
EEC	European Economic Community
ESR	Existing Substances Regulations (793/93/EEC)
EU	European Union
GI	Gastro-intestinal Tract
IOELV	Indicative Occupational Exposure Limit Value
IUPAC	International Union of Pure and Applied Chemistry
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest-Observed Adverse Effect Level
NAEL	No Adverse Effect Level
NEDB	National Exposure DataBase at HSE in UK
NIOSH	The National Institute of Occupational Safety and Health
NOAEC	No-Observed Adverse Effect Concentration
NOAEL	No-Observed Adverse Effect Level
OEL	Occupational Exposure Limit
OC	Operational Conditions
PBT	Persistent, Bioaccumulative and Toxic
PC	Polycarbonate
PPE	Personal Protective Equipment
PVC	Polyvinyl Chloride
RAR	Risk Assessment Report
RCR	Risk Characterisation Ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (EC No. 1907/2006)
RMM	Risk Management Measure
RRS	Risk Reduction Strategy
RWC	Reasonable Worst Case
SCOEL	Scientific Committee on Occupational Exposure Limits
STEL	Short-term Exposure Limits
TCNES	Technical Committee for New and Existing Substances
TGD	Technical Guidance Document

TWA	Time-Weighted Average
vPvB	Very Persistent Very Bioaccumulative