

**UPDATED RISK ASSESSMENT
OF
4,4'-ISOPROPYLIDENEDIPHENOL
(Bisphenol-A)**

**CAS Number: 80-05-7
EINECS Number: 201-245-8**

**Final human health draft for publication
(to be read in conjunction with published EU RAR of BPA, 2003)**

April 2008

Introduction

A risk assessment of 4,4'-isopropylidenediphenol (Bisphenol-A, BPA) produced in accordance with Council Regulation (EEC) 793/93¹ was published in 2003². In relation to human health, conclusion (i) "There is need for further information and/or testing" was reached for developmental toxicity. Further research was needed to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development at low doses. The information requirements were a 2-generation study in mice according to OECD 416 (with some specific modifications).

This 2-generation study has now been submitted to the Rapporteur (UK) for evaluation. The UK has updated the original risk assessment, reviewing the requested study and the new data on human exposure and effects of BPA that have become available since the original risk assessment report was completed.

The format of the report is broadly in line with that of the original risk assessment. Significant new information is summarised in this updated risk assessment and a comment is added to indicate how this affects the findings from the original risk assessment.

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¹ O.J. No. L 084, 05/04/1993 p. 0001 - 0075

² European Union Risk Assessment Report: 4,4'-isopropylidenediphenol (BPA) – 3rd Priority List, Volume 37. European Commission Joint Research Centre, EUR 20843 EN.

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0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No: 80-05-7

EINECS No: 201-245-8

IUPAC name: 2,2-bis(4-hydroxyphenyl)propane (also known as Bisphenol-A, or BPA)

0.1 HUMAN HEALTH

0.1.1 Human health (toxicity)

0.1.1.1 Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

This conclusion is reached in relation to repeated dose systemic effects and for reproductive toxicity during the manufacture of BPA and the manufacture of epoxy resins. In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact with high concentrations (>30%) of BPA.

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

This conclusion is reached in relation to repeated dose systemic effects and reproductive toxicity for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, thermal paper manufacture and manufacture of tin plating additive. This conclusion also applies in relation to eye and respiratory tract irritation and repeated dose local effects in the respiratory tract for all scenarios.

0.1.1.2 Consumers

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

This conclusion is reached for all consumer scenarios in relation to all endpoints.

0.1.1.3 Humans exposed via the environment

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

This conclusion is reached for both local and regional exposure scenarios in relation to all endpoints.

0.1.1.4 Combined exposure

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

This conclusion is reached for combined exposure scenarios in relation to all endpoints.

0.1.2 Human health (risks from physico-chemical properties)

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

This conclusion is reached because there are no risks from physico-chemical properties arising from the use of BPA.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 Occupational exposure

4.1.1.1.1 Summary of original risk assessment report

The total number of persons occupationally exposed to BPA is not known, but due to its widespread use in epoxy resins and polycarbonate it is expected to be thousands. However, the exposure is likely to be negligible in many cases as the residual BPA in epoxy resins and polycarbonate is low.

Most of the data used in this assessment have been supplied by industry, either directly or through trade organisations. The HSE has no BPA exposure data on its NEDB (National Exposure Database) and no data were available from any of the other competent authorities. There is little data available from published papers although two were found relating to (i) use of epoxy resin-based paint and (ii) the use of epoxy resin-based powder paints.

The occupational exposure to BPA is discussed in 10 scenarios:

- manufacture of BPA
- manufacture of PC
- manufacture of articles from PC
- manufacture of epoxy resins and moderated epoxy resins
- use of BPA in PVC manufacture
- manufacture of liquid epoxy paints, lacquers and powder coatings
- use of epoxy resin-based powder coatings, paints and lacquers
- manufacture of thermal papers
- manufacture of tin-plating additive
- manufacture of tetrabrominated flame retardants (TBBA)

Some uses of BPA have been identified but not discussed in the following sections as these uses do not apply in the European Union or because information on some of the minor uses was not available. These include tyre manufacturing, brake fluid manufacturing, polyols/polyurethane manufacturing and polyamide processing.

In a number of instances, companies supplying information stated that personal protective equipment and/or respiratory protective equipment was used. However, unless stated otherwise in the text, details of the type were not provided.

BPA Manufacture

The industry from which the highest inhalation exposures were reported was the BPA manufacturing industry with 8hr TWAs ranging from “none detected” to 23.3 mg/m³.

Reasonable worst case scenarios were estimated using the 90th percentile. This was calculated where there is sufficient data. Where insufficient data were available, professional judgement was used to estimate the 90th percentile. A reasonable worst case 8hr TWA for BPA manufacturing was estimated at 5 mg/m³.

Short term exposures varied considerably, ranging from “none detected” to 43.6mg/m³. Generally, short term exposures rarely exceeded 10 mg/m³.

Dermal exposure ranged from 0 to 5 mg/cm²/day (EASE estimation). Bag filling and maintenance activities gave rise to the highest estimates for dermal exposure. A reasonable worst case scenario was decided as 5 mg/cm²/day.

The inhalation exposure results on which this assessment was based can be found summarised in **Tables 4.1** (8hr TWAs) and **4.2** (short term exposures). The BPA manufacturing process is largely an enclosed system with breaches for product sampling, product bagging and tanker/silo filling and some maintenance activities. Product sampling is a short term activity typically lasting about 3 to 5 minutes, and may be carried out once or twice per shift. There were no short term sample results available so EASE was used to estimate exposures during this activity giving a three minute exposure range of 0 to 5 mg/m³ and a short term exposure level of 0 to 1 mg/m³. Short term results for bagging gave results of 14 and 15 mg/m³, although these results were reported not to reflect the current occupational exposure. Data provided by SPI (USA) gave short term task-specific results between none detected and 0.96 mg/m³. A reasonable worst case scenario for short term exposures was agreed as 10 mg/m³.

8hr TWA exposures for operators varied widely, both in the way they were sampled and analysed, and in the range of the results reported. Many operators measured total inhalable particulate or respirable dust, with some samples being analysed specifically for BPA. The results ranged from none detected (nd) to 23.3 mg/m³ 8hr TWA. Product bagging and tanker/silo filling were reported to be full shift activities. Exposures for these activities were generally below 5 mg/m³. All the EASE results predicted exposure ranges below 5 mg/m³ for the above activities. The highest results were obtained where maintenance activities or cleaning was carried out during the sampling period, although information regarding the types of tasks carried out were not available. Sampling results for more recent maintenance activities (1998-2000) ranged from less than 0.05 to 0.62 mg/m³. A reasonable worst case scenario for 8hr TWA for manufacturing activity was agreed as 5 mg/m³. *Polycarbonate manufacture*

It was reported that there was little or no opportunity for exposure to BPA during the manufacture of polycarbonate, as the BPA entered the plant as a solution and was piped directly into a closed system. However, four respirable dust samples for PC dust had been collected in 1990-1991, although they were not analysed for BPA. Further dust sampling was undertaken from 1993 to 1996. These were for total inhalable particulate (TIP) and were not analysed for BPA. These results ranged from 0.1 to 1.1 mg/m³. The 90th percentile for these figures was 1.0 mg/m³. It was reported by industry that there is a maximum of 100 ppm residual BPA in the PC polymer. Taking this into account the reported results range from 7x10⁻⁷ to 1.1x10⁻⁴ mg/m³, 8hr TWA with a 90th percentile of 1x10⁻⁴ mg/m³, 8hr TWA. In 2000, the same company took a personal sample to confirm that there was no exposure to BPA in the PC manufacturing plant. The sample was analysed for BPA. The result was less than 1x10⁻³ mg/m³, 8hr TWA. EASE modelling resulted in a range of 0 to 1x10⁻⁴ mg/m³, 8hr

TWA. A reasonable worst case scenario for this activity was agreed as $1 \times 10^{-3} \text{ mg/m}^3$, 8hr TWA.

There is reported to be no opportunity for exposure to BPA during the manufacture of articles from polycarbonate, due to the stability of the polymer, and the retention of any residual BPA within the polymer matrix. As the manufacturing process does not use any higher temperatures than those used for extrusion in the PC manufacturing industry, the same results were used to represent exposure in the manufacture of articles from PC. The reasonable worst case scenario was agreed as $1 \times 10^{-3} \text{ mg/m}^3$, 8hr TWA. A number of responses from companies manufacturing epoxy resins and modified epoxy resins highlighted the charging of vessels with BPA prills or flakes as the main source of exposure in this industry. Short term exposures during this activity ranged from 0.32 to 17.5 mg/m^3 , with 8hr TWAs of up to 1.2 mg/m^3 . A reasonable worst case scenario was agreed as an 8hr TWA of 0.7 mg/m^3 . A reasonable worst case scenario for short term exposure was agreed as 11 mg/m^3 .

PVC Manufacture

The use of BPA in PVC manufacture is being phased out. As handling of BPA is considered to be similar to industries such as thermal paper manufacturing, the EASE data for that scenario were used to generate data for PVC manufacturing. A reasonable worst case scenario was estimated to be 0.1 mg/m^3 8hr TWA. A short term reasonable worst case exposure was estimated to be 1 mg/m^3 .

Manufacture of epoxy resins

Manufacture of liquid epoxy resin-based paints is not reported to be a source of significant exposure to BPA given the very low (10 ppm) quantity of residual BPA in the uncured epoxy resin, most of which would be retained within the resin matrix.

Use of epoxy resin-based paints

The residual amount of BPA in epoxy resins for powder paints is reported to be about 300 ppm. Calculations made using this figure and total inhalable particulate exposure measurements from the HSEs NEDB, gave an estimated exposure of up to 0.02 mg/m^3 , 8hr TWA. Industry supplied data for personal exposure across all activities ranging from 0.3 to 10 mg/m^3 , 8hr TWA for total inhalable particulate. This was calculated to give a range of personal exposures to BPA of 9×10^{-5} to $3 \times 10^{-3} \text{ mg/m}^3$. Given that the amount of residual BPA in powder paints is likely to be lower than that calculated, a reasonable worst case scenario of 0.01 mg/m^3 8hr TWA was estimated. A short term reasonable worst case estimate of 0.3 mg/m^3 was made based on data from SPI

Exposure to total inhalable particulate during the use of powder paints has been reported to be across a higher range than for manufacturing. The percentage of BPA in the coating powder is up to 40%. The estimated range of 8hr TWAs is up to 0.02 mg/m^3 . Actual measured exposure results were reported in a NIOSH paper. The range of 8hr TWAs reported was 0.003 to 1.063 mg/m^3 . A reasonable worst case scenario for an 8hr TWA was estimated to be 0.5 mg/m^3 for spraying coating powders and 0.005 mg/m^3 for dip-painting.

Manufacture of thermal papers

Thermal paper manufacturers reported only one exposure result for BPA, which was lower than the limit of detection for an hour-long sample. An 8hr TWA calculated from this result gave a figure of less than 0.25 mg/m^3 . Enough information was available to allow EASE estimations to be made. The estimated range predicted was 0 to 0.04 mg/m^3 . A reasonable worst case scenario for an 8hr TWA for this industry was estimated to be 0.1 mg/m^3 . A reasonable worst case scenario for short term exposure would be 4 mg/m^3 .

Manufacture of tin plating additives

Small quantities of BPA are used in the manufacture of tin plating additives. No exposure data were available but sufficient information was supplied to allow an EASE prediction to be made. This gave an exposure range of 0.02 to 0.05 mg/m^3 8hr TWA, with the only source of exposure identified being the charging of the reactor vessel with BPA. A reasonable worst case scenario would be an 8hr TWA of 0.05 mg/m^3 .

Manufacture of TBBA

One company was manufacturing TBBA using BPA. No exposure data were available, but EASE was used to estimate exposure during the packaging process. This gave an estimated exposure range of 6×10^{-6} to $1.5 \times 10^{-5} \text{ mg/m}^3$ 8hr TWA.

In summary, 8hr TWAs rarely exceeded 5 mg/m^3 in BPA manufacturing facilities, and rarely exceeded 0.5 mg/m^3 in the other industries discussed. Short term exposures could reach as high as 43.6 mg/m^3 , but were more usually less than 10 mg/m^3 .

Table 4.1 Summary table of occupational inhalation exposure data (8hr TWA) from the 2003 published RAR

Work activities	No of samples	Type of sample	Range 8hr TWA (mg/m ³)	Mean 8hr TWA (mg/m ³)	90th percentile 8hr TWA (mg/m ³)	RWC exposure inhal. BPA (mg/m ³)	Source
BPA manufacturing							
Sampling and filling (1988-1992)	24	resp. part.	0.04 to 5.01	0.59	1.23		Industry
Filling big bags (1998)	3	inhal. BPA	0.21 to 1.79	0.81	1.61		Industry
Filling silo tankers (1998)	3	inhal. BPA	less than 0.5 to 1.61	0.89			Industry
Various (1998)	8	inhal. BPA	0.13 to 0.62	0.3			
Various (1997)	8	TIP	less than 0.1 to 0.9	0.38	1.79		
Various (1993-1996)	15	TIP	less than 0.1 to 6	0.94			Industry
Filling (1988-1992)	4	TIP	0.42 to 1.79	1.1			
Packaging	9	TIP	0.002 to 7.5	1.1	n/a		Industry
Reworking	8	TIP	0.002 to 23.3	7.9	n/a		Industry
Plant operator	12	TIP	less than 0.1 to 0.8	0.3	n/a		Industry
Plant operator	13	*BPA	0.02 to 2.13	0.61	2.12		Industry
Maintenance operator	2	*BPA	0.04 to 2.08	1.06			Industry
Plant operator	7	inhal. BPA	0.21 to 1.04	not known	n/a		Industry
Maintenance	3	inhal. BPA	0.52 to 1.35	not known	n/a		Industry
Maintenance (1998-2000)	8	*BPA	Less than 0.05 to 0.62		n/a		Industry
Charging big bags	5	inhal. BPA	0.02 to 0.93	0.35	n/a		Industry
Various	not known	*BPA	nd to 2.6	not known	n/a		SPI (USA)
Operator incl. sampling	n/a	inhal. BPA	0 to 0.03	n/a	n/a		EASE
Product silo filling	n/a	inhal. BPA	0 to 1	n/a	n/a		EASE
Bag filling	n/a	inhal. BPA	0 to 5	n/a	n/a	5	EASE
PC manufacturing							
Plant operator	4	resp. part (BPA)	0.07 to 0.27 (7x10 ⁻⁷ to 2.7x10 ⁻⁵)	0.2 (2x10 ⁻⁵)	n/a	1x10 ⁻³	Industry
Plant operator	16	TIP (BPA)	0.1 to 1.1 (1x10 ⁻⁵ to 1.1x10 ⁻⁴)	0.43 (4.3x10 ⁻⁵)	1x10 ⁻⁴		Industry

Work activities	No of samples	Type of sample	Range 8hr TWA (mg/m ³)	Mean 8hr TWA (mg/m ³)	90th percentile 8hr TWA (mg/m ³)	RWC exposure inhal. BPA (mg/m ³)	Source
Plant operator	Not known	Resp. part (BPA)	Not known	Less than 0.1 (less than 1x10 ⁻⁵)	Not known		Industry
Plant operator	1	Inhal. BPA	n/a	Less than 1x10 ⁻³	n/a		Industry
Plant operator	n/a	Inhal. BPA	0 to 1x10 ⁻⁴	n/a	n/a		EASE
Manufacture of articles from PC							
Plant operator	n/a	Inhal. BPA	0 to 1x10 ⁻⁴	n/a	n/a	1x10 ⁻³	EASE /Industry
Epoxy resin manufacturing							
Charging reactors	not known	various	less than 0.01 to 1.09	not known		0.7	Industry
Various	96	*BPA	less than 0.1 to 2.8	0.24	0.7		SPI (USA)
Container unloading	n/a	inhal. BPA	0 to 0.25	n/a			EASE
Use of BPA in PVC manufacture							
Charging reactors	n/a	Inhal. BPA	0 to 0.04	n/a	n/a	0.1	EASE
Manufacture of epoxy resin-based paints, lacquers, and coating powders							
Coating powders manufacturing							
Various	28	inhal. BPA	3.3x10 ⁻⁴ to 0.02 (calc)	0.01	0.008	0.01	HSE
Various	210	Inhal. BPA	9x10 ⁻⁵ to 3x10 ⁻³	Not known	Not known		Industry
Use of epoxy resin-based paints, lacquers and coating powders							
Coating powders use							
Spraying, loading, cleaning	53	inhal. BPA	2.4x10 ⁻⁵ to 0.02 (calc)	1.6x10 ⁻³	0.005	0.5	HSE
Spray painters	6	inhal. BPA	0.173 to 1.063	0.6			NIOSH
Spray painters	n/a	Inhal. BPA	6x10 ⁻⁴ to 6x10 ⁻³	n/a	n/a		EASE
Dip painters	2	inhal. BPA	0.004 to 0.005	0.0045		0.005	NIOSH
Dip/spray painters	7	resp. BPA	0.003 to 0.131	0.04			NIOSH
Dip painters	n/a	Inhal. BPA	6x10 ⁻⁴ to 6x10 ⁻³	n/a	n/a		EASE

Work activities	No of samples	Type of sample	Range 8hr TWA (mg/m ³)	Mean 8hr TWA (mg/m ³)	90th percentile 8hr TWA (mg/m ³)	RWC exposure inhal. BPA (mg/m ³)	Source
Thermal paper manufacturing							
Charging reactor	1	inhal. BPA	less than 0.25	less than 0.25		0.1	Industry
Charging reactor	n/a	inhal. BPA	0 to 0.04	n/a			EASE
Manufacture of tin plating additive							
Manufacture of tin plating additive - charging vessel	n/a	inhal. BPA	0.02 to 0.05	n/a		0.05	EASE
Manufacture of TBBA							
Packaging final product	n/a	inhal. BPA	6x10 ⁻⁶ to 1.5x10 ⁻⁵	n/a		1.5x10 ⁻⁵	EASE

TIP = total inhalable particulate

Inhal. Part = inhalable particulate

Inhal. BPA = inhalable BPA

Resp. part. = respirable particulate

(BPA) = calculated BPA concentration in particulate

Table 4.2 Summary table of short term, task specific occupational inhalation exposures to BPA from the 2003 published RAR

Work activities	No of samples	Range (mg/m ³)	Mean (mg/m ³)	RWC exposure (mg/m ³)	Source
Bagging machine operator 1990	2	14 to 15	14.5	10	Industry
BPA manufacturing Various	15	nd to 0.96	not known		SPI (USA)
PC manufacturing Connecting BPA chargepoint	6	nd to less than 0.64	0.29	0.5	SPI (USA)
Epoxy resin manufacture- charging reactor	12	0.32 to 17.5 (inhalable dust)	1.52	11	Industry
Epoxy resin manufacture various	68	nd to 43.6	1.81		SPI (USA)
Manufacture of coating powders	2	Nd to 0.3	0.15	0.3	SPI (USA)
Use of BPA in PVC manufacture – charging reactors	n/a	0 to 1	n/a	1	EASE

Work activities	No of samples	Range (mg/m ³)	Mean (mg/m ³)	RWC exposure (mg/m ³)	Source
Thermal paper manufacture charging reactor	1	less than 4	less than 4	4	Industry

The results of dermal exposure predictions can be found in **Table 4.3**.

Dermal exposure to BPA can occur during manufacturing and use of BPA. During manufacturing, operators can come into contact during product sampling and during bag filling and other filling operations. Using the EASE model, dermal exposure during sampling was estimated to be in the range 0 to 0.1 mg/cm²/day. Exposure is likely to be towards the lower end of the range as the activity takes less than five minutes to complete. It is estimated that 420 cm² of skin may be exposed during this activity.

Filling operations are full shift activities, so the potential for dermal exposure is greater. The EASE estimation gave a range of 1-5 mg/cm²/day. The operators are reported to wear personal protective equipment, including gloves. PPE, properly selected and worn will significantly reduce exposure. A reasonable worst case exposure would be 1 mg/cm²/day. It is estimated that 420 cm² of skin may be exposed during this activity.

The only potential for dermal exposure during PC manufacturing was during the bagging of PC granules. The EASE estimation gave a range of 1x10⁻⁵ to 1x10⁻⁴ mg/cm²/day.

The same exposure range was used to estimate exposure during the manufacture of articles from PC, when loading PC granules from the big bags to the extruder.

The main source of exposure identified during epoxy resin manufacturing was the charging of reactors. The EASE estimation gave a range of 0.1 to 1 mg/cm²/day. The use of PPE during this task was reported. PPE, properly selected and worn will significantly reduce exposure.

Estimations of dermal exposure during two maintenance activities were carried out using EASE as an illustration of the potential dermal exposures during general maintenance activities. The EASE prediction gave a range of 0.1 to 1 mg/cm²/day for both activities.

For PVC manufacturing, a reasonable worst case scenario of 0.1 mg/cm²/day was estimated for dermal exposure using EASE data. It was estimated that an area of skin equivalent to 420 cm² may be exposed during this activity.

EASE was used to predict dermal exposures during the manufacture and use of epoxy resin-based powder coatings. Although controls are generally poorer in these industries, the potential for exposure is lower due to the small amount of residual BPA in the epoxy resin (approximately 300 ppm). The range of dermal exposure predicted using EASE during epoxy resin-based powder coating manufacture was 3 x 10⁻⁴ to 1.5 x 10⁻³ mg/cm²/day. The figure of 1.5x10⁻³ mg/cm²/day was taken to be a reasonable worst case dermal exposure. The range estimated using EASE for powder coating application was 6 x 10⁻⁴ to 1.8 x 10⁻³ mg/cm²/day given a maximum BPA content of 120 ppm. Charging reactors was the only activity identified by the thermal paper manufacturers and the tinplating additive manufacturers where the potential for dermal exposure arises. This activity takes about 5 to 10 minutes per shift. EASE was used to estimate a range of dermal exposure. The range predicted was 0 to 0.1 mg/cm²/day.

Dermal exposure during bag filling of TBBA was estimated using EASE. The range predicted, taking into account the fact that there is only 3 ppm BPA in the final product, is 3x10⁻⁷ mg/cm²/day to 3x10⁻⁶ mg/cm²/day.

In summary, dermal exposure was estimated to be highest during filling operations during BPA manufacture, which is a full shift activity. The estimated range of exposures were the same for charging reactors and maintenance activities during epoxy resin manufacturing, but these tasks were shorter lived, so exposures are likely to be lower. The lowest dermal exposure range predicted was for PC manufacturing, which has a very low percentage of residual BPA.

Table 4.3 Summary table of estimated dermal exposures using EASE from the 2003 published RAR

Work activities	Extent of area of dermal contamination	Range of dermal exposures (mg/cm ² /day)	RWC for dermal exposure (mg/cm ² /day)
BPA manufacturing			
Product sampling	420	0 to 0.1	0.1
Bag filling/other filling operations	420	1 to 5	1
Manufacture of PC			
Bag filling of PC granules	420	1x10 ⁻⁵ to 1x10 ⁻⁴	1x10 ⁻⁴
Manufacture of articles from PC			
Loading PC granules from big bags	420	1x10 ⁻⁵ to 1x10 ⁻⁴	1x10 ⁻⁴
Epoxy resin manufacturing			
Charging reactors	420	0.1 to 1	1
Maintenance - changing filter socks	840	0.1 to 1	1
Maintenance - emptying weigh vessel	840	0.1 to 1	1
Use of BPA in PVC manufacture			
Charging reactors	420	0 to 0.1	0.1
Manufacture of coating powders			
Manufacturing	1300	3x10 ⁻⁴ to 1.5x10 ⁻³	1.5x10 ⁻³
Use of coating powders			
Powder coating	1300	6x 10 ⁻⁴ to 1.8x10 ⁻³	1.8x10 ⁻³
Thermal paper manufacturing			
Charging reactors	420	0 to 0.1	0.1
Manufacture of tin-plating additive			
Charging reactor	420	0 to 0.1	0.1
Manufacture of TBBA			
Bag filling	420	3x10 ⁻⁷ to 3x10 ⁻⁶	3x10 ⁻⁶

Occupational exposure limits

Table 4.4 Occupational exposure limits for bisphenol-A (from the 2003 published RAR)

Country	8-hour TWA exposure (mg/m ³)	Source
Germany	5 (inhalable)	List of MAK and BAT values 1997
Holland	5 (respirable)	The National MAC-list 1999
USA*	5	Proposed WEEL - AIHA

* This information was provided by personal communication and no information was available with respect to whether the limit would be for inhalable or respirable dust.

These limits are provided for information and not as an indication of the level of control of

exposure achieved in practice in workplaces in these countries.

4.1.1.1.2 Updated information

Some applications of BPA have been discontinued in the EU. The uses of BPA as an inhibitor in PVC polymerisation and in the manufacture of TBBA have ceased. Therefore these scenarios are not considered further.

New information and exposure data have been provided by industry for BPA manufacture and epoxy resin manufacture.

New Industry Data for BPA Manufacture

Inhalation exposure

In 2007 Plastics Europe provided HSE with some new exposure data (Tables 4.5 and 4.6) and information on control measures. The data cover all companies (A, B, C and D) that manufacture PBA in the EU and are believed to be representative of all the sites that undertake this process.

Table 4.5 Industry inhalation exposure data for BPA manufacture

Industry inhalation exposure data for BPA manufacture (8hr-TWA) Company/ Site	Date	Parameter	Job	Number of samples	Range 8 hr TWA (mg/m ³)	Mean 8hr TWA (mg/m ³)
C/1	2000	Dust		14	1.36-3.45	2.18
C/1	2001	Dust		15	0.35-0.43	0.39
C/1	2002	Dust		12	0.27-2.48	1.13
C/1	2003	Dust		20	<0.1-0.8	0.24
C/1	2004	Dust		16	<0.1-3.9	1.34
C/1	2005*	Dust		15	<0.1-1.1	0.53
C/1	2006*	Dust		8	<0.01-0.1	<0.1
B/1	2003 - 2004	Total Dust	Bisphenol A (general operations)	6	0-3.6**	0.8
B/1	2003 and 2007	Total Dust (mostly BPA)	Bagging	2	0-0.33	0.17
B/1	2003 and 2007	Total Dust (mostly BPA)	Line Openings	2	0-2.9	1.7
A/1	Jan 07	BPA dust (respirable)	Shift foreman		<0.0002	
A/1	Jan 07	BPA dust	Shift personnel		0.0010	

		(respirable)				
A/1	Jan 07	BPA dust (respirable)	Lab assistant		<0.0006	
A/1	Jan 07	BPA dust (respirable)	Lab personnel		<0.0006	
A/1	Jan 07	BPA dust (respirable)	PCT personnel		<0.0002	
A/1	Jan 07	BPA dust (respirable)	Sampling personnel		0.0182	
A/1	Jan 07	BPA dust (respirable)	Maintenance foreman		0.0050	
A/1	Jan 07	BPA dust (respirable)	Safeguard		0.0050	
A/2	Jul 07	BPA dust (respirable)	Sampling personnel		0.1322	
A/2	Jul 07	BPA dust (respirable)	Tank truck filler		0.0762	
A/2	Jul 07	BPA dust (respirable)	Big bag filler		1.0810	
A/2	Aug 07	BPA dust (respirable)	Big bag filler		1.0317	
A/2	Aug 07	BPA dust (respirable)	Big bag filler		1.1502	

* Modifications to bagging facilities in 2005 led to further reductions in exposure

** The determination was for total dust measured by personal samplers on workers in a variety of occupations. The upper end of the range is believed to be an outlier in a data set which includes three determinations below the limit of detection. Company D also stated that they considered the data given above to be representative for their workplace. The newer data from Company A, which measured BPA dust, indicate that exposures for a range of jobs within the plant can be controlled to less than 1.1 mg/m³ (8hr-TWA). The results from Company C are harder to interpret as they have measured 'dust' with no indication of how much of that dust is BPA. Based on these newer data it is proposed that the RWC for 8hr TWA inhalation exposures for BPA manufacture should be 3 mg/m³.

Plastics Europe also provided data from one company (B) on short term exposures. This is given in Table 4.6.

Table 4.6 Industry short term inhalation exposure data for BPA manufacture

Work Activities	No of Samples	Sample Analysis	Range (15 min TWA)	Mean (15 min TWA)
Loading of tankers (2004)	2	Total dust (mostly BPA)	0.2 – 0.5	0.4
Loading of tankers (2007) Reduced dust levels due to	3	Total dust (mostly BPA)	not known	< 0.5

improvement of ventilation system)				
Line Openings 2005 – 2007	4	Total dust (mostly BPA)	0 – 0.5	0.38
Sampling	4	Total Dust (mostly BPA)	0 – 0.83	0.2
Bagging of Big Bags 2005-2007	3	Total Dust (mostly BPA)	0.31 – 0.5	0.43

Although these data are from a much more recent time period than those quoted in the original RAR, they are only from one company (B). The short term data given in the RAR were also only from one company, but a different one (C). In the original RAR there was a general lack of contextual data, particularly sampling period. Therefore it is difficult to know how comparable the data are. We also have no information as to how representative these data are for the industry as a whole.

Given this uncertainty, professional judgement has been used to determine the short term inhalation RWC. This was also done in the original RAR, and there it was agreed that the RWC should be 10 mg/m³. Following consideration of this new data, it is now proposed that the short term inhalation RWC should be 6 mg/m³.

Dermal Exposure The RAR used EASE predictions to determine dermal exposure and no sampling data have been provided by industry to use instead of the EASE predictions. However, industry have provided information on control measures used to allow the mitigating effect of PPE to be taken into account when determining the RWC dermal exposure for BPA manufacture. The use of PPE can be taken into account if two conditions are met:

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

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

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

The standard PPE is:

- working shoes;
- working suit with long sleeved jacket and long leg trousers;

- safety helmet;
- safety goggles and
- protective gloves – type (nitrile butadiene rubber coated cotton , latex, leather) varies depending on the company and the task.

All of this information taken together gives confidence that the mitigating effects of PPE can be taken into account when determining the RWC dermal exposures for BPA manufacture. Therefore following the guidance given in the TGD exposures values have been reduced by 90%. Modified values are given in Table 4.7

Table 4.7 Modified dermal exposure values for BPA manufacture

Work activities	Extent of area of dermal contamination (cm ²)	RWC for dermal exposure (mg/cm ² /day)	Modified RWC for dermal exposure (mg/cm ² /day)
BPA manufacture			
Product sampling	420	0.1	0.01
Bag filling/other filling operations	420	1	0.1

New Industry Information for Epoxy Resin Manufacture

There are 15 sites across the EU which manufacture epoxy resins. Industry has provided information on risk management measures from 5 of these. The RAR identifies charging of reactors as a potential source of dermal exposure and a predicted exposure was estimated. Information from five companies indicate that on their sites this exposure is likely to be negligible as either the BPA is manufactured on site and charged via a closed system or where delivered by tanker, unloading is done under a nitrogen blanket. Charging at some sites is done from hoppers within a closed system. However, some sites charge from bags (of varying sizes). Although some information is available on control measures from these sites, information is not available from all sites manufacturing epoxy resins, so it is not possible to say how representative of the industry they are. Therefore it is not possible to modify the RWC values given in the RAR.

4.1.1.1.3 Impact of new information

Some applications of BPA have been discontinued in the EU. The uses of BPA as an inhibitor in PVC polymerisation and in the manufacture of TBBA have ceased. Therefore these scenarios are not considered further.

Industry has supplied new information which has allowed the modification of RWC exposure values for BPA manufacture only.

For inhalation exposures the revised RWCs proposed are 3mg/m³ 8hr-TWA and 6 mg/m³, short term. For dermal exposures during BPA manufacture the RWC for product sampling is 0.01 mg/cm²/day over 420 cm² and for bag filling it is 0.1 mg/cm²/day over 420 cm².

Occupational exposure limits

In 2004 the EU DG Employment Scientific Committee on Occupational Exposure Limits (SCOEL) recommended an Indicative Occupational Exposure Limit Value (IOELV) for BPA of 10 mg/m³ (8h – TWA). The value is expected to be included in a forthcoming IOELV Directive. The justification for the value is as it follows (SCOEL SUM 113, May 2004):

“In relation to establishing a recommended occupational exposure limit (OEL), SCOEL began by considering the available data relating to inhalation exposure. In rats exposed daily to airborne BPA for 13 weeks there was a clear NOAEL of 10 mg/m³, with mild olfactory epithelium inflammation at 50 and 150 mg/m³. There was no evidence of systemic toxicity in this study; if it is assumed that all of the inhaled BPA was retained and absorbed, exposure to 150 mg/m³, a level at which no systemic effects were observed, would equate to a body burden in the rat of about 34 mg/kg/day.

If one then considers the surrounding toxicological evidence, most of which arises from oral dosing studies in rodents, there are no findings that preclude the recommendation of a health-based occupational exposure limit. In long-term repeated oral dosing studies NOAELs of 74 mg/kg/day in rats and 80 mg/kg/day in dogs have emerged; in mice liver toxicity was seen at 120 mg/kg/day, the lowest dose level used. The use of these results to make predictions of dose-response characteristics for inhalation exposure, via route-to-route extrapolation, is hampered by the knowledge that following oral dosing there is extensive first-pass metabolism of BPA absorbed and transported directly to the liver. Nevertheless, the available data from oral dosing studies support the contention that no systemic toxicity arises in experimental animals with inhalation exposures of 10 mg/m³.

Dramatically contrasting results have been reported in different laboratories conducting standard and non-standard developmental toxicity studies in rats and mice. This has been an area of much dispute, centred on the alleged endocrine-modulating potency of BPA. Although a 2-generation study is being conducted in an attempt to clarify the situation, the judgement of SCOEL was to regard the 50 mg/kg/day NOAEL established in a standard multigeneration study in rats, as the most appropriate reference point for OEL considerations. Set against the analysis above, this suggests no concern for reproductive toxicity in experimental animals with exposures in the region of 10 mg/m³.

Returning to the repeated inhalation NOAEL of 10 mg/m³ in rats, with mild nasal olfactory epithelium inflammation arising at 50 mg/m³, and considering extrapolation of these findings to humans, one would expect that humans could be less sensitive than rats to this effect, based on what is understood of general differences in inhaled particle deposition between the two species. SCOEL thereby arrived at a conclusion that repeated inhalation exposure to 10 mg/m³ BPA (as inhalable dust) would pose no concern for local or systemic toxicity and therefore recommended an 8h TWA OEL at this level. In humans inhaling 10 m³ of air, if it assumed that all of the inhaled BPA would be retained and absorbed (a worst-case assumption), this would result in a body burden of just a little over 1 mg/kg/day.

There is no toxicological basis for recommending an additional specific short-term OEL; nor are “Sk” or “Sen” notations appropriate.”

4.1.1.2 Consumer exposure

As indicated in section 2.1 of the updated environment report, the EU usage of BPA is estimated to be approximately 1,149,870 tonnes/year. The largest quantities are used in the production of polycarbonates and epoxy resins, which have many applications in consumer goods, such as food contact containers, adhesives and protective coatings. A description of the major uses of BPA is given in section 2.2.2.

In these consumer applications, BPA is contained within or generated from a polymer matrix. Potential consumer exposure can therefore arise only under conditions where residual monomer in the polymer matrix becomes available for exposure or where breakdown of the polymer occurs, to generate additional monomer which is available for exposure. Under certain conditions, for example, at elevated temperature or extreme pH, hydrolysis of the polymer may occur, resulting in the regeneration of BPA from the polymer and thus increasing the amount of BPA which may be available for exposure. The products that are likely to have the potential for the highest exposure of consumers to BPA are those that are used in applications which involve direct contact with foodstuff. These include food and beverage containers which have epoxy resin internal coatings, and polycarbonate tableware and bottles, such as those used for infant formula milk. Exposure to BPA arising from use of these products is determined by the migration of BPA from the polymer into the food with which it is in contact, under the particular conditions of use. Migration of BPA from these products into food or beverages stored in them may occur if conditions are created which allow hydrolysis of the polymer during food or beverage storage or if there is residual monomer in the polymer. Consumption of the food or beverage will then result in ingestion of BPA. Inhalation and dermal exposure is considered to be negligible.

Other relatively minor sources of consumer exposure to BPA that are considered in this consumer exposure assessment arise from its use in dental fissure sealants and in epoxy-based surface coatings and adhesives. The use of BPA in dental fissure sealants will result in oral exposure. For epoxy-based surface coatings and adhesives, the main route of exposure is dermal.

Other uses of BPA, such as in printing inks and thermal paper, are considered to result in negligible potential for consumer exposure in comparison with the other sources considered and therefore will not be addressed further in this assessment.

4.1.1.2.1 Summary of original risk assessment report

BPA polycarbonate - food contact applications

There are many applications of BPA polycarbonates which involve direct contact with food. These include returnable beverage bottles, infant feeding bottles, tableware such as plates and mugs and food-storage containers. These main uses will be considered in this exposure assessment. A number of studies have been conducted which investigate the potential consumer exposure to BPA as a result of using these products. These studies have addressed the potential for exposure to residual BPA contained within the polycarbonate and have also explored the conditions which are necessary to initiate hydrolysis of the polymer to generate BPA which is then available for migration.

There is relatively limited good quality information on the levels of BPA in food and drink resulting from migration from polycarbonate tableware. A small number of studies have been

conducted to measure BPA concentrations in the contents of polycarbonate infant feeding bottles. Two of these studies have measured levels of up to about 50 ppb (50 µg/l; 0.05 mg/kg assuming a density of 1 g/ml) BPA in the food simulants contents of used bottles, in tests which represent realistic worst case exposure conditions. This value will be used as the basis for calculating consumer exposure for this scenario.

Using these values, estimates of daily ingestion of BPA can be calculated. Table 4.5 shows the estimates of daily ingestion for infants, arising from the use of polycarbonate feeding bottles. Estimates are derived for infants aged 1-2 months and 4-6 months. The estimates for daily intake of milk are taken from MAFF (1998).

Table 4.8 Estimates of infant ingestion of BPA from the use of polycarbonate feeding bottles (from the 2003 published RAR)

Age of baby	Daily intake of milk (l)	Concentration of BPA in milk (µg/l)	Daily ingestion of BPA (µg/day)
1 – 2 months	0.699	50	35
4 – 6 months	0.983	50	50

These values of 35 µg/day (0.035 mg/day) for a 1-2 month baby and 50 µg/day (0.05 mg/day) for a 4-6 month baby will be taken forward to the risk characterisation.

In relation to polycarbonate tableware and food storage containers, a number of well-reported studies have found no detectable levels of BPA in the food or drink contents of the tableware. Where detectable migration levels have been reported, the data derive from reports of limited detail and reliability and in studies in which food simulants have been used; migration into actual foodstuffs has not been detected. The highest reported level of BPA in food simulants detected as a result of migration from polycarbonate tableware is 5 ppb (5 µg/kg; 5×10^{-3} mg/kg). Although there is some uncertainty about the reliability of this value, it will be used as the basis of calculating consumer exposure for this scenario.

For exposure arising from the use of polycarbonate tableware, the most realistic scenario is considered to be that of a young child, for whom the total daily food and drink intake may be taken from polycarbonate tableware. The total daily intake of food and drink for a young child (1.5-4.5 years) is estimated to be 2 kg. This value is based on UK data for the consumption of solid and liquid food by young children, and represents the 97.5th percentile consumption (HMSO, 1995). Therefore, assuming that the concentration of BPA in the foodstuff is 5 µg/kg (5×10^{-3} mg/kg), total daily ingestion of BPA is 10 µg (0.01mg/day). This value will be taken forward to the risk characterisation.

BPA epoxy resins - food contact applications

BPA based epoxy resins are formulated with curing agents to yield high-performance crosslinked coatings. Heat cured epoxy coatings are, due to their favourable properties such as toughness, adhesion and chemical resistance, used as protective linings for metal sanitary cans to maintain the quality of canned food and beverages.

Epoxy resins with differing molecular weights have different applications. High molecular weight epoxy resins are used in heat cured protective interior coatings for food and beverage

containers; liquid and low molecular weight epoxy resins are typically used in ambient cured industrial protective coatings, adhesives, floorings or fillers. The majority of exterior coating applications are industrial and therefore negligible consumer exposure is expected. However, there are some consumer applications for these products and therefore these scenarios will be addressed in this exposure assessment.

BPA epoxy resins are used as binders in protective linings in food and beverage cans and in wine storage vats. Migration levels from epoxy coatings are governed by a variety of parameters such as coating composition, coating weight, curing conditions, sterilisation time and temperature and type of foodstuff. Carbonated soft drinks are the predominant type of beverage distributed in cans. These cans are typically filled at room temperature, and stored at or below room temperature. Canned foods are mostly sterilised at high temperatures, up to 135°C. The sterilisation time will vary, with shorter residence times for higher temperatures. Typically, sterilisation at 120°C is performed for 90 minutes. The canned foods are subsequently stored at room temperature.

Approximate coating weights for typical beverage cans are 250 mg/330 ml (1.06 mg/cm²) for a tinplate can and 125 mg/330 ml for an aluminium can; for food cans, coating weight may vary between 0.4 and 2.5 mg/cm² (Nehring Institute, 1998).

A number of studies which have investigated migration of BPA from epoxy resin coated cans and a single study of migration into wine vats, are available, and have been considered in this exposure assessment.

Three studies provide consistent evidence for migration of BPA from epoxy resin linings of food cans into the can contents. Two of these studied migration under conditions which represent the sterilisation process which would normally occur. Migration of BPA in these studies results in levels of up to about 70-90 ppb (70-90 µg/kg) in the can contents, from studies using fatty foods or simulants which mimic fatty foods. As migration is likely to be greatest into fatty foods, these results are considered to be representative of realistic worst case conditions. Rounding this up, a value of 100 ppb (100 µg/kg; 0.1 mg/kg) BPA in the contents of a typical food can will be used in the calculation of total daily ingestion of BPA in this scenario. This value will be taken forward to the risk characterisation.

A study into the migration of BPA from epoxy resins used as coating materials for wine vats (Larroque *et al.*, 1989) calculated that based on a level of BPA migration of 100 mg/kg resin, for a 1500 l vat lined with 10 kg resin, the amount of BPA in the wine will be 650 ppb (650 µg/l). No other information is available on this exposure scenario. Given the conditions of the single study available (newly applied resin, with extended contact time), it is likely that the level of migration and resultant estimated levels of BPA in the wine contents of the vat will be over-estimated, although it is not known to what extent. The value of 650 ppb (650 µg/l) wine will be taken forward to the risk characterisation as a very worst-case estimate for this scenario.

Table 4.9 provides the estimates of daily ingestion of BPA, as a result of food contact applications of epoxy-resins. Intake for adults is based on consumption of one bottle (0.75 l) of wine per day and consumption of all other food and drink from canned sources. Based on UK data, the estimate of total daily food and drink consumption for an adult is 4.5 kg (of which 2 kg is expected to be water); this represents the 97.5th percentile of consumption (HMSO, 1990). Of the 2.5 kg of food consumed daily, it is assumed that 1 kg is from canned

food (recommendation by the SCF). A combined adult intake for consumption of wine and all other food is also given.

Intake is also calculated for infants aged 6-12 months, for whom a high quantity of food may come from canned products. Intake is calculated for young children, in the age group 1.5-4.5 years. This age group has been chosen to represent the group with the highest potential food intake per kg bodyweight. In calculating BPA intake for infants, estimated intake of canned food is based on UK survey data, which indicated that the 97.5th percentile daily consumption of canned foods of the type which could contain a source of BPA, for this age group (including baby foods) is 0.375 kg (FSA, 2001; HMSO, 1992). In calculating intake for young children, there is no reliable information on canned food intake. The only information available is an estimated daily intake of food and drink, again based on 97.5 percentile values obtained from UK data (HMSO, 1995). Therefore, for the purposes of risk characterisation, a value of 2 kg for total intake is assumed. It should however be noted that as this intake includes drink and assumes that all food could come from sources resulting in BPA exposure, it will result in an overestimate of actual intake, although the degree of overestimation is unknown.

Table 4.9 Estimates of daily ingestion of BPA from food contact applications of epoxy-resins (from the 2003 published RAR)

Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of BPA in wine ($\mu\text{g/l}$) or food ($\mu\text{g/kg}$)	Daily ingestion of BPA ($\mu\text{g/day}$)
Wine	0.75	650	500
Canned food (infant 6-12 months)	0.375	100	40
Canned food (young child 1.5-4.5 years)	2	100	200
Canned food (adult)	1.0	100	100
Canned food + wine (adult)	0.75 l wine 1.0 kg food	650 $\mu\text{g/l}$ wine 100 $\mu\text{g/kg}$ food	600

The value of 500 $\mu\text{g/day}$ (0.5 mg/day) for ingestion of BPA resulting from consumption of wine will be carried forward to the risk characterisation.

The values of 100 $\mu\text{g/day}$ (0.1 mg/day) for an adult, 200 $\mu\text{g/day}$ (0.2 mg/day) for a young child and 40 $\mu\text{g/day}$ (0.04 mg/day) for an infant, for ingestion of BPA resulting from the consumption of canned food will be carried forward to the risk characterisation as a worst case scenario. In addition, a combined adult intake of 600 $\mu\text{g/day}$ (0.6 mg/day), for consumption of wine in addition to food, will be carried forward.

BPA epoxy resins - marine antifouling paints

Marine antifouling paints are used in the consumer sector for the protection and decoration of yachts and boats. The paints are applied by brush or roller. In the UK these paints are typically applied once per year. There are some measured data on consumer exposure arising from the brush application of these products.

Calculations of BPA exposure as a result of brush application of antifouling paints are based on a paint containing 40% epoxy-resin and a residual level of 10 ppm BPA in the resin. Exposure occurs via the inhalation and dermal routes. Although exposure to BPA vapour would be low in these applications (because of low vapour pressure), exposure to BPA in an aerosol is possible. Values of 3×10^{-4} μg (3×10^{-7} mg) for inhalation exposure and 29 μg (0.03 mg) for dermal exposure to BPA per event, resulting from brush application of paint without protective clothing will be taken forward to the risk characterisation.

BPA epoxy resins - wood varnish

There are no data on consumer exposure arising from the application of wood varnish. However, measured data are available for the professional application of wood preservatives. Given that the application methods are similar, these data are likely to be representative of the exposure arising from the application of wood varnish and therefore have been used to derive consumer exposure estimates for this scenario. As before, estimates of exposure to BPA are calculated on the basis of its content in the product; resin content is 40% w/w, with a residual level of BPA in the resin of 10 ppm. Exposure occurs via the inhalation and dermal routes. The amount of wood varnish used per event ranges from 1.0-8.5 litre, with a median value of 4 litre. Values of 0.02 μg (2×10^{-5} mg) for inhalation and 3.6 μg (0.0036 mg) for dermal exposure to BPA per event, for brush application without the use of protective clothing and gloves, will be carried forward to the risk characterisation.

BPA epoxy resins - wood fillers

BPA is present in some wood fillers sold for consumer use. Information provided by industry indicates that a typical product on the market contains approximately 20% of epoxy resin with a residual BPA content of 10 ppm. Exposure occurs to the hands only. A value of 9 μg (0.009 mg) BPA per event, resulting from the handling of wood filler without gloves, will be taken forward to the risk characterisation.

BPA epoxy resins - adhesives

Epoxy resin based adhesives are available to consumers. These adhesives are sold in '2-pack' systems. Potential dermal exposure to residual BPA in the epoxy resin can therefore arise from consumer use of these 2-pack products. In 2-pack adhesives, residual BPA content is less than 1 ppm. Based on a residual level of 1 ppm BPA in the adhesive, dermal exposure to BPA arising from the use of adhesives is calculated to be 0.014 mg per event.

Dental fissure sealant

BPA is a component of restorative materials such as fissure sealant, used in dentistry. It is not an active ingredient in any dental sealant or composite, but derivatives of BPA used in dentistry include bis-glycidyl dimethacrylate (bis-GMA) and BPA-dimethyl acrylate (bis-DMA). BPA may be present as an impurity in these substances, or may be formed as a result of degradation. It has been demonstrated that BPA can be released from sealants which contain bis-DMA but not those containing bis-GMA. Sealants consist of an organic resin matrix, whereas resin based composites (or fillings) consist of an organic resin matrix with an inorganic filler. According to information from the British Dental Association, filled composites would result in substantially less exposure than sealants, possibly because they contain proportionally less resin. Most sealants contain only bis-GMA.

Consumer exposure occurs during the polymerisation process following application of the resin. The resin matrix is initially present as a fluid monomer that is converted into a rigid polymer by a free radical initiated addition. Once applied to tooth cavities, composites and sealants are polymerised *in-situ*; the polymerisation reaction may be initiated chemically or by photo-initiation using UV or visible light. The degree of formation of oligomers into polymers varies depending on the composition of the resin and its distance from the tooth surface. Conversion of 60-75% is expected with most common composites. Lower levels of conversion may be associated with greater migration of free components from the composites.

A number of studies have been conducted looking at the release of BPA from commercially available dental sealants under a variety of exposure conditions. The information suggests that release of BPA is most likely only under conditions where degradation of the parent monomer (bis-DMA or bis-GMA) could occur. The data also suggest that degradation of bis-GMA does not occur and therefore only those sealants which contain bis-DMA are likely to release BPA.

Three studies have shown the release of BPA into the saliva of humans following placement of dental sealant. The results of these three studies provide somewhat different estimates of BPA concentration in saliva measured 1 hour post treatment (5.8 - 105.6 ppb, 3-31 ppm or 0.3-2.8 ppm). However, it appears possible that the higher estimates of BPA concentration in saliva may overestimate the actual concentrations which could be expected to arise following dental treatment, as a result of interference in the analytical method used to determine BPA.

Given the uncertainties surrounding the reliability of the higher estimates of BPA concentration in saliva, the concentration of BPA in saliva following dental treatment is considered more likely to be in the range 0.3-3 ppm. This concentration of saliva was measured at 1 hour post treatment. When saliva samples were analysed for BPA concentration at time points later than 1 hour post treatment, in two studies, no measurable levels were detected. This suggests that any exposure to BPA as a result of dental treatment will be an acute event.

4.1.1.2.2 Updated information

BPA polycarbonate – tableware and food storage containers

Since the finalisation of the RAR, the European Food Standard Authority (EFSA) has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the migration of BPA into foods and food simulants have been evaluated.

Recent studies suggest that migration of BPA from polycarbonate tableware and food storage containers may increase when receptacles are used for heating or cooking foods, for example in the case of microwave heating (Nerin *et al.*, 2003). However, quantitative data to estimate BPA migration under these conditions are not available. Therefore, the value of 5 µg/kg already identified in the original RAR is still valid. Based on a daily adult consumption of 3 kg of food or beverages, a potential dietary exposure of 15 µg/day (0.25 µg/kg bw/day; bw = 60 kg) from polycarbonate tableware and food storage containers can be derived for an adult. For a young child (1.5 – 4.5 years), based on a daily consumption of 2 kg of food or

beverages, a potential dietary exposure of 10 µg/day (0.9 µg/kg bw/day; bw = 11 kg) from polycarbonate tableware and food storage containers can be derived.

BPA polycarbonate – infant feeding bottles

Since the finalisation of the RAR, the European Food Standard Authority (EFSA) has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the migration of BPA from polycarbonate infant feeding bottles have been evaluated.

In a recent study by Wong et al. (2005), 30 new commercial plastic baby milk bottles available on the market in Singapore were cut into pieces and tested for migration into 10% ethanol at 70°C or corn oil at 100°C. After 240 h incubation, BPA migration into 10% ethanol was detected in 21 samples whereas BPA migration into corn oil was detected in 12 samples. BPA concentration values calculated from this study are much higher than those based on other migration studies but the test conditions were so far removed from any normal conditions of use of baby bottles that they were not considered by EFSA to assess potential exposure to BPA.

Brede *et al.* (2003) subjected polycarbonate baby feeding bottles to repeated washing/boiling/brushing. When 12 different bottles from the Norwegian market were tested by filling them with hot water (100°C) for 1 hour, the mean BPA level from new bottles was 0.23 µg/l (range of 0.11 to 0.43 µg/l) while the mean level from bottles subjected to simulated repeated use was 8.4 µg/l (ranging from 3.7 to 17 µg/l) after 51 dishwasher cycles and 6.7 µg/l (ranging from 2.5 to 15 µg/l) after 169 dishwasher cycles. While all 12 bottles released higher levels of BPA after 51 cycles compared to new, there was no trend between 51 and 169 cycles. Migration from 5 of the bottles remained the same, it decreased significantly for 5 bottles, and it increased significantly for 2 bottles. The authors commented that the effects seen could be due to depolymerisation of the polycarbonate.

In another study by Tan and Mustafa (2003), BPA leaching was measured in 30 new baby feeding bottles collected on the Malaysian market and in 100 baby feeding bottles used for more than 3 months collected from Malaysian families. The authors noted that most of the bottles collected in families were used or passed from one child to the next as long as they were not cracked or rendered useless. Mean BPA leaching from the new bottles filled with water was 0.18 ng/cm² (ranging from non detectable to 1.34 ng/cm²) at 80°C. Mean BPA leaching from the used bottles filled with water was 3.37 ng/cm² (ranging from 0.11 to 25.51 ng/cm²) at 80°C. EFSA noted that based on these data and considering a contact area of 172 cm² (for a standard feeding bottle filled to the 200 ml mark), BPA leaching would range from non-detectable to 1.15 µg/l in new bottles and from 0.1 to 21.9 µg/l in used bottles.

In a study performed in the UK (CSL, 2004), migration from samples of two different brands of polycarbonate feeding bottles was measured. The migration tests were performed on the virgin bottle (after sterilization), and after 20 and 50 cycles of dishwashing and bottle-brushing. One hour contact at 70°C was applied with 10% ethanol to simulate infant formulae and 3% acetic acid to simulate acidic fruit juice. As prescribed by Directive 93/8/EEC for repeated use articles, three successive contacts were performed. No BPA migration was detected in the new bottles. Migration was observed at the first contact with concentrations varying from non-detectable (<1.1 µg/l) to 4.5 µg/l in 10% ethanol and from non-detectable (<0.3 µg/l) to 0.7 µg/l in acetic acid for washed bottles. The dishwasher reagents (detergent, rinse aid and salt) were

tested and found not to be the source of the elevated migration levels. In all but one sample, migration was not observed at the second and third contact.

To summarise the above reported data, EFSA noted that BPA migrates from polycarbonate feeding bottles and that migration can increase with repeated use of the bottle due to the cleaning treatments (dishwashing, sterilization, brushing, etc). In recent years, the decrease in the limit of detection has allowed determination of BPA leaching that was not detected previously. The migration levels observed vary according to studies in relation to varying experimental conditions (temperature, time of contact, migrant). The degree of leaching may vary according to the bottle brand (due to varying manufacturing process and raw material); new studies made available recently suggest that it may also vary according to the age of the bottle, probably in relation to the number and type of cleaning treatment performed at household level. Infants are likely to be fed everyday using bottles of the same brand cleaned in the same way. For this reason an estimate of average BPA migration values would not capture the exposure of infants who are fed every day with bottles leaching more BPA than the average. EFSA noted that migration testing by 3 successive contacts, as prescribed by Directive 93/8/EEC, did not enable the identification of an increased release of BPA whereas cycles of dishwashing and, even more, domestic use for 3 months or more did.

EFSA noted that according to the two migration studies conducted since 2003 under conditions mimicking realistic conditions of use, levels of BPA migration in used polycarbonate bottles were respectively up to 22 µg/l (Tan & Mustafa, 2003) and up to 14 µg/l (Brede, 2003). These upper values were lower than the upper value of 50 µg/l identified in the EU RAR. Although based on a limited number of bottles, the studies used in the RAR mimicked realistic conditions of treatment of commercially available used bottles and were of sufficient quality from an analytical point of view. The concentration value of 50 µg/l of infant formulae in used bottles was therefore used by EFSA as the basis to calculate a conservative estimate of exposure in infants.

Therefore, despite this new data, the original estimates from the RAR of 35 µg/day (8 µg/kg bw/day; bw = 4.5 kg) for a 1-2 month baby and 50 µg/day (7 µg/kg bw/day; bw = 7 kg) for a 4-6 month baby for infant ingestion of BPA from the use of polycarbonate feeding bottles, are still valid.

EFSA also considered a scenario in which powdered infant formulae may be packed in food cans with epoxy-phenolic resins used as internal surface. Kuo and Ding (2004) determined the content of BPA in 6 brands of canned powdered infant formulae and follow up formulae available on the market in Taiwan. BPA was detected in all samples at concentrations ranging from 45 to 113 µg/kg. Based on a reconstitution ratio of 135 g/l of liquid formula and on a concentration value of 100 µg BPA/kg, the above mentioned 1-2 and 4-6 month infant consuming 155 and 140 ml/kg bw/day of reconstituted infant formulae respectively would consume 21 and 19 g/kg bw of powdered infant formulae, leading to a potential exposure of up to 2.1 and 1.9 µg BPA/kg bw/day. EFSA noted that this potential source of exposure is quantified on the basis of a very limited number of samples from a non-EU market. These figures will not be used in the risk characterisation as in the EU infant formulae are not packed in food cans.

In the case of breastfed infants, BPA in human milk occurs as a consequence of exposure of the mother through oral and dermal routes. In a study by Sun et al. (2004), twenty-three human milk samples of healthy lactating women living in Japan were analysed for BPA. BPA was

detected in all samples (limit of detection 0.11 µg/l) with values in the range from 0.28 to 0.97 µg/l. The mean value was 0.61 µg/l. Considering the consumption of 174 ml/kg bw of human milk per day in infants exclusively breastfed, EFSA calculated a potential dietary exposure of 0.1 µg/kg bw/day at the mean and 0.2 µg/kg bw/day at the highest BPA concentration observed. EFSA noted that this estimate is based on a limited number of samples of human milk collected in Japan and may not be representative of the EU situation.

BPA epoxy resins - food contact applications

Since the finalisation of the RAR, the European Food Standard Authority (EFSA) has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the migration of BPA into foods and food simulants have been evaluated (see Table 4.10).

Table 4.10 BPA concentrations in canned commercial products according to recent published studies (from EFSA, 2006)

Reference/Country	Type of product	LOD/LOQ (µg/kg)	Number of products analysed	Percent samples above LOQ	Percent samples above 100 µg/kg	Minimum (µg/kg)	Maximum (µg/kg)	Average concentration of values above LOQ (µg/kg) ⁽¹⁾
FSA (2000)/UK	Beverages	2/7	11	0%	0%	<2	<7	-
	Foods	2/7	46 ⁽²⁾	78%	0%	7	70	23
Goodson et al (2004)/UK	Foods	2 ⁽⁴⁾	10 ⁽³⁾	100%	0%	9	91	40
Braunrath et al (2005)/Austria	Beverages	0.1 – 0.9 ⁽⁴⁾	7	86%	0%	0.1	3.4	1.1
	Vegetables	1.1 – 7.4 ⁽⁴⁾	6	100%	0%	8.5	35	23.9
	Fruits	1.2 – 5.4 ⁽⁴⁾	4	100%	0%	5	24	10.5
	Fat-containing products	0.2 – 9.3 ⁽⁴⁾	9	100%	0%	4.8	17.6	10.7
Horie et al (1999)/Japan	Beverages	<1/1	80	n.a.	n.a.	<1	212	18
Imanaka et al (2001) ⁽⁶⁾ /Japan	Meat	<1/1	8	100%	n.a.	17	602	n.r.
	Vegetables	<1/1	14	100%	n.a.	2	25	n.r.
Yoshida et al (2001)/Japan	Foods	10 ⁽⁷⁾	12	50%	0%	<10	95	44
Kang & Kondo (2003)/Japan	Dairy products	1 ⁽⁴⁾	3	100%	0%	21	43	31
Thomson & Grounds (2005)/New Zealand	Food	10-20 ⁽⁷⁾	79	32%	2%	<10	191	34
	Beverages	10 ⁽⁷⁾	4	0%	0%			-

n.r. not reported ; n.a. not available

(1) Calculated by EFSAI.

(2) Two high values from a meat product were excluded since they related to a technology no longer in use in the EU (Association of Plastics Manufacturers in Europe, 2006a).

(3) In this study, ten retail food cans were analysed for BPA before studying the effect of heating and storing.

(4) LOD only.

(5) Average of all samples, including undetectable levels which were given a value of zero.

(6) Abstract only available.

(7) LOQ only.

According to EFSA (2006), in both food and beverages, concentrations of BPA above 100 µg/kg were rarely observed. Three surveys performed on the EU market covered a limited number of products: 18 beverages with concentrations in the range of non-detectable to 3 µg BPA/kg and 65 solid foods with concentrations in the range of 5 to 91 µg BPA/kg. BPA concentrations above 100 µg/kg were not observed in these surveys. In other surveys, the highest observed concentration in canned beverages was 212 µg/kg in a coffee sold in Japan (Horie *et al.*, 1999). The highest observed concentrations of BPA in canned foods were

reported in Japan for meat products: up to 602 µg BPA/kg in corned beef and 212 µg BPA/kg in chicken (Imanaka *et al.*, 2001). In New Zealand, individual samples of tuna, corned beef and coconut cream reached up to 191 µg BPA/kg (Thomson & Grounds, 2005).

A number of migration studies of BPA from epoxy resin linings into the can contents have also been performed. BPA migration from cans of beverages was assessed by Kang and Kondo (2002) in Japan. BPA migration into water, decaffeinated and non-decaffeinated coffee averaged 14 µg BPA/l (range 9 to 31 µg /l), 66 µg BPA/l (range 33 to 107 µg/l) and 84 µg BPA/l (range 50 to 134 µg/l), respectively.

BPA migration into water was assessed for 9 different food cans differing in shape, size and material, used for packaging of fruits and juice in Japan (Takao *et al.*, 2002). All cans were filled with bottled spring water and sealed with a seamer. Cans were either not heated or heat-treated for 30 minutes. Low levels of BPA migration (less than 2 µg/l) were found in all unheated cans. When the heat treatment was performed, the migration was up to 5 µg BPA/l at 80°C and up to 30 µg BPA/l at 100°C.

In a study by Goodson *et al.* (2004), experiments were conducted to investigate the effects of different storage conditions and damage (experimentally produced denting) to cans on the migration of BPA into foods by filling epoxy-phenolic coated cans with four foods (soup, minced beef, evaporated milk and carrots) and one food simulant (10% ethanol). Filled cans of each food type or simulant were then sealed and processed before storage at three different temperatures. It was found that 80–100% of the total BPA present in the coating had migrated to foods or simulant directly after heat processing. The level was not changed during extended storage (up to 9 months) or in damaged cans or if canned foods were then heated in the can to make ready to eat. This indicates that most migration occurs during the can retorting step.

EFSA noted that migration values vary according to a number of factors (heating time, temperature, food or simulant). Each consumer is likely to consume a variety of canned foods and beverages that will not always have the same BPA concentrations. On this basis, single high migration values observed were not considered in the assessment of chronic dietary exposure.

EFSA considered whether to use a migration value of 100 µg BPA/kg both for canned solid food and for canned beverages, but concluded that this would not be representative of beverages and would provide an overly conservative assessment of chronic dietary exposure for adults and children with a varied diet. EFSA noted that in the 3 surveys conducted in the EU in canned commercial products, BPA in canned beverages was always less than 7 µg BPA/kg and that the average concentration of BPA in solid foods in which it was quantified was up to 40 µg BPA/kg. Although limited, these data were used to develop an exposure scenario considering 10 µg BPA/kg as the value for canned beverages and 50 µg BPA/kg as the value for canned solid foods. This scenario was used by EFSA to provide a conservative assessment of exposure to BPA through canned products in adults and children consuming a variety of products. These estimates, which are slightly lower than that (100 µg/kg) identified in the original RAR, will be used to calculate revised adult dietary intakes of BPA from canned food and beverages to be taken forward to the risk characterisation.

A different scenario was considered for infants since they tend to consume a limited number of commercial products and may be more likely to consume the same products which may

have a high BPA concentration. Thus, for infants aged 0-6 months and 6-12 months, a value of 100 µg BPA/kg canned foods and beverages was used. This value is the same as that identified in the original RAR.

BPA was recently determined in samples of wine available on the Austrian market and sourced from vats (steel, wood and plastic), glass bottles and carton packages (Brenn-Struckhofova & Cichna-Markl, 2006). Reported storage time varied from 0.25 to 11 months. In 13 of the 59 wine samples, the BPA concentration was below the LOQ of 0.2 µg/l. Mean BPA concentrations for all wine samples above the LOQ was 0.58 µg/l. In seven samples, BPA levels ranged from 0.2 to 0.5 µg/l. Only in one sample (stored 10.5 months in a steel vat) was a significantly higher BPA level of 2.1 µg/l found. The mean and median for all wine samples with BPA concentrations above the LOQ were 0.58 and 0.40 µg/l respectively. These values are far lower than previously published BPA levels (650 µg/l) derived from migration experiments using wine simulants (Larroque et al., 1989 – see original RAR). EFSA noted that even though this survey is limited and further information on the possible deterioration of epoxy resins in wine vats used for many years would be desirable, potential residues of BPA in wine appear to be in the same range as those found in canned beverages (within 10 µg/l). Therefore, rather than the very conservative estimate of 650 µg/l, the more realistic value of 10 µg/l will be used to calculate the adult dietary intake of BPA from wine to take forward to the risk characterisation.

The Association of Plastics Manufacturers in Europe (2006) confirmed that epoxy resins, in which BPA is used as an accelerator in amine-based hardeners, may be used for tanks holding alcoholic beverages and that these are multiple-use applications with containers continuously filled, emptied and refilled over long time periods in use – sometimes many years. On the other hand the Association stated that these are relatively minor applications for such products and that surface-to-volume ratios are extremely small.

Table 4.8 provides the estimates of daily ingestion of BPA, as a result of food contact applications of epoxy-resins. Intake for adults is based on consumption of one bottle (0.75 l) of wine per day and consumption of 1 kg of canned food and 2 litres of canned beverages, including wine (recommendation by EFSA). A combined adult intake for consumption of canned beverages and canned food is also given.

Intake is also calculated for infants aged 6-12 months, for whom a high quantity of food may come from canned products. Intake is calculated for young children, in the age group 1.5-4.5 years. This age group has been chosen to represent the group with the highest potential food intake per kg bodyweight. In calculating BPA intake for infants, estimated intake of canned food is based on UK survey data, which indicated that the 97.5th percentile daily consumption of canned foods of the type which could contain a source of BPA, for this age group (including baby foods) is 0.375 kg (FSA, 2001; HMSO, 1992). In calculating intake for young children, there is no reliable information on canned food intake. The only information available is an estimated daily intake of food and drink, again based on 97.5 percentile values obtained from UK data (HMSO, 1995). Therefore, for the purposes of risk characterisation, a value of 2 kg for total intake is assumed.

Table 4.11 Estimates of daily ingestion of BPA from food contact applications of epoxy-resins.

Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of BPA in wine (µg/l) or food (µg/kg)	Daily ingestion of BPA (µg/day)
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Source of exposure	Daily intake of wine (l) or canned food (kg)	Concentration of BPA in wine ($\mu\text{g/l}$) or food ($\mu\text{g/kg}$)	Daily ingestion of BPA ($\mu\text{g/day}$)
Wine	0.75	10	7.5
Canned beverages (adult)	2	10	20
Canned food (adult)	1.0	50	50
Canned food + beverages (adult)	1 kg food 2 l beverages (including wine)	50 $\mu\text{g/kg}$ food 10 $\mu\text{g/l}$ beverages (including wine)	70
Canned food and beverages (infant 6-12 months)	0.375	100	37.5
Canned food and beverages (young child 1.5-4.5 years)	2	50	100

For an adult the worst-case dietary intake of 70 $\mu\text{g/day}$ BPA (1.25 $\mu\text{g/kg}$ bw/day; bw = 60 kg) resulting from consumption of canned food and canned beverages (including wine) will be carried forward to the risk characterisation. For a 6-12 month infant and for a young child, dietary intakes of 37.5 and 100 $\mu\text{g/day}$ BPA (4.3 $\mu\text{g/kg}$ bw/day; bw = 8.7 kg and 9 $\mu\text{g/kg}$ bw/day; bw = 11 kg) resulting from consumption of canned food and canned beverages will be carried forward to the risk characterisation.

Another potential source of BPA exposure is migration from polycarbonate tableware and storage containers. Potential dietary exposures of 15 $\mu\text{g/day}$ (0.25 $\mu\text{g/kg}$ bw/day; bw = 60 kg) and 10 $\mu\text{g/day}$ (0.9 $\mu\text{g/kg}$ bw/day; bw = 11 kg) were derived for an adult and a young child (1.5 – 4.5 years) respectively for this scenario.

Therefore, the overall potential dietary exposure to BPA in the adult population would be 1.50 (1.25 + 0.25) $\mu\text{g/kg}$ bw/day and in young children 9.9 (9 + 0.9) $\mu\text{g/kg}$ bw/day.

BPA epoxy resins - marine antifouling paints

There is no significant new information on this consumer use.

BPA epoxy resins - wood varnish

There is no significant new information on this consumer use.

BPA epoxy resins - wood fillers

There is no significant new information on this consumer use.

BPA epoxy resins – adhesives

There is no significant new information on this consumer use.

Dental fissure sealant

There is no significant new information on this consumer use.

4.1.1.2.3 Impact of new information

New information on levels of BPA in polycarbonate tableware and food storage containers, polycarbonate infant feeding bottles and food contact applications of epoxy resins has led to slight changes in the exposure estimates taken forward to the risk characterisation. These are summarised in the table below and compared to the values of the original RAR.

Table 4.12 Exposure estimates taken forward to the risk characterisation for the different oral consumer scenarios

Source of exposure	Daily ingestion of BPA (mg/day)	Estimated body burden (mg/kg/day)	Daily ingestion of BPA (mg/day) from original RAR	Estimated body burden (mg/kg/day) from original RAR
Infant feeding bottles (1-2 month baby)	0.035	0.008	0.035	0.008
Infant feeding bottles (4-6 month baby)	0.050	0.007	0.050	0.007
Canned food and beverages (infant 6-12 months)	0.0375	0.0043	0.040	0.0046
Canned food and beverages (young child 1.5-4.5 years)	0.100	0.009	0.200	0.018
Canned food (adult)	0.050	8×10^{-4}	0.100	1.6×10^{-3}
Canned beverages (adult)	0.020	3×10^{-4}	n.a.	n.a.
Wine (adult)	0.010	1.7×10^{-4}	0.500	8.3×10^{-3}
Canned food and beverages including wine (adult)	0.070	0.00125	0.600	0.01
Polycarbonate tableware (young child, 1.5-4.5 years)	0.010	9×10^{-4}	0.010	9×10^{-4}
Polycarbonate tableware (adult)	0.015	2.5×10^{-4}	n.a.	n.a.
Canned food and beverages + polycarbonate tableware (young child, 1.5-4.5 years)	0.110	0.01	0.210	0.019
Canned food and beverages + polycarbonate tableware (adult)	0.085	0.00150	0.600	0.01

n.a. not available

4.1.1.3 Humans exposed via the environment

4.1.1.3.1 Summary of original risk assessment report

Table 3.9 from the environment section of R325_0207_env_hh has been repeated here (Table 4.13) and gives the predicted environmental exposures to BPA and the daily human doses arising from releases from production, processing and manufacture of BPA, epoxy resins, PVC and thermal paper, and for releases at the regional level.

It can be seen that the daily human intake via the environment based upon typical human consumption and inhalation rates at the regional level is 1.78×10^{-5} mg/kg/day and the highest local exposure (use as an inhibitor in PVC production) is 0.059 mg/kg/day. These two figures were taken forward to the risk characterisation.

Table 4.13 Concentrations for indirect exposure of humans via the environment (from the 2003 published RAR)

	Concentration in drinking water (mg/l)	Concentration in wet fish (mg/kg)	Concentration in plant roots (mg/kg)	Concentration in plant leaves (mg/kg)	Concentration in milk (mg/kg wet weight)	Concentration in meat (mg/kg wet weight)	Concentration in air (mg/m ³)	Total daily intake (mg/kg day)
Site specific								
BPA production	3.93×10 ⁻⁴	0.027	1.49×10 ⁻³	1.96	2.64×10 ⁻³	8.35×10 ⁻³	3.61×10 ⁻⁴	0.0338
Epoxy resin production	0.012	0.074	0.3	0.065	4.85×10 ⁻⁵	1.53×10 ⁻⁴	2.08×10 ⁻¹⁰	3.22×10 ⁻³
Thermal paper production	1.88×10 ⁻³	0.127	1.4×10 ⁻⁴	3.14×10 ⁻⁵	2.11×10 ⁻⁶	6.67×10 ⁻⁶	2.08×10 ⁻¹⁰	2.65×10 ⁻⁴
Generic scenarios								
Phenoplast cast resin processing	1.29×10 ⁻³	0.0875	0.013	2.81×10 ⁻³	2.98×10 ⁻⁶	9.42×10 ⁻⁶	2.08×10 ⁻¹⁰	3×10 ⁻⁴
Thermal paper recycling	0.187	12.6	2.03	0.441	4.45×10 ⁻⁴	1.41×10 ⁻³	2.08×10 ⁻¹⁰	0.0448
PVC – Inhibitor during production process	0.227	15.4	2.97	0.643	6.01×10 ⁻⁴	1.9×10 ⁻³	2.08×10 ⁻¹⁰	0.0591
PVC – Anti-oxidant during processing	2.19×10 ⁻⁴	0.0148	1.51×10 ⁻³	3.28×10 ⁻⁴	4.45×10 ⁻⁷	1.41×10 ⁻⁶	2.08×10 ⁻¹⁰	4.46×10 ⁻⁵
PVC – Preparation of additive packages	8.8×10 ⁻³	0.595	0.114	0.0246	2.3×10 ⁻⁵	7.3×10 ⁻⁵	2.08×10 ⁻¹⁰	2.27×10 ⁻³
PVC – Anti-oxidant in plasticiser production	0.0014	0.0964	0.0173	0.00374	3.63×10 ⁻⁶	1.15×10 ⁻⁵	2.08×10 ⁻¹⁰	3.58×10 ⁻⁴
PVC – Plasticiser use	1.88×10 ⁻⁴	0.0127	1.1×10 ⁻³	2.39×10 ⁻⁴	3.62×10 ⁻⁷	1.15×10 ⁻⁶	2.08×10 ⁻¹⁰	3.64×10 ⁻⁵
Regional	1.14×10 ⁻⁴	7.74×10 ⁻³	1.96×10 ⁻⁴	4.37×10 ⁻⁵	1.85×10 ⁻⁷	5.86×10 ⁻⁷	2.08×10 ⁻¹⁰	1.78×10 ⁻⁵

4.1.1.3.2 Updated information

Overall environmental exposure to BPA was recently assessed in Japan (Miyamoto and Kotake, 2006). Exposure levels from different possible sources (atmosphere, water, food, tableware, toys, etc.) were estimated and aggregated in different age classes. Children aged 1–6 years had the highest average estimated level of exposure (1.2 µg/kg bw/day) due to relatively high dietary consumption per unit body weight and the use of polycarbonate tableware for this age class. Daily BPA exposure was also estimated, based on 24 h urines collected in 58 adult subjects. The 95% confidence intervals for average daily exposure were estimated to be 0.028–0.049 µg/kg/day for adult males and 0.034–0.059 µg/kg bw/day for adult females. The 95% confidence intervals for high-exposure (95th percentile) were estimated to be 0.037–0.064 µg/kg bw/day for adult males and 0.043–0.075 µg/kg/day for adult females. EFSA (2006) considered these data but since no details were available in relation to the characteristics of the diet of subjects from which urines were collected, EFSA agreed that the relevance of this information to the EU situation remained uncertain. The authors of the survey pointed out that in Japan, in recent years, industries voluntarily reduced the amount of BPA used as an additive in the production of PVC, thermal paper manufacturers voluntarily substituted BPA used as a developing agent and polycarbonate tableware and polycarbonate feeding bottles were substituted with non-polycarbonate articles (with less than 6% of feeding bottles currently being made with polycarbonate).

Because of changes in the environmental emissions of BPA (see updated environmental RAR; draft of May 2007; R325_0705_env), the predicted daily human doses for the local and regional scenarios have been subject to revision. For the production of PVC additive packages containing BPA, section 3.1.2.2 of R325_0705_env indicates that industry has carried out two sampling exercises at sites operating this process. As data are now available for all sites, specific rather than generic calculations have been carried out in estimating human exposure for this local scenario. For washing of polycarbonate bottles, figures for human exposure via the environment have now been added. As indicated in section 3.1.2.1 of R325_0705_env, the use of BPA as an inhibitor in PVC production ceased voluntarily in the EU in 2003. Therefore, as there are no longer any emissions from this application, it is not considered further.

Revised figures of human exposure via the environment (see table 4.14) have all been calculated with EUSES by using typical human consumption and inhalation rates. It can be seen that the daily human intake via the environment at the regional level is 9.1×10^{-6} [it was 1.78×10^{-5} in the original RAR] mg/kg/day and the highest local exposure (BPA production) is 0.041 [it was 0.06 for PVC production in the original RAR] mg/kg/day. These two figures will be taken forward to the risk characterisation.

It should be pointed out that although there is a large amount of measured data of BPA concentrations in surface water (see section 3.1.4.5.3 of R325_0705_env) and some data on measured levels in sewage sludge (see section 3.1.4.6.3 of R325_0705_env), these cannot be related to any of the local scenarios. Therefore the risk assessment will be based on the calculated values. The measured levels in surface water show that BPA is present at concentrations below 1 µg/l. Comparing the measured and calculated surface water levels, these are in quite good agreement in general - certainly the higher end of measured levels are of a similar order to the calculated values. It should be noted however that the drinking water concentrations included in Table 4.11 assume no removal of BPA from surface water in the production of drinking water (the default assumption based on BPA's properties). There are a small number of studies which have measured BPA in water before and after treatment to

produce drinking water, and these show a significant reduction in concentration. They also show lower levels of BPA in drinking water than those calculated. The levels at a treatment plant in Spain were all below the quantification limit of 6.5 ng/l (Rodriguez-Mozaz et al., 2004). Wenzel et al. (2003) summarised levels in drinking water in Europe; from three studies these were 0.5 – 2.0 ng/l at one site and <8.8 or <11 ng/l at two others. Although these cannot be related to specific scenarios, they indicate that the actual concentrations in drinking water are likely to be much lower than those calculated in this assessment.

Table 4.14 Concentrations for indirect exposure of humans via the environment estimated using EUSES

	Concentration in drinking water (mg/l)	Concentration in wet fish (mg/kg)	Concentration in plant roots (mg/kg)	Concentration in plant leaves (mg/kg)	Concentration in milk (mg/kg wet weight)	Concentration in meat (mg/kg wet weight)	Concentration in air (mg/m ³)	Total daily intake (mg/kg day)
Site-specific								
BPA production	7.1x10 ⁻⁵	2.2x10 ⁻³	1.8x10 ⁻³	2.4	3.2x10 ⁻³	0.01	4.4x10 ⁻⁴	0.041
Epoxy resin production	8.2x10 ⁻⁴	0.056	8.7x10 ⁻³	1.9x10 ⁻³	2.0x10 ⁻⁶	6.2x10 ⁻⁶	1.6x10 ⁻¹⁰	1.9x10 ⁻⁴
Thermal paper production	8.0x10 ⁻⁴	0.055	1.4x10 ⁻⁴	3.1x10 ⁻⁵	9.2x10 ⁻⁷	2.9x10 ⁻⁶	1.6x10 ⁻¹⁰	1.1x10 ⁻⁴
PVC – preparation of additive packages	2.3x10 ⁻⁴	0.016	2.3x10 ⁻³	5.1x10 ⁻⁴	5.6x10 ⁻⁷	1.8x10 ⁻⁶	1.6x10 ⁻¹⁰	5.4x10 ⁻⁵
Generic scenarios								
Phenoplast cast resin processing	1.0x10 ⁻³	0.069	0.013	2.8x10 ⁻³	2.7x10 ⁻⁶	8.5x10 ⁻⁶	1.6x10 ⁻¹⁰	2.6x10 ⁻⁴
Polycarbonate bottle washing	3.4x10 ⁻⁵	2.2x10 ⁻³	1.4x10 ⁻⁴	3.1x10 ⁻⁵	7.8x10 ⁻⁸	2.5x10 ⁻⁷	1.6x10 ⁻¹⁰	5.8x10 ⁻⁶
PVC – Anti-oxidant during processing	1.4x10 ⁻⁴	9.3x10 ⁻³	1.5x10 ⁻⁴	3.3x10 ⁻⁴	3.5x10 ⁻⁷	1.1x10 ⁻⁷	1.6x10 ⁻¹⁰	3.3x10 ⁻⁵
PVC – Anti-oxidant in plasticiser production	2.8x10 ⁻⁴	0.019	3.4x10 ⁻³	7.3x10 ⁻⁴	7.3x10 ⁻⁷	2.3x10 ⁻⁶	1.6x10 ⁻¹⁰	7.0x10 ⁻⁵
PVC – Plasticiser use	1.1x10 ⁻⁴	7.2x10 ⁻³	1.1x10 ⁻³	2.4x10 ⁻⁴	2.7x10 ⁻⁷	8.6x10 ⁻⁷	1.6x10 ⁻¹⁰	2.5x10 ⁻⁵
Thermal paper recycling – deinking	b: 3.8x10 ⁻⁵ p: 0.016 c: 0.014	2.2x10 ⁻³ 2.2x10 ⁻³ 2.2x10 ⁻³	9.7x10 ⁻⁴ 0.41 0.35	2.1x10 ⁻⁴ 0.089 0.075	1.8x10 ⁻⁷ 6.6x10 ⁻⁵ 5.6x10 ⁻⁵	5.8x10 ⁻⁷ 2.1x10 ⁻⁴ 1.8x10 ⁻⁷	1.6x10 ⁻¹⁰ 1.6x10 ⁻¹⁰ 1.6x10 ⁻¹⁰	1.4x10 ⁻⁵ 4.2x10 ⁻³ 3.6x10 ⁻³
Thermal paper recycling – no deinking	b: 4.8x10 ⁻⁵ p: 9.0x10 ⁻⁴ c: 7.7x10 ⁻⁴	2.3x10 ⁻³ 2.3x10 ⁻³ 2.3x10 ⁻³	1.2x10 ⁻³ 0.029 0.019	2.6x10 ⁻⁴ 4.9x10 ⁻³ 4.2x10 ⁻³	2.2x10 ⁻⁷ 3.7x10 ⁻⁶ 3.2x10 ⁻⁶	7.0x10 ⁻⁷ 1.2x10 ⁻⁵ 1.0x10 ⁻⁵	1.6x10 ⁻¹⁰ 1.6x10 ⁻¹⁰ 1.6x10 ⁻¹⁰	1.6x10 ⁻⁵ 2.4x10 ⁻⁴ 2.0x10 ⁻⁴

Regional	3.2×10^{-5}	2.2×10^{-3}	4.9×10^{-4}	1.1×10^{-4}	1.8×10^{-7}	5.8×10^{-7}	1.6×10^{-10}	9.1×10^{-6}
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Notes for thermal paper recycling: b = biological sludge only; p = paper sludge only; c = combined sludges

Levels of BPA in human blood and excretion of BPA and BPA-metabolites in unintentionally exposed humans

Since the finalisation of the RAR, EFSA has issued a further opinion of BPA (EFSA, 2006). In this opinion, the more recent studies on the concentration of BPA in human fluids have been evaluated (see Tables 4.15 and 4.16).

A number of methods to quantitate low concentrations of BPA in biological samples have been developed. These methods were applied to determine BPA concentrations in blood or urine samples from human subjects without intentional exposures to BPA. The analytical methods applied include ELISAs, single trace chromatographic separations such as HPLC with fluorescence detection (both with and without fluorophore derivatisation), and HPLC with electrochemical detection. Recently, results from studies using more specific methods for BPA-quantitation based on mass spectrometry using both single and triple quadrupol instruments were published.

Moreover, the studies used widely different sample workup procedures. These included simple dilution of aqueous samples with polar organic solvents, extraction of BPA into ethyl acetate or ether, and solid phase extractions. Some studies included treatment with glucuronidase and/or sulphatase to cleave the expected major metabolites of BPA, or applied specific methods to quantitate BPA-glucuronide. The results of the many studies available for evaluation are summarised in Tables 4.9 and 4.10.

The studies on BPA blood levels in humans without intentional exposure to BPA report concentrations of up to 10 µg/l blood (Fung et al., 2000; Fukata et

Table 4.15. Reported plasma or blood concentrations of bisphenol A in human subjects without known specific exposures to BPA (from EFSA, 2006)

Reference and sampling region	Analytical method, sample workup	No. of samples analyzed	Concentration ranges reported	Comments
(Yamada <i>et al.</i> 2002) Japan	ELISA (EcoAssay Bisphenol A kit from Otsuka pharmaceuticals, Tokyo, Japan), solid phase extraction,	248 samples of maternal serum and amniotic fluid	0.64 to 6.63 µg BPA/l in maternal serum (90th percentile) < LOD of 0.81 µg BPA/l in amniotic fluid	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody, LOD of 0.2 µg BPA/l, Background below LOD
(Ikezuki <i>et al.</i> 2002) Japan	ELISA (EcoAssay Bisphenol A kit from Otsuka pharmaceuticals, Tokyo, Japan)	Blood samples from 13 healthy pre-menopausal women, 37 women with early pregnancy, 37 late pregnancy, 32 umbilical cord blood samples, 36 ovarian follicular fluid samples	2.0 ± 0.8 µg BPA/l (non-pregnant) 1.5 ± 1.2 µg BPA/l (early pregnancy); 2.4 ± 0.8 µg BPA/l (follicular fluid); 2.2 ± 1.8 µg BPA/l (fetal serum); 8.3 ± 8.9 µg BPA/l (amniotic fluid)	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody, LOD of 0.5 µg BPA/l
(Takeuchi and Tsutsumi 2002) Japan	ELISA (EcoAssay Bisphenol A kit from Otsuka pharmaceuticals, Tokyo, Japan), solid phase extraction	14 healthy women, 16 women with polycystic ovary syndrome (PCOS) and 11 healthy men	0.64 ± 0.1 µg BPA/l (normal women) 1.49 ± 0.11 µg BPA/l (healthy men); 1.04 ± 0.1 µg BPA/l (women with PCOS)	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody, LOD not given, very small standard deviations
(Inoue <i>et al.</i> 2001) Japan	LC/MS, electrospray ionization, glucuronidase treatment, solid phase extraction	Only 3 blood samples analyzed	0.1 to 1 µg BPA/l	Very limited number of samples analyzed
(Ohkuma <i>et al.</i> 2002)	competitive ELISA	100 samples analyzed, no details about sample workup	Many < 0.3 µg BPA/l, up to 1 µg BPA/l (no details given)	Some results with antibody confirmed by GC/MS determination of BPA, no assessment of BPA-glucuronide
(Fung <i>et al.</i> 2000) Japan	HPLC with fluorescence detection solid phase extraction	Serum samples from 18 men and 22 women after application of dental sealant containing BPA	None above detection limit of 5 ppb (5 µg BPA/l)	No assessment of BPA-glucuronide
(Schonfelder <i>et al.</i> 2002b) Germany	GC/MS after derivatisation by silylation, solvent extraction with ethylacetate	37 maternal and fetal plasma samples, and placenta tissue levels	Median BPA conc. in maternal plasma 3.1 µg BPA/l (range from 0.3 to 18.9 µg BPA/l); 2.3 µg BPA/l in fetal plasma (range from 0.2 to 9.2 µg BPA/l); median of 12.7 µg BPA/l placenta tissue (range	Glucuronidase cleavage not applied; contradictory statements and results presented regarding background of BPA in blanks; LOQ 0.1 µg BPA/l

			from 1 to 104.9 µg BPA/kg)	
(Takeuchi <i>et al.</i> 2004) Japan	ELISA (no source specified, presumably EcoAssay Bisphenol A kit)	73 blood samples analyzed from women with different endocrine status	1.17 ± 0.16 µg BPA/l to 0.71 ± 0.09 µg BPA/l in obese resp. non-obese women (mean ± SD)	No assessment of BPA-glucuronide; unknown cross-reactivity of the antibody
(Volkel <i>et al.</i> 2005) Germany	LC/MS/MS with and without glucuronidase treatment	Randomly collected blood samples from 7 males and 12 females	All samples below LOD of 0.5 µg BPA/l	LOD 0.5 µg BPA/l, no background after method adjustment
(Fukata <i>et al.</i> 2006) Japan	ELISA with three different kits, same samples analyzed by HPLC with electrochemical detection	Randomly collected from 21 male and 31 female subjects, age 22 – 51 years	Two of the ELISA kits indicated BPA concentrations of 0.66 + 0.29 resp. 0.71 + 0.49 µg/l, LC with electrochemical detection all samples < LOD	LC with electrochemical detection had LOD of 0.2 µg/l
(Sajiki <i>et al.</i> , 1999) Japan	HPLC with electrochemical detector, solid phase extraction	Randomly collected blood samples from 12 adult women and nine adult men	Average BPA-concentrations of 0.33+0.54 µg/l in females (range from 0 - 1.6 µg/l) and 0.59+0.21 µg/l in males (range 0.38-1 µg/l)	No glucuronidase treatment, LOD of 0.2 µg/l, use of glass vessels for sampling

Table 4.16. Reported urine concentrations of BPA in human subjects without known specific exposures to BPA (from EFSA, 2006)

Reference and sampling region	Analytical method sample workup	No. of samples analyzed	Concentration ranges reported	Comments
<i>al.</i> 2004) Japan	GC/MS/MS, glucuronidase treatment, solvent extraction followed by solid phase extraction	Samples from 5 health adults, on 5 consecutive days, in addition, 24h urine samples from 36 male subjects	< 0.58 to 13 µg BPA/day (median of 1.3 µg/day) in five subjects observed over 5 days; < 0.21 µg BPA/day to 14 µg BPA/day for the 36 other subjects (median of 1.2 µg BPA/day)	Detection limit 0.38 µg BPA/l of urine, no information on contamination of solvents or leaching of BPA
(Matsumoto <i>et al.</i> 2003) Japan	HPLC with fluorescence detection, glucuronidase treatment, solvent extraction	Morning spot urine from 46 male and 4 female students,	Up to 30 µg BPA/g creatinine/app. 18 µg BPA/l, 39 % of samples collected in 1999 were below LOD of 1.7 µg BPA/g creatinine (1 µg BPA/l)	Single trace method, no information on contamination
(Hanaoka <i>et al.</i> 2002) Japan	HPLC with electrochemical detection, glucuronidase	Spot urine samples from 42 individuals without intentional	BPA > 1 µmol/mol creatinine in controls (< app. 1.2 µg BPA/l), no further details	Single trace method, no information on contamination.

	treatment followed by protein precipitation,	BPA-exposure, 42 males exposed to BADGE		
(Kim <i>et al.</i> 2003) Korea	HPLC with fluorescence detection, separate assessments with and without glucuronidase treatment, solvent extraction	Spot urine samples collected from 15 healthy men and 15 healthy women	BPA from 0.28 to 2.36 µg/l (mean 0.58 + 0.14) in males and 0.068 – 1.65 µg/l (mean 0.56 + 0.1) in females; BPA glucuronide from 0.16 to 11.67 µg/l (mean 2.34 + 0.85) in males and < LOD to 4.34 (mean 1.0 + 0.34) in females; BPA sulphate from LOD to 1.03 µg/l (mean 0.49 + 0.27) in males and < LOD to 3.4 (mean 1.2 + 0.32) in females	Single trace method, no information on contamination
(Ye <i>et al.</i> 2005) United States	LC/MS/MS with column switching, glucuronidase treatment	30 spot urine samples from adults	Mean conc. of 3.5 µg BPA/l, (95 percentile of 11.5 µg BPA/l)	LOD of 0.4 µg/l, reagent blank gives response of app. 0.1 µg BPA/l
(Yang <i>et al.</i> 2003) Korea	HPLC with fluorescence detection; glucuronidase treatment, solvent extraction	Morning spot urine from 34 adult males and 39 adult females	Geometric mean of 8.91 + 8.32 µg BPA/l	LOD of 0.34 µg/l, no background
(Kawaguchi <i>et al.</i> 2005) Japan	GC/MS with EI ionization, thermal desorption; glucuronidase treatment, sorptive extraction after derivatisation	Urine samples from 5 health subjects, no further information	Range from < LOD to 5.41 µg BPA/l	LOD 0.1 µg/l, reagent background not detailed
(Mao <i>et al.</i> 2004) China	HPLC after fluorophore derivatisation with p-nitrobenzoyl chloride; acid hydrolysis to cleave conjugates followed by solid phase extraction	10 healthy male and 10 healthy female subjects, no information on urine collection	Range from < LOD to 3.95 mg BPA/l, mean 1.22 + 1.38 mg BPA/l;	LOD 2.7 µg BPA/l, but poorly resolved peak for BPA, peak assignment on retention time only, very high concentrations of endogenous hormones indicated by assay suggest systematic error in evaluation
(Kuklennyik <i>et al.</i> 2003) United States	GC/MS with chemical ionization and electrophore derivatisation, negative ion detection; glucuronidase treatment and extractive derivatisation	30 urine samples from individuals painting houses and 6 unexposed individuals used as controls	Quantitative evaluation of BPA levels in urine of unexposed controls not detailed, based on graphic presentation estimated as below 2 µg/l	LOD of 0.1 µg BPA/l, no information on BPA-contamination of blanks
(Calafat <i>et al.</i> 2005) United States	GC/MS with chemical ionization and electrophore derivatisation, negative ion detection;	Spot urine samples from 394 adults in the US, collected at different times of the day	Geometric mean of 1.21 µg BPA/l for urban and of 1.56 µg BPA/l for rural residents	LOD of 0.1 µg BPA/l; no information on BPA-contamination of blanks

	glucuronidase treatment and extractive derivatisation			
(Ouchi and Watanabe 2002) Japan	HPLC with electrochemical detector and column switching, determination with and without pretreatment with glucuronidase	Morning spot urine samples from 48 female students	BPA below LOD except for one sample with 0.2 µg BPA/l; BPA-glucuronide detected in all samples with concentrations from 0.2 to 19.1 µg BPA-gluc/l (median of 1.2 µg/l)	Background of 0.26 µg BPAglucuronide/l
(Volkel <i>et al.</i> 2005) Germany	LC/MS/MS with and without glucuronidase treatment	Randomly collected urine samples from 7 males and 12 females without known BPAexposure	All samples below LOD of 1.14 µg BPA/l LOD 1.14 µg BPA/l	Background below LOD after method adjustment
(Ye <i>et al.</i> 2005) United States	LC/MS/MS with column switching, separate analysis for free BPA without enzymatic hydrolysis, after glucuronidase and after sulfatase treatment	Randomly collected urine samples from 30 adult individuals without known BPA-exposure	Means for free BPA were < LOD, for BPAglucuronide 3.1 µg/l, BPA-sulphate 0.5 µg/l	LOD 0.3 µg BPA/l
(Fukuta <i>et al.</i> 2006) Japan	HPLC with electrochemical detection for total and free BPA; BPA-glucuronide concentrations in some samples confirmed by LC/MS-MS	Randomly collected urine samples from 21 male and 31 female subjects, age 22 – 51 years	2 samples showed free BPA (0.24 and 0.35 µg/l); mean of total BPA was 1.92 + 1.99 µg/l. ELISA kits gave total BPA concentrations of 15.9 + 9.9; 16.7 + 19.5; and 18.6 + 23.7 µg/l	LOD 0.5 µg BPA/l for HPLC

al., 2006; Ikezuki et al., 2002; Inoue et al., 2001; Ohkuma et al., 2002; Schonfelder et al., 2002b; Takeuchi and Tsutsumi 2002; Takeuchi et al., 2004; Volkel et al., 2005; Yamada et al., 2002). The studies reporting detection of BPA in human blood in concentrations higher than 1 µg/l have usually determined BPA, without prior enzymatic cleavage of BPA glucuronide. Based on toxicokinetics of BPA in humans, BPA glucuronide is expected to be present in higher concentrations as compared to BPA (Teeguarden et al., 2005; Volkel et al., 2002). The fate of BPA glucuronide under the conditions of the diverse sample processing conditions and a possible cross-reactivity of the antibodies with BPA glucuronide is not reported, leaving the possibility that reported BPA levels actually reflect BPA glucuronide levels.

EFSA also noted that blood levels for BPA in unintentionally exposed human subjects (reported as up to 10 µg/l) are higher than the peak BPA concentrations determined in blood of monkeys (5 nM, app. 1.1 µg/l) after oral administration of a dose of 100 µg/kg bw or in blood of humans given oral doses of 60 – 80 µg/kg bw. In these human subjects, free BPA in plasma was not detected even within a short time after doses much higher than the doses of BPA received by the general population from the diet. Furthermore, these reported concentrations of BPA in blood of unintentionally exposed human subjects of up to 10 µg/l are orders of magnitude above the maximal concentrations of BPA predicted in blood by PBPK models on the basis of human BPA toxicokinetics after oral administration (see below, app. 40 pmol/l or 9 ng/l) (Filser et al., 2003; Teeguarden et al., 2005). Based on the PBPK model, these maximal blood levels will be reached after oral uptake of BPA at a daily dose of 1 µg/kg bw and after simulation of a dietary exposure pattern (Filser et al., 2003; Teeguarden et al., 2005).

A number of other confounders have also been reported. Regarding the use of ELISA to quantify BPA, the cross-reactivity of the antibodies to other constituents in serum is unknown and may result in an overestimation of BPA concentrations. Attempts to confirm BPA concentrations indicated by ELISA using instrumental analytics have failed, and consistently indicate a large overestimation of BPA concentrations by ELISA (Inoue *et al.*, 2002; Tominaga, *et al.*, 2006). In addition, studies have reported contamination of reagents with BPA or leaching of BPA from the materials for sample collection, storage and processing (Sajiki et al., 1999; Sajiki, 2001). The background may interfere with the analytical quantitation of BPA in low concentrations, suggesting higher BPA concentrations than actually present. Due to all these confounders, the reported analytical results on BPA blood concentrations most probably considerably overestimate real blood concentrations actually present.

A recent paper (Fukata *et al.*, 2006) compares LC/MS/MS, LC/ECD, and three commercially available ELISA kits for measurement of BPA in 52 matched human urine and serum samples. The LC/MS/MS method, which positively identifies BPA and BPA-monoglucuronide, correlates very well with the LC/ECD method. However, the three ELISA kits not only have poor correlation with the reliable LC-based methods, but they also have poor correlation with each other. From this set of data, it can be concluded that the ELISA kits not only produce inaccurately high values for BPA, but they are not apparently measuring BPA at all.

Considering the evidence as a whole, EFSA concluded that the validity of the reported high blood levels of BPA in unintentionally exposed human subjects is questionable.

The studies on human urinary concentrations of BPA metabolites show peak levels of 15 µg/l and confirm that BPA is mainly present as BPA glucuronide in urine. The more recent studies

analyzing BPA concentrations in human urine often applied sensitive and selective mass spectrometry and are considered useful to assess daily exposures to BPA in humans. While spot urine samples may not be totally appropriate, due to the dietary exposure pattern and the rapid excretion, the BPA concentrations in spot urine samples and in 24 h pooled urine samples correlate reasonably well. The cumulative daily human exposures can be derived from urinary excretion of BPA and/or BPA metabolites since orally administered BPA is almost completely recovered in urine within 24 h after an oral exposure (Volkel et al., 2002). Mean urinary (total) BPA concentrations in the USA and in Japan are reported to range from 1.2 to 3.5 µg/l, while samples from a cohort in Germany did not contain detectable concentrations of BPA with a detection limit of 1.1 µg/l (Volkel et al., 2005).

A study in the USA, quantifying BPA in the urine of 394 subjects from the general population, detected BPA in 95% of the urine samples in concentrations up to 5.18 µg/l, 95th percentile) (Calafat et al., 2005).

Supplementary information supporting that exposure to BPA is in the above-mentioned range is available from the CDC biomonitoring program. BPA was included in the most recent CDC biomonitoring program, which is a part of the broader National Health and Nutrition Examination Survey (NHANES). The BPA biomonitoring raw data is now available to the public on the CDC website (http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003-2004/nhanes03_04.htm). Total BPA (after hydrolysis of conjugates) was measured in urine samples from 2,517 individuals (age 6-85 years). Initial preliminary analysis of these data indicate that this study is in agreement with previous CDC studies; overall weighted statistics of these BPA concentrations in urine have produced a median of 2.8 ng/ml, a geometric mean of 2.6 ng/ml, a 5th percentile of ND (0.36 ng/ml LOD) and a 95th percentile of 16 ng/ml (detection frequency: 93.5%).

Based on a total urine volume of 2 litres excreted over 24 h, the available data give an estimate of an average daily dietary exposure of BPA of up to 7 µg/adult and upper range dietary exposures up to 10 µg per adult (0.16 µg/kg bw for a 60 kg person). A recent Japanese assessment of BPA exposure of the general population used urinary excretion data and estimated (95 % confidence interval) daily BPA exposure as 0.037 to 0.064 µg/kg bw/day for male and 0.043 – 0.075 µg/kg bw/day for female adults (Miyamoto and Kotake, 2006).

EFSA (2006) noted that exposure assessed from urinary excretion measured in groups of subjects from the general population in the USA, Japan and Korea could be used to assess the order of magnitude of overall average BPA exposure. The discrepancy between the levels of exposure estimated through biomarkers and the levels of exposure assessed by combining food consumption data with BPA concentration in the diet is likely to be due to the highly conservative assumptions performed in the latter, which are aimed at assessing exposure in the most exposed population groups.

4.1.1.4 Combined exposure

4.1.1.4.1 Summary of original risk assessment report

The worst case combined exposure would be someone exposed via the environment near to a PVC production plant, and who is also exposed via food contact materials as described in section 4.1.1.2.

The exposures for these component parts are presented below. The maximum combined exposure from these sources is 0.009 and 0.069 mg/kg/day for the regional and local scenarios respectively.

Table 4.17 Components of combined exposure (from the 2003 published RAR)

Source of exposure	Exposure (mg/kg/day)
As a consumer (oral exposure via food and wine)	9×10^{-3}
Indirect exposure via the environment:	
Regional	1.78×10^{-5}
Local	0.06
Total : Regional	9×10^{-3}
Local	0.069

The value of 9×10^{-3} for consumer exposure is based on an adult consumer receiving exposure via canned food and wine. The values of 1.78×10^{-5} and 0.06 for regional and local environmental exposure respectively, have been taken from Table 4.10. The main route of exposure from environmental sources is the oral route. The average body weight of 70 kg has been assumed.

4.1.1.4.2 Updated information

The worst case combined exposure would be for someone exposed via the environment near to a BPA production plant, and who is also exposed via food contact materials as described in section 4.1.1.2.

The exposures for these component parts are presented below. The maximum combined exposure from these sources is 1.45×10^{-3} [it was 9×10^{-3} in the original RAR] and 0.043 [it was 0.069 in the original RAR] mg/kg/day for the regional and local scenarios respectively.

Table 4.18 Components of combined exposure

Source of exposure	Exposure (mg/kg bw/day)
As a consumer (oral exposure from canned food and canned beverages and from polycarbonate tableware and storage containers)	1.45×10^{-3}
Indirect exposure via the environment:	
Regional	9.1×10^{-6}
Local	0.041
Total : Regional	1.45×10^{-3}

Source of exposure	Exposure (mg/kg bw/day)
Local	0.043

The value of 1.45×10^{-3} for consumer exposure is based on an adult consumer receiving exposure via canned food and canned beverages and from polycarbonate tableware and storage containers. The values of 9.1×10^{-6} and 0.041 for regional and local environmental exposure respectively, have been taken from Table 4.11. The main route of exposure from environmental sources is the oral route. Average body weights of 60 and 70 kg have been assumed for consumers and humans exposed via the environment.

4.1.2 Effects assessment: Hazard identification and dose (concentration) - response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Summary of original risk assessment report

The limited data available in humans, from a single study, indicates that BPA does not accumulate in endometrium or body fat (the only tissues tested). In experimental animals, toxicokinetic data are available from three oral studies in a single species, the rat and from an *in vitro* dermal absorption study, using human skin. These studies provide the basis for a general understanding of the main features of the toxicokinetic profile. Following oral administration, absorption from the gastrointestinal tract is rapid and extensive, although it is not possible to reliably quantify the extent of absorption. Following dermal exposure, the available data suggest that there is limited absorption, in the region of about 10% of the applied dose. BPA was removed rapidly from the blood, and metabolism data indicate extensive first pass metabolism following absorption from the gastrointestinal tract. A clear sex difference was observed in the clearance of parent compound from the blood. In females parent compound was present in the blood at much later sampling times. There are no data available to explain why this sex difference was observed. In view of this first pass metabolism, the bioavailability of unconjugated BPA is probably limited following oral exposure, at no more than 10-20% of the administered dose. Limited data are available for the distribution of BPA following oral administration: an *in vivo* DNA adduct study shows that BPA reaches the liver, an *in vivo* micronucleus study suggests that BPA or a metabolite reaches the bone marrow, a limited toxicokinetic study suggests that BPA or a metabolite reaches the testes, and a repeat dose study in pregnant rats suggests that BPA reaches the liver of both the dam and fetus. However, because of first pass metabolism, it is likely that the distribution and bioavailability of unconjugated BPA is limited following oral exposure. There is also evidence of enterohepatic circulation occurring.

The major metabolic pathway in rats involves glucuronide conjugation; limited sulphate conjugation may also occur. Approximately 10% and 20% of the administered dose was recovered in the urine as the glucuronide metabolite in males and females, respectively. There are no data available to explain why this sex difference was observed. Comparative *in vitro* studies of metabolism suggest some quantitative differences in the rate of metabolism between rats, mice and humans. In general, human liver samples show slower rates of glucuronidation compared with either rats or mice. Estimates of overall liver metabolic capacity suggest that human liver may have greater metabolic capacity than either rats or

mice and that capacity is lowest in the mouse. However, these estimates are based on limited kinetic data and are therefore of uncertain reliability. *In vitro* data in rats also indicate that fetuses do not metabolise BPA as extensively as immature and adult animals. In addition, data from cell free systems and *in vivo* studies on the interaction of BPA with DNA, supported by a chemical photodecomposition study, suggest that limited oxidation of BPA to bisphenol O-quinone by cytochrome P450 may occur.

The major route of excretion is via the faeces with the urinary route being of secondary importance: over 7 days post dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post dose. A sex difference was also observed in elimination, with females excreting approximately twice as much radioactivity in the urine (24-28%) than males (14-16%). Again, there are no data available to explain why this sex difference was observed. In addition, a strain difference was observed in elimination, with female F344 rats excreting approximately twice as much radioactivity in the urine than female CD rats. Data from a number of studies suggest limited excretion of BPA in the milk. However, the data do not allow a reliable quantitative determination to be made.

The first pass metabolism and extensive and rapid elimination of BPA suggest that the potential for transfer to the foetus and bioaccumulation may be limited. This is supported by data from toxicokinetic studies in pregnant rats that suggest limited distribution of BPA to the foetus, but no evidence for accumulation, and results from a repeat dose study in pregnant rats which show limited distribution to the fetal liver, with no evidence to indicate accumulation in the liver, the only organ tested.

There are no data on the toxicokinetics of BPA following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

4.1.2.1.2 Updated information

A number of recent publications addressing the toxicokinetics of BPA in primates and in pregnant and non-pregnant rodents of different ages have become available since the original RAR was finalised.

Studies in humans and non-human primates

A study in human volunteers investigated the toxicokinetics of a low dose of BPA given orally (Volkel *et al*, 2002). In the first half of the study, designed to provide information on the metabolic fate of BPA, a single oral dose of 5 mg deuterium-labelled BPA was given to three healthy adults of each sex; mean body weight was 67 kg (range 57 – 82 kg). Samples of blood were taken at intervals of 4 hours, and urine produced during 6-hour periods was collected. These samples were assayed for BPA and its metabolites by HPLC with mass spectrometry; the assay limit of detection for free BPA was 6nM for urine and 10nM for blood samples. Measurements of individual metabolites were confirmed by gas chromatography, and by enzymatic hydrolysis of samples. In the second half of the study,

designed to investigate the toxicokinetics of BPA in plasma in more detail, the same dose of deuterium-labelled BPA was given orally to four male subjects, including one from the first half of the study. The mean body weight was 78 kg (range 63 – 92 kg). Samples of blood were taken at intervals of 40 minutes and urine produced over 6-hour periods was collected for assay of BPA and BPA metabolites as described above.

In both parts of the study BPA-glucuronide was the only metabolite detected in samples of plasma and urine. Neither free BPA nor other BPA metabolites were found.

The first part of the study showed that the dose of deuterium-labelled BPA administered orally was completely recovered in urine as BPA-glucuronide. The urinary concentration of this metabolite fell rapidly from a peak at 6 hours after dosing with a terminal half-life ($t_{1/2}$) of 5 hours. The concentration of BPA-glucuronide in the plasma also fell rapidly after dosing. There were no apparent sex differences in the profiles of BPA-glucuronide in either plasma or urine.

In the second part of the study it was found that in plasma the C_{max} of BPA-glucuronide was approximately 800nM and occurred 80 minutes after dosing. The plasma concentration of BPA-glucuronide declined biexponentially with a half-life of 89 min, and the clearance from the plasma (0.13 litres/minute) was found to be similar to that reported for plasma creatinine, signifying that BPA-glucuronide probably enters the urine by filtration, with no renal secretion or reabsorption. The apparent distribution volume (37 litres) was similar to the total body water volume, suggesting that BPA-glucuronide distributes throughout the extra- and intracellular aqueous media and does not bind to plasma proteins.

In summary, this study found that in humans low doses of BPA (in the range 54 to 88 $\mu\text{g}/\text{kg}$ bw) were absorbed rapidly and completely from the GI tract after oral administration. BPA was efficiently conjugated to the glucuronide by first-pass-metabolism, with free BPA and other metabolites not being detected in plasma from 40 minutes after dosing onwards. The BPA-glucuronide formed was cleared from the circulation within 24 hours by urinary excretion. No evidence of enterohepatic recirculation was found.

The same authors also carried out a study in which BPA and BPA-glucuronide were measured by a sensitive technique (high performance liquid chromatography/tandem mass spectrometry) in plasma and urine of human volunteers (3 males and 3 females) given a single oral dose of 25 μg (~ 0.35 $\mu\text{g}/\text{kg}$ bw) BPA and in human subjects (7 males and 12 females) without intentional exposure to BPA (Volkel et al., 2005).

In urine samples from human volunteers administered BPA, BPA was below the limit of detection (LOD = 2.5 pmol/ml urine) with the exception of two samples (~ 1 $\mu\text{g}/\text{l}$) in which BPA was less than 2% of the administered dose; 75-85% of the administered dose was recovered as BPA-glucuronide within 5 hours after application. An elimination half-life of 4 hours was calculated. In urine or blood samples from human subjects without intentional exposure to BPA, BPA concentrations were always below the LOD of ~ 1.14 $\mu\text{g}/\text{l}$; the concentration of BPA-glucuronide in urine was always below the LOQ of 65 pmol/ml. Due to the rapid and complete elimination of BPA in humans as BPA-glucuronide in urine, the concentration of BPA determined in the urine of the 19 subjects without intentional exposure to BPA investigated in this study suggests that human exposure to BPA in these subjects is less than 2.3 $\mu\text{g}/\text{person}/\text{day}$ (0.04 $\mu\text{g}/\text{kg}$ bw/day; bw = 60 kg) based on a volume of 2 litres of urine excreted.

A GLP-compliant study investigated the toxicokinetics of a low dose of ^{14}C -labelled BPA given orally or intravenously to cynomolgus monkeys (Kurebayashi *et al*, 2002). Three males and three females were administered 0.1 mg/kg ^{14}C -labelled BPA intravenously, and then two weeks later they were given the same dose orally by gavage. Blood samples were taken 5 minutes after dosing and at selected intervals for up to one week after dosing. Urine, including cage-washings, and faeces were collected over 12 or 24 hour periods up to one week after dosing. Metabolites were separated and quantitated using HPLC, and the nature of metabolites was investigated by enzymatic hydrolysis.

Following intravenous (iv) administration, most of the radioactivity (79 – 86%) was excreted in the urine, with only 2% being excreted in the faeces. Plasma radioactivity declined with a terminal half-life of approximately 14 hours. The proportion of blood radioactivity bound to plasma proteins was relatively high (94%). The total body clearance and distribution volume were calculated to be 4.5 ml/min/kg body weight and 1.6-1.8 litres/kg body weight respectively.

Following oral dosing, as after iv dosing, most of the administered dose (82-85%) was excreted in urine, with a high proportion appearing in the urine within 12 hours of administration, suggesting that absorption from the GI tract was rapid and relatively complete. Only a small proportion of the dose of BPA administered (2-3%) was excreted in the faeces. Plasma radioactivity rose to a maximum value between 0.25 and 2 hours after dosing, and subsequently fell with a terminal half-life of approximately 10 hours. No radioactivity was detected in plasma 72 hours after dosing. The terminal elimination half-life of ^{14}C -BPA-derived radioactivity in plasma following iv dosing was larger (13.5 hours in males and 14.7 hours in females) than that following oral dosing (9.6 hours in males and 9.8 hours in females). It has been suggested by the authors that this might have been due to deposition of the lipophilic free BPA in adipose tissue after iv dosing, in contrast to first-pass metabolism after oral dosing.

HPLC analysis recognised 5 radioactive species in samples of urine and plasma following both iv and oral dosing: free BPA, BPA-glucuronide, BPA-diglucuronide and two unidentified minor metabolites. In urine samples, free BPA was found at low levels (between 0.0 and 1.5% of the radioactivity detected) following both iv and oral dosing. The largest fraction of the radioactivity detected was due to BPA-glucuronide (73-81% of urinary radioactivity), BPA-diglucuronide and other BPA metabolites, suggested by the study authors to be BPA-sulphate and 5-hydroxy BPA.

After oral dosing, the major radioactive species in plasma was found to be BPA-glucuronide (up to 95-100% of plasma radioactivity) with unconjugated BPA representing only up to 1.4% of the total plasma radioactivity. After iv dosing, most (between 57 and 82%) of the plasma radioactivity was due to BPA-glucuronide, with unconjugated BPA representing 27-29% of the total plasma radioactivity. It was noted that after iv dosing the plasma levels of unconjugated BPA declined more rapidly in females than in males with a fast phase terminal half-life of 0.4 hours in females and 0.6 hours in males. Since there were no significant sex differences in urinary excretion, this difference was thought to be related to female animals having a higher adipose tissue content than males, suggesting that circulating free BPA can partition into the adipose tissue.

In summary, this study in cynomolgus monkeys found that a low oral dose of BPA was extensively absorbed from the GI tract (up to 85% of the administered dose), subsequently undergoing rapid first-pass metabolism to BPA-glucuronide and a slower conversion to BPA-diglucuronide. Both metabolites were excreted into the urine, and, consistent with the data in humans but in contrast to rats, the urine was a much more important route of elimination of

BPA-derived radioactivity than the faeces. This study also suggested that there could be preferential distribution of lipophilic unconjugated BPA to adipose tissue after iv dosing, in contrast to first-pass metabolism after oral dosing.

EFSA (2006) noted that rapid elimination from blood and extensive first-pass metabolism of orally administered BPA in humans and primates is also indicated by results of a recent study on the blood toxicokinetics of BPA in chimpanzees and monkeys (Tominaga et al., 2006).

Overall, the results on the toxicokinetics of BPA in humans and primates show a rapid first-pass biotransformation and elimination of orally administered BPA and indicate that, after oral uptake, only low levels of unmodified BPA may reach the systemic circulation. The data also show that enterohepatic recirculation of BPA does not occur in humans and primates.

Studies in rodents

A GLP-compliant study (Domoradzki et al, 2003) was recently conducted with the aim of comparing the metabolism and toxicokinetics of BPA (BPA) in pregnant rats at different stages of gestation and in non-pregnant rats. The study also investigated the distribution of BPA or its metabolites to the embryo/foetal tissues. The experimental study design consisted of three parts.

In the first part which was conducted to evaluate any impact of stage of pregnancy on BPA metabolism, ¹⁴C-labelled BPA was administered via oral gavage in corn oil at 10 mg/kg to groups of 4 animals/gestational stage of pregnant Sprague-Dawley (SD) rats on gestational days (gd) 6, 14 and 17, and to non-pregnant, 11 week-old female SD rats. Rats were placed in Roth-type metabolism cages immediately post-dosing to allow for the separate collection of urine and faeces up to 96 hours post-dosing.

Blood samples were collected at the expected free, unconjugated BPA T_{max} time point (15 minutes post administration) and at additional selected time points over the 96-hour period post administration. Levels of total plasma radioactivity for each time point and amounts of free, unconjugated BPA and BPA-glucuronide metabolite were quantified in pooled plasma samples by liquid scintillation counting or by HPLC with radiochemical detection. Levels of BPA, BPA-glucuronide and other metabolites were also quantified in pooled excreta samples from selected groups and collection intervals. Specific structural identification of free, unconjugated BPA and BPA-glucuronide was confirmed by LCMS for selected plasma, urine and faecal samples. At sacrifice (96 hours post-dosing), selected tissues were collected and analysed for radioactivity, levels of free, unconjugated BPA and metabolites. The tissues analysed included maternal blood, brain, fat, liver, kidneys, ovaries, uterus, skin, placenta, embryo/foetus (6 individual embryos/foetuses and 1 pooled embryo/foetus sample for each gd 6, gd 14 and gd 17 dam) and remaining carcass. A final cage wash was collected and mass balance of radioactivity determined.

In the second part which was conducted to study further the distribution of BPA and BPA-glucuronide to the embryo/foetal tissue, ¹⁴C-BPA was administered via oral gavage at 10 mg/kg in corn oil to groups of 5 animals/sacrifice time point/gestational stage of pregnant SD rats on gd 11 (embryonic stage), 13 (foetal stage) and 16 (foetal stage). Rats were then sacrificed at one of three different time points. Sacrifice time point 1 was 15 minutes post-dosing, the anticipated peak plasma radioactivity time point in the maternal animals. Sacrifice time point 2 was 12 hours post-dosing, the time of the secondary maximum of plasma

radioactivity in maternal animals and sacrifice time 3 was 96 hours post-dosing, when BPA was anticipated to be non quantifiable in the maternal blood of most animals. The timing of the three sacrifices meant that the experiment covered stages in development from embryo to foetus to perinatal. Samples of maternal blood, embryos/foetuses and yolk sacs/placentas collected at each of the three sacrifice points for each of the three gestational stages were analysed for radioactivity, free, unconjugated BPA and/or metabolite profile, including quantitation of BPA monoglucuronide. In addition, from gd 20 (96 hours post dosing of gd 16 rats) sacrificed dams, samples of foetal blood, plasma, liver and brain were also collected for analysis.

In the third part, 5 pregnant SD rats were dosed by gavage with 0 or 10 mg/kg ^{14}C -BPA in corn oil on gd 16, using a radioactivity level of approximately 500uCi per rat. This higher specific activity of ^{14}C -BPA was employed to improve the limit of detection of analytes. Fifteen minutes post-dosing, all animals were sacrificed and maternal blood, placentas and foetuses were collected for analysis. The levels of radioactivity, free, unconjugated BPA and BPA-glucuronide were determined in these samples.

Part 1 - There were no signs of treatment-related toxicity observed and no deaths occurred during part 1 of the study. At 96 h post-dosing, the faeces were the major elimination route of ^{14}C -BPA-derived radioactivity for all 3 gestational groups and for the non-pregnant animals, accounting for 65-78% of the administered dose. The mean percentage of the administered dose that was eliminated via the urine was also similar for all groups, ranging from 14-22%. The ^{14}C -BPA-derived radioactivity in tissues (brain, fat, liver, kidney, ovary, uterus and skin) and remaining carcass was 1-6% of the administered dose across all groups, and the embryo/foetus samples accounted for <0.1% of the administered dose in the gd 17 group and were non-quantifiable in the gd 14 and gd 6 groups. In general, liver and kidney had the highest concentrations of tissue radioactivity. Brain did not contain quantifiable levels of radioactivity, and levels in fat were only occasionally quantifiable in individual animals. Placentas (only available from the last two gestational groups as the placenta is not formed yet on gd 6) had quantifiable radioactivity only in the last gestational group, representing 0.01% of the administered dose. These data showed that the pregnancy status had no effect on the overall disposition of orally administered ^{14}C -BPA-derived radioactivity in SD female rats. Generally, the concentration of ^{14}C -BPA-derived radioactivity quantified in embryo/foetus and placenta samples was equal to or less than the levels found in other tissues analysed for radioactivity, such as liver or skin or ovaries. This suggests that orally administered ^{14}C -BPA-derived radioactivity does not preferentially accumulate in the embryo/foetal or placental compartment of pregnant SD rats.

Plasma ^{14}C -BPA-derived radioactivity was quantifiable from 15 minutes post-dosing through to 48 hours post-dosing for all groups. Plasma radioactivity in all groups was highest at 15 minutes post-dosing but reached a secondary maximum between 12 and 24 hours post-dosing. The pattern of a primary peak and a secondary peak in total plasma radioactivity, which was reproducible across all groups, is considered to be evidence of enterohepatic recirculation of ^{14}C -BPA-derived radioactivity in pregnant and non-pregnant SD rats. By 72 hours post-dosing, radioactivity was not quantifiable in the plasma of non-pregnant rats or from gd 14 dams. ^{14}C -activity was not quantifiable in the plasma from gd 17 dams by 96 hours post-dosing, while plasma from gd 6 dams still contained quantifiable levels of radioactivity at terminal sacrifice (96 hour).

Unchanged BPA was only quantifiable at a few time points (15 and 45 minutes post-dosing) in most groups with typical concentrations close to the levels of detection. Only the late stage pregnancy group (gd 17-21) had low but quantifiable levels of free, unconjugated BPA in plasma for the first 12 hours post-dosing.

BPA-glucuronide represented the largest fraction of quantifiable plasma radioactivity for all groups, up to 100% in some cases. Two BPA-glucuronide maximum occurred; these correlated with the peaks of plasma ^{14}C -BPA-derived radioactivity observed at 15 minutes and 12-24 hours post-dosing, clearly indicating that BPA-glucuronide is the predominant form of ^{14}C -BPA-derived radioactivity in the systemic circulation, and that enterohepatic circulation is a major factor in the metabolism of BPA. The BPA-glucuronide concentration-time profiles were very similar to those for plasma radioactivity, with BPA-glucuronide reaching non quantifiable levels at the same time, by either 72 or 96 hours post-dosing (non-pregnant, gd 14 and gd 17) or remaining quantifiable at terminal sacrifice (gd 6). Two other metabolites of BPA were observed in plasma but both were minor and were not structurally identified.

Up to nine separate peaks were identified in the urine samples, with the majority of the urinary radioactivity identified as BPA-glucuronide (0.3 – 5% of the administered dose and 62-70% of the radioactivity recovered from the urine over the 96-hour collection period for all groups). Approximately 19 to 23% of the radioactivity recovered from the urine over the 96-hours collection period was determined to be BPA for all groups. No marked differences were observed between the urinary metabolite profiles in non-pregnant and pregnant animals.

Up to seven peaks were identified in faecal samples with the majority of the faecal radioactivity identified as free, unconjugated BPA (83-89% of the radioactivity recovered from the faeces over the 96-hour collection period for all groups). Approximately 2 to 3% of the radioactivity recovered from the faeces over the 96-hour collection period was found to be BPA-glucuronide for all groups. No marked differences were observed between the faecal metabolite profiles in non-pregnant and pregnant animals.

Part 2 - The mean percent of the administered dose recovered in maternal blood for animals dosed with 10 mg/kg at gd 11,13 and 16 were in the same range (0.11 – 0.25%) as observed before in other studies conducted with SD rats. In general, BPA was non-detectable in maternal plasma from all groups. The average peak maternal plasma concentrations of BPA-glucuronide at 15 minutes post-dosing were similar for the animals dosed on gd 11 or 13, but were 1.7-2 times higher in rats dosed on gd 16. By 12 hours post-dosing, the concentration of BPA-glucuronide was about 7- to 11-fold less than that seen at 15 minutes post-dosing for all groups. At 96 hour post-dosing there was insufficient radioactivity for analysis.

There was no apparent selective affinity of either placenta or embryo/foetus for ^{14}C -BPA-derived radioactivity. Analysis of the yolk sacs and embryos from gd 11 animals indicated that BPA was non-detectable at all times post-dosing. BPA-glucuronide was detected only at 15 minutes post-dosing in pooled samples of yolk sac, but not in pooled embryos. For gd 13 and 16 animals, BPA and BPA-glucuronide were detected in yolk sac/placenta at 15 minutes and 12 hours post-dosing. BPA was detected in embryonic tissue only at 15 minutes post-dosing from gd 13 animals. BPA and BPA-glucuronide were detected only in pooled samples of foetal tissue from gd 16 animals at 15 minutes post-dosing.

Part 3 - Maternal plasma, placental and foetal concentrations of free, unconjugated BPA and BPA-glucuronide were confirmed in a separate experiment (part 3 of the study) where gd 16 animals received a higher specific activity of BPA. At 15 minutes post-dosing, the percentage of the administered dose recovered in maternal plasma, placenta (combined value per dam) and foetus (combined value per dam) ranged from 0.12 – 0.24%, 0.05 – 0.13% and 0.005 – 0.020%, respectively. These data show that the distribution of the total ^{14}C -BPA-derived material to the placenta was only about $\frac{1}{4}$ of that found in the maternal plasma and that the distribution of the total ^{14}C -BPA-derived material to the foetal tissue was about $\frac{1}{10}$ of that found in the placenta.

The concentrations of BPA detected in maternal plasma, placenta and foetus at 15 minutes post-dosing were 0.064, 0.0953 (1.5 times the concentration in maternal plasma) and 0.0176 (18% of the concentration in the placenta) $\mu\text{g/g}$ plasma-tissue respectively, and the concentrations of BPA-glucuronide measured in maternal plasma, placenta and foetus at 15 minutes post-dosing were 1.699, 0.3421 (20% of the concentration in maternal plasma) and 0.0130 (4% of the concentration in the placenta) $\mu\text{g/g}$ plasma-tissue respectively. These values were in agreement with those obtained in part 2 of the study where a lower specific activity was used. It can be seen that BPA-glucuronide in maternal plasma distributes to placenta and foetus only to a limited degree, 20% to placenta and 4% to the foetal tissue, and that the distribution of BPA-glucuronide corresponds to the distribution of ^{14}C -BPA radioactivity from maternal plasma to placental and foetal tissue. BPA from maternal plasma appears to be better distributed to placental tissue, and to a reduced degree to foetal tissue. Accordingly, the ratio of BPA-glucuronide to BPA is altered in these tissues as compared to plasma, the ratios being 27, 4 and 0.7 for maternal plasma, placental tissue and foetal tissue, respectively. This decrease in the ratio of BPA-glucuronide to BPA concentrations in the placenta as compared with the maternal plasma may have been a result of the ample glucuronidase activity found in the rat placenta which could hydrolyse some of the BPA-glucuronide that is distributed from maternal plasma to placental tissue back to BPA, rather than a result of increased distribution of free, unconjugated BPA from maternal plasma. It is also reported in the literature that UDP-glucuronosyltransferase is not fully developed in foetal liver; therefore, conversion of BPA to BPA-glucuronide may be saturated in the foetus after exposure to 10 mg/kg BPA. This would provide an explanation for the decreased ratio of BPA-glucuronide to BPA concentrations observed in the foetal tissue as compared with the placental tissue after exposure to a high dose of BPA.

Overall, these findings clearly demonstrate that very low levels of BPA are distributed to the foetus following a relatively high oral dose of BPA (10 mg/kg). However, the distribution of BPA to the foetus (or placenta) did not alter the overall pharmacokinetic fate of BPA in pregnant rats as compared to non-pregnant rats. Therefore, the rates of transfer in and out of the foetus were apparently not the rate-limiting processes in determining the overall elimination half-life of BPA from the maternal-foetal "unit".

The disposition of oral doses of BPA in pregnant SD rats was similar to that found in non-pregnant rats. ^{14}C -BPA-derived radioactivity in plasma was quantifiable through 48 hours post-dosing. The time course of radioactivity in plasma demonstrated two peaks of radioactivity over the first 24 hours post-dosing in both pregnant and non-pregnant animals. This pattern of a primary and a secondary peak in total plasma radioactivity was clear evidence that enterohepatic recirculation of ^{14}C -BPA-derived radioactivity occurred in both pregnant and non-pregnant SD rats.

Free, unconjugated BPA in plasma was quantifiable only at a few time points in most groups, if at all, and when detected, was very close to the level of quantitation. In contrast to the level of unchanged BPA, BPA-glucuronide represented the largest fraction of quantifiable plasma radioactivity, up to 100%, for all groups. Plasma concentrations of BPA-glucuronide also demonstrated two peaks with time in both pregnant and non-pregnant animals. These data again offer clear evidence of enterohepatic recirculation as a major factor in the metabolism of BPA in both pregnant and non-pregnant SD rats. Clearly, BPA-glucuronide is the predominant form that is circulating and systemically available.

In summary, the tissue distribution, metabolism, or the rates or routes of excretion of BPA, or the plasma concentration-time profiles of BPA-glucuronide did not appear to be altered at any stage of gestation investigated as compared to non-pregnant rats. In addition, no selective

affinity of either yolk sac/placenta or embryo/foetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in this study. Maternal and embryo/foetal exposure to free, unconjugated BPA did occur following oral administration, but systemic levels were found to be low due to extensive first pass metabolism.

The same research group also carried out a GLP-compliant study investigating the toxicokinetics of BPA in neonatal rats (Domoradzki *et al*, 2004). In this study, a single dose of ^{14}C -labelled BPA was administered by oral gavage to neonatal rats at post-natal day (pnd) 4, 7, and 21, and also to 11-week old adult rats for reference.

Neonatal Sprague-Dawley animals of both sexes were dosed with ^{14}C -BPA at either 1 or 10 mg/kg, and at each of 8 time points between 15 minutes (the time point expected for the peak plasma concentration of free, unconjugated BPA) and 24 hours after dosing, animals (3 per age/sex/dose) were anaesthetised and sacrificed to provide samples of blood plasma, brain, kidney, liver, carcass/skin and testes or uterus and ovaries.

Groups of male and female adult animals of the same strain (4 animals/sex) were dosed at 10 mg/kg and housed in metabolism cages to enable collection and analysis of urine and faeces produced over 12-hour or 24-hour time periods. Samples of blood were collected from these adult animals by in-dwelling jugular vein cannula at 11 time points between 15 minutes and 96 hours after dosing for provision of plasma. At the end of this 96-hour time period these animals were sacrificed, and samples of blood, brain, carcass, fat, kidneys, liver, skin and testes or uterus and ovaries were collected.

Samples of plasma, urine and faeces from individual animals taken at these sampling times were assayed for total ^{14}C -BPA-derived radioactivity by liquid scintillation counting, and the mass balance of radioactivity determined. As in the previous study, samples of plasma taken from animals of the same age at the same time-point were pooled, and the concentrations of radiolabelled compounds in these pooled samples were measured by HPLC with radiochemical detection. The HPLC peaks produced by free, unconjugated BPA and BPA-glucuronide were identified by mass spectrometry/electrospray ionisation.

During the study there were no deaths, and no signs of treatment-related toxicity were observed. At the dose of 10 mg/kg, ^{14}C -BPA-derived radioactivity in the plasma of animals of all ages rose to a peak value within an hour of administering BPA, and then declined over the next few hours. In neonatal animals after the initial peak, plasma radioactivity declined over the subsequent 24 hours, and mean half-lives were in the region of 4 to 10 hours. In adult animals plasma radioactivity fell during the 6 hours after dosing, but over the next 18 hours rose slightly before falling to low levels at 96 hours. The highest peak levels of ^{14}C -BPA-derived radioactivity were found in the plasma of pnd 4 neonates, where peak values were between 20 and 100 times higher than in adults. In neonates at pnd 7 and pnd 21, peak values were between 10 and 20 times higher than at 11 weeks. The reason for the much higher peak values of ^{14}C -BPA-derived radioactivity in the plasma of young neonates than in adult animals was not discussed by the study authors, but it is likely to reflect an immaturity in the development of hepatic excretory functions in neonatal rats. In adult females plasma ^{14}C -BPA-derived radioactivity fell more rapidly than in adult males, and the calculated half-life ($t_{1/2}$) in females was 15 hours, compared with 22 hours in males. In neonates at pnd 7 and pnd 21 this sex difference in the decline of plasma radioactivity was less marked, and was not seen at pnd 4. At the 1 mg/kg dose of BPA, radioactivity was not detected in the plasma of adult animals at any time point, but the kinetics of plasma radioactivity in neonatal animals followed the profile seen at the higher dose.

Following administration of BPA at 10 mg/kg, HPLC showed the presence in the plasma of at least 13 different radiolabelled compounds. Of these, the two compounds present in the largest amounts were peaks 6 and 8, which were identified as BPA-glucuronide and free, unconjugated BPA respectively. The other radiolabelled compounds detected in the plasma were not characterised. In general, fewer individual radiolabelled compounds were detected in the plasma of older animals. The number of radioactive peaks detected in the plasma at pnd 4 was 12 in male animals and 11 in female animals, whereas at pnd 7 in male and female animals 7 and 8 different peaks respectively were detected. In animals at pnd 21 and at 11 weeks only 3 or 4 different radioactive peaks were detected. The study authors concluded that this reflected the conversion of BPA to a larger number of metabolites in younger neonates than in older animals and indicated saturation of the BPA glucuronidation pathway. However, since the total concentration of radiolabelled compounds appearing in the plasma was found to be considerably higher at pnd 4 and at pnd 7 than at pnd 21 and at 11 weeks, an alternative explanation for the larger number of peaks in younger neonates might be that in the plasma of animals at pnd 21 and at 11 weeks some minor metabolites had been present below the limit of detection. These data also indicate a dose dependency in the metabolism and pharmacokinetics of BPA administered to neonates with nearly complete metabolism of BPA to BPA-glucuronide (94-100% of the plasma radioactivity) at a dose of 1 mg/kg and with up to 13 different plasma metabolites observed at the 10 mg/kg dose.

At 10 mg/kg ^{14}C -BPA, the concentration of free, unconjugated BPA in the plasma of all animals rose to a peak within 2 hours of dosing, and subsequently fell over the period from 2 to 24 hours after dosing. Peak plasma concentrations of free, unconjugated BPA were highest in pnd 4 neonates, and lowest in adults; the difference between the peak values at the two ages was over 150 times. The reason for the much higher peak values of free, unconjugated BPA in the plasma of young neonates than in adult animals was not discussed by the study authors, but it is likely to be partly due to increased activity of the glucuronidation pathway in adults. In neonates, free, unconjugated BPA in the plasma fell with a half-life in the region of 4 to 17 hours. After the administration of 1 mg/kg ^{14}C -BPA, the kinetics of free, unconjugated BPA in animals at pnd 4 and 7 tended to follow the pattern seen at the higher dose. Free, unconjugated BPA was not detected in the plasma of animals at pnd 21 or at 11 weeks after administration of the lower dose.

BPA-glucuronide was detected in the plasma of animals of all ages at all time-points during the 24 hours after dosing with 10 mg/kg ^{14}C -BPA. In neonates, plasma BPA-glucuronide peaked within 2 hours after dosing and then fell over the period between 2 and 24 hours after dosing. The decay followed first-order kinetics, with a half-life in the region of 4 to 10 hours. In adult animals the plasma concentration of BPA-glucuronide fell during the one-hour period after dosing, but rose to a second peak between 12 and 24 hours after dosing. After administration of 1 mg/kg ^{14}C -BPA, BPA-glucuronide was not detected in the plasma of adult animals. In neonatal animals, the kinetics of BPA-glucuronide followed the pattern seen at the higher dose. These profiles of BPA-glucuronide in the plasma suggest that in adults, but not in neonates, BPA undergoes enterohepatic circulation. In neonates, there were no sex differences in the kinetics of BPA-glucuronide, but the plasma concentration of BPA-glucuronide fell more rapidly in adult females than in adult males, with calculated half-lives of 11 and 22 hours respectively. These data indicate that the half-lives for the elimination of BPA-glucuronide in plasma were more rapid in neonatal animals than in adults, likely due to reduced microflora β -glucuronidase activity and an absence of enterohepatic recirculation in neonates. As it had already been found for BPA-derived radioactivity and for free, unconjugated BPA, the peak concentration of BPA-glucuronide in the plasma was higher in neonates than in adults; the difference was between 9-fold and 22-fold. As for total ^{14}C -BPA-

derived radioactivity, the reason for the higher peak values of BPA-glucuronide in the plasma of neonates than in adult animals was not discussed by the study authors, but it is likely to reflect poorly-developed excretory functions.

The ratio of BPA-glucuronide to free, unconjugated BPA in the plasma was calculated by comparing the areas under the curve (AUC) for the plots of BPA-glucuronide concentration and free, unconjugated BPA concentration against time, although in adult animals given 10 mg/kg ^{14}C -BPA, and in adult and pnd 21 animals given 1 mg/kg ^{14}C -BPA, the ratio could not be determined. For the 10 mg/kg dose of ^{14}C -BPA, the AUC ratio of BPA-glucuronide to free, unconjugated BPA was found to be in the region of 3 to 7 at pnd 4, between 31 and 36 at pnd 7 and in the region of 55 to 56 at pnd 21. For the 1mg/kg dose, in animals at both pnd 4 and pnd 7, this ratio was found to be in the region of 45 to 96. The fact that for the pnd 4 animals the AUC ratio of BPA-glucuronide to free, unconjugated BPA was found to be an order of magnitude lower at the high dose than at the lower dose, suggests that the glucuronidation pathway may have been subject to substrate saturation at the higher dose. On the other hand, with the pnd 7 animals, the AUC ratios at the two dose levels were similar, suggesting that substrate saturation of the pathway had not occurred.

In relation to BPA excretion, this study did not investigate this kinetic parameter in neonates but only in adult animals. In adult rats dosed with 10 mg/kg ^{14}C -BPA, the major route of excretion was in the faeces. During the 96-hour period after dosing 75% of the radioactivity administered appeared in the faeces. The rate of excretion appeared to be higher in females than in males; in the 24 hours after dosing 41% of the administered dose appeared in the faeces of females, compared with 24% in males. During the 96 hours after dosing 15% of the administered dose was excreted in the urine of both males and females. The initial rate of urinary excretion of radioactivity also appeared to be higher in females than in males; in the 12 hours after dosing 4.1% of the administered dose was excreted in the urine of females compared with 1.5% in males. Ninety-six hours after giving ^{14}C -BPA at 10 mg/kg, the proportion of the administered dose which remained in the tissues and carcass of adult animals was 4%. In this study, the contributions of individual radiolabelled compounds to ^{14}C -BPA-derived radioactivity in excreta were not investigated. However, historical data were presented which showed that in previous studies of adult rats given the same oral dose, most of the faecal radioactivity was found to be in the form of free, unconjugated BPA, while most of the urinary radioactivity was in the form of BPA-glucuronide.

At sacrifice, in animals of all ages, the concentration of ^{14}C -BPA-derived radioactivity expressed per unit tissue weight was lowest in the brain, slightly higher in the testes and tended to be higher still in the kidney and liver. In neonatal animals, radioactivity was found to be highest in the skin/carcass. In the tissues of adult animals, the skin, carcass and fat were sampled separately; the carcass was found to contain the highest concentration of radioactivity, with the skin and fat containing only low concentrations.

The kinetics of ^{14}C -BPA-derived radioactivity in individual tissues tended to follow those of plasma radioactivity, with tissue concentrations peaking within 2 hours of dosing and subsequently falling with a half-life of several hours. In general in animals at pnd 4 and pnd 7 the concentrations of radioactivity in the tissues tended to be higher, and half-lives tended to be longer, than in animals at pnd 21. The tissue/plasma ratio of ^{14}C -BPA-derived radioactivity in all groups of neonatal animals was lowest in the brain where the ratio was less than 0.25. In the testes, the ratio was also low, being less than 0.6 in all groups of neonatal animals. The tissue/plasma ratio tended to be in the region of 1 in liver and kidney, and between 1 and 3 in the skin/carcass across all groups of neonatal animals. Regarding the ovaries and uterus, the tissue/plasma ratio tended to be in the region of 1, although in pnd 4

animals at 15 minutes, 1.5 hours or 3 hours after treatment with 10 mg/kg ^{14}C -BPA, the tissue/plasma ratio was between 3 and 5. However this finding of high radioactivity levels in ovaries and uterus was not reproduced in pnd 4 animals given 1 mg/kg ^{14}C -BPA.

In summary, this study found that BPA was rapidly absorbed from the GI tract and metabolised to BPA-glucuronide in both neonatal and adult rats. The capacity of the glucuronidation pathway was more prone to substrate saturation at pnd 4 than at pnd 7, suggesting an age-dependent increase in metabolic capacity consistent with an age-dependent development of glucuronyl transferases. The plasma concentrations of free, unconjugated BPA and BPA-glucuronide peaked within 2 hours of dosing, and peak concentrations were several times higher in the youngest neonates than in adults, suggesting that neonates have poorly-developed excretory functions. In adult animals, but not in neonates, plasma levels of BPA-glucuronide underwent a secondary rise between 12 and 24 hours after dosing, suggesting that BPA was subject to enterohepatic circulation. There were no sex differences in the kinetics of BPA-glucuronide in neonates, but in adult animals BPA-glucuronide was removed from the circulation more rapidly in females than in males, probably due to faster excretion. A number of minor metabolites of BPA were detected in plasma but not identified. In general, fewer metabolites were detected in older animals reflecting the conversion of BPA to a larger number of metabolites in younger neonates than in older animals and indicating a likely saturation of the glucuronidation pathway in the younger animals. In both neonatal and adult animals, ^{14}C -BPA-derived radioactivity tended to concentrate in the skin/carcass, but concentrations in the brain and testes were found to be lower than in the plasma.

Another recent study investigated the contribution of biliary excretion to the elimination of oral and intravenous doses of BPA in rats (Kurebayashi *et al*, 2003). In the first part of the study, adult male Fischer-344 rats (3 per dosing schedule) were given a single, low dose of ^{14}C -labelled BPA (0.1 mg/kg) administered either by oral gavage or intravenously. In addition, for each of these dosing schedules, 6 animals (3 males and 3 females) had the bile duct cannulated, and bile was collected over 2-hour periods till 6 hours after dosing. In the second part of the study, designed to characterise in detail BPA metabolites, 3 adult male rats of the same strain were given a single high dose (100 mg/kg) of deuterium-labelled BPA or unlabelled BPA by oral gavage. For this high dose experiment, another 3 male animals had the bile duct cannulated for collection of bile over the 18-hour period after dosing. Animals were housed individually in metabolic cages for the collection of faeces and urine produced over the first and second 24-hour periods after dosing.

Blood samples were taken at 9 selected time points between 15 minutes and 48 hours after dosing. Total ^{14}C -BPA-derived radioactivity was measured in samples of blood, plasma and excreta from low dose rats by scintillation counting. In addition, samples were assayed for free, unconjugated BPA and individual BPA metabolites by HPLC with scintillation counting, mass spectrometry/electrospray ionisation or NMR spectroscopy. BPA metabolites isolated by HPLC were subjected to enzymatic hydrolysis with glucuronidase or sulphatase to confirm their composition.

In the male rats given a low dose of BPA by intravenous administration, blood levels of ^{14}C -BPA-derived radioactivity initially fell rapidly with an elimination half-life of 0.6 hours. This phase was followed by a second longer phase with a terminal elimination half-life of 40 hours. The clearance from the circulation was 8 ml/min/kg body weight. The distribution volume (27 litres/kg bw) was high, suggesting substantial binding to plasma proteins/and or tissues. In fact, partition measurements showed that the proportion of ^{14}C -BPA-derived radioactivity in the blood bound to plasma proteins was relatively high (95%). In the male animals given the low dose of BPA orally, ^{14}C -BPA-derived radioactivity in the blood

initially rose to a peak 0.4 hours after dosing, and subsequently fell with a terminal elimination half-life of 45 hours. An analysis of the AUCs for blood ^{14}C -BPA-derived radioactivity over the 48-hour period after dosing showed an oral bioavailability of 86%. During the 48-hour period after oral or intravenous dosing, most (78-82%) of the ^{14}C -BPA-derived radioactivity was found to be eliminated in the faeces, with a relatively small proportion (10-12%) appearing in the urine.

Measurements in the rats with cannulated bile ducts showed that a sizeable fraction (45-66%) of the administered radioactivity was excreted into the bile within 6 hours of oral or intravenous dosing. The proportion of the administered dose excreted was slightly higher in males than in females. HPLC analysis of the bile produced during this period following oral or intravenous administration suggested that 84-88% of biliary radioactivity was in the form of BPA-glucuronide. The nature of the other radiolabelled species in the bile was not reported.

In experiments where rats were given a high dose of unlabelled BPA by the oral route, 41% of the administered dose was excreted in the bile during the 18-hour period after dosing. HPLC analysis revealed that most of this (>99%) was in the form of BPA-glucuronide. Free, unconjugated BPA was present in trace amounts and BPA-sulphate was not detected. Analysis of the excreta produced within 72 hours of dosing revealed that most (61%) of the administered dose was eliminated from the body in the faeces, with only 8% of appearing in the urine. HPLC analysis revealed that the faeces contained only free, unconjugated BPA, with BPA glucuronide and BPA-sulphate not being detected. The urine contained mostly (82%) BPA-glucuronide, with free, unconjugated BPA and BPA-sulphate making minor contributions (14% and 4% respectively).

In summary, in this study the toxicokinetics and fate of BPA were found to be similar when free, unconjugated BPA was given at a low dose either intravenously or orally, and at a high dose orally. After oral administration BPA was absorbed rapidly and efficiently from the GI tract (up to 86% of the administered dose). A high proportion of the dose (45-66%) was rapidly excreted in the bile in the form of BPA-glucuronide, with the rate of biliary excretion tending to be higher in males than females. With all three dosing schedules most (78-82%) of the administered dose was eliminated from the body in the faeces, with only a small proportion of the dose appearing in the urine (10-12%). In the low dose experiments the chemical identity of this form was not investigated, but in the high dose experiments most of the faecal radioactivity was found to be in the form of free, unconjugated BPA. Since BPA has a high oral bioavailability, the free, unconjugated BPA in the faeces is more likely to be derived from BPA glucuronide excreted in the bile and hydrolysed to free BPA in the GI tract, rather than representing unabsorbed free BPA which might have passed along the GI tract into the faeces unchanged.

EFSA (2006) noted that most of the studies examining the toxicokinetics of BPA in rodents used rats as experimental models. In contrast to primates, in adult rats several studies using oral doses of BPA ranging from 20 $\mu\text{g}/\text{kg}$ bw to 100 mg/kg bw have confirmed that BPA-glucuronide formed in the liver and the intestinal wall after oral administration undergoes enterohepatic recirculation after cleavage of the glucuronide back to BPA and most of the dose is slowly excreted with faeces (Kurebayashi *et al.*, 2005; Sakamoto *et al.*, 2002). Urinary excretion of BPA and its metabolites in rats is strain-specific and accounts for 10 to 40 % of applied dose; the major metabolite present is BPA-glucuronide, but a small percentage of the applied dose is recovered in urine as parent BPA. In faeces of rats dosed orally with ^{14}C -BPA, the majority of the BPA-derived radioactivity was attributed to the

parent compound. Other BPA metabolites, such as a diglucuronide or a mixed sulphate/glucuronide represent minor identified BPA metabolites in rats.

EFSA (2006) commented that, in summary, these results, in combination with results from previous publications (Pottenger *et al.*, 2000; Snyder *et al.*, 2000), confirm that BPA in rats is mainly metabolised to BPA glucuronide and excreted from the liver with the bile. This is in contrast to the situation in humans where BPA-glucuronide is excreted through the urine because the threshold for biliary elimination (MW of 350 D) is lower in rats than primates (MW of 500 D). Thus, BPA in rodents is subject to enterohepatic recirculation irrespective of dose and route of administration, resulting in slow elimination with apparent terminal elimination half-lives between 19 and 78 h (Domoradzki *et al.*, 2004; Kurebayashi *et al.*, 2003; Kurebayashi *et al.*, 2005; Pottenger *et al.*, 2000).

No study on the disposition and biotransformation of BPA in mice after oral administration was identified. One study addressed the biotransformation of a low dose (20 µg/kg bw) of ³H-BPA in pregnant CD-1 mice (Zalko *et al.*, 2003) after subcutaneous (sc) injection. BPA was extensively metabolised in CD-1 mice. Identified metabolites included BPA-glucuronide as major metabolite, but also several double conjugates, and conjugated methoxylated derivatives. Fetal radioactivity (more than 4% of the administered radioactivity 24 hours after administration of BPA) was associated with free BPA, BPA-glucuronide, and a disaccharide conjugate. While these data suggest a more intensive biotransformation of BPA in CD-1 mice and a higher contribution of metabolic oxidation to this biotransformation, a quantitative comparison of differences in rat and mouse biotransformation of BPA is not possible due to differences in study design, route of exposure and periods of observation after BPA administration. Furthermore, formation of metabolites attributable to a metabolic oxidation of BPA has not been observed in studies in rats or in primates *in vivo*.

In studies in which subcellular fractions from the liver of both mice and rats were incubated with BPA, a number of BPA-metabolites were reported to be formed by oxidative biotransformation. These were identified as 4-methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, isopropyl-hydroxyphenol, a BPA glutathione conjugate, glutathionyl-phenol, glutathionyl 4-isopropylphenol, and BPA dimers (Jaeg *et al.*, 2004; Yoshihara *et al.*, 2004). One of the BPA metabolites formed by S-9 catalyzed oxidation, 4-methyl-2,4-bis(phydroxyphenyl)pent-1-ene, has an approximately two orders of magnitude greater affinity to the oestrogen receptor alpha as compared to BPA (Yoshihara *et al.*, 2004). However, there is no evidence that these metabolites are formed to a larger extent in rats or in primates *in vivo* or in intact hepatocytes from rodents or humans (Pritchett *et al.*, 2002), likely due to an effective glucuronidation of BPA (Domoradzki *et al.*, 2003; Kurebayashi *et al.*, 2003; Kurebayashi *et al.*, 2002; Kurebayashi *et al.*, 2005; Pottenger *et al.*, 2000; Snyder *et al.*, 2000; Volkel *et al.*, 2005; Volkel *et al.*, 2002).

PBPK and PBPK modelling

In the EFSA (2006) opinion, some consideration is given to the available PBPK and PBD models for BPA.

Blood concentrations of BPA after human dietary exposures were estimated using two

physiologically based toxicokinetic models developed for BPA. These models successfully predicted experimental BPA toxicokinetics in humans (Volkel *et al.*, 2002; Teeguarden *et al.*, 2005). Experimentally determined partition coefficients, plasma protein binding, binding of BPA to the oestrogen receptor alpha and its oestrogenic activity in competition with oestradiol, and the kinetics of BPA elimination by glucuronidation after oral administration in humans were incorporated into a physiological toxicokinetic-toxicodynamic model to predict age dependently the concentrations of free (non-protein bound) BPA in blood and other tissues. When simulating a daily dietary uptake of 1 µg BPA/kg bw separated into three meals, peak concentrations of free (not bound to plasma proteins) BPA in blood were predicted as 3 pmol/l in a one year old child and as 3.7 pmol/l in 50 year old adults. Normalised for the oestrogenic activity of endogenous 17-β-oestradiol, the highest increase in the oestrogenic activity induced by this dose of BPA was calculated to be 0.22% (Filser *et al.*, 2003) for 11-year old boys (lowest circulating 17-β-oestradiol levels).

Computational modeling of the possible effects of plasma protein binding of oestradiol and BPA, incorporating affinities of oestradiol and BPA to different binding proteins and physiologic concentrations of these proteins in rodents and in male and female humans, predicts that unless very high concentrations (> 100 nM) of BPA are reached in blood, oestradiol binding to the receptor will always dominate. Therefore, under realistic blood concentrations expected in humans from oral exposure to BPA from diet in the range of up to 0.05 nM, only a very small fraction of the oestrogen receptor will be occupied by BPA. Occupancy of the oestrogen receptor by BPA is predicted to be further decreased when the rapid elimination of BPA is incorporated into the modelling (Teeguarden and Barton, 2004; Teeguarden *et al.*, 2005).

4.1.2.1.3 Impact of new information and summary of toxicokinetics

The new information on the toxicokinetics of BPA in humans and in pregnant and non-pregnant rodents of different ages provides an important contribution to our knowledge of the kinetic properties of BPA. However, the most significant impact of the new information for risk assessment purposes arises from the studies in humans. These studies have demonstrated that at comparable exposure levels the blood concentrations of free BPA in humans are much lower than those in rodents, indicating that there are important quantitative differences in the fate of BPA between humans and rodents.

The toxicokinetics of BPA have been well studied in rats both *in vivo* and *in vitro*, and have been investigated to a lesser extent in mice and cynomolgus monkeys. Two studies have investigated the toxicokinetics and fate of an oral dose of labelled BPA in human volunteers.

Absorption

In the species studied (rats, mice, monkeys, humans), the available evidence suggests that following oral administration, BPA is rapidly and extensively absorbed from the gastrointestinal tract. Analysis of plasma AUC values suggests that the extent of absorption from the GI tract is up to 86% in rats and up to 85% in monkeys. The only relevant human studies suggest that, on the basis of the recovery of labelled BPA-glucuronide from the urine, a relatively low dose of BPA (54-88 µg/kg) was completely absorbed after oral dosing.

An *in vitro* dermal absorption study using human skin found limited absorption of BPA at millimolar concentrations; the extent of absorption was in the region of 10% of the applied dose.

There are no data on the toxicokinetics of BPA following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and the high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first-pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

Metabolism

The available data indicate that BPA is subject to extensive first-pass metabolism following absorption from the gastrointestinal tract.

In all species studied, the major metabolic pathway involves conjugation of BPA to BPA-glucuronide. Studies conducted in rats suggest that in neonates the glucuronidation pathway is more susceptible to saturation than in adults indicating an age-dependent increase in metabolic capacity. *In vitro* studies with microsomal preparations also suggest species differences, with the rank order for the metabolic clearance rate per unit weight of tissue being mice > rats > humans. When the total clearance rates for the whole liver were calculated, the rank order was reversed (humans > rats > mice).

In addition to the glucuronidation pathway, *in vivo* and *in vitro* studies suggest that in the rat, BPA may be subject to limited oxidation to bisphenol O-quinone by cytochrome P450, and also to conjugation to BPA-sulphate and 5-hydroxy-BPA.

A study in pregnant mice given subcutaneous doses of BPA also found that glucuronidation was the major pathway for the metabolism of BPA, although dehydrated, sulphated and methoxylated conjugates of BPA were also produced. Some minor metabolites were double conjugates, such as a double conjugate of BPA with glucuronide and N-acetyl galactosamine which was found in the intestine, placenta, amniotic fluid and foetal tissue. A study in cynomolgus monkeys showed that BPA-glucuronide was the major metabolite, although there was evidence for production of a minor metabolite, possibly BPA-sulphate or 5-hydroxy-BPA. Studies conducted in humans provide evidence for the glucuronidation of BPA in man; some studies also found evidence for the sulphation of BPA.

Distribution

Most studies investigating the distribution of BPA measured tissue radioactivity levels after giving labelled BPA to experimental animals. An oral dosing study in rats found that the tissue concentrations of BPA-derived-radioactivity were highest in the liver, kidney and carcass, and lowest in the brain and testes, and there were no large differences between adult and neonatal animals. A number of studies in rats suggest that BPA metabolites and especially free BPA have a limited distribution to the embryo/foetal or placental compartments following oral administration. No selective affinity of either yolk sac/placenta or embryo/foetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in a recent study in rats after oral dosing. However, maternal and embryo/foetal

exposure to free BPA did occur, but systemic levels were found to be low due to extensive first-pass metabolism.

Regarding the distribution of free, unconjugated BPA to tissues after oral dosing, since free BPA is removed rapidly from the blood after absorption by first pass metabolism, it has been suggested that in animals the availability of free BPA to extrahepatic tissues is likely to be limited following oral exposure. In adult rats it has been estimated that no more than 5-10% of the administered dose of free BPA is available to the tissues, although this figure may be higher in neonates. In humans, the systemic availability of free BPA is very low as enterohepatic recirculation of BPA does not occur.

In summary, there are differences between humans and rodents in the distribution of BPA. After oral administration, BPA is rapidly metabolised in the gut wall and the liver to BPA-glucuronide. This metabolite is devoid of endocrine activity. In humans, the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion. Due to the rapid biotransformation and excretion ($t_{1/2} = 5$ hours) and plasma protein binding, peak free BPA concentrations in humans after oral exposure that are available for estrogen receptor binding are very low. In contrast, BPA glucuronide is eliminated in bile in rodents and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic recirculation results in slow excretion ($t_{1/2} = 15-22$ hours) and increased systemic availability of free BPA in rodents.

This conclusion is supported by the observation that in urine of rats dosed orally with BPA, a part of the dose was excreted as free BPA in urine (1 -4 % of applied dose, whereas BPA-glucuronide in urine accounted for 20-40 % of applied dose). In both of the human studies and the monkey study free BPA was below the limit of detection in all urine and blood samples (equivalent to a ratio of free BPA to BPA-glucuronide of < 0.5 %). Since free BPA found in urine is translocated from blood to urine in the kidney, these observations of higher free BPA levels in urine of rats compared with primates further support the existence of species differences in blood levels of free BPA between rodents and humans with higher AUCs for free BPA in rats.

Excretion

The major route of elimination in the rat is via the faeces. The available data indicate that the percentage of the administered dose recovered in the faeces is in the range 50% to 83%. Urinary excretion is of secondary importance in the rat, with 13% to 42% of the administered dose being recovered in the urine. Over 7 days post-dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post-dosing. A sex difference was also observed for urinary elimination, with females excreting approximately twice as much radioactivity (24-28%) than males (14-16%). A study in female SD rats found that excretion was not affected by pregnancy at 3 different stages of gestation. Data from a number of studies suggest limited excretion of BPA in the milk. However, the data do not allow a reliable quantitative determination to be made.

Following oral administration to rats, a high proportion of the administered dose (45-66%) was rapidly excreted in the bile in the form of BPA-glucuronide, with the rate of biliary excretion tending to be higher in males than females. Most of the faecal radioactivity was found to be in the form of free BPA. Since BPA has a high oral bioavailability in the rat, the free BPA found in the faeces is more likely to be derived from BPA-glucuronide excreted in the bile and hydrolysed to free BPA in the gastrointestinal tract rather than representing unabsorbed BPA which might have passed along the gastrointestinal tract into the faeces

unchanged. Most of the urinary radioactivity was found to be in the form of BPA-glucuronide (82%) with free BPA and BPA-sulphate making minor contributions (14% and 4% respectively).

In contrast to the findings in rodents, in cynomolgus monkeys given BPA orally most of the administered dose (82–85%) was recovered in the urine, with only 2-3% of the dose being recovered in the faeces. In two studies in human volunteers given a low dose of BPA orally, the administered dose was completely recovered in the urine as BPA-glucuronide. No free BPA was detected and no gender differences in the kinetics of BPA-glucuronide in plasma and urine were reported.

4.1.2.2 Acute toxicity

4.1.2.2.1 Summary of original risk assessment report

No useful information is available on the effects of single exposure to BPA in humans. Oral LD₅₀ values beyond 2,000 mg/kg are indicated in the rat and mouse, and dermal LD₅₀ values above 2,000 mg/kg are evident in the rabbit. Few details exist of the toxic signs observed or of target organs. For inhalation, a 6-hour exposure to 170 mg/m³ (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed. These data indicate that BPA is of low acute toxicity by all routes of exposure relevant to human health.

4.1.2.2.2 Updated information

There is no significant new information on the acute toxicity of BPA.

4.1.2.3 Irritation

4.1.2.3.1 Summary of original risk assessment report

Limited human anecdotal information of uncertain reliability is available from written industry correspondence suggesting that workers handling BPA have in the past experienced skin, eye and respiratory tract irritation. It cannot be determined whether the reported skin reactions were related to skin sensitisation or irritation. However, a recent well conducted animal study clearly shows that BPA is not a skin irritant. A recent well conducted animal study shows that BPA is an eye irritant; effects persisted until the end of the study (day 28 postinstillation) in 1 of 3 rabbits. Overall, taking into account the animal and human evidence, BPA has the potential to cause serious damage to the eyes.

Slight and transient nasal tract epithelial damage was observed in rats exposed to BPA dust at 170 mg/m³ for 6 hours. Slight local inflammatory effects in the upper respiratory tract were observed in rats exposed to 50 mg/m³ and 150 mg/m³ of BPA in 2 and 13 week repeat inhalation studies, but were not observed at 10 mg/m³ in the same studies. Increased duration of exposure did not increase the severity of the response at 50 and 150 mg/m³. Taken together with anecdotal human evidence, these data suggest BPA has a limited respiratory irritation potential.

4.1.2.3.2 Updated information

There is no significant new information on the irritation of BPA.

4.1.2.4 Corrosivity

The data available and summarised above show that BPA is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Summary of original risk assessment report

There are several reports of patients with dermatitis responding to BPA in patch tests. However, it is unclear whether BPA or related epoxy resins were the underlying cause of the hypersensitive state. Anecdotal information indicates skin inflammation in workers handling BPA, although given the uncertain reliability of this information no conclusions can be drawn from it. In animals, a skin sensitisation test performed to current regulatory standards is not available. The available studies are negative, but the test reports lack detail and no reliable justifications were given for the choice of concentrations used. In the study using the highest challenge concentration, 50% in a guinea pig closed-patch test, a sensitisation rate of 12.5% was obtained. It is possible that the concentrations used in all the available studies were not maximised and a greater response might have been obtained with higher induction and challenge concentrations. Based on the findings from the most robust study, BPA may possess a skin sensitisation potential, albeit a limited one. BPA in the presence of UV light can also elicit skin responses in humans, and reproducible positive results for photosensitisation have been obtained in mouse ear swelling tests. Mechanistic studies in mice have suggested this is an immune-mediated process. Therefore, examination of the available human and experimental animal studies leaves the picture somewhat unclear as to whether one or more of the following are properties of BPA; (1) orthodox skin sensitisation (2) photosensitisation (3) BPA eliciting a response in people previously skin sensitised to another substance (e.g. epoxy resins).

Overall, it is clear that skin reactions can be a potential consequence of repeated skin exposure in humans. Thus, taking all of these data available into account, BPA is considered capable of producing skin sensitisation responses in humans. There are no data from which to evaluate the potential of BPA to be a respiratory sensitiser.

4.1.2.5.2 Updated information

Animal data

In a GLP-compliant, modified Local Lymph Node Assay (LLNA) recently submitted by industry (Vohr, 2002), the skin sensitisation potential of BPA was investigated according to a slight modification of the OECD TG 429. This modification involved measurement of cell proliferation in the draining lymph nodes by cell count determinations instead of radioactive labelling, and measurement of ear swelling and ear weight (due to increased vascular

permeability in the associated inflammatory irritative response) as indicators of acute skin irritation reactions of the test substance (Homey et al., 1998). Groups of 6 female NMRI mice were treated epicutaneously on to the dorsal part of both ears with 25 µl of BPA in DAE 433 (40% dimethylacetamide, 30% acetone, 30% ethanol) at concentrations of 0, 3, 10 or 30%. No information is provided in the study report on how these concentrations were selected and why 30% was chosen as the highest concentration. DAE 433 was the vehicle of choice because of its photostability which made it suitable for the subsequent photo-LLNA (see below). This treatment was repeated on three consecutive days. One day after the last application, the animals were sacrificed and the auricular lymph nodes removed for cell count determinations. Ear swelling and ear weights were also measured. Stimulation indices for cell counts were then calculated by dividing the number of cell counts of the treated lymph nodes by the number of cell counts obtained from the control (vehicle) animals.

No systemic toxicity and no local irritation were observed at any of the concentrations tested. No dose-related or statistically significant increase in the stimulation indices was observed at any of the concentrations tested (stimulation indices of 1.25, 1.35 and 1.24 were obtained at 3, 10 and 30% respectively). No increase above controls was also noted for the ear swelling and the ear weight in any of the treated animals. The positive control, alpha hexyl cinnamic aldehyde (HCA) applied at concentrations of 3, 10 and 30% in DAE 433 produced the appropriate response. Although the positive level (stimulation index ≥ 1.3) was reached at a concentration of 10% BPA, this was neither dose-dependent nor statistically significant.

Overall, under the conditions of this LLNA, BPA did not show either a skin sensitisation or a skin irritation potential up to a concentration of 30%.

In a GLP-compliant LLNA (Vohr, 2003) modified to test for photoreactivity, 0, 3, 10 or 30% BPA in DAE 433 was applied to the dorsal part of both ears (25 µl/ear) of groups of 6 female NMRI mice on 3 consecutive days. The animals were irradiated with UV-light immediately after application (20J UV-A/cm²). A control group of 6 mice were treated with 30% BPA without exposure to UV-light. Again, no information is provided in the study report on how these concentrations were selected and why 30% was chosen as the highest concentration. On day 1 and 4 of the study, the ear swelling of the animals was measured using a spring-loaded micrometer, and mean ear swelling calculated. On day 4 of the study, the ear weight of the animals was also measured. These two parameters, ear swelling and ear weight were used as indicators of unspecific irritation reactions by the test substance. The mice were sacrificed on the fourth day and the auricular lymph nodes excised and transferred to sterile physiological saline. The lymph nodes were weighed and cell counts per ml determined. The stimulation indices were calculated by dividing the absolute weight or cell counts of the treated lymph nodes by the vehicle ones.

A "positive level" was considered by the author to be a stimulation index ≥ 1.3 . In this study, both the weight and cell count indices were less than 1 in all dose groups and hence were considered to be negative. A "positive level" for ear swelling was stated to be a 2×10^{-2} mm increase compared to the vehicle UV-A irradiated animals, and in this study the changes in ear swelling were less than this value and hence considered to be negative. The positive control, 8-methoxypsoralen applied at concentrations of 0, 0.1 and 0.3% in DAE 433 with UV-A irradiation and at 0.3% without UV-A irradiation produced the appropriate response.

Overall, under the conditions of this photo-LLNA, BPA did not show either a skin photo-sensitisation or a skin photo-irritation potential up to a concentration of 30%.

Human data

Medical surveillance information obtained from 5 out of the 6 BPA manufacture plants present in the EU was recently provided by industry (PlasticsEurope, 2007). During BPA manufacture, workers may be exposed to phenol, acetone and BPA. As phenol and acetone are not skin sensitisers, the assessment of the potential skin sensitising activity of BPA is not confounded by exposure to other chemicals in these factories. In company A, no cases of skin sensitisation were identified among 110 workers examined since 1991 (site 1) and among 190 workers examined since 1984 (site 2). In company B, no cases of dermatitis were identified among 500 workers examined since 1976, and in company C no cases were identified among 75 workers. Employees are examined every 1 to 3 years by a physician or a nurse. During examination, the condition of the skin is checked. Workers are also asked to report any work-related health problem.

4.1.2.5.3 Impact of new information and summary of sensitisation

A recent LLNA study has shown that BPA does not possess skin sensitisation potential. However, in this study the concentration of BPA was not maximised. Therefore, there remains some uncertainty as to whether high concentrations (> 30%) of BPA can still exert skin sensitising activity. Similarly, a recent photo-LLNA has shown that BPA does not possess skin photo-sensitisation potential. However, again, in this study the concentration of BPA employed was not maximised. Although there are sporadic reports showing that BPA in the presence of UV light can elicit skin responses in humans, comprehensive medical surveillance data obtained from BPA manufacture plants has shown that no cases of skin sensitisation have been identified among approximately 875 employees examined for several years. Due to the nature of these data, although it can be concluded that the risk of skin sensitisation is low under the exposure conditions experienced by these workers, a potential skin sensitisation hazard cannot be completely excluded.

Overall the new information does not confirm the previously reported evidence of a skin sensitisation potential of BPA. While the data do not exclude a skin sensitising activity of BPA at high concentrations (> 30%), there is no evidence that this is a concern for workers in current BPA manufacturing plants (such workers are believed to represent the group most likely to be exposed to BPA dust).

There are no data from which to evaluate the potential of BPA to be a respiratory sensitiser. However, based on the lack of reports of cases of respiratory sensitisation, there are no grounds for concern for this endpoint.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Summary of original risk assessment report

No useful information on the effects of repeated exposure to BPA in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in

rats following exposure to 50 mg/m³ (6 hours/day, 5 days/week for 13 weeks). There was no significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m³. A NOAEL of 10 mg/m³ was identified in rats in this 13-week study.

Dietary studies in rats produced a decrease in body weight gain and minor changes in the weights of several organs at higher doses probably of no toxicological significance, especially given the absence of other related pathological findings. However, in one study in male rats, reductions in the weight of several reproductive organs and testicular toxicity were seen following dietary exposure to 235 mg/kg for 44 days. A NOAEL was not established from this study. Although these effects on the reproductive organs have not been seen in any other robust repeated dose toxicity study in rats or mice (including a 2-year study in F344 rats), the severity of effects was generally dose-related and therefore cannot be disregarded. The only other finding was an inconsistent observation of caecal enlargement in some 90-day studies. The caecal enlargement was observed at 25 mg/kg and above and was without any associated histological abnormalities. In addition, it was not observed in a 2-year study at doses up to about 140 mg/kg or a multigeneration study at doses up to 500 mg/kg/day. Consequently, this is not regarded as a toxicologically significant observation of relevance to humans. A NOAEL of 74 mg/kg has been established for rats from a 2-year study based on marginal effects on bodyweight gain at the next dose level of 148 mg/kg. Chronic inflammation of the liver was seen from 50 mg/kg in a 3-generation study, but with no convincing dose-response relationship. These liver effects in rats were thus considered to be background variation and not treatment-related. Renal tubule degeneration of the kidney was also seen in this 3-generation study in females at 500 mg/kg but not at 50 mg/kg.

Dietary studies in mice indicated that the liver is a target organ in this species, with changes being observed in the size and nucleation state of hepatocytes in 2-year and 90-day studies. The incidence and severity of these treatment-related multinuclear giant hepatocytes was greater in males than in females, and it was not possible to identify a no effect level for males. The effect was observed at all dose levels used in males from 120 mg/kg. In females, a no-effect level of 650 mg/kg was identified for these cellular changes in the 2-year study. The only other findings in mice were significant reductions in body weight gain at dose levels of approximately 650 mg/kg/day and above. Thus, LOAELs of 120 mg/kg in males for multinuclear giant hepatocytes and 650 mg/kg in females for a reduction in body weight gain of unknown magnitude were identified in a 2-year study.

In a 90-day dietary study in dogs, a no effect level of approximately 80 mg/kg was identified, with increases in relative liver weight being the only other finding observed at approximately 270 mg/kg: in the absence of histopathology this finding is of doubtful toxicological significance.

There are no animal data available for repeated dermal exposure.

4.1.2.6.2 Updated information

Information on repeated dose toxicity can be derived from a well conducted and reported 13 week rangefinding study for a 2-generation study (Tyl *et al.* 2005) and the subsequent 2-generation study (Tyl *et al.*, 2007, see Section 4.1.2.9.2 for full details of this study, including information on findings in the reproductive organs) in mice.

In the rangefinding study, groups of 10 male and 10 female CD-1 mice were exposed to BPA in the diet at concentrations of 0, 500, 2000, 2500 or 3500 ppm, continuously for 13 weeks. The resulting average BPA intakes were, respectively, 0, 74, 298, 373 and 541 mg/kg/day for

males and 0, 100, 370, 487 and 728 mg/kg/day for females. Clinical signs, bodyweights and food consumption were monitored throughout the study. At the terminal necropsy the kidneys and liver were removed, weighed, and processed for histopathological examination. There were no treatment-related clinical signs of toxicity, mortality or effects on bodyweight gain. For males only, relative liver weight was significantly increased at 2000, 2500 and 3500 ppm, although the differences did not follow a dose-related pattern. Histological examination of the liver revealed a dose related increase in the incidence of centrilobular hepatocyte hypertrophy in all BPA-exposed groups of males. In females the incidence of hepatocyte degeneration or necrosis was increased at 2500 and 3500 ppm. In males only relative kidney weight was significantly increased at 500, 2000, 2500 and 3500 ppm, although the differences did not follow a dose-related pattern. The only histopathological findings in the kidney that were considered to be treatment related were an increased incidence of nephropathy at 3500 ppm. This study confirmed that the liver and kidney are targets for BPA toxicity.

In the subsequent 2-generation study (Tyl et al., 2007), eight groups of 28 male and 28 female CD-1 mice (F_0 generation) were exposed to BPA in the diet at concentrations of 0, 0.018, 0.18, 1.8, 30, 300 or 3500 ppm, which resulted in a BPA intake close to the target doses of 0, 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg/day, respectively. For the BPA exposed F_0 and F_1 parental/retained animals there were no treatment-related mortalities or clinical signs of toxicity. Evidence of general toxicity was observed in 300 ppm and 3500 ppm groups. At 300 ppm, this evidence was limited to an increased incidence of centrilobular hepatocyte hypertrophy of minimal to mild severity in F_0 males (40% vs. 11% in controls) and females (10% vs. 2%) and F_1 parental/retained males (30% vs. 10%). There were no increases in liver weight at this dose level. At 3500 ppm, bodyweight gain was reduced among the F_1 parental/retained males; at termination mean bodyweights of the parental and retained males were 4% and 10%, respectively, less than the vehicle controls. Kidney weights were increased in F_0 males and in F_1 parental/retained males. Histological examination of the kidney revealed an increased incidence of minimal to mild nephropathy in the F_0 males and F_1 parental/retained animals at 3500 ppm. Absolute liver weights were significantly increased in F_0 males (by 18%) and females (by 20%) and in F_1 parental males (by 17%) at 3500 ppm. Histological examination of the liver revealed an increased incidence of minimal to mild centrilobular hypertrophy in the F_0 males (100% vs. 11% in controls) and females (60% vs. 2%) and F_1 parental/retained males (65% vs. 10% in controls) and parental females (70% vs. 4%) at 3500 ppm. The increased incidence of centrilobular hypertrophy at 300 ppm (50 mg/kg/day) was not accompanied by an increase in the group mean liver weight, suggesting that the liver changes seen at this dose level were minor and without toxicological significance. Therefore, the study NOAEL for general toxicity can be set at 50 mg/kg/day on the basis of the observation of toxicologically significant effects on bodyweight gain, kidney and liver at the next highest dose level of 600 mg/kg/day (3500 ppm).

4.1.2.6.3 Impact of new information and summary of repeated dose toxicity

A recent oral 2-generation study in mice has confirmed that the repeated dose toxicity of BPA involves effects on bodyweight gain, liver and kidney. A NOAEL of 50 mg/kg/day was identified from this study. No useful information on the effects of repeated exposure to BPA in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in rats following exposure to 50 mg/m³ (6 hours/day, 5 days/week for 13 weeks). There was no

significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m³. A NOAEL of 10 mg/m³ was identified in rats in this 13-week study.

Oral studies in rats and mice have shown that the repeated dose toxicity of BPA involve effects on bodyweight gain, liver and kidney. A NOAEL of 50 mg/kg/day has been identified in a recent 2-generation study in mice for these effects. This NOAEL rather than the original LOAEL of 120 mg/kg/day for liver effects from the published report is taken forward to the risk characterisation.

There are no animal data available for repeated dermal exposure.

4.1.2.7 Mutagenicity

4.1.2.7.1 Summary of original risk assessment report

No human data regarding mutagenicity are available. However, BPA appears to have demonstrated aneugenic potential *in vitro*, positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems there is information that shows BPA disrupts microtubule formation. BPA has been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. However, some deficiencies in the conduct of these studies have been noted and the negative results cannot be taken as entirely conclusive. BPA does not appear to be aneugenic *in vivo*, since a recently conducted, standard mouse bone marrow micronucleus test has given a negative result. BPA was negative in a briefly reported dominant lethal study in rats but, given the limited details provided, this is not regarded as an adequate negative result. The only other data in somatic cells *in vivo* are from a ³²P-postlabelling assay, which showed that BPA is capable of producing DNA adduct spots in rat liver following oral administration. These adduct spots were not characterised fully.

Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies, it does not appear that BPA has significant mutagenic potential *in vivo*. Any aneugenic potential of BPA seems to be limited to *in vitro* test systems and is not of concern. The relevance of the finding that BPA can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health.

4.1.2.7.2 Updated information

In vivo studies

A recent study (Hunt et al., 2003) has investigated the effects of short-term, low-dose exposure to BPA on the meiotic processes of female mice during the final stages of oocyte growth. The investigation was triggered by the sudden, spontaneous increase (from 1-2% to 40%) in the background rate of meiotic disturbance in oocytes from female mice, observed as misalignment of chromosomes on the metaphase spindle (termed 'congression failure') of the first meiosis (MI). Coincident with this was an increase (from 0.5-1% to 5.8% determined by pooling together incidence values from control animals of different ages, strains/genotypes and derived from different breeding stocks) in the background level of aneuploidy

(hyperploidy, i.e. cells with >20 chromosomes). According to the authors, these observations were associated with the accidental damage to caging material by the inadvertent use of harsh alkaline detergents. Subsequent investigations led the authors to postulate that the changes could be due to exposure to BPA leaching from the damaged cages and water bottles. It should be noted that these data cannot be unambiguously associated with BPA itself. While release of BPA is one possibility, other degradation products or oxidation products could also be involved. No information was provided in the paper on the active and inert ingredients present in the detergents that were used.

The study focused primarily on the first discovery of this change in the levels of congression failure and hyperploidy, and the subsequent investigations to identify the exposure source producing the effect. Details of experiments conducted with BPA itself are limited.

Groups (size not stated) of juvenile (20- to 22-day-old), sexually immature (prepuberal) female C57BL/6 mice were treated with oral gavage doses of 0, 20, 40 or 100 µg/kg bw/day BPA in corn oil for 6-8 days. The animals were then sacrificed and germinal vesicle (GV)-stage oocytes (meiotically competent oocytes) were liberated from antral follicles (they contain a large number of oocytes). These oocytes were then cultured overnight, and only those exhibiting a polar body the following morning, i.e. metaphase II (MII)-arrested oocytes were embedded in fibrin clots, fixed and prepared (immunostained with tubulin antibodies to visualise the spindle and counterstained with DAPI to visualise the chromosomes) for analysis (46-255 oocytes were examined per group). MII-arrested oocytes were preferred to MI oocytes (those analysed when the change in congression failure levels was first observed) since this static arrest phase alleviates the problem of variation due to differences in cell cycle rate.

A dose-related increase in congression failure was observed among the treated animals. Congression failure was seen in 2/115 (1.7%), 10/172 (5.8%), 19/255 (7.5%) and 5/46 (10.9%) oocytes at 0, 20, 40 and 100 µg/kg bw/day respectively. It is unclear from the study report whether or not this increase was statistically significant at all three exposures or whether the dose-response showed a statistically significant trend. Furthermore, to determine the shortest exposure that produced detectable effects, an additional set of experiments using a dose of BPA of 0 or 20 µg/kg bw/day for 3, 5 or 7 days prior to oocyte analysis was conducted. Again, the number of animals per group was not stated. A total of 67, 138 and 234 oocytes in treated animals and 61, 70 and 140 oocytes in controls were examined on days 3, 5 and 7 respectively. All three exposures resulted in increased levels of defects of the alignment of the chromosomes on the meiotic spindle, although only the 7-day treatment was statistically significantly elevated above control values. Percentages (values taken from a graph) of congression failure of 1.7, 3 and 2% were obtained from the three different control groups, and of 3.2, 5.4 and 8.5% from the 3-, 5- and 7-day treatment groups, respectively.

The dose levels of BPA employed in the experiments (20-100 µg/kg bw) were selected based on the levels of BPA measured by GC-MS in the water from damaged bottles. However, despite this attempt to mimic the exposure dose resulting from damaged caging materials (up to ~ 70 µg/kg bw), the congression failure levels measured in the BPA dosing studies were lower (up to 10.9%) than those obtained using damaged cages and damaged bottles together (41.4%), damaged bottles only (26.9%) or even damaged cages only (8.7-20.1%). It has been speculated by the authors that the explanation for this discrepancy was the lower bioavailability of BPA following single bolus dosing (the dosing regime employed in the BPA exposure studies), due to rapid first-pass elimination, in comparison to that obtained following continuous exposure via drinking water (the dosing regime that was believed to be operative in those experiments using damaged caging materials). However, this hypothesis is

not consistent with the known toxicokinetics and metabolism of BPA, and therefore this discrepancy raises some uncertainties as to the cause of the original observed changes in background levels of congression failure. Also, it is unclear why BPA was not administered in drinking water. This method of administration would have been more directly relevant to the original observations. Further uncertainties arise as a result of the observation that the level of hyperploidy in oocytes of control mice did not return to the typical background level (0.5-1%) after elimination of all damaged caging materials (2%).

The principal finding of this study was an increase in congression failure, a misalignment of chromosomes during the metaphase stages of meiosis. Experiments to demonstrate that BPA, following congression failure, actually induced aneuploidy (hyperploidy) were not conducted. While one might expect that a misalignment of chromosomes during meiotic metaphase would be associated with improper chromosome segregation, this has not been conclusively demonstrated for meiotic cells. The alignment of chromosomes at metaphase is a dynamic process. Chromosomes that appear to be mis-aligned at one point can quickly become properly aligned and segregate properly. As a result, chromosome misalignment or aberrant congression represent cellular effects that may eventually lead to aneuploidy but are not considered definitive genotoxic effects. Similar studies of chromosome alignment during mitosis have provided examples of cases where, under conditions where high frequencies of abnormal metaphases were induced, relatively few cells exhibited lagging chromosomes during anaphase or telophase (Schuler, et al., 1999).

The hyperploidy results, which represent a more important and established genotoxic endpoint, were generated only for control animals before, during and after the inadvertent use of damaged cages and water bottles, by pooling incidence values from animals of different ages, strains/genotypes and derived from different breeding stocks. It is questionable whether it is appropriate to pool and compare the hyperploidy results from these groups. For example, since the incidence of aneuploidy increases with age in many cell types, it would not seem appropriate to combine or compare the incidence in the 8-12 month old mice with those that are 4 weeks of age.

Normally, the statistical analysis of *in vivo* mutagenicity studies compares the frequency of abnormal events per animal. In this study, however, the number of oocytes and aberrant oocytes appears to have been pooled from several animals and analysed without accounting for animal-to-animal variability. This approach is not ideal as it may mask animal to animal variability and give erroneous results.

Historical background frequency for congression failure in meiosis II was not provided. In the absence of these data, the true biological significance of the results obtained with BPA treatment (congression failure in meiosis II) cannot be assessed.

Overall, there are several methodological weaknesses in this study, namely lack of direct evidence that BPA induces aneuploidy, a non-standard, non-validated methodology, discrepancies between the original observations and the findings with BPA, absence of standardisation in the number of oocytes examined, inappropriate pooling of data, problems with the statistical evaluation, small sample size, lack of historical control data, along with reporting inadequacies.

Furthermore, these results appear to differ in significant ways from what is known of the mutagenic and toxicological profile of BPA. For example, BPA has been shown in *in vitro* studies to interfere with chromosome segregation in mitotic cells (Ochi, 1999; Parry, et al., 2002; Tsutsui, et al., 2000; Tsutsui, et al., 1998) at concentrations ranging from 20 to 150µM. However, given the very low doses of BPA administered in this study and the efficient

conjugation of BPA during first-pass metabolism, one would expect the plasma concentrations achieved in these experiments to be much lower than those that have shown effects *in vitro*.

BPA is negative in standard bone marrow micronucleus tests in mice, so it would be unlikely to induce micronuclei (possibly by an aneugenic effect) in germ cells.

Lastly, if BPA significantly affected chromosome segregation in 10% of the affected oocytes, one would expect that it would also affect reproduction with decreases in litter size being seen in the treated animals. However, no effects on litter size have been seen in a comprehensive multi-generation study in mice covering a wide array of doses (10^{-3} to 10^{+2} mg/kg/day), lower, equal, and substantially higher than those used in this investigation.

Overall, therefore, in view of a number of weaknesses and flaws identified in the study along with the reporting inadequacies and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis.

A follow up study was published by the Hunt lab (Susiarjo *et al.*, 2007). This publication used a different study design but suffers from similar weaknesses.

Noting the Hunt *et al* (2003) data, Attia *et al* (2004) reported on a series of preliminary experiments that have examined the potential aneugenic activity of BPA in germ cells *in vivo*. The study information is currently available only as an abstract. These studies showed no increase in hyperploidy in mouse spermatocytes or oocytes. The authors concluded that the aneuploidy predicted by Hunt *et al* (2003) could not be confirmed in these other studies and that further investigations were being conducted.

CERHR (Center for the Evaluation of Risks to Human Reproduction) interim draft (CERHR, 2007) states that the Attia study is now available as a draft-publication (Pacchierotti *et al.*, 2007). In this publication the potential aneugenic effects of BPA were investigated in mouse somatic and germ cells. C57Bl/6 female mice were superovulated using pregnant mare serum and hCG and subsequently gavaged with BPA at 0.2 or 20 mg/kg bw. Metaphase II oocytes were collected after 17 hours and evaluated using C-banding. Additional female mice were gavaged with BPA at 0.04 mg/kg bw/day for 7 days or were given BPA in drinking water at a concentration of 0.4 mg/l for 7 weeks. These mice were superovulated at the end of the 7-day or 7-week treatment period and housed overnight with untreated males. Females without vaginal plugs were killed for evaluation of oocytes by C-banding. Females with vaginal plugs were treated with colchicine to prevent the first embryonic cleavage, and zygotes were collected the next morning for evaluation by C-banding. There were no effects of BPA on induction of aneuploidy. There was a statistically significant increase in premature centromere separation in the group treated for 7 weeks, but there was no effect of BPA treatment on the proportion of zygotes with structural or numeric chromosome changes.

4.1.2.7.3 Impact of new information and summary of mutagenicity

The new information on the mutagenicity of BPA deals with the effects of short-term, low-dose exposure to BPA on the meiotic processes of female mice during the final stages of oocyte growth. These new data have shown that BPA produces an increase in congression

failure, a misalignment of chromosomes during the metaphase stages of meiosis II. However, in view of several methodological weaknesses and flaws identified in the study along with the reporting inadequacies, and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis. Furthermore, these findings have not been confirmed in more recent publications.

Therefore, the original conclusion from the published assessment that BPA has no significant mutagenic potential *in vivo*, is still valid.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Summary of original risk assessment report

There are no human data contributing to the assessment of whether or not BPA is carcinogenic. In animals, a dietary carcinogenicity study in two species is available; F344 rats and B6C3F₁ mice. A small increased incidence of leukaemias was seen in male and female F344 rats along with increases in the frequency of mammary gland fibroadenomas in male rats. These increases were not statistically significant, were slight and in a strain prone to these tumours. An increased incidence in benign Leydig cell tumours seen in male rats was within historical control limits. In mice, a small increased incidence in lymphomas was observed in males, but was not statistically significant and there was no dose-related trend. No increased incidence in any tumour type was observed in female mice. Overall, all of these tumour findings in rats and mice are not considered toxicologically significant. Consequently, it is concluded that BPA was not carcinogenic in this study in both species. No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, BPA did not exhibit properties that raise concern for potential carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m³ in a 13 week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m³. Taking into account all of the animal data available the evidence suggests that BPA does not have carcinogenic potential.

4.1.2.8.2 Updated information

The effects of transplacental and lactational exposure to BPA on the development of prostate cancer in rats were assessed using a model for reproductive organ carcinogenicity (Ichihara *et al.*, 2003). Modulating effects of BPA on prostate cancer incidence in male offspring exposed transplacentally and lactationally to BPA were investigated in female F344 rats (~8–15 dams/group) administered 0, 0.05, 7.5, 30, and 120 mg BPA/kg/day by gavage during pregnancy and lactation. Dam body weight and food intake were monitored during the study. Gestation duration and implantation sites were evaluated. Pups were counted and sexed at birth. Litters were randomly culled to 8 pups on PND 4, and pups were weaned on PND 21. At 5 weeks of age, 21 male rats/group were injected subcutaneously with 50 mg/kg bw 3,2-dimethyl-4-aminobiphenyl (DMAB) 10 times at 2-week intervals. An additional 12 rats/group in the 0, 0.05, 7.5, and 120 mg/kg bw/day BPA groups were injected with corn oil during the same time period. Surviving male offspring were killed and necropsied at 65 weeks of age. Blood was collected for analysis of serum testosterone levels in 5 rats/group. Reproductive organs were examined for gross abnormalities, weighed, and fixed in 10% buffered formalin. A histopathological examination of the prostate was conducted.

Body weights of dams in the 120 mg/kg bw/day group were significantly lower than control values from GD 14 to 20. There were no consistent or dose-related effects on dam body weights during lactation. Exposure to BPA had no effect on gestation duration or number of implantation sites. In pups exposed to BPA, there were no differences in number of live births, sex ratio, external anomalies, or body weights during the lactation period. Exposure to BPA had no effect on weights of prostate, testis, or epididymis. Without DMAB treatment, incidences of prostatic intraepithelial neoplasia (PIN), carcinoma, and atypical hyperplasia were not increased by exposure to BPA, and there were no increases in tumours of the non-reproductive organs. No effect was observed on serum testosterone levels. This screening study showed that exposure of rat dams up to 120 mg/kg bw/day BPA during the gestation and lactation periods did not predispose their offspring to prostate cancer development later on in life (65 weeks of age). However, it is noted that the sample sizes were inadequate for the assessment of the cancer endpoint.

In another study on the prostate gland, the effect of short-term neonatal exposure to BPA on susceptibility of Sprague Dawley rats to prostate cancer were investigated (Ho *et al.*, 2006). On PND 1, 3, and 5 (day of birth = PND 0), 20–30 male pups/group were subcutaneously injected with corn oil vehicle, BPA at 0.1 µg/pup (0.010 mg/kg bw), or estradiol benzoate (EB) at 0.001 µg/pup (0.1 µg/kg bw) or 25 µg/pup (2500 µg/kg bw). Pups were weaned on PND 21. At PND 90, half of the rats from each treatment group were implanted with Silastic capsules containing 17β-estradiol (E) and testosterone (T) and the other half were implanted with empty capsules; the capsules were left in place for 16 weeks. The treatment was designed to result in a serum E level of ~75 pg/ml and T level of ~3 ng/ml, levels reported to induce prostatic intraepithelial neoplasia (PIN) in 33% of Sprague Dawley rats. Rats were killed at 28 weeks (PND 200; 6-7 months) of age. Prostates were removed, and histopathological evaluations were conducted on each lobe. Immunohistochemistry techniques were used to measure proliferation. Apoptosis was measured using the TUNEL technique. In addition, PCR techniques were used to study methylation pattern and expression changes in prostate cell signaling proteins on PND 10, 90 (before adult E+T treatment), and 200.

The study authors stated that similar responses were observed in each of the 3 prostate lobes; and thus results were presented only for dorsal prostate. In animals that did not receive E+T in adulthood, BPA exposure had no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index. In animals treated with E+T in adulthood, BPA exposure resulted in a statistically significant increased incidence and severity of PIN (100 [10/10] vs. 40% [4/10] incidence in controls). In the BPA/E+T group compared to the E+T group, the proliferation index was increased and the apoptosis index was decreased in regions where PIN was observed.

In the investigation of a molecular basis for increased susceptibility to PIN, neonatal exposure to estrogenic (either BPA or EB) compounds altered methylation patterns in several cell signaling genes. Phosphodiesterase type 4 (PDE4), an enzyme involved in cyclic AMP breakdown, was selected for further investigation. Neonatal BPA exposure resulted in hypomethylation of the PDE4 gene and increased expression of that gene at 90 and 200 days of age, with or without E+T exposure in adulthood. Similar responses in PDE4 gene methylation and expression were observed with exposure to the low and high EB doses.

Overall, this study showed that short-term neonatal exposure of male rats to 10 µg/kg bw BPA by subcutaneous administration had no effect on the prostate gland later on in life (at 6-7 months of age). This is consistent with the findings by Ichihara *et al.* (2003). The

observation of hypomethylation of the cell signalling gene, PDE4, in the BPA-treated animals, in itself does not represent an adverse effect. However, when in adulthood the animals were given implants of E and T for 16 weeks, BPA exposure appeared to increase prostate gland susceptibility to hormonally-induced histopathological lesions (PIN). Although the study authors claim that PIN is a precancerous lesion leading to prostate cancer, as the animals were sacrificed at 6-7 months of age, this could not be verified and, hence, the toxicological significance of PIN in animals remains unknown. It is also noted that no information was provided on the background variation of PIN in this strain of rats and on the experimental variation of E+T-induced PIN. Overall, therefore, due to the small sample sizes, use of a single dose level (and hence no dose-response information) and lack of information on the background variation of PIN and E+T-induced PIN, it is difficult to establish whether the increased incidence of E+T-induced prostrate lesions was a real, treatment-related effect. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures. The kinetics of BPA following subcutaneous administration, including the extent of absorption and its rapid metabolism in the liver to the endocrine inactive conjugate, BPA-glucuronide, are likely to differ from the kinetics of BPA by relevant routes of exposure.

Effects of maternal exposure to BPA on uterine carcinogenesis were studied in offspring of Donryu rats (a strain of rats with a high rate of spontaneous and ENNG-induced uterine tumours) administered BPA (0, 0.006 and 6 mg/kg bw/day, n = 12, 15 and 19/group respectively) daily by gavage from GD 2 to PND 21 (Yoshida *et al.*, 2004). After delivery, offspring were sexed, weighed, and examined for external abnormalities on PND 1 and then weighed weekly through PND 21. Litters were adjusted to 8–10 pups at PND 4 or 6 (day of birth = PND 0). Dams were weighed, and observed during the study and killed following weaning of litters on PND 21. Implantation sites were examined and organs including uterus, vagina, and ovaries were examined histologically. All female offspring were examined for vaginal opening, and following vaginal opening, vaginal smears were taken for the remainder of the study. Three to 5 offspring/group from different litters were killed on PND 10, 14, 21, or 28 and at 8 weeks of age. At most time periods, uteri were weighed and examined histopathologically to determine development of uterine glands. Ovaries and vagina were also examined histologically. ER α was determined using an immunohistochemical method. Serum was collected for measurement of FSH and LH by RIA. Four offspring/group from different litters were killed at 8 weeks of age on the morning of estrus to examine ovulation by counting ova in oviducts. At 11 weeks of age, 35-36 animals/group were injected in the uterine horn with N-ethyl-N'-nitro-nitrosoguanidine (ENNG) to initiate uterine carcinogenesis. At ~24 weeks following cancer initiation (~15 months of age), the 24–30 surviving animals/group were killed and uteri were examined histologically to determine the presence of tumors and other lesions.

In dams exposed to BPA, there were no clinical signs of toxicity or effects on body weight, implantation sites, or gestation length. BPA exposure had no effect on litter size, pup body weight at birth and through PND 21, external abnormalities in pups or age of vaginal opening. In uteri of BPA-exposed offspring, there were no effects on weight, gland development, ER α , or cell proliferation. BPA exposure had no effect on ovulation, estrous cyclicity, or serum FSH or LH levels. There were no effects on ovarian histopathology following BPA treatment. The incidence of uterine preneoplastic or neoplastic lesions induced by ENNG was not increased by BPA exposure. Overall, transplacental and lactational exposure of rats to BPA did not exert an influence on uterine development and maturation and on ENNG-induced uterine carcinogenesis up to 15 months of age. However, it is noted that the sample sizes were inadequate for the assessment of the cancer endpoint.

Takashima *et al.* (2001) examined the effect of BPA exposure during development on multi-organ carcinogenicity (including thyroid and lungs) induced by N-nitrosobis (2-hydroxypropyl)amine (BHP). Female Wistar rats (12/group) were fed a diet containing 0 or 10,000 ppm BPA for 10 weeks prior to mating, and through mating, gestation and lactation. Intakes of BPA were reported to be about 400-600 mg/kg bw/day. The rats were mated to untreated males and GD 0 was defined as the day of the vaginal plug. Endpoints associated with pregnancy, delivery, and nursing were evaluated. Dam body weight and food intake were measured. Offspring were not culled and were weaned at 3 weeks of age. Dams were killed following weaning of offspring. Serum levels of thyroid hormones were measured in 2-4 dams/group. Implantation sites were evaluated. Weights of several organs, including ovary, were measured. The organs were fixed and processed for histopathological evaluation. Offspring (n = 32-50/group) were evaluated for body weight gain, preputial separation, and vaginal opening. Beginning at 5 weeks of age and continuing for 12 weeks, offspring in each group were subdivided into 2 groups (n = 17-21/group/sex) that received either undosed tap water or tap water containing 2000 ppm BHP. Offspring were killed at 25 weeks of age. Serum thyroid hormone levels were measured. Organs, including testis, ovary, and uterus were weighed. In 5-19 offspring/sex/group, histopathological examinations were conducted in organs targeted by BHP (lungs, thyroid, esophagus, liver, and thymus).

Dam body weight was lower in the BPA group compared to the control group during the gestation period and at weaning. Food intake and maternal serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone were unaffected by BPA exposure. There were no changes in weights or histopathological alterations of maternal organs, including uterus and ovary. BPA had no significant effect on mating, fertility, duration of gestation, live-born pups, implantation loss, or offspring viability through PND 21. In pups from dams exposed to BPA compared to pups from control dams, body weights were higher (by 11%) in females at 3 days of age and lower in males and females at 10 days and 2 weeks of age (16-22% decreases in males and 12-19% decreases in females). Prenatal and postnatal exposure to BPA did not affect preputial separation or vaginal opening. No effects were observed on thyroid hormones and offspring organ weights. Prenatal and postnatal BPA exposure was not associated with significant differences in the development of BHP-induced neoplasms in the offspring. The results of this study indicate that oral exposure to 400-600 mg/kg bw/day BPA during development does not exert promoting effects on BHP-induced thyroid, lung, liver, thymus and esophagus carcinogenesis in rats. However, it is noted that the sample sizes were inadequate for the assessment of the cancer endpoint.

The effects of prenatal BPA exposure on susceptibility to mammary tumor induced by N-nitroso-N-methylurea (NMU) were examined in Wistar rats (Durando *et al.*, 2007). On GD 8-23 (GD 1 = day of vaginal sperm), 11-14 dams/group were subcutaneously dosed with 0 or 0.025 mg/kg bw/day BPA. Pups were delivered on GD 23 and weaned on PND 21. During the study, body weights and day of vaginal opening were monitored. Offspring were killed before puberty (PND 30), after puberty (PND 50), or in adulthood (PND 110 and 180). In mammary gland stroma and epithelium, proliferation was assessed by BrdU incorporation and apoptotic cells were identified by the TUNEL method. Morphometric analyses were conducted in sectioned mammary glands. Mast cells were identified by immunostaining for proteinase. At least 6 offspring/group/time point were evaluated. No littermates were used in the evaluation at any given time point. Additional offspring were examined for responsiveness to chemically-induced mammary preneoplastic or neoplastic lesions. On PND 50, the established carcinogen N-nitroso-N-methylurea (NMU) was administered

intraperitoneally to 10–16 offspring from the vehicle control group at 25 or 50 mg/kg bw and to 21 offspring from the BPA group at 25 mg/kg bw. Based on findings from a pilot study, 25 mg/kg bw NMU was considered a subcarcinogenic dose and 50 mg/kg bw NMU was considered a positive control. During the study, rats were palpated for tumours. Rats that received 50 mg/kg bw NMU were killed on PND 180 and rats that received 25 mg/kg bw NMU were killed on PND 110 or PND 180. Whole-mounted mammary glands were examined for tumours. Immunostaining was conducted to identify cytokeratin 8 (an epithelial marker) and p63 (a myoepithelial marker). Data were statistically analyzed using the Mann-Whitney *U* test. It was not clear if the litter or offspring were considered the statistical unit.

BPA exposure did not affect successful pregnancies, dam weight gain, pregnancy duration, number of pups/litter, or percent females/litter. Anogenital distance on PND 1 or 5 and postnatal body weights were unaffected in pups exposed to BPA. Vaginal opening was accelerated in pups from the BPA group (mean 34 days of age compared to 39 days of age in controls). On PND 50, the BrdU/apoptosis ratio was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma of BPA-exposed animals; the effects were not observed on PND 30 or PND 110. Significantly increased percentages of hyperplastic ducts, density of stromal nuclei, and numbers of mast cells were observed in the BPA group on PND 110 and PND 180. Exposure to BPA resulted in formation of a dense stroma layer around mammary epithelial structures and replacement of normal adipose tissue with a fibroblastic stroma. In rats exposed to 25 mg/kg bw NMU on PND 50, incidence of hyperplastic lesions on PND 180 was significantly higher in the group with prenatal BPA exposure compared to controls (mean incidence of 35.5% compared to 15.7% in controls). Although statistical significance was not achieved, exposure to 25 mg/kg bw NMU resulted in tumors in 2 of 15 rats in the prenatal BPA group and 0 of 10 rats in the prenatal control group on PND 180. Cytokeratin 8 immunostaining revealed no invasion by stromal epithelial cells. The study authors concluded that in rats prenatal exposure to a low dose (0.025 mg/kg bw/day) of BPA perturbs mammary gland histoarchitecture and increases its carcinogenic susceptibility to a chemical carcinogen (NMU) administered 50 days after the end of BPA exposure. However, due to the small sample size, lack of clarity on statistical analysis and use of a single dose level, it is difficult to establish whether the effects reported were due to chance or were real, treatment-related effects. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures.

In a similar study, Murray *et al.* (2007) examined the effect of prenatal BPA exposure on *in situ* induction of mammary tumors in rats. From GD 9 (GD 1 = day of vaginal sperm) through PND 1 (PND 0 = day of birth) Wistar-Furth rat dams received subcutaneous injections of 0, 0.0025, 0.025, 0.250, or 1 mg/kg bw/day BPA. Number of dams treated was not reported. Based on a limited amount of information provided on the number of offspring examined, it appears that ≤ 6 dams/group were treated. Pup viability was assessed on PND 1. On PND 2 pups were sexed and litters were culled to 8 pups. Anogenital distance was measured on PND 4. Litters were weighed during the lactation period. Female offspring were monitored for body weight and vaginal opening in the post-weaning period. Female offspring were killed on PND 50 or PND 95. Mammary glands were collected and whole-mounted or sectioned for histopathological examination. Morphometric analyses were conducted to examine possible presence of preneoplastic lesions. Mammary glands were examined for ER- α and Ki-67 protein by an immunohistochemistry technique. One female/litter was included in the histological examinations. Apparently, ≤ 6 offspring/group were examined

histopathologically. The number of offspring examined for the other endpoints was not reported. It was not clear if dams or offspring were considered the statistical unit.

BPA exposure did not affect offspring viability, sex ratio, age at vaginal opening, or female anogenital distance. Anogenital distance was reduced on PND 4 in males from the 0.250 mg/kg bw/day group. Percent hyperplastic ducts was increased in all dose groups on PND 50; the study authors noted that the effect on PND 50 was quantitatively similar in all dose groups (i.e. 3–4-fold increase). Cribriform structures classified as carcinomas-in-situ were observed in the 0.25 and 1 mg/kg bw/day groups. The incidence of these structures in the controls and lower dose groups were not reported. Although the study authors classified the cribriform structures as carcinoma in situ because of their hallmarks, it is difficult to establish whether or not these histopathological findings are clear neoplastic lesions of the mammary gland. The study authors concluded that fetal BPA exposure at dose levels of 0.250 and 1 mg/kg bw/day in rats is able to induce development of preneoplastic and neoplastic mammary lesions. However, again, due to the small sample size, lack of clarity on the statistical analysis, absence of a dose-response relationship and uncertainty about the incidence of the cribriform-like lesions in the controls it is difficult to establish whether the effects reported were due to chance or were real, treatment-related effects. In addition, because of the uncertainty about the significance of the cribriform structures, it is unclear whether real neoplasia actually occurred. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures.

4.1.2.8.3 Impact of new information and summary of carcinogenicity

BPA has not shown any significant carcinogenic activity in two standard oral cancer bioassays in rats and mice.

The new information principally concerns the potential promoting effects of prenatal and/or neonatal exposure of rats to BPA on the carcinogenesis induced by established carcinogens/initiators in specific organs (prostate, uterus, thyroid, lungs, liver, thymus, esophagus, liver and mammary gland). One single study (Murray *et al.*, 2007) examined the potential full carcinogenic activity of prenatal exposure to BPA on the mammary gland. Three studies were conducted by the oral route of exposure and three by subcutaneous administration. Although not conclusive, the studies involving oral administration showed that BPA does not exert promoting activity up to relatively high levels of exposure on DMAB-induced prostate cancer (up to 120 mg/kg bw/day), ENNG-induced uterus cancer (up to 6 mg/kg bw/day) and BHP-induced thyroid, lung, liver, thymus and esophagus cancer (up to 400-600 mg/kg bw/day). The studies involving subcutaneous administration showed that BPA at relatively low doses (in the µg/kg bw/day range) does increase the incidence of E+T-induced preneoplastic and neoplastic lesions of the prostate and the incidence of NMU-induced hyperplastic lesions of the mammary gland and does induce hyperplastic and cribriform lesions of the mammary gland. However, these studies had several limitations and methodological weaknesses which make difficult to establish whether the reported findings were real, treatment-related effects. Furthermore, because of the subcutaneous route of administration, it is questionable whether they are relevant to normal routes of exposures.

Overall, there is only one new study in which the full carcinogenic potential of BPA on the mammary gland has been examined in a prenatal model. Although this study claims that prenatal exposure to BPA induces preneoplastic and neoplastic lesions of the mammary

gland, its validity is hampered by serious methodological limitations. It is also noted that these findings are inconsistent with the absence of preneoplastic lesions of the mammary gland in the offspring of several standard multi-generation studies in rats and mice.

Regarding the other new studies, it can be concluded that prenatal and/or neonatal exposure to BPA does not exert promoting activity on the carcinogenesis induced by established carcinogens/initiators in specific organs.

Overall, therefore, the new information on the potential carcinogenic and/or promoting effects of BPA in prenatal and neonatal rat models supports the original conclusion from the published report that BPA does not possess any significant carcinogenic potential.

4.1.2.9 Reproductive toxicity

4.1.2.9.1 Summary of original risk assessment report

No human data are available. BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with BPA glucuronide *in vitro*. The available data also indicate that there is a marked strain difference in the response to BPA in rats. However, there are no data to indicate the underlying reasons for such differences.

It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories. Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of BPA on fertility and reproductive performance have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. Although no effect on fertility was seen in the rat two-generation study, low dose levels were employed (0.2-200 µg/kg/day). In the multigeneration study, an effect on fertility (reduction in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse, it is prudent to assume that BPA may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg.

The continuous breeding study in the mouse provides some evidence that BPA can cause adverse effects on fertility. In the F₀ generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter

tested at each dose level from the F₁ generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F₁ generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F₀ generation) there was no effect on epididymal weight. In spite of the uncertainty, the epididymis is associated with sperm transport and storage, and any reduction in the weight of this organ would be of concern. Although no effects were seen in the 2-generation rat study, it is not considered suitable for use in the risk characterisation due to the low dose levels employed (0.2-200 µg/kg/day). However, these data combined with that for the multigeneration study does provide a comprehensive dose-response range for evaluating effects on fertility in the rat. In addition, comparing the rat and mouse data it can be seen that similar toxicological profiles were observed for effects on fertility; effects were seen in both species at approximately the same dose level (i.e. reductions in litter size at 500 mg/kg/day in the rat and at 600 mg/kg/day in the mouse). Consequently, it is considered that the NOAEL of 50 mg/kg/day identified in the rat multigeneration study is also likely to produce no adverse effects in mice for which there is only a LOAEL of 300 mg/kg/day (for a small but statistically significant decrease in epididymal weight in F₁ males only). Therefore, the NOAEL of 50 mg/kg/day identified from the multigeneration study will be used for risk characterisation purposes, in relation to effects on fertility.

No evidence that BPA is a developmental toxicant was observed in standard development studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F₁-F₃). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg.

However, additionally, some studies have investigated the potential of BPA to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at dose levels in the range 2 – 50 µg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. It is noted that in contrast to the studies showing effects on the male reproductive tract, the studies that did not find an effect of BPA also did not show any effects of DES. Furthermore, no functional changes in reproductive parameters or reproductive organ development were observed in a recent rat two-generation study using similar dose levels. The reasons for the differences in these results are unclear. Recent evidence from one study suggests that there are differences in the sensitivity of different mice strains to the effects of oestrogens, which may be related to the selection of strains for large litter size. This difference in sensitivity may in part explain some of the differences in the current database, although the relevance of these rodent strain differences in relation to human health remains unclear.

Overall, in standard developmental studies in rodents, there is no convincing evidence that BPA is a developmental toxicant. However, the available and apparently conflicting data from studies conducted using low doses (in the µg/kg range) do raise uncertainties. Overall,

the majority of EU member states felt that the studies reporting effects at low doses could not be dismissed. However, the member states disagreed on how these studies should be used, if at all, in the risk characterisation for this endpoint. The disagreements were based on differing views about the uncertainties surrounding the reproducibility of the findings and their biological significance, if any, to human health.

This issue was referred to the Competent Authorities in June 2001. It was agreed unanimously by the Competent Authorities that further work was required to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development at low doses. In addition, it was agreed that a provisional NOAEL of 50 mg/kg/day for developmental effects, derived from the rat multi-generation study, should be used in the risk characterisation in the interim, whilst awaiting the outcome of further testing, with the aim of identifying those scenarios which are clearly of concern irrespective of the outcome of the further testing.

4.1.2.9.2 Updated information

Member States required that a 2-generation study in the mouse involving exposure to low ($\mu\text{g}/\text{kg}$ bw/day range) and high (mg/kg bw/day range) doses of BPA be conducted. This study has now become available (Tyl *et al.* 2007) and is summarised below.

In addition to this comprehensive 2-generation study, a large number of studies investigating the reproductive toxicity of BPA have become available since the finalisation of the RAR. Among these studies, several (approximately 40-50) have investigated the same standard reproductive and developmental endpoints as those examined by Tyl *et al.* (2007). These studies have been performed on a range of animal species and strains, at different life stages, over a wide array of doses, using a variety of exposure routes, for varying exposure durations, and have investigated a large assortment of endpoints (for a detailed review see Gray *et al.*, 2004; Goodman *et al.*, 2006; EC SCF, 2002; and EFSA, 2006). The majority of these studies have reported only small changes (unrelated to dose) in organ weight, tissue architecture, receptor expression or hormone levels of unknown pathophysiological consequences. Some have found no effect, but, overall, no consistent, reproducible, adverse effects have been observed. Furthermore the results from these studies have been in contrast to the results of investigations conducted according to internationally recognised guidelines and in compliance with GLP, including the recent 2-generation study in the mouse by Tyl *et al.* (2007). As we consider this investigation by Tyl *et al.* (2007) as the gold-standard, definitive study of the reproductive toxicity of BPA (for the endpoints examined), all the other recent publications investigating the same standard reproductive and developmental endpoints have not been evaluated in detail in this report.

However, there are also numerous recent studies which have investigated the potential developmental neurotoxicity of BPA. As these endpoints were not examined by Tyl *et al.* (2007), these publications have been considered in detail in this evaluation.

Additionally, one study investigated the effects of neonatal exposure to BPA on the morphology of the reproductive tract at 18 months of age in female mice (Newbold *et al.* 2007). Because the potential for the expression of developmental effects in old-age has not been assessed in the standard reproductive toxicity studies, an appraisal of this study is included below.

One relevant human study has been published since the finalisation of the RAR and is included in this update (Sugiura-Ogasawara *et al.* 2005). This is an investigation of the possible association between BPA exposure and recurrent miscarriage.

2-generation study in mice

The effects of BPA on fertility and reproductive performance in mice have been investigated in a two-generation study, conducted in compliance with GLP (Tyl *et al.*, 2007). The study design and interpretation of the results were supervised by a Steering Group, that was chaired by a representative of the European Chemicals Bureau and included experts from several EU Member States. The overall design of this study was based on OECD Test Guideline 416, enhanced by incorporation of a second vehicle control group, a positive control group, a total of 6 exposure levels of BPA, the retention of additional F₁ male offspring for organ weight and other assessments, and extending histopathological examinations to all treatment groups. This study was conducted in response to an ESR risk assessment conclusion that further research is needed to resolve the uncertainties surrounding the potential for BPA to produce adverse effects on development of the male reproductive tract at low doses (0.002- 0.05 mg/kg/day) in mice.

Eight groups of 28 male and 28 female CD-1 mice (F₀ generation) were exposed to BPA in the diet (Purina Certified Ground Rodent Diet®, No. 5002) at concentrations of 0 (2 vehicle control groups), 0.018, 0.18, 1.8, 30, 300 or 3500 ppm, which resulted in a BPA intake close to the target doses of 0, 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg/day, respectively. CD-1 strain mice were used as there have been claims that this strain is specifically sensitive to low doses of BPA. Two vehicle control groups were used to better characterise the natural variability in mice of the parameters evaluated in the study. The exposure levels were selected as a range that would make possible a comprehensive assessment of the dose-response relationship for reproductive toxicity. The lowest BPA dietary concentration was selected to provide a BPA intake of about 0.003 mg/kg/day, close to that at which effects on the development of the male reproductive system have been reported by Nagel *et al.* 1997 and vom Saal *et al.* 1998. The next two higher concentrations of 0.18 and 1.8 ppm were selected as 10-fold incremental increases. Concentrations of 30 and 300 ppm were selected to produce intakes that matched the NOAEL and LOAEL, respectively, for general parental toxicity in a 3-generation dietary study in the rat (Tyl *et al.* 2002). The highest concentration of 3500 ppm was selected as an exposure level that would cause mild general parental toxicity, based on the results of a 13-week rangefinding study (Tyl *et al.* 2005). The phytoestrogen content of the batches of diet used were: genistein 177-213 ppm, daidzein 173-181 ppm, glycitein 39-55 ppm and total isoflavones 390-449 ppm.

The positive control group of 28 male and 28 female mice was exposed to 17 β -oestradiol (E2) in the diet at a concentration of 0.5 ppm (resulting in an E2 intake of about 0.08 mg/kg/day), to confirm the sensitivity of the mouse model to a potent endogenous oestrogen. This exposure level was selected as one that would result in effects on oestrogen sensitive reproductive parameters, based on the findings of a E2 rangefinding (Tyl *et al.* 2004a) and 2-generation study (Tyl *et al.* 2004b, 2006 - this study is briefly summarised below).

Exposure of the F₀ generation commenced at 6 weeks of age and continued throughout an 8 week pre-breed exposure period, a 2 week mating period (each male was paired with a female from the same exposure group) and gestation. Exposure of F₀ females to BPA continued throughout lactation until weaning on post-natal day (pnd) 21. At weaning, 28 male and 28

female F₁ animals were selected from each exposure group for retention and were similarly exposed for a pre-breed, mating gestation and lactation period. An additional one F₁ male from each litter was retained (termed 'F₁ retained males') with continued exposure for 3 months until sacrifice concurrently with the parental F₁ males. The remaining F₁ animals were sacrificed at weaning. Parental males were sacrificed at the end of their respective delivery period and parental females were sacrificed at weaning of their litters. The study was terminated with the sacrifice at weaning of the F₂ generation.

For the parental F₀ and F₁ generation and retained F₁ males, clinical signs of toxicity, body weights and food consumption were recorded. Oestrous cycles were monitored in the last 3 weeks of the pre-breed exposure period and during the mating period for both the F₀ and F₁ parental animals. A necropsy was conducted on all adult animals in which reproductive (including the prostate) and other selected organs were removed and weighed. Histology of selected tissues was conducted on all vehicle control animals and on 10 parental males and females from each of the F₀ and F₁ BPA and E2 groups. Histology was also conducted on all vehicle control and E2 F₁ retained males and on 10 randomly selected F₁ retained males from each BPA group. For all parental and retained males, epididymal sperm number, motility and morphology were assessed, testicular homogenisation-resistant spermatid head count was recorded, and daily sperm production and efficiency of daily sperm production was calculated. For all parental females the number of ovarian primordial follicles was counted. Parameters assessed in the young offspring included litter size, body weight, survival, gross appearance, anogenital distance (on PND 0 and 21), vaginal patency and preputial separation. For offspring killed at weaning a gross necropsy was conducted on all; selected organs were removed and weighed from two randomly selected pups/sex/litter and histopathology was conducted on the reproductive organs of one pup/sex/litter and on all selected organs from the other selected pup/litter.

All statistical comparisons to each BPA group and the E2 positive control group values were made against the pooled values for the two vehicle control groups.

For the BPA exposed F₀ and F₁ parental/retained animals there were no treatment-related mortalities or clinical signs of toxicity. Evidence of general toxicity was observed in 300 ppm and 3500 ppm groups. At 300 ppm, this evidence was limited to an increased incidence of centrilobular hepatocyte hypertrophy of minimal to mild severity in F₀ males (40% vs 11% in controls) and females (10% vs 2%) and F₁ parental/retained males (30% vs 10%). There were no increases in liver weight at this dose level. At 3500 ppm, bodyweight gain was reduced among the F₁ parental/retained males; at termination mean bodyweights of the parental and retained males were 4% and 10%, respectively, less than the vehicle controls. Kidney weights were increased in F₀ males and in F₁ parental/retained males. Histological examination of the kidney revealed an increased incidence of minimal to mild nephropathy in the F₀ males and F₁ parental/retained animals at 3500 ppm. Absolute liver weights were significantly increased in F₀ males (by 18%) and females (by 20%) and in F₁ parental males (by 17%) at 3500 ppm. Histological examination of the liver revealed an increased incidence of minimal to mild centrilobular hypertrophy and minimal to mild nephropathy in the F₀ males (100% vs 11% in controls) and females (60% vs 2%) and F₁ parental/retained males (65% vs 10% in controls) and parental females (70% vs 4%) at 3500 ppm. The increased incidence of centrilobular hypertrophy at 300 ppm (50 mg/kg/day) was not accompanied by an increase in the group mean liver weight, suggesting that the liver changes seen at this dose level were minor and without toxicological significance. Therefore, the study NOAEL for general toxicity can be set at 50 mg/kg/day on the basis of the observation of toxicologically significant effects on bodyweight, kidney and liver at the next highest dose level of 600 mg/kg/day (3500 ppm).

Concerning the reproduction system, there were no effects on F₀ or F₁ adult reproductive organ weights (F₁ prostate weights are further discussed below), sperm parameters, ovarian primordial follicle count, oestrous cyclicity or histopathology. There were no effects on F₀ or F₁ mating performance or fertility. However, gestational length was statistically significantly increased for both the F₀ and F₁ generations at 3500 ppm (19.3 days vs. 19.0 days for the vehicle controls, both generations), although the health implications of this marginal difference are questionable.

There were no statistically significant differences and no treatment related changes in prostate weight in either the F₁ parental or retained males. Prostate weights appeared slightly increased at 0.018 ppm in the F₁ parental males but because this difference was not also seen in the F₁ retained males, and statistical significance was not achieved, this was considered not to be treatment related.

F₁ and F₂ litter size, pup survival at birth and during lactation were not affected by BPA exposure. There were no treatment-related malformations or clinical signs during lactation. However, several effects on the offspring were apparent at 3500 ppm only. F₁ pup bodyweights were significantly less than vehicle controls on pnd 7, 14 and 21, but F₂ pup bodyweights were not affected. Absolute and bodyweight- or brain-related testes and spleen weights were reduced in the F₁ and F₂ males sacrificed at weaning. The effects on testes weight correlated with an increased incidence of hypoplasia of the seminiferous tubules at 3500 ppm (F₁: 12% vs. 1% in vehicle control; F₂: 35% vs. 4% in vehicle control). Similar effects, however, were not seen in the F₁ parental and retained males. Also, acquisition of preputial separation was slightly delayed in the F₁ parental (by 2 days, compared with negative control, adjusted for bodyweight at time of acquisition) and F₁ retained (by 1.8 days) males at 3500 ppm. Additionally, at 3500 ppm there was a slightly increased incidence of undescended testes observed at weaning sacrifice (a condition with a high and variable background rate in CD-1 mice) of the F₁ and F₂ generations, but there were no indications in adult males of a permanent effect on testes descent. Anogenital distance (AGD) was significantly reduced in F₁ pups on pnd 21 at 300 and 3500 ppm, although this was considered unlikely to be treatment-related because the effect was not also seen on pnd 0 in the F₁ generation, on pnd 0 or 21 in the F₂ offspring; in addition the fact that AGD was not consistently affected in E2 two-generation studies (Tyl *et al.* 2006, Biegel *et al.* 1998) shows that AGD is not an oestrogen regulated endpoint.

In the E2 positive control group no general toxicity was observed in the F₀ and F₁ parental animals. Reproductive toxicity was expressed as changes in a number of parameters, demonstrating the sensitivity of the mouse model to an oestrogenic substance. F₀ and F₁ gestational length was increased. There was a reduced number of live F₁ litters born, reduced F₁ litter sizes and F₁ fertility was reduced. Onset of puberty (vaginal patency) was accelerated in the F₁ females, preputial separation was delayed in F₁ males, female reproductive organ weights in adults and offspring of all generations were increased. Testes and epididymal weights were decreased and the incidence of seminiferous tubule hypoplasia of the testes was increased among F₁ and F₂ weanlings. Anogenital distance was reduced in male offspring of the F₁ and F₂ generations, although this is considered not to be an oestrogen regulated endpoint. Finally, there was an increased incidence of vaginal epithelial keratinization and bilateral luminal dilatation of the uterus in F₁ and F₂ weanlings. Overall, with the exception of reduced anogenital distance in male offspring, the findings with the E2 positive control group were consistent with those from the E2 2-generation study (Tyl *et al.* 2004b, 2006, briefly summarised below).

To conclude, BPA caused effects on pregnancy and the offspring (observed as a slightly increased duration of gestation, reduced pup bodyweight during lactation, a slight increase in the incidence of undescended testes at weaning, seminiferous tubule hypoplasia in offspring at weaning, and delayed acquisition of preputial separation), occurring only at the highest dietary concentration 3500 ppm (intake approximately 600 mg/kg/day), an exposure level that also caused mild parental toxicity. Fertility was not affected by BPA exposure. There was no evidence of an adverse effect on the development of the male reproductive tract at low doses of BPA. Overall, the study NOAEL for both general and reproductive toxicity is 50 mg/kg/day.

The design of the E2 2-generation study (Tyl *et al.* 2004b, 2006) was based on OECD Test Guideline 416. Six groups of 25 male and 25 female CD-1 mice (F₀ generation) were exposed to E2 in the diet (Purina Certified Ground Rodent Diet®, No. 5002) at concentrations of 0, 0.001, 0.005, 0.05, 0.15 or 0.5 ppm, which resulted in E2 intakes of about 0.2, 1.0, 10, 30 or 100 µg/kg/day, respectively. The exposure concentrations were selected as a range likely to include both effect and no-effect levels for E2 reproductive toxicity, based on the results of a rangefinding study (Tyl *et al.* 2004a). The F₀ and F₁ exposure periods, mating and sacrifice schedules and experimental observations were essentially the same as for the BPA 2-generation study (Tyl *et al.* 2007). The study was terminated with the sacrifice at weaning of the F₂ generation.

There were no treatment-related mortalities or clinical signs of toxicity among F₀ and F₁ parental/retained animals. Evidence of general toxicity in the parental/retained animals was limited to significantly reduced bodyweight gain during gestation and food consumption during lactation for F₀ females at 0.5 ppm. Reproductive effects were seen at the three highest exposure levels. At 0.5 and 0.15 ppm, F₀, F₁ and F₂ uterus weights were increased, F₁ and F₂ weanling testes and epididymides weights were decreased, F₁ and F₂ litter size was reduced, the timing of vaginal patency was accelerated in F₁ females and the timing of preputial separation was delayed in F₁ males. At 0.05 ppm, uterus weights were increased in F₁ and F₂ generations. No general or reproductive toxicity was observed at 0.005 or 0.001 ppm. Overall, the study NOAEL for reproductive toxicity in this E2 study is 0.005 ppm (about 1 µg/kg/day) and the study LOAEL is 0.05 ppm (approx. 10 µg/kg/day).

Developmental neurotoxicity endpoints

The effect of prenatal and perinatal exposure to BPA on neurological development has been investigated in a large number of recent studies. Many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. Thirty-one studies conducted using the oral route and three studies using the subcutaneous route of exposure are summarised below. The impact of these recent studies on the hazard assessment for BPA is assessed using a weight of evidence approach that focuses on the reliability and consistency of the evidence. This overall assessment, which is presented after the individual study summaries, draws attention to a low level of confidence in the reliability of the studies and a lack of consistency in the results, such that no firm conclusions can be drawn.

The literature search also located several developmental neurotoxicity studies in which BPA was administered by intracisternal injection. These studies have been omitted because this route is clearly of no relevance to the risk assessment for human health.

To ensure transparency with respect to any connections between investigations, the study summaries have been grouped according to the investigating team.

Studies by collaborating researchers, mainly from Universities of Florence, Siena, Rome, Calabria and Parma, Italy

The effects of prenatal and post natal exposure to BPA at maternal dose levels of 0.01 - 0.4 mg/kg/day on a range of behaviours and on receptor expression in the brain were investigated in a series of 12 studies conducted by the Italian collaborating researchers.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in male and female Sprague-Dawley rats (Farabollini *et al.* 1999). Dosing was by the oral route, using a micropipette. A group of 11 Sprague-Dawley females was fed BPA dissolved in arachis oil at 0.04 mg/kg/day from 10 days prior to mating to the day of weaning (pnd 21) of their offspring. A second group of 11 females received the vehicle from 10 days prior to mating to gd 13, then 0.4 mg/kg/day BPA from gd 14 to pnd 6 and then the vehicle only until pnd 21. A control group received the vehicle from 10 days prior to mating to pnd 21. Twelve-15 pups/gender/group were selected for behavioural testing, conducted between pnd 85 and 87. The order of testing with respect to treatment group was counterbalanced to avoid confounding due to circadian rhythm and variations in testing conditions. Activity during a 5 min session in a holeboard box was recorded, immediately followed by a 5 min session in an elevated-plus maze. These tests are thought to provide measures of 'anxiety' and locomotion. The statistical analysis was probably conducted using the individual pup as the experimental unit. This is a weakness in the study design, which is present in many of the other BPA developmental neurotoxicity studies.

There were no signs of maternal toxicity or foetal malformations (data not presented). In the holeboard test, the frequency and duration of head dipping for females was significantly reduced (2-4-fold, in comparison with controls) in both BPA groups, with the effects being more marked at 0.4 mg/kg/day. Among males, the only head dipping parameter affected was the frequency, which was reduced at 0.4 mg/kg/day. Reduced head dipping frequency is thought to indicate an increased state of anxiety. No other holeboard parameters were affected by BPA treatment. Among females, the proportion of time spent in the centre of the maze was significantly reduced at both 0.04 and 0.4 mg/kg/day and the frequency of self grooming was increased at 0.04 mg/kg/day. For males in both BPA exposed groups, the percentage of entries that were to open arms and the frequency of stretched posture (stretching the body forward without moving the paws) was significantly increased. A relatively higher proportion of entries to open arms is thought to indicate a decreased state of anxiety, so the results of maze test for males appear in conflict with the holeboard findings.

The effect of prenatal or neonatal exposure to BPA on social and sexual behaviour was investigated in the Sprague-Dawley rat (Farabollini *et al.* 2002). Groups of 7 females were administered 0.04 mg/kg/day BPA via the oral route, by feeding from a micropipette, either during gestation or the lactation period. A group of 13 control females received the arachis oil vehicle throughout gestation and lactation. On pnd 2 the offspring were culled to a standard litter size of 4/sex and cross-fostered to establish the following groups of 12 pups/sex:

- (1) Prenatal exposure group: born to BPA treated mothers and nursed by vehicle control mothers
- (2) Postnatal exposure group: born to control mothers but fostered to BPA treated dams

(3) Control group: born to and nursed by mothers exposed to only the vehicle

Behavioural testing commenced at about 14 weeks of age when the response of each rat to the introduction of an unfamiliar ('intruder') rat of the same sex and bodyweight was assessed over a 15 min period. The results for females not in dioestrus were excluded from the analysis. At 15 weeks, each rat was placed between two cages, one containing a sexually mature male and the other a sexually responsive female, and the time spent ('sexual preference') in the area adjacent to each cage was recorded. In a second phase of this test, each male was given access to a sexually responsive female and its sexual performance was scored. At 16 weeks, the sexual behaviour of each female, when paired with a mature male, was examined. On the day of the test, the stage of the oestrous cycle was determined. Behaviour was video recorded for later scoring by an observer blinded to treatment group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably based on the individual pup, and did not take account of the litter of origin.

In the intruder test, the proportion of prenatally exposed males displaying defensive behaviour was greater (9 vs. 4, out of 12) and displaying ambivalent behaviour was less (3 vs. 8) in comparison with the control males. Also, the ratio of defensive/antagonistic was increased in this BPA group (by 280%). For the BPA postnatal males, and both BPA female groups, intruder test behaviour was similar to the control animals. There were no differences in sexual preference behaviour between the control and BPA exposed groups of either sex. In the sexual performance testing of males, there were statistically significant differences for several parameters in the BPA groups in comparison with the controls; in the post natal group the mean number of intromissions required for ejaculation was increased (15 vs. 11 in the controls) and in the prenatal group the mean latency to first intromission (120 vs. 40 sec) and duration of female genital sniffing (40 vs. 15 sec) were increased. However, the numbers of mounts, latency to ejaculation and time between ejaculation and the next mount were similar for all groups. For the females, the results of the prenatal and post natal BPA groups were combined; for BPA females in pro-oestrus the time taken to enter the arena occupied by the male was reduced (20 vs. 120 sec) and the mean number of displays of lordosis was increased (12 vs. 4).

The effect of prenatal and neonatal exposure to BPA on the behavioural response to pain of offspring was investigated (Aloisi *et al.* 2002). Groups of 7 Sprague Dawley female rats were administered 0.04 mg/kg/day BPA via the oral route, by feeding from a micropipette, during either gestation or the lactation period. As a control group 13 females received the peanut oil vehicle throughout gestation and lactation. Within 48 h of birth the offspring were cross-fostered to form the following groups:

- (1) Prenatal exposure group: 11 males and 9 females born to BPA exposed mothers and nursed by vehicle control mothers
- (2) Postnatal exposure group: born to vehicle control mothers but fostered to BPA exposed mothers
- (3) Control group: 16 males and 11 females, born to and nursed by mothers exposed to only the vehicle

At 22 weeks of age, each pup received a subcutaneous injection of either formalin (half the animals in each group) or saline in a hind paw. Each animal was then observed in an open field for 60 mins. Behaviours such as licking, flexing and jerking of the paw were recorded.

The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis did not take account of the litter of origin.

The frequency of paw jerking was significantly reduced 40-60 min after the injection in the males of the post-natal BPA exposure group. Duration of paw flexing was increased during the first 30 mins in males and females from the pre-natal BPA exposure group. The statistical analysis of the other behaviours observed in the open field, such as duration of exploration activity or rearing, revealed no effect of BPA exposure. Overall, only slight inter-group differences in the pain response were seen in this study.

The effect of prenatal and neonatal exposure to BPA during pregnancy and lactation on play behaviour was investigated in the Sprague-Dawley rat (Dessi-Fulgheri *et al.* 2002). Dosing was by the oral route, using a micropipette. A group of 11 females was fed BPA dissolved in arachis oil at 0.04 mg/kg/day from 10 days prior to mating through to the day of weaning (pnd 21) of their offspring. A second group of 11 females received the vehicle from 10 days prior to mating to gd 13, 0.4 mg/kg/day BPA from gd 14 to pnd 6 and then the vehicle until pnd 21. A control group received the vehicle from 10 days prior to mating to pnd 21. For behavioural testing, either 12 or 15 pups of each sex/group were randomly chosen at weaning from different litters and housed by group in cages containing 3 males and 3 females. Behavioural observations were made at three ages, pnd 35, 45, and 55. Six cage mates were transferred to an arena and observed for 6 minutes; scoring of the various social and non-social behaviours was conducted by an observer blinded to treatment group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. Presumably the individual pup was taken as the experimental unit.

The results for the three ages were pooled for analysis. Eight types of behaviour were identified by principal component analysis, and statistically significant differences between the control and treated groups were reported for four of these. The frequency of play directed towards females was significantly increased in the females of the BPA 0.04 mg/kg/day group. Behaviours considered to represent low-intensity mating elements were significantly decreased in both sexes of the BPA 0.4 mg/kg/day group. Sociosexual exploration was significantly decreased in males of both BPA groups and females at 0.4 mg/kg/day. The frequency of social interest behaviour was significantly decreased in both sexes at BPA 0.4 mg/kg/day, but increased among 0.04 mg/kg/day males.

The effect of prenatal and neonatal exposure to BPA on the somatostatin receptor subtype 2 (sst₂) in the limbic regions of the brain was investigated in the Sprague-Dawley rat (Facciolo *et al.* 2002). Females were fed BPA dissolved in arachis oil via the oral route at 0.04 or 0.4 mg/kg/day from an unspecified time point prior to mating to the day of weaning (pnd 23) of their offspring. A vehicle control group was included. The number of females in each group was not specified, although the report indicated that a total of 32 females were used. At birth, all litters were culled to 8 pups and one pup/litter was cross-fostered to another litter of the same dose group. Receptor binding was assessed using ¹²⁵I-Tyr⁰-somatostatin-14 as a ligand in animals killed on pnd 10 or 23 (4-6 animals/group/age). At the same ages, interactions of sst₂ with α -containing γ -aminobutyric acid (GABA) receptors, using the agonists zolpidem and Ro 15-4513, were examined in 12-13 rats/group. Results were reported for only the 0.40 mg/kg day because higher affinity was obtained for receptor ligand binding. The statistical analysis was probably conducted using the individual pup as the experimental unit, and did not take account of the litter of origin.

At pnd 10, significantly lower sst₂ levels of the low-affinity state receptor were found in the gyrus dentate of the hippocampus and basomedial nucleus of the amygdala. Significantly

higher $ss2_2$ levels were observed only for the high-affinity state, in the periventricular nucleus of the hypothalamus. A similar trend was seen at pnd 23, with the exception that there were much lower levels of the high-affinity $ss2_2$ receptor subtype in the amygdala nucleus and ventromedial hypothalamic nucleus. These differences were potentiated when the binding activity of $ss2_2$ was measured in the presence of two selective agonists (zolpidem and Ro 15-4513) specific for the α -containing GABA type A (α GABA_A) receptor complex. The authors consider that these results provide evidence for a possible role of a $ss2_2$ subtype α -containing GABA type A receptor system in the promotion of oestrogen-like activities of BPA.

In a subsequent study, the effect of prenatal and neonatal exposure to BPA on the expression of somatostatin receptor subtype 3 ($ss3_3$) mRNA receptors in the limbic regions of the brain was assessed (Facciolo *et al.* 2005). Dosing was by the oral route, using a micropipette. Groups of 12 Sprague-Dawley females were fed BPA dissolved in arachis oil at 0.04 or 0.4 mg/kg/day from 8 days prior to mating to the day of weaning (pnd 23) of their offspring. A vehicle control group of 8 females was included. At birth, one female pup/litter was cross-fostered to another litter of the same BPA dose group, but the report does not state that this procedure was also conducted in the control group. On pnd 7 and at 55 days of age, 4 females/group/age were killed. Brains were sectioned and a ³²S-labeled probe was used in an in situ hybridization method to measure $ss3_3$ mRNA expression. The effects of α GABA_A receptor subunits on expression of $ss3_3$ mRNA was examined by incubating the brain sections in 1 nM–100 μ M of α GABA_A receptor agonists (zolpidem, flunitrazepam, RY 080, and RO 15-4513). Additional brain sections from high-dose rats were used to determine interactions between $ss3_3$ with $\alpha 1$ and $\alpha 5$ subunits with or without addition of 5–500 nM zolpidem or RY 080.

In the BPA 0.4 mg/kg/day group killed at 55 days of age, $ss3_3$ mRNA levels in level V of the frontoparietal cortex were reduced in comparison with the control group. In the BPA 0.4 mg/kg/day group killed on pnd 7, $ss3_3$ mRNA levels were reduced in the hypothalamic periventricular nuclei and increased in the ventromedial nuclei. The expression of $ss3_3$ mRNA showed more marked upregulation and downregulation in the presence of agonists specific for α GABA_A receptors. According to the authors, these results suggest that BPA exposure has an influence on cross-talking mechanisms that are implicated in the plasticity of neural circuits.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in the Sprague-Dawley rat (Adriani *et al.* 2003). Groups of 9 mated females were dosed by the oral route, using a micropipette, with 0 (arachis oil vehicle control) or 0.04 mg/kg/day BPA from the day of mating to the day of weaning of their offspring. One male and one female from each litter were retained and subjected to the following series of tests: ‘novelty preference’ behaviour at pnd 30-45; ‘impulsivity’ at pnd >70; open field activity following amphetamine treatment at pnd >70. Testing of the experimental groups was reported to have been counterbalanced across time for the novelty preference test, but it was not stated if this was done for the impulsivity or open field testing.

Novelty preference was tested in an opaque Plexiglas box, subdivided into two compartments that were connected via a closable door. One compartment had a wide-mesh floor and the other a narrow mesh floor. Activity in the apparatus was monitored from video recordings by counting the number of times imaginary lines were crossed. During a familiarisation phase (days 1-3 of the testing schedule) each rat was placed in one compartment for 20 minutes on each day. On day 4 each animal was placed in the familiar compartment for 5 minutes and then the connecting door was opened and the rat was able to explore the whole apparatus for

a 24 minute period. Time spent in each compartment and activity rate (number of lines crossed per minute) were recorded.

Impulsivity testing was conducted during a food-deprivation period. Each animal was placed in box containing two nose-poking holes for 30 minute daily sessions. Nose-poking was detected by a photocell. One hole was termed the ‘immediate and small (IAS)’ hole, poking of which triggered the release of one food pellet. The other was a ‘large and delayed (LAD) hole’ that triggered the release of five food pellets. For a 1 week training period, when food pellet release was immediate, a chamber light was turned on for one second after nose poking and for 25 seconds after food delivery, during which time nose poking had no consequences. At the start of a 1 week testing session a 10 second delay was introduced for the LAD hole between nose-poking and food delivery. This delay was progressively increased to 100 seconds during the week. The light was turned on during the delay, during which time nose-poking (termed ‘inadequate responding’) had no consequence. The percentage choice between the LAD and IAS holes and frequency of inadequate responding was recorded.

The open field response to amphetamine investigation was conducted 1 week after the impulsivity testing. Animals were observed for 30 minutes in an open field, 15 minutes after receiving a subcutaneous injection of either saline (4/sex/group) or d-amphetamine (1 mg/kg, 4/sex/group). Rearing, grooming, and number of lines crossed were recorded.

Graphical representation of the novelty preference test results for day 4 show that males from the control and treated groups spent a similar proportion of time in the novel compartment, whereas for BPA treated females there was a marked, statistically significant, reduction in the proportion of time spent in the novel compartment. The analysis of the activity while in the novel compartment showed statistically significantly increased counts for both males and females of the BPA treated group, particularly towards the end of the 24 minute session.

In the impulsivity test, both treated and control animals of both sexes developed a preference for the LAD hole during the training sessions. During the testing session preference progressively shifted towards the IAS hole, although the BPA group retained a consistently higher preference for the LAD hole compared to controls throughout the testing week. There were no differences in preference between the males and females. Concerning the pattern of inadequate responding, as the length of delay was increased the frequency of inadequate poking in the LAD hole progressively decreased and that for the IAS hole progressively increased. For BPA treated males the progressive increase in inadequate responding at the IAS hole was less marked than for the controls and very similar to the behaviour shown by the female groups. For females, the pattern of inadequate responding for the control and treated groups was similar. The authors’ interpretation of these findings was that the BPA group was exhibiting reduced impulsivity.

The open field behaviour for saline treated animals of the control and BPA groups was similar. But for males only of the BPA treated groups there was a partial inhibition of an amphetamine-stimulated increase in rearing and number of lines crossed.

The effect of prenatal and neonatal exposure to BPA on play behaviour of female offspring was investigated in the Sprague-Dawley rat (Porrini *et al.* 2005). Dosing was by the oral route, using a micropipette. A group of 12 females was fed BPA at 0.04 mg/kg/day from mating through to the day of weaning (pnd 21) of their offspring. A second group of 10 females received the peanut oil vehicle for the same period. Cross-fostering between mothers of the same treatment group was conducted on pnd 2. At weaning, the pups were housed by treatment group in cages containing 3 male and female sibling pairs. Play behaviour was assessed in six cage mates placed together in an arena for 6 min on pnd 35, 45 and 55, as

described by Dessi-Fulgheri *et al.* (2002). The results for 18 females from each exposure group were analysed. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably conducted using the individual pup as the experimental unit, and litter of origin was not taken into account.

Six types of behaviour were identified by principal component analysis, and statistically significant differences between the control and the BPA group were reported for three of these. The score for social and non-social exploration in the BPA group was significantly increased (by 34%) at 35 days and (by ~25%) at 45 days, in comparison with controls. Play with males was significantly decreased (by a factor of 2) at 45 days. Duration of grooming behaviour was reduced (by a factor of 2) on day 45.

The effect of BPA exposure after weaning on the behaviour of juvenile males was investigated in the Sprague-Dawley rat (Della Seta *et al.* 2006). Groups of 26 males were dosed via the oral route, by feeding from a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.04 mg/kg/day from pnd 23 to 30. Another group of 26 males were similarly exposed to 0.0004 mg/kg/day ethinyl oestradiol. The behaviour of 12 males from each group was examined on pnd 45 and at pnd >90. The report did not state whether the order of testing was counterbalanced with respect to treatment group.

At the younger age, the response to the introduction of a PVC tube into the home cage of males housed in groups of 4 was videorecorded and later scored by an observer blind to treatment status. As young adults, the sexual behaviour and performance of each male in the presence of a receptive adult female rat was similarly recorded and scored. Plasma 17 β -oestradiol and testosterone level were measured in 5-8 males/group on pnd 37 and 105. It is not clear from the report if littermates were present in each treatment and age group and, if so, whether the statistical analysis took account of this.

There was no treatment-related effect on bodyweight. Three types of juvenile behaviour were identified by principal component analysis, and statistically significant differences between the control and the BPA group were reported for one. Biting/sniffing/climbing behaviours directed at the PVC tube were significantly lower in the BPA group. In the sexual behaviour assessment, 10/12 controls and 9/12 BPA treated males were active, and the data analysis was restricted to these animals. Only one element of sexual performance was significantly affected; latency to first intromission was reduced. Plasma testosterone levels were significantly lower than controls on pnd 37 (by 33%) and 105 (by 61%). Plasma 17 β -oestradiol was not affected. In the ethinyl oestradiol group exploring, behaviours directed at the PVC tube and sexual performance were reduced, but there were no changes in hormone levels.

The effect of prenatal exposure to BPA on maternal nursing behaviour was investigated in CD-1 mice (Palanza *et al.* 2002). Groups of 10-12 mated females were exposed to BPA via the oral route, by feeding from a micropipette, to levels of 0 (corn oil vehicle control) or 0.01 mg/kg/day from gd 14-18. Pups were weaned on pnd 20. At 2-2.5 months of age female offspring (F₁ generation) were mated, and dosed with either the vehicle or 0.01 mg/kg BPA from gd 14-18, creating four treatment groups as follows: 20 control F₁ females receiving the vehicle only; 15 control F₁ females receiving BPA; 15 BPA F₁ females receiving the vehicle only; 15 BPA F₁ females receiving BPA. Maternal behaviour was monitored over a 2 hour period on each of pnd 2-15. The report did not state whether the order of testing was counterbalanced with respect to treatment group. Additionally, F₂ litter size, pup

bodyweights, cliff drop aversion and righting reflex were recorded. The statistical analysis provided an adjustment for litter effects.

F₁ females exposed to BPA either only prenatally or only as an adult spent significantly less time nursing and in the nest and more time nest building, resting alone, grooming and out of the nest. The only significant effect observed in F₁ females exposed to BPA both in utero and as an adult was increased time resting. There were no significant differences for the F₂ generation parameters. The lack of consistency between the effects seen the groups exposed either prenatally or as an adult and the group exposed during both periods suggests that these intergroup differences were unlikely to have been caused by BPA exposure.

The effect of prenatal exposure to BPA on *d*-amphetamine reinforcing effects was investigated in CD-1 mice (Laviola *et al.* 2005). Groups of 10-12 mated females were exposed to BPA via the oral route, by feeding from a syringe, to levels of 0 (corn oil vehicle control) or 0.01 mg/kg/day from gd 11-18. At pnd 60, 3 males and 3 females from each litter (1/sex/dose level of *d*-amphetamine) were subjected to conditioned place preference testing. The order of testing with respect to treatment group was counterbalanced. On the first day of the test procedure, animals were familiarised to the apparatus. On days 2 and 4 each animal received an intraperitoneal injection of 0, 1, or 2 mg/kg *d*-amphetamine and were confined to one compartment of the test apparatus for 20 minutes. On days 3 and 5 each animal was injected with saline and confined in another compartment for 20 minutes. On the final day of testing, each animal was given free access to the entire apparatus for 10 minutes without *d*-amphetamine or saline treatment and the time spent in each compartment and total locomotion activity was recorded.

Conditioned place preference occurred in control females following injection with either *d*-amphetamine dose, but was not observed in the BPA exposed females. In males, a preference displayed for the *d*-amphetamine-associated compartment was similar for the BPA exposed and control animals. There were no significant differences in locomotor activity between the BPA and control groups.

Ceccarelli *et al.* (2007) investigated the effect of juvenile BPA exposure on brain development of Sprague-Dawley rats. Groups of 14 juveniles/group (sex distribution not stated) were dosed by the oral route, using a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.04 mg/kg/day BPA from pnd 23 to 30. Another group of 14 juveniles were similarly exposed to 0.0004 mg/kg/day ethinyl oestradiol. Half the animals were killed on pnd 37 and half on pnd 90. Females killed on pnd 90 were killed in oestrus. Blood samples were taken and brains were processed for immunohistochemistry. ER α levels were analysed in three sexually dimorphic regions of the hypothalamus: arcuate nucleus, ventromedial nucleus and medial preoptic area. Serum testosterone and 17 β -estradiol were determined.

The ER α analysis revealed just one statistically significant observation for comparisons between the control and same sex BPA groups; levels were higher (~2-fold) in the ventromedial nucleus among BPA females killed on pnd 37. On day 37, serum testosterone levels in the BPA males were significantly lower (by ~30%). 17 β -estradiol levels were not affected by BPA treatment. In the ethinyl oestradiol group there were occasional differences in ER α levels, which were not consistent with the change in the BPA group. Also, testosterone levels were increased in males on pnd 37 and 17 β -estradiol levels were increased in females on pnd 90 in the ethinyl oestradiol group. Overall, some evidence of differences in ER α in one of three sexually dimorphic regions of the hypothalamus was seen following BPA exposure to juvenile females.

Studies by researchers associated with Hoshi University, Japan

A series of five studies designed to investigate the effect of prenatal and postnatal exposure to BPA at maternal dose levels ranging from 0.006 - 250 mg/kg/day on the central dopaminergic system in mice were conducted by the Hoshi University investigators.

Suzuki *et al.* (2003) investigated the effect of prenatal and neonatal exposure to BPA on the dopamine D1 receptor-dependent rewarding effect, locomotion stimulation of methamphetamine and dopamine D₁ receptor activity in male ddY mice. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 2.5, 60, 250 mg/kg/day during gestation and lactation. A place-preference conditioning test was conducted on 6-10 male offspring per group (age not reported). In a six day conditioning period animals received either an injection of methamphetamine and immediately placed in a particular compartment for 50 min (days 1, 3, 5) or saline and placed in the other compartment (days 2, 4, 6) for the same time period. On day 7 each animal was, untreated, given free access to the entire apparatus for 15 min and the time spent in each compartment was recorded. Locomotor activity after either a saline or 2 mg/kg methamphetamine subcutaneous injection was automatically measured over a 3 hour period in 9-10 offspring/group. Further injections of methamphetamine were given at 7 day intervals to investigate if sensitisation to the locomotion-stimulating effects of methamphetamine was induced. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The effects on dopamine D1 receptor-mediated G-protein activation (indicating up-regulation of these receptors) by dopamine in limbic forebrain homogenates taken from three control and three 250 mg/kg/day animals (age when killed was not reported) were measured in a ³⁵S-guanosine-5' [γ-thio]-triphosphate binding assay. Protein levels of dopamine and vesicle monoamine transporters in the brain were determined by Western blot and mRNA levels of dopamine D1 receptor in the brain were determined by RT-PCR, also in three control and three 250 mg/kg/day animals. It is not clear if the statistical analysis used the litter or the pup as the experimental unit.

BPA treatment had no effect on maternal bodyweight or clinical condition. In place-preference conditioning testing, there was a dose-dependent and statistically significant increase in all BPA exposed groups in preference for the compartment associated with methamphetamine. Preference for this compartment was eliminated by injecting the animals with SCH23390, a dopamine D1 receptor antagonist. The stimulation of locomotion by methamphetamine was significantly enhanced (by ~80% at peak) in the BPA 250 mg/kg/day group, and this stimulation was more pronounced when methamphetamine injection was repeated. Dopamine-induced binding of ³⁵S-guanosine-5' [γ-thio]-triphosphate to membranes was increased in the BPA groups, indicating enhanced G-protein activation, which was eliminated following injection with SCH23390 or sulpiride, a dopamine D2 receptor antagonist. No changes were observed for expression of dopamine and vesicle monoamine transporter proteins. Expression of dopamine D1 receptor mRNA was significantly up-regulated to 130% of control levels in the high-dose BPA group.

Mizuo *et al.* (2004a) investigated the effect of prenatal and neonatal exposure to BPA on the rewarding effects, locomotion activity and receptor activity induced by morphine in male ddY mice. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 2.5, 60, 250 mg/kg/day during gestation and lactation. Place conditioning testing was conducted in 6-10 male offspring/group (litter distribution was not reported). Each animal was placed in one compartment of a testing apparatus following saline injection and in

a second compartment of the apparatus following morphine injection. On the next day each animal was given free access to both compartments and the time spent in each compartment was measured. Locomotor activity was automatically measured over a 3 hour period in 9-10 offspring/group after either a saline or 10 mg/kg morphine injection. The order of testing with respect to treatment group was counterbalanced. Morphine stimulated ^{35}S -guanosine-5' [γ -thio]-triphosphate binding to membranes (an indication of μ -opioid receptor mediated G protein activation) and expression of μ -opioid receptor mRNA were measured in midbrain homogenates taken from three control and three BPA 250 mg/kg/day animals.

BPA treatment had no effect on maternal bodyweight or clinical condition. In place-preference conditioning testing, there was a dose-dependent and statistically significant increase at 60 and 250 mg/kg/day in preference for the compartment associated with morphine. Locomotion at 250 mg/kg/day only was significantly increased (130 activity counts vs. 10 in controls) following morphine injection. Although place preference differences were observed, there were no effects of BPA on ^{35}S -guanosine-5' [γ -thio]-triphosphate binding or expression of μ -opioid receptor mRNA.

The effect of prenatal and neonatal exposure to BPA on functional changes in dopamine D3 receptors was investigated in male ddY mice (Mizuo *et al.* 2004b) Females (group size not reported) received BPA via the diet at estimated dose levels of 0 or 250 mg/kg/day during gestation and lactation. Offspring were killed (numbers and age not reported) and limbic forebrain homogenates were prepared. The effects on dopamine D3 receptor-mediated G-protein activation by 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT, a D3 receptor agonist) were measured in a ^{35}S -guanosine-5' [γ -thio]-triphosphate binding assay, and a dopamine D₃ receptor binding assay using [3H] PD128907 was conducted. The expression of dopamine D3 receptor mRNA was measured in a RT-PCR assay. Results for 3 animals/group (litter distribution not described) were reported.

BPA treatment had no effect on maternal bodyweight or clinical condition. It was found that G-protein activation by the D3 receptor agonist was markedly reduced in the BPA group, in comparison with controls. In the D₃ receptor binding assay, a decrease of the receptor density in the brain was found in the BPA exposed group.

Narita *et al.* (2006) investigated the effect of prenatal and neonatal exposure to BPA on the dopaminergic system of ddY strain mice, in a very poorly written report. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 0.006, 0.06, 0.6, 100, or 400 mg/kg/day during gestation and lactation. A series of investigations were conducted. Firstly, a place conditioning test was conducted in 6-14 mice/group (gender not specified). In the preconditioning phase mice were placed in compartment of the apparatus immediately after a saline injection and in another compartment following sc injection of 1 mg/kg morphine. On the day of testing, the amount of time spent in each compartment was recorded. Secondly, locomotor activity following a subcutaneous injection of 10 mg/kg morphine was measured for 3 hours in 5-15 mice from the 0, 0.006, 0.6 or 400 mg/kg groups. Also, dopamine induced binding of ^{35}S -guanosine-5' [γ -thio]-triphosphate was measured in limbic forebrain homogenates from 3 offspring of the 0, 0.006, 0.6 or 400 mg/kg groups. The order of testing with respect to treatment group was counterbalanced. It is not clear from the report if the statistical analysis was conducted using the litter or the individual pup as the experimental unit.

There were no effects on maternal bodyweight gain. In the place conditioning test, mice from the 0.006, 100 and 400 mg/kg/day groups spent more time (about 8-fold) in the compartment cage associated with morphine injection, compared with controls; no place preference was

shown by the BPA 0.06 and 0.6 mg/kg/day groups. In the locomotion test, activity after the morphine injection was increased (about 10-fold) at 0.006 and 400 mg/kg/day. In the binding assay, dopamine-induced binding was increased at 0.006, 0.6 or 400 mg/kg/day (by about 32, 18, and 56%, respectively), compared with controls. According to the authors, these findings suggest that prenatal and neonatal exposures to low BPA doses may potentiate central dopamine receptor dependent neurotransmission in the mouse. However, in the absence of a conventional dose-response relationship it is plausible that the observed inter-group differences were due to background variation.

Narita *et al.* (2007) provided a further investigation into the effect of prenatal and neonatal exposure to BPA on the dopaminergic system of male ddY strain mice. Mated females (group size not reported) received BPA via the diet at estimated dose levels of 0 or 400 mg/kg/day from either gd 0-7, gd 7-14, gd 14-20 or pnd 0-20. A similar series of investigations to those described by Narita *et al.* (2006) were conducted in 6-16 male offspring/group, at 7-9 weeks of age. In the place preference test, a clear preference for the compartment associated with morphine was seen in the males exposed for the periods gd 7-14 and pnd 0-20, but not for gd 0-7 or gd 14-20. In the locomotion test, activity after the morphine injection was markedly increased in the 7-14 and pnd 0-20 groups, but again the gd 0-7 or gd 14-20 groups were not affected. Similarly, dopamine-induced binding was potentiated in the gd 7-14 and pnd 0-20 groups, but not in the gd 0-7 or gd 14-20 groups.

Studies from Chemical Industry Institute for Toxicology (CIIT), USA

Three studies, focussing on brain structure, have been conducted at CIIT.

Kwon *et al.* (2000) examined the effect of prenatal and neonatal exposure to BPA on SDN-POA (Sexually Dimorphic Nucleus of the PreOptic Area) volume in female Sprague-Dawley rats (other reproductive parameters were also assessed). Groups of 8 mated females were dosed orally by gavage with 0 (vehicle control) 3.2, 32 or 320 mg/kg/day BPA from gd 13 to pnd 21. A positive control group received diethylstilbestrol at 1.5 mg/kg/day. On pnd 10, 1-3 female pups per litter were killed for measurement of SDN-POA volume. Other parameters measured in offspring included reproductive organ weights and histopathology in males and oestrous cyclicity, vaginal opening and lordosis behaviour in females. The statistical analysis was conducted using the litter as the experimental unit.

There was no evidence of maternal toxicity, based on bodyweight and organ weight analysis. BPA treatment had no effect on SDN-POA volume, or any of the other parameters investigated. In the positive control group there was an increase in maternal liver weight, increased SDN-POA volume and disrupted oestrous cycling. Thus, this study provided no evidence of an effect on SDN-POA volume of prenatal and neonatal BPA exposure maternal dose levels of up to 320 mg/kg/day.

The effect of short-term neonatal exposure to BPA on the development of the anteroventral periventricular nucleus of the hypothalamus (AVPV) was investigated in the Sprague Dawley rat (Patisaul *et al.* 2006). Group size was 5-8 pups/sex. BPA was administered by subcutaneous injection on pnd 1 and 2 at a dose level of approximately 100 mg/kg/day. A control group received the sesame oil vehicle. On pnd 19 the pups were killed and the brains were removed for immunohistochemical processing. The numbers of cells in the AVPV immunoreactive for ER α and/or tyrosine hydroxylase (TH) were counted.

As expected, based on earlier investigations, TH expression was sexually dimorphic; higher numbers were present in females. In the BPA group, the numbers of TH positive cells for females were similar to the female controls, but for males the numbers were increased (by ~75%) in comparison with the control males. For ER α expression, which is not sexually dimorphic, there were no differences between the control and BPA treated groups. The number of cells positive for both TH and ER α was substantially reduced in the medial AVPV for BPA females, reducing the marked sexual dimorphism normally present in this area. There were no differences in the numbers of double-positive cells among the males.

In a further study, the effect of short term neonatal exposure on the development of the AVPV and SDN-POA was investigated in male Sprague Dawley rats (Patisaul *et al.* 2007). Group size was 5-8 male pups. BPA was administered by subcutaneous (sc) injection on pnd 1 and 2 at a dose level of approximately 100 mg/kg/day. A vehicle control group received the sesame oil vehicle on these days. On PND 85, males were gonadectomised. Six age-matched female rats were ovariectomised and assigned to the study for comparative purposes. After a recovery period, the rats were given sc injections of 10 μ g estradiol benzoate, and 48 hours later, a sc injection of 500 μ g progesterone. The authors note that this protocol has consistently induced fos gene expression in GnRH neurons, leading to LH release in females. About 8 hours later the animals were killed and the brains removed for processing. The volumes of the AVPV, SDN-POA and calbindin-immunoreactive regions of the SDN-POA and the numbers of immunoreactive calbindin, GnRH and fos cells were counted.

BPA had no effect on the volumes of the SDN-POA, AVPV and immunoreactive regions of the SDN-POA. Also, there was no effect on the numbers of GnRH reactive cells. There was no fos gene expression in the males, indicating the absence of GnRH activation, as is normal for males. However, there was a significant increase in SDN-POA calbindin-positive nuclei (by ~40%), in comparison with controls.

Studies by researchers mainly from University of Tokyo, Japan

The Tokyo group has published two studies investigating the effects of prenatal and postnatal BPA exposure on behaviour, either at dose levels of 4 - 400 mg/kg/day, or 0.1 mg/kg/day.

The effect of prenatal and neonatal exposure to BPA on behaviour was investigated in F344 rat (Negishi *et al.* 2003). Groups of 8 or 9 mated females were dosed orally by gavage with 0 (olive oil vehicle control), 4, 40 or 400 mg/kg/day BPA from gd 10 to pnd 20. Maternal bodyweights, litter size and pup bodyweights were recorded. Eight pups/sex/group were randomly selected for necropsy at 8 weeks of age and selected organ weights were recorded. Three behavioural evaluations were conducted in all offspring: spontaneous motor activity at about 4 weeks of age, an active avoidance test at about 4 or 8 weeks of age and open field behaviour at about 8 weeks of age. Spontaneous activity was measured using a heat sensor mounted above the examination cage, over 12 hours during a dark phase of the daily light cycle. The active avoidance test was conducted in a two compartment shuttle box. The animals were each subjected to 50 daily trials, with 50 seconds between each trial, on 3 consecutive days. For each trial a 5 second buzzer and light stimulus (the conditioned stimulus) was provided, followed by a 5 second electric shock (the unconditioned stimulus) delivered through the cage floor. A correct response was recorded when the animal moved to the other compartment during the 5 second buzzer/light stimulus. Open field behaviour was monitored over 5 minutes, during a dark phase of the daily cycle. Grooming, locomotion, stretching, rearing, or other activity was analysed using a computer assisted system. The

report did not state whether the order of testing was counterbalanced with respect to treatment group. It is not clear from the report if the statistical analysis was conducted using the litter or the individual pup as the experimental unit.

Maternal weight gain at 40 and 400 mg/kg/day was significantly reduced throughout most of the treatment period. The bodyweights of male offspring at 400 mg/kg/day were slightly, though significantly, lower than controls throughout much of the lactation (by ~10% on pnd 21) and post-lactation periods. For male offspring at 40 mg/kg/day and females at 40 and 400 mg/kg/day there were occasional statistically significantly lower bodyweights. Organ weights were not affected by maternal BPA treatment.

Spontaneous motor activity, in terms of activity 'counts', was not influenced by maternal BPA treatment; the authors drew attention to longer periods of inactivity in the BPA groups, but these differences did not follow a dose-related pattern. In the shuttle box active avoidance test, at 4 weeks of age the males at 40 and 400 mg/kg/day recorded a significantly greater proportion of correct responses, but a consistent dose-related response was not apparent across the 1st, 2nd and 3rd days of the trial. At 8 weeks of age the proportion of correct responses at 40 and 400 mg/kg/day was similar to the controls, although at 4 mg/kg/day the proportion of correct responses was lower than controls on the 1st day of the trial. Active avoidance behaviour for the females was similar for all four experimental groups. In the open field test there were no inter-group differences in locomotion, stretching, rearing or other behaviour. However, the proportion of time spent grooming for males at 4 mg/kg/day was significantly greater than the controls, but in the absence of a dose-related response this difference was considered unlikely to be due to BPA exposure.

The effect of prenatal exposure to BPA on behaviour of males was investigated in the F344 rat (Negishi *et al.* 2004). Groups of 10 or 11 mated females were dosed orally by gavage with 0 (corn oil vehicle control) or 0.1 mg/kg/day BPA from gd 3 to pnd 20. One male pup/litter was selected for a series of behavioural tests. These were: open field at 8 weeks of age, spontaneous activity at 12 weeks, passive avoidance at 13 weeks, elevated plus-maze at 14 weeks, active avoidance at 15 weeks and a monoamine-disruption test (a comparison of open field behaviour following intraperitoneal injections of 0.9% saline and the monoamineoxidase inhibitor trans-2-phenylcyclopropyl-amine hydrochloride (Tcy)). The report did not state whether the order of testing was counterbalanced with respect to treatment group.

There were no effects on maternal or pup bodyweights. Behaviour in the open field and elevated-plus maze, and spontaneous activity were not affected by BPA exposure. In the passive avoidance test there were no statistically significant differences between the control and BPA group for any of the parameters recorded, although the BPA exposed rats tended to delay entry into a dark compartment in the retention trial. In the active avoidance test BPA-treated offspring showed significantly fewer correct avoidance responses during the 1st, 2nd and 3rd of the four training sessions (each comprising of 25 trials), indicating slower learning. Also, among BPA-treated offspring there was an increase in the number of failures to respond to both the conditioned stimulus (buzzer and light) and unconditioned stimulus (electric shock) compared with the controls (2.5% failures, vs. ~0.3 in controls). In the monoamine disruption test, BPA exposed males failed to show the significant increase in the proportion time in locomotion behaviour in response to Tcy that was seen in controls (14 vs. 24%). However, a Tcy-induced significant reduction in rearing frequency among controls was also seen in the BPA group.

Studies by researchers associated with Kushi academic institutes, Fukuoka, Japan

The Kushi group has conducted five studies, investigating the effects of prenatal and postnatal BPA exposure at maternal exposure levels of 0.002 - 1.5 mg/kg/day on behaviour or brain structure.

The effect of prenatal and neonatal exposure to BPA on behaviour and brain development was investigated in the Wistar rat (Kubo *et al.* 2001). Groups of 5 mated females were administered BPA via the drinking water at 0 (vehicle control) or approximately 1.5 mg/kg/day during gestation and the lactation period. Open field behaviour was investigated in offspring (11-14/group, sex and litter distribution not reported) at 6 weeks of age. The report did not state whether the order of testing was counterbalanced with respect to treatment group. A passive avoidance test was conducted at 7 weeks (11-14/group). At 20 weeks of age the volumes of the sexually dimorphic nucleus of the preoptic area (SDN-POA) and locus coeruleus were measured (6-7/sex/group). It was not clear if the statistical analysis was conducted using the litter or individual pup as the experimental unit.

In the open field, the distance moved, rearing frequency and time spent in centre of the field were all significantly greater for control females than control males. In the passive avoidance test the latency to enter the dark chamber following a shock was significantly longer in control males compared with control females of the same group. For the BPA group, these gender-related differences were not present. This was due to differences in the behaviour of both sexes; the three open field parameters were higher and the passive avoidance test latency period shorter for BPA males in comparison with control males, and vice versa for the BPA females. However, there were no statistically significant differences when the results for BPA males and females were compared with their gender controls. The volume of the SDN-POA in the control group was significantly greater in males than females, and a similar difference was also present in the BPA group. In contrast, a gender difference observed in the control group for the volume of the locus coeruleus (~14% greater for females) was reversed in the BPA group (volume was greater for males). However, it should be noted that the difference between control males and BPA males was not statistically significant, and neither was the difference between control females and BPA females.

Kubo *et al.* (2003) investigated the effect of prenatal and neonatal exposure to BPA on behaviour and brain development in a second study. Groups of 5-6 mated female Wistar rats were administered BPA via the drinking water at 0 (vehicle control), 0.03 or 0.3 mg/kg/day (estimated intakes). As positive controls, groups of 5 mated females received either diethylstilbestrol (~0.0065 mg/kg) or resveratrol (~1.5 mg/kg/day) via the drinking water. The animals were probably exposed throughout gestation and the lactation period, although the report is not clear on this. Open field testing was conducted at 6 weeks of age on 24 animals/group; the sex and litter distribution of the tested pups was not reported. Sexual behaviour of males (in the presence of a receptive untreated female) and females (in the presence of a sexually vigorous untreated male) was assessed at 11-12 weeks of age in 7-13 animals/sex/group. The report did not state whether the order of testing was counterbalanced with respect to treatment group. At 14 weeks of age the volume of the SDN-POA and locus coeruleus was measured (7-8/sex/group). Also, the number of neurones in the locus coeruleus was estimated. The study was conducted as 3 replicated blocks. The data were analysed using the individual pup as the experimental unit.

As with the previous Kubo *et al.* (2001) study, in the open field the distance moved, rearing frequency and time spent in centre of the field for control females were all significantly

greater than control males. With the exception of the distance moved in the BPA 0.03 mg/kg/day group where the normal significant sex difference was present, these sex differences were less marked and did not achieve statistical significance in the BPA group. The masking of the sex difference was due primarily to a greater distance moved and rearing frequency among BPA males and a reduced amount of time spent in the centre for the BPA females, in comparison with their gender controls. With regard to the assessment of sexual behaviour there was no evidence of a treatment-related effect in either males or females. In the diethylstilbestrol group, the open field distance moved, rearing frequency and time spent in centre of the field were significantly increased for both sexes. In the resveratrol group there were no effects on open field behaviour. Consistent with the Kubo *et al.* (2001) study, the volume of the SDN-POA was similar in both the control and BPA exposed groups and the gender difference observed in the control group for the volume of the locus coeruleus (greater for females) was reversed in the BPA group. However, a 'conventional' dose response relationship was not apparent for the locus coeruleus differences as the extent of the reversal was greater in the 0.03 mg/kg/day group. Differences in the locus coeruleus neurone count matched those observed for volume of this brain. In both the diethylstilbestrol and resveratrol groups the normal locus coeruleus sex difference was reversed but SDN-POA was not affected.

Fujimoto *et al.* (2006) conducted a range of behavioural testing in the offspring of mothers exposed to BPA during the last week of gestation. Groups of 6 Wistar rats received BPA via the drinking water from gd 13 to pnd 0 at 0 (vehicle control) or approximately 0.015 mg/kg/day. The following behavioural tests were conducted in the offspring (20-24/sex/group in each test): open field at 6 weeks of age, elevated plus maze at 7 weeks, passive avoidance at 8 weeks and forced swimming test at 9 weeks. The report did not state whether the order of testing was counterbalanced with respect to treatment group. The statistical analysis was probably conducted using the individual pup as the experimental unit.

In the open field test, treatment related differences were observed for rearing behaviour. The duration of rearing was significantly greater for BPA males (by ~50%) in comparison with the control males. Among the control offspring, the rearing frequency and duration was significantly greater for the females as compared with males, but this gender difference was not present in BPA group, due to both a comparative increase in rearing activity in males and a slight decrease in females of the BPA group. BPA treatment had no effect on the other parameters measured in the open field test. Behaviour in the elevated plus maze and passive avoidance test was similar for controls and the BPA group. In the forced swimming test, the duration of immobility was significantly greater (by ~75%) and the duration of limb movements was significantly less (by ~8%) for BPA males compared with control males. For females, the duration of diving was significantly greater (by ~28%) in the BPA group. Also, the significant gender difference in duration of struggling seen in the controls (longer for females) was not seen in the BPA group, due to both a comparative increase in duration for males and a slight decrease for females of the BPA group.

Kawai *et al.* (2003) investigated the effect of prenatal exposure on 'aggressive' behaviour in male CD-1 mice. Groups of 7-9 females were dosed by the oral route, using a micropipette, with BPA at 0 (corn oil vehicle control), 0.002 and 0.02 mg/kg/day from gd 11-18. 'Aggression' testing was conducted 8, 12 and 16 weeks of age in groups initially comprising of 26-32 male offspring randomly selected from each treatment group. Each male was placed in a cage with an 'opponent' mouse (a male specially selected from the control group, used for testing once/day) and their behaviour was observed for 7 mins. The report did not state whether the order of testing was counterbalanced with respect to treatment group. About 10

of the test mice from each group were killed at 9, 13 and 17 weeks of age for testes weight and serum testosterone level measurement. It was not clear from the report whether the statistical analysis had been conducted using the litter or the individual pup as the experimental unit.

'Aggression' scores, as determined by contact time with the 'opponent', were significantly increased (about 2-fold) at 8 weeks at both 0.002 and 0.02 mg/kg/day, compared to the control group at 8 weeks. No effect on aggression score was seen at 12 and 16 weeks. The lack of a conventional dose response relationship and absence of an effect at older ages suggests that the increased aggression score at 8 weeks was not related to BPA treatment.

Kawai *et al.* (2007) investigated the effect of prenatal exposure to BPA on the expression of ER α and ER β on male ICR mice. Groups of 18 pregnant females were dosed by the oral route, using a micropipette, with BPA at 0 (peanut oil vehicle control) or 0.002 mg/kg/day from gd 11-17. The pups were weaned pnd 21. At 4-5, 8-9 or 12-13 weeks of age, 8-12 randomly selected male offspring for each group were killed. Serum testosterone levels were measured. Brains were removed and processed for immunostaining with antibody to ER α , ER β , serotonin and serotonin transporter. Immunoreactivity levels in sections of the dorsal raphe nucleus were assessed. It is not clear from the report if the analysis took account of litter effects.

The number of neurons expressing ER α and ER β was significantly greater (by ~40-140%) in the BPA group at 5 and 13 weeks but not at 9 weeks, in comparison with controls. There were no differences in the numbers of serotonin immunoreactive neurones or in the immunoreactivity of serotonin transporter in the BPA group. Overall, this study suggested that prenatal BPA exposure might influence the numbers of ER α and ER β in the brain of males, but in the absence of differences at 9 weeks of age this evidence is questionable.

Studies from Kyoto Prefectural University of Medicine

Two studies, investing brain structure using immunohistochemical techniques, were conducted by the Kyoto group.

Nakamura *et al.* (2006) investigated the effect of prenatal exposure to BPA on brain (neocortex) development in ICR/Jcl mice. Females (group size not reported) were dosed by subcutaneous injection with BPA at 0 (sesame oil vehicle control) 0.02 mg/kg/day from gd 0 to 16. Pregnant mice received an intraperitoneal dose of 0.5 mg 5-bromo-2'-deoxyuridine on either gd 10, 12, 14, or 16; the embryos were removed either 1 hour or 2-3 days later (10 embryos/group). The embryonic forebrains were dissected out and processed for immunohistochemistry using a range of primary antibodies. Morphometric analysis of the neocortex was conducted on 10 embryos from two or more dams/group/day of kill. Also, the expression of various genes within the forebrain was determined by RT-PCR.

BrdU-labelled cells examined 1 hour after BrdU injection showed no differences between the BPA and control groups, which indicated that the proliferation of precursor cells was not affected. The BrdU-labeled cells, analysed 2 days after BrdU injection, were decreased in the ventricular zone of BPA group killed on gd 14 and 16, whereas they were increased in the cortical plate at gd 14, compared with controls. Furthermore, the expression of Math3, Ngn2, Hes1, LICAM, and THR α was significantly upregulated at E14.5 in the BPA-treated group. According to the authors, these results suggest that BPA might disrupt normal neocortical development by accelerating neuronal differentiation/migration.

Tando *et al.* (2007) investigated the effect of prenatal and neonatal BPA exposure on brain development in ddY mice. Females (group size not reported) received BPA via the diet at estimated dose levels of 0, 4.5 or 1200 mg/kg/day during gestation and lactation until pnd 21. At 8-11 weeks of age offspring were killed and the brains were removed and processed for immunohistochemical detection of the following proteins: tyrosine hydroxylase (assessed in the substantia nigra), calbindin D-28 K, calretinin, and parvalbumin (assessed in the somatosensory cortex). Also, cell death was assessed using terminal transferase dUTP nick end labelling (TUNEL) staining.

The distribution and density of immunopositive staining for calbindin D-28K, calretinin, and parvalbumin (regarded as markers for GABAergic neurons) in the BPA groups was similar to controls. The volume and density of tyrosine hydroxylase-positive (regarded as a marker for dopaminergic neurons) nuclei and fibres in the substantia nigra were reduced (by about 18%) in female offspring at 4.5 mg/kg/day BPA (but not at 1200 mg/kg/day), compared with controls. TUNEL staining did not reveal the presence of degeneration or cell death.

Studies from other Institutes

Five independent studies from other institutes, investigating the effects of BPA on various aspects of neurodevelopment, are available.

The effect of prenatal and postnatal exposure to BPA on behaviour was investigated in a conventional (similar to OECD test guideline 416) 2-generation study (Ema *et al.* 2001). Groups of 25 male and female Sprague Dawley rats (F₀ generation) were administered BPA by oral gavage at 0 (water vehicle control), 0.0002, 0.002, 0.02 or 0.2 mg/kg/day for 10 (males) or 2 (females) weeks prior to mating. Dosing of the females continued throughout gestation and lactation. At weaning, 1-2 F₁ pups/sex/litter were retained for continued dosing, as for their respective parents. At 5-6 weeks of age, 1 pup/sex/litter was observed in an open field for 3 min. At 6-7 weeks of age, 6 pups/sex/group was tested in a water-filled T-maze; time from start of run to touching the escape ramp and number of errors was recorded. The results were not presented in the report, but the authors stated that there were no BPA-related differences in open field behaviour or T-maze performance.

The effects of neonatal exposure to bisphenol A on Morris water maze performance were investigated in F344 rats (Carr *et al.* 2003). Groups of 10 neonatal rats of each sex were dosed orally, by gavage, with 0 (safflower oil vehicle control), 0.1 or 0.25 mg/kg/day BPA dissolved in safflower oil from pnd 1 to pnd14. An additional group of 10 neonates of each sex was similarly dosed with 17 β -estradiol (E₂) at 0.072 mg/kg/day. Litter-mates were assigned to treatment groups such that there was one member of each treatment group for each sex in each litter.

On pnd 33 the rats were tested in a straight swimming channel, 150 cm in length. A trial consisted of placing each rat facing the wall at one end of the channel and the time taken to swim to a wire escape ramp at the other end of the channel was recorded. Each rat was subjected to 4 trials on one day, with a one minute interval between each trial. On pnd 34, Morris water maze testing commenced. The report did not state whether the order of testing was counterbalanced with respect to treatment group.

There were no treatment-related effects on bodyweight or on performance in the straight channel swimming test. In the Morris maze there were no statistically significant differences

between the control and BPA treated groups for the mean time taken to find the escape platform during the acquisition phase. However, in the memory retention test the time spent in the escape quadrant was less (23 vs. 29 seconds for males, 14 vs. 22 seconds for females) for animals from the BPA 0.25 mg/kg/day group when compared with controls, with the difference achieving statistical significance for the females. This was interpreted by the authors as indicating an impairment of the retention of spatial memory in the BPA group. For the E₂ group the only significant difference relative to controls was a prolonged time for males to find the platform on the 3rd day of the acquisition phase.

The effect of prenatal and neonatal exposure to BPA on numbers of corticotrophin releasing hormone (CRH) neurones in the brain was investigated using immunohistological techniques in the Wistar rat (Funabashi *et al.* 2004). Groups of 8-11 mated females received BPA via the drinking water during gestation and until weaning on gd 21 at 0 (vehicle control) or approximately 2.5 mg/kg/day. The study was conducted as two series of experiments, the results of which were combined. Offspring (8-11/sex/group) were killed at between 4 and 7 months of ages for brain examination; females were killed in the pro-oestrus phase of the oestrous cycle. It was not clear from the report if 1/sex/litter was selected, or if control and exposed animals of each gender were killed at similar ages. The numbers of CRH-immunoreactive neurones in the anterior and posterior areas of the bed nucleus of the stria terminalis (BST) and in the preoptic area were counted.

In both the anterior and posterior BST the numbers of CRH-immunoreactive neurones for each sex were not significantly different in comparison with their respective controls. However, the statistically significant gender difference in numbers of neurones in the control group (numbers were about 2-fold higher in females) was not seen in the BPA exposed group. This was due to the presence of relatively higher numbers of neurones in males and lower numbers females. In the preoptic area there were no differences between the control and BPA exposed group in the numbers CRH-immunoreactive neurones.

Honma *et al.* (2006) investigated the effect of prenatal and neonatal exposure to BPA on brain development in female Sprague-Dawley rats. Groups of 6 mated female rats were dosed orally, by gavage, with BPA at dose levels of 0 (corn oil vehicle control), 4 or 40 mg/kg/day from gd 6 to pnd 20, when the offspring were weaned. Female offspring were killed, usually 4-6/sex/group, at 1, 3, 6 or 9 weeks of age and the brains were processed for analysis. The levels of the following neurotransmitters in a number of areas of the brain were analysed by HPLC: noradrenalin, dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid, serotonin and its metabolite 5-hydroxyindoleacetic acid, acetyl choline and its precursor/metabolite choline. No differences were reported at 1 week of age. At 3 weeks, levels of the dopamine metabolites, serotonin and its metabolites were increased in some brain areas of BPA exposed offspring. At 6 and 9 weeks of age there were few statistically significant differences in the BPA groups when compared with the controls. Overall, there were no discernable patterns to the variations of neurotransmitter levels and no conclusions could be drawn from this study.

Ryan and Vandenberg (2006) investigated the effect of prenatal and neonatal exposure to BPA on aspects of the non-reproductive sexually dimorphic behaviour of ovariectomised female C57/B1/6 mice. Ovariectomised animals were used because, according to the authors, the potential confounding effects of oestrous cycling in sexually dimorphic behaviours are eliminated. Groups of mated females (group size probably 14-16) were dosed orally, by gavage, with 0 (corn oil vehicle control), 0.002 or 0.2 mg/kg/day BPA from gd 3 to pnd 21, when the offspring were weaned. Another group was similarly dosed with 0.005 mg/kg/day

ethinyl oestradiol. Litter size, anogenital distance, and pup bodyweight were recorded at weaning. At pnd 28 one randomly chosen female pup from each litter was surgically ovariectomised and retained for behavioural testing, which commenced at pnd 44. The following behavioural tests were conducted on 14-16 females/group: elevated plus maze, light-dark preference chamber (regarded as tests for anxiety), radial arm maze and modified Barnes maze (regarded as tests of spatial memory). The report did not state whether the order of testing was counterbalanced with respect to treatment group. The day on which cornified cells were first seen in a vaginal smear (regarded as onset of puberty) was recorded.

There were no effects of treatment on litter size, anogenital distance, and pup bodyweight at weaning. The onset of puberty was significantly earlier in the BPA 0.2 mg/kg/day group (by 4.5 days). There were no statistically significant differences in the parameters recorded in the elevated plus maze for the BPA groups, in comparison with the vehicle controls. In the light-dark preference chamber, the time spent in the light chamber was significantly less (by 52%) than vehicle controls at 0.2 mg/kg/day, indicating higher levels of anxiety. Performance in the spatial memory tests for the BPA groups was similar to that of the vehicle controls. In the ethinyl oestradiol group, time spent in the open arms of the elevated plus maze and in the light chamber was decreased and the number of errors in the radial arm and Barnes mazes was increased, regarded by the authors as indicative of a masculinisation of behaviour.

Weight of evidence assessment of developmental neurotoxicity studies

This assessment focuses on the reliability and consistency of the evidence. To help the reader, a summary table of the available developmental neurotoxicity studies has been added below.

Table 4.19 Summary of developmental neurotoxicity studies, using oral route unless otherwise stated

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Studies by linked researchers mainly from Universities of Florence, Siena, Rome, Calabria and Parma, Italy					
Farabollini <i>et al.</i> 1999 Rat n = 9 - 11	0, 0.04 mg/kg (prior to conception - pnd 21, 0.4 mg/kg (gd14 - pnd 6), by micropipette	Hole board, elevated-plus maze	Anxiety-related behaviour increased in hole board test in males and females. Anxiety-related behaviour decreased in elevated- plus maze in males.	Possibly inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques
Farabollini <i>et al.</i> 2002 Rat n = 8 - 13	0, 0.04 mg/kg, gd 0 - pnd 21, by micropipette. Cross fostering to create prenatal and post natal exposure groups	'Sociosexual' behaviour	Slight intensification of sexual behaviour in females. Slightly reduced sexual performance in males	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Aloisi <i>et al.</i> 2002 Rat n = 7	0, 0.040 mg/kg, by pipette, gd 0 - pnd 21. Cross fostering to create prenatal and post natal exposure groups	Response to pain (caused by sc injection of formalin) at 22 weeks of age	Response very marginally diminished	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Dessi- Fulgheri <i>et al.</i> 2002 Rat n = 9-11	0, 0.04 (prior to conception- pnd 21), 0.4 mg/kg (gd 14 - pnd 6), by pipette	Play behaviour	Differences in 4 out of 8 types of behaviour, in both sexes.	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Facciolo <i>et al.</i> 2002 Rat n = ?	0, 0.04, 0.4 mg/kg, oral, exposure period not stated	Effects on somatostatin receptor subtype 2 (sst ₂) in limbic region of brain	Expression of some sst ₂ states increased	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	
Facciolo <i>et al.</i> 2005 Rat n = 12	0, 0.04, 0.4 mg/kg by pipette, 8 d pre-mating- pnd 21 lactation	Effects of expression of somatostatin receptor subtype 3 (sst ₃) mRNA in brain of females	Expression of sst ₃ reduced in some areas and increased in an other. Differences were increased in presence of αGABA _A agonist.	Possibly inappropriate statistical methods. A mechanistic study of limited value for hazard assessment.	

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Adriani <i>et al.</i> 2003 Rat n = 9	0, 0.04 mg/kg, gd 0 - pnd 21, by micropipette	Novelty seeking, impulsivity. Open field with and without amphetamine challenge	Reduced novelty seeking behaviour in females and decreased impulsive behaviour in males. Partial inhibition of amphetamine stimulated open field activity. No open field differences without challenge.	Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit
Porrini <i>et al.</i> 2005 Rat n = 10	0, 0.04 mg/kg, gestation - pnd 21, by micropipette	Play behaviour in females	Some aspects of female behaviour differed	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Della Seta <i>et al.</i> 2006 Rat, pups, n = ~25 males	0, 0.04 mg/kg, pnd 23 - 30, micropipette	Males juvenile play (response to black PVC tube placed in cage), sexual behaviour	Some marginal differences in play and sexual behaviour	Possibly inappropriate statistical methods. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques
Palanza <i>et al.</i> 2002 Mouse, n = 9 (F ₀)	0, 0.01 mg/kg, gd 14 -18 to F ₀ and F ₁ females, by micropipette	Maternal nursing behaviour of F ₁	No convincing evidence of an effect on nursing behaviour.	Possibly inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques
Laviola <i>et al.</i> 2005 Mouse n = 10 - 12	0, 0.01 mg/kg, gd 11 - 18	d-amphetamine-reinforcing effects at pnd 60 using conditioned place preference testing	Conditioned place preference not present in females. Males not affected	Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit
Ceccarelli <i>et al.</i> 2007 Rat, pups n = 14	0, 0.04 mg/kg, pnd 23 - 30, oral	ER α levels in three sexually dimorphic region of the brain on pnd 37 and 90	Increase in ER α levels in medial preoptic area of females at pnd 37, but not at pnd 90. No differences in ER α levels among males	Single BPA treatment group. A mechanistic study of limited value for hazard assessment.	Analysis of results used appropriate statistical unit
Studies by researchers associated with Hoshi University, Japan					

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Suzuki <i>et al.</i> 2003 Mouse n = ?	0, 0.4, 100, 250 mg/kg bwt, in diet, mating to pnd 21	Male offspring: methamphetamine -induced rewarding effects and hyperlocomotion. G-protein activation in limbic forebrain, expression of dopamine D ₁ receptor mRNA.	Preference for methamphetamine- associated compartment. Increased locomotion. G- protein activation increased and dopamine D ₁ receptor mRNA upregulated at high dose level.	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	Behavioural testing conducted according to acceptable techniques.
Mizuo <i>et al.</i> 2004a Mouse n = ?	0, 2, 500, 2000 ppm in diet (~ 2.5, 60, 250 mg/kg), gest & neonatal period	Male offspring: morphine-induced rewarding effects and hyperlocomotion. G-protein activation and expression of μ - opioid receptor mRNA.	Preference for morphine- associated compartment at 60 and 250 mg/kg. Increased locomotion at 250 mg/kg. No effect on G-protein activation and mRNA	Possibly inappropriate statistical methods. Group size not reported.	Behavioural testing conducted according to acceptable techniques.
Mizuo <i>et al.</i> 2004b Mouse n = ?	0, 2000 ppm in diet (250 mg/kg) gd 0 - weaning	Male offspring: functional changes in dopamine D ₃ receptors	Attenuation of dopamine D ₃ receptor-mediated G- protein activation by 7- OH-DPAT in the mouse limbic forebrain. Decrease in D ₃ receptor density	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	
Narita <i>et al.</i> 2006 Mouse n = ?	0, 0.006, 0.06, 0.6, 100, 400 mg/kg/day, in diet, gest and lact timing not reported	Effects on dopaminergic system- place conditioning and locomotion test, [³⁵ S]GTP γ S binding assay in response to morphine	Potential of dopamine receptor-dependent neurotransmission	Possibly inappropriate statistical methods. Group size not reported. A mechanistic study of limited value for hazard assessment.	Behavioural testing conducted according to acceptable techniques.
Narita <i>et al.</i> 2007 Mouse n = ?	0, 400 mg/kg/day, gd 0 - 7, 7 - 14, 14 - 20 or pnd 0 - 20	Males: effects on dopaminergic system- place conditioning and locomotion test, [³⁵ S]GTP γ S binding assay in response to morphine	Potential of dopamine receptor-dependent neurotransmission in gd 7-14 and pnd 0-20 groups, but not for gd 0-7 or gd 14-21	Possibly inappropriate statistical methods. Group size not reported. Single BPA treatment group. A mechanistic study of limited value for hazard assessment. Report poorly written.	Behavioural testing conducted according to acceptable techniques.
Studies from Chemical Industry Institute for Toxicology, USA					

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Kwon <i>et al.</i> 2000 Rat n = 8	0, 3.2, 32, 320 mg/kg/day, gavage, gd 13- pnd 21	SDN-POA volume	No effects		Analysis of results used appropriate statistical unit
Patisaul <i>et al.</i> 2006 Rat, pups n = 5 - 8	100 mg/kg/day pnd 1 - 2, subcutaneous route	Brain immunohistochemistry at pnd 19	Interference with normal testosterone associated masculinisation of the anteroventral periventricular nucleus	Single BPA treatment group. Small group size. A mechanistic study of limited value for hazard assessment.	Analysis of results used appropriate statistical unit
Patisaul <i>et al.</i> 2007 Rat, pups n = ?	66 mg/kg/day, pnd 1 - 2, subcutaneous route	Brain immunohistochemistry as adults	No effects on SDN-POA volume. Increase in calbindin +ve nuclei.	Single BPA treatment group. Group size not reported. A mechanistic study of limited value for hazard assessment.	Analysis of results used appropriate statistical unit
Studies by researchers mainly from University of Tokyo, Japan					
Negishi <i>et al.</i> 2003 Rat n = 8-9	0, 4, 40, 400 mg/kg, gavage, gd 10 - pnd 20	Spontaneous motor activity, active avoidance, open field	No consistent effects on behaviour.	Possibly inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques.
Negishi <i>et al.</i> 2004 Rat n = 8 - 10	0. 0.1 mg/kg, gavage, gd 3 - pnd 20	Males: spontaneous motor activity, passive and active avoidance, open field, elevated-plus maze, monoamine disruption test	Differences in active avoidance behaviour.	Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit
Studies by researchers associated with Kushi academic institutes, Fukuoka, Japan					
Kubo <i>et al.</i> 2001 Rat n = 5	0, 1.5 mg/kg (some uncertainty), drinking water gd 0 - pnd 21	Open field, passive avoidance testing, also volume of SDN-POA and locus ceruleus, serum hormone levels	Anxiety-related behaviour slightly reduced and avoidance memory increased in males, opposite effect in females. Normal locus ceruleus volume gender difference reversed	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques.
Kubo <i>et al.</i> 2003 Rat n = 5	0, 0.03, 0.3 mg/kg, drinking water, probably gd 0 - pnd 21	Open field, sexual behaviour, volume of SDN-POA and locus ceruleus.	Anxiety-related behaviour slightly reduced in males and slightly increased in females. Normal locus ceruleus volume gender difference reversed.	Inappropriate statistical methods. Small group size.	Behavioural testing conducted according to acceptable techniques.

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Fujimoto <i>et al.</i> 2006 Rat n = 6	0, 0.015 mg/kg drinking water , gd 13 - pnd 0	Open field, elevated plus maze, passive avoidance, forced swimming test, 20-24/gp tested	Open field rearing activity increased in males, decreased in females. Some differences in forced swimming test. No effects in elevated plus maze or passive avoidance tests.	Possibly inappropriate statistical methods. Small group size. Single BPA treatment group.	Behavioural testing conducted according to acceptable techniques.
Kawai <i>et al.</i> 2003 Mouse n = 7 - 9	0, 0.002, 0.02 mg/kg, gd 11- 17, by micropipette	Aggressive behaviour in males, at 8, 12, 16 weeks of age	No convincing dose- related effects on aggression.	Possibly inappropriate statistical methods. Small group size.	
Kawai <i>et al.</i> 2007 Mouse n = 19	0, 0.002 mg/kg, gd 11 - 17, by micropipette	Brain expression of ER α and ER β , in males	Number of neurones expressing ER α and ER β increased at 5 and 13 weeks of age, but not at 9.	Single BPA treatment group. Statistical methods not clear. A mechanistic study of limited value for hazard assessment.	Analysis of results used appropriate as statistical unit
Studies from Kyoto Prefectural University of Medicine					
Nakamura <i>et al.</i> 2006 Mouse n = ?	0, 0.02 mg/kg, gd 0 - 16, subcutaneous route	Brain immunohistochem ical investigation	Evidence of accelerated neuronal differentiation	Single BPA treatment group. Group size not reported and statistical approach not clear. A mechanistic study of limited value for hazard assessment.	
Tando <i>et al.</i> 2007 Mouse n = ?	0, 4.5, 1200 mg/kg/day, gd 0 - pnd 21, dietary	Brain immunohistochem ical investigation for tyrosine hydroxylase, calbindin D-28 K, calretinin, and parvalbumin proteins	Reduction in number of tyrosine hydroxylase- positive nuclei and fibres in the substantia nigra among females exposed to the low dose. Other parameters not affected.	Group size not reported and statistical approach not clear. A mechanistic study of limited value for hazard assessment.	
Studies from other institutes					
Ema <i>et al.</i> 2001 Rat n = 25	0, 0.0002, 0.002, 0.02, 0.2 mg/kg/day, gavage, prenatal, postnatal	Conventional 2- generation study, with added endpoints for open field and water- filled T maze	No effects on open field behaviour or T-maze performance declared by authors	Actual results of behavioural testing not included in report.	GLP study. Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit.

Study/species/ group size (usually number of litters)	Dosing regime	DNT endpoints investigated	Reported findings in BPA exposed offspring	Weaknesses in study design	Remarks
Carr <i>et al.</i> 2003 Rat, pups, n = 10/sex	0, 0.1, 0.25mg/kg/day, pnd 1 - 14, gavage	Morris water maze	Reduction in memory retention in males and females at 0.25 mg/kg		Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit
Funabashi <i>et al.</i> 2004 Rat n = 8- 11	0, 2.5 mg/kg drinking water, prenatally and to pnd 21	Immunohistochem istry investigations of brain	Reduction in normal gender difference in numbers of corticotrophin-releasing hormone neurones in bed nucleus of stria terminalis	Possibly inappropriate statistical methods. Single BPA treatment group. A mechanistic study of limited value for hazard assessment.	
Honma <i>et al.</i> 2006 Rat n = 6	0, 4, 40 mg/kg/day, gd 6-pnd 20, gavage	Neurotransmitter levels in various regions of brain at 1, 3, 6, 9 weeks in female offspring	Results had no discernable pattern	Possibly inappropriate statistical methods. Small group size. A mechanistic study of limited value for hazard assessment.	
Ryan and Vandenberg h 2006 Mouse n = 14-16	0, 0.002, 0.2 mg/kg/day, gd 3 - pnd 21, gavage	Ovariectomised female offspring investigated in elevated plus maze, light-dark preference chamber, radial arm and modified Barnes mazes	No differences in special memory tests. Increased anxiety in light-dark preference testing.		Behavioural testing conducted according to acceptable techniques. Analysis of results used appropriate statistical unit.

n = group size gd = gestational day pnd = postnatal day

Reliability

A number of weaknesses in the available BPA developmental neurotoxicity studies can be identified.

With the exceptions of a conventional (similar to OECD test guideline 416) 2-generation study by Ema *et al.* (2001) and the behavioural studies of Della Seta *et al.* (2006) and Ryan and Vandenberg (2006), the studies generally used relatively small group sizes, typically 7-10 litters. For some studies (Suzuki *et al.* 2003, Mizou *et al.* 2004, Narita, 2006, 2007, Tando *et al.* 2007, Patisaul *et al.* 2007) the group size was not stated in the report. Although an important influence of small group size is to reduce the power of the study to detect an effect, and as such is less of an issue for a study that claims a treatment-related effect, this does reduce confidence in the reliability of the study.

For many studies involving maternal dosing and the selection of more than one pup/sex/litter for testing the statistical analysis appeared to have been conducted using the individual pup as the experimental unit. This is inappropriate because it is the litter, not the individual pup, which has been randomly assigned to the treatment groups. The failure to use the litter as the experimental unit will inflate the number of independent observations considered in the statistical analysis and increase the risk of a false positive (Type 1 error) outcome. For some studies it was not stated whether the litter or individual pup was selected as the experimental unit. Also, it is not clear from many of the study reports whether the statistical analysis took account of the non-independence of repeated measures in the same individual, as recommended in draft OECD Guideline 426 (Developmental neurotoxicity study). Failure to take account of this non-independence will, again, increase the risk of Type 1 errors.

All the study reports were obtained from the published peer-reviewed scientific literature and reporting was therefore necessarily brief. Thus, comprehensive information on the experimental conditions, methods, results and natural background variation of the parameters measured are not available. While this does not invalidate the studies, the capacity of the reader to critically appraise each study and assess the validity of the authors' conclusions may be restricted, and in such cases have a negative influence on confidence in each study.

With exception of the 2-generation study by Ema *et al.* (2001), none of the studies declared a compliance with the principles of GLP. Again, this does not invalidate the studies, but confidence in their reliability might have been greater if they had been conducted according to GLP.

When differences between control and treated groups are observed in toxicology studies, the results will usually be examined for dose-response relationships. The presence of consistent dose-response relationships will increase confidence that the observed differences were caused by the experimental treatment and not due to natural variation. A number of studies, notably most of the behavioural studies from the Italian team (Farabollini *et al.* 2002, Aloisi *et al.* 2002, Adriani *et al.* 2003, Della Seta *et al.* 2006, Palanza *et al.* 2002, Laviola *et al.* 2005), used just one exposure level of BPA and so there is no opportunity to evaluate observed differences between control and treated groups in the light of a dose response assessment. Consequently, confidence in the validity of claims of a causal effect of BPA exposure is reduced.

Developmental neurotoxicity testing is a relatively new and not fully established area in regulatory toxicology (evidenced by the fact that an OECD Guideline for developmental neurotoxicity testing has not yet been finalised) and therefore experience in the conduct and interpretation of the studies is limited. However, many of the behavioural tests conducted in the BPA studies are established tests that have been used extensively for a number of years in pharmacological research and are the types of tests (with the exception of those involving pharmacological challenge) recommended by draft OECD Guideline 426. These tests are considered relevant to human health. Concerning the brain receptor expression and immunohistochemical investigations, these techniques do not have an established role in regulatory toxicology and should be regarded tools for mode of action and mechanistic investigation rather than hazard identification tests.

Overall, many limitations have been identified. The reliability of the development neurotoxicity database is considered to be low because of the collective impact of these limitations.

Consistency of evidence: behavioural endpoints

The effect of prenatal and postnatal exposure to BPA on various aspects of behaviour (locomotory and exploratory activity, grooming, cognitive, emotional, social, sexual, response to pain and response to pharmacological challenge) have been investigated in rats or mice in 22 studies. A large range of maternal exposure levels, 0.0002-400 mg/kg/day, were investigated. The consistency of evidence for a genuine treatment-related effect on each of these behavioural endpoints will be considered in turn.

Open field testing for general locomotory and exploratory activity, grooming and anxiety was conducted in the following studies: Ema *et al.* (2001), Kubo *et al.* (2001), Kubo *et al.* (2003), Adriani *et al.* (2003), Negishi *et al.* (2003), Negishi *et al.* (2004) and Fujimoto *et al.* 2006. No effects were reported by Ema *et al.* (2001), in which exposure was to 0.0002-0.2 mg/kg/day, throughout the prenatal and postnatal periods. This study used the largest group size (n = 25 litters) of all the BPA studies reviewed and as such is likely to be the most reliable, but the weight that can be given to this study is limited because of incomplete reporting. The two Kubo *et al.* (2001 and 2003) studies used higher exposure levels of 0.03-1.5 mg/kg/day, dosing throughout gestation and lactation, and reported a decrease in anxiety-related behaviour (time spent away from the centre) in males and an increase in females, although the differences were slight. Adriani *et al.* (2003) reported no effects in open field testing following a saline injection in animals exposed during the prenatal and neonatal period at 0.004 mg/kg/day. Negishi *et al.* (2003) found no effects on open field behaviour at the relatively high exposure levels 4-400 mg/kg/day during the prenatal and postnatal periods. Also, Negishi *et al.* (2004) found no effects on open field behaviour of males (females not tested) at the lower exposure level of 0.1 mg/kg/day during the same periods. Finally, Fujimoto *et al.* (2006) reported increased rearing activity in males and females exposed to 0.0015 mg/kg/day during late gestation. Overall, there appears to be no convincing evidence of a consistent BPA-related effect on open field behaviour

Anxiety and related behaviour was also tested by other methods in the following studies: Farabollini *et al.* (1999, hole board test, elevated plus maze), Negishi *et al.* (2004, elevated plus maze), Fujimoto *et al.* (2006, elevated plus maze, forced swimming test), and Ryan and Vandenberg (2006, elevated plus maze, light-dark preference chamber). Farabollini *et al.* (1999) reported increased anxiety-related behaviour in males and female rats in a hole board

test, and decreased anxiety-related behaviour in the elevated plus maze in males following prenatal and neonatal exposure to 0.04 or 0.4 mg/kg/day. Negishi *et al.* (2004) reported no changes in anxiety related behaviour in male rats (females not tested) at 0.1 mg/kg/day during the prenatal and neonatal periods. Fujimoto *et al.* (2006) also found no changes in anxiety related behaviour in males and female rats exposed to the very low exposure level of 0.0015 mg/kg/day during late gestation. Ryan and Vandenberg (2006) found that anxiety behaviour was increased in females (males not tested) at 0.2 mg/kg/day prenatally and neonatally, but not at 0.002 mg/kg/day. Thus, the studies by Farabollini *et al.* (1999) and Ryan and Vandenberg (2006) provide evidence of increased anxiety in rats (males and females, hole board test) and mice (females), respectively, at doses levels of 0.04-0.4 mg/kg/day, but evidence of decreased anxiety in male rats (elevated plus maze) was also seen in the study of Farabollini *et al.* (1999) and no evidence of an effect on anxiety in males was reported by Negishi *et al.* (2004) at similar dose levels. Overall, there does not appear to be a consistent pattern across species and gender in the results of the tests for anxiety.

Cognitive (learning and memory) testing was conducted in the following studies: Ema *et al.* (2001, T water maze), Kubo *et al.* (2001, passive avoidance), Negishi *et al.* (2003, active avoidance), Negishi *et al.* (2004, active and passive avoidance), Fujimoto *et al.* (2006, passive avoidance) and Carr *et al.* (2003, Morris water maze). No effects were reported by Ema *et al.* (2001), in which exposure was to 0.0002-0.2 mg/kg/day throughout the prenatal and postnatal periods. Kubo *et al.* (2001) reported an enhancement of avoidance memory in males, with the opposite effect in females at 0.03 or 0.3 mg/kg during the prenatal and neonatal periods. Negishi *et al.* (2003) found no consistent effects in active avoidance testing at the relatively high exposure levels of 4-400 mg/kg/day. Negishi *et al.* (2004) tested only males, exposed to 0.1 mg/kg/day during the prenatal and neonatal periods, and found no differences in a passive avoidance test. However, avoidance memory appeared to be decreased in an active avoidance test. Fujimoto *et al.* (2006) reported no effects in a passive avoidance test in males and females exposed to the very low dose of 0.0015 mg/kg/day during late gestation. Carr *et al.* (2003) noted changes in the memory testing phase in males and females exposed to 0.25 mg/kg/day during the neonatal period, but not at 0.1 mg/kg/day, indicative of an effect on spatial memory retention. Overall, a consistent picture does not emerge from the cognitive testing results.

Other aspects of behaviour investigated were as follows: Farabollini *et al.* (2002) investigated social and sexual behaviour in rats exposed to 0.04 mg/kg/day and noted some marginal differences, interpreted as a slight intensification of sexual behaviour in females and a slightly reduced sexual performance in males. Aloisi *et al.* (2002) investigated the response to pain in rats exposed to 0.04 mg/kg/day during the prenatal and neonatal periods and reported only very slight differences. Adriani *et al.* (2003) also conducted behavioural tests in rats also exposed to 0.04 mg/kg/day during these periods and reported changes interpreted as reduced novelty seeking in females and increased impulsivity in males. Porrini *et al.* (2005) used an identical exposure regime and noted some difference in play behaviour in females (males not tested). Dessi-Fulgheri *et al.* (2002) reported some differences in the play behaviour of rats exposed to 0.04 mg/kg during the prenatal and neonatal periods or 0.4 mg/kg/day during the late prenatal and early neonatal periods. Della Seta *et al.* (2006) exposed 3-4 week old juvenile male rats to 0.04 mg/kg/day and reported only very marginal changes in play and, at a later age, sexual behaviour. In mice, Palanza *et al.* (2002) found no convincing evidence of an effect on maternal nursing behaviour in females exposed during the prenatal period and/or as adults at 0.01 mg/kg/day. Kawai *et al.* (2003) reported no convincing effect on aggressive behaviour in male mice exposed during the late prenatal period to 0.002 or 0.02 mg/kg/day

BPA. These studies looked at different aspects of behaviour so it is not possible to assess the study results for consistency. However, it is noted that the reduced novelty seeking seen in females (Adriani *et al.* 2003) was not confirmed by changes in open field behaviour in females in the same study or in other studies.

A number of other studies investigated the behavioural response to pharmacological challenge. The aim of these studies was to detect possible effects of BPA on the organisation of the brain at the receptor or neurotransmitter level. Adriani *et al.* (2003) reported a partial inhibition of an amphetamine-stimulated increase in male rats, but not females, exposed to 0.04 mg/kg/day during the prenatal and neonatal period. Laviola *et al.* (2005) found that conditioned place preference for an amphetamine-associated compartment was abolished in female mice, but not in males, exposed to 0.01 mg/kg/day during late gestation. Negishi *et al.* (2004) found that a monoamine oxidase inhibitor (Tcy)-stimulated increase in open field activity was reduced in male rats (females not tested) exposed to 0.1 mg/kg/day during the prenatal and neonatal periods. Suzuki *et al.* (2003) found that the stimulating effects on locomotion of methamphetamine (which has very similar actions to amphetamine) and place preference for a methamphetamine-associated compartment was enhanced in male mice (females not tested) exposed to higher levels of BPA, 0.4-250 mg/kg/day, during the prenatal and neonatal periods. As both amphetamine and Tcy cause an increase in the levels of dopamine and noradrenalin, the results of the Adriani *et al.* (2003) and Negishi *et al.* (2004) studies can be regarded as showing a consistent effect. However, there is an inconsistency with the study of Laviola *et al.* (2005) because females only, rather than males, were affected. Suzuki *et al.* (2003) reported an enhancement, rather than an inhibition, of methamphetamine response, which is another inconsistency.

Several studies have investigated the behavioural response to morphine challenge. Mizou *et al.* (2004a) found a place preference for a morphine-associated compartment in male mice (females not tested) exposed 60 or 250 mg/kg/day BPA during the prenatal and postnatal periods, although the μ -opioid receptor activity was not affected. Narita *et al.* (2006, 2007) also reported some evidence for place preference for a morphine-associated compartment in mice exposed to the 0.006-400 mg/kg/day during the prenatal and neonatal periods. However, because of the absence of a conventional dose response relationship and lack of consistency of response within the Narita *et al.* (2007) study, the interpretation of these results is uncertain. Overall, the consistency of results for effects on the behavioural response to morphine cannot be assessed because of limited investigation.

Consistency of evidence: receptor expression in brain

Effects of BPA exposure at the receptor level have been investigated in nine studies.

Facciolo *et al.* (2002, 2005) reported some changes in expression of somatostatin receptor subtypes 2 and 3 following maternal exposure to 0.04 or 0.4 mg/kg/day, but these endpoints have not been investigated in other independent studies.

ER expression has been investigated in two recent studies, but the extent of investigation is too limited to assess consistency. Ceccarelli *et al.* (2007) reported an increase in ER α levels in the medial preoptic area of brain in females exposed to BPA at 0.04 mg/kg/day from pnd 23-30. Kawai *et al.* (2007) reported an increase in brain expression of ER α and ER β following prenatal exposure to a maternal BPA level of \approx 0.002 mg/kg/day at 5 and 13 weeks of age but, inconsistently, not at 9 weeks.

The possible influence of prenatal and postnatal BPA exposure on different aspects of the central dopaminergic system was investigated in five studies conducted by the Hoshi University group. Suzuki *et al.* (2003) reported an upregulation of dopamine D₁ receptors in the male limbic forebrain at maternal exposure levels of 400 mg/kg/day in a study that also reported a BPA-associated enhanced response to methamphetamine. Mizou *et al.* (2004a) found no evidence of an effect on μ -opioid receptor expression in males. Mizuo *et al.* (2004b) found that the response to a dopamine D₃ agonist and D₃ receptor density in the male brain were both decreased at a maternal BPA dose level of 250 mg/kg/day. Narita *et al.* (2006, 2007) reported evidence of an activation of dopamine receptor mediated G-protein, but the interpretation of these results is uncertain because of the absence of a conventional dose response relationship and lack of consistency of response within the Narita *et al.* (2007) study. Overall, the extent of investigation of possible effects on the central dopaminergic system is too limited to allow an assessment for consistency.

Consistency of evidence: brain morphology and brain chemistry

SDN-POA volume was measured in five studies. Kwon *et al.* (2000) found no effects following perinatal and neonatal exposure at maternal dose levels of 3.2-320 mg/kg/day. Patisaul *et al.* (2006, 2007) reported no effects on SDN-POA volume following subcutaneous administration at 100 mg/kg on pnd 1 and 2. At a lower range of maternal exposure levels, 0.03-1.5 mg/kg/day, Kubo *et al.* (2001, 2003) also reported no effects on SDN-POA volume. Kubo *et al.* (2001, 2003) also measured the volume of the locus coeruleus and reported a reversal of the normal gender difference in both studies. However, a conventional dose response relationship was not present and control group comparisons did not achieve statistical significance, so it is possible that these differences were due to chance rather than BPA exposure.

The following studies examined aspects of brain development that were not looked at in other studies: Funabashi *et al.* (2004) found differences in the numbers of CRN neurones; Honma *et al.* (2006) investigated neurotransmitter levels in various regions of the brain; Tando *et al.* (2007) reported reduced numbers of dopaminergic neurons in the substantia nigra at maternal exposures of 4.5 mg/kg/day and no differences in the numbers of GABAergic neurones in the cerebral cortex in females at 4.5 or 1200 mg/kg/day. Nakamura *et al.* (2006) reported evidence of accelerated neuronal differentiation at a maternal exposure, using the subcutaneous route, of 0.02 mg/kg/day.

Overall, with the exception of SDN-POA volume, the extent of brain morphology and brain chemistry investigations are very limited and so consistency between studies cannot be assessed.

Conclusions to weight of evidence assessment of developmental neurotoxicity studies

Confidence in the reliability of the developmental neurotoxicity database is low because of limitations in the design and reporting in all of the available studies. These limitations include small group size, inappropriate statistical analysis, brief reporting of methods and results, lack of compliance with GLP and use of one BPA dose level. The receptor/neurotransmitter level studies are regarded as mode of action or mechanistic investigations and cannot be used as the primary support for conclusion regarding the hazardous properties of BPA.

The consistency assessment shows that there is no discernable and reproducible pattern to the behavioural testing results. Most of the studies investigating effects at the receptor/neurotransmitter level and brain morphology have not been replicated by independent laboratories; so consistency cannot be assessed.

Overall, taking together the low confidence in the reliability of the developmental neurotoxicity studies and the lack of consistency in the results of behavioural testing, no conclusions can be drawn from these studies³. This opinion is very similar to that of EFSA (2006), who reviewed nine of the developmental neurotoxicity studies.

Developmental effects on female reproductive tract expressed in old-age.

In a study designed to investigate the effects of BPA on the development of the reproductive tract, groups of 24 neonatal CD-1 mice received subcutaneous injections of BPA at dose levels of 0 (corn oil vehicle control), 0.01, 0.1 or 1 mg/kg/day from pnd 1 to 5 (Newbold *et al.* 2007) The neonatal mice were drawn from the pooled offspring of an unspecified number of dams. The neonates were randomly fostered to litters each comprising of 8 female pups per dam. It was not stated whether fostered litter-mates received the same experimental treatment. The female mice were weaned on pnd 21 and maintained without further experimental treatment until sacrifice at 18 months of age. The uterus and ovaries/oviducts were removed and processed for examination by light microscopy. At 18 months, 18, 23, 20 and 16 out of 24 females from the control, 0.01, 0.1 and 1 mg/kg/groups, respectively, survived. Group mean bodyweights at 18 months were similar. The incidence of histopathological changes in the ovaries and uterus is presented below:

Table 4.20 Incidence (%) of histopathological changes in reproductive tract (Newbold et al. 2007)

Histopathological finding		Dose level (BPA mg/kg/day)			
		Control	0.01	0.1	1
Ovary/ oviduct	Presence of corpora lutea	100	96	90	88
	Ovarian cysts	39	35	70*	38
	Parovarian cysts	0	4	10	6
	Progressive proliferative lesion of oviduct	0	13	15	6
Uterus	Cystic endometrial hyperplasia	6	22	45**	25
	Adenomyosis	6	9	20	19
	Wolffian duct remnants in uterine wall	0	13	10	19
	Leiomyoma	0	4	10	6
	Atypical hyperplasia	0	4	5	0
	Stromal polyp	6	4	25	6

³ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007.

* significantly different from control, Fisher's exact test, $p \leq 0.05$ ** significantly different from control, Fisher's exact test, $p \leq 0.01$

As shown in the above table, the incidence of a number of findings was increased in the BPA-treated groups. In particular, the incidence of cystic endometrial hyperplasia and ovarian cysts was statistically significantly increased at 0.1 mg/kg/day, in comparison with controls. However, these differences did not follow a dose-related pattern and could not therefore be attributable to BPA treatment. It is also noted that these findings are inconsistent with the results of a study by Yoshida et al (2004) (summarised in the carcinogenicity section) in which no effects of transplacental and lactational exposure to BPA (up to 6 mg/kg/day) were seen on ovarian and uterine histopathology in 15 months-old female rats.

Human case-control study

Sugiura-Ogasawara *et al.* (2005) investigated the possible association between recurrent miscarriage and serum BPA levels in a case-control study. A group of 45 women (mean age 31.6 years) with a history of 3 or more consecutive first-trimester miscarriages who attended Nagoya City University Hospital during a 17 month period were selected for the study. The subject selection method was not reported, although it was stated that cases with uterine abnormalities or chromosome abnormalities in either partner were excluded from the study. Thirty-two healthy non-pregnant women (mean age 32.0 years), with no history of pregnancy or infertility served as controls. The occupation of most of the cases was either housewife (20 subjects) or office worker (17 subjects), whereas the control subjects were doctors, nurses and secretaries employed by Nagoya Hospital. Immunological tests for parameters such as antinuclear bodies (ANAs, a sensitive marker for systemic lupus erythematosus), antiphospholipid antibodies (aPLs) and natural killer (NK) activity and blood tests for hypothyroidism, diabetes mellitus and hyperprolactinaemia were conducted for all cases, but not for controls. Serum BPA levels were measured using an ELISA method in single fasting blood samples taken from all subjects 5-9 days after ovulation (Sugiura-Ogasawara, 2006).

The mean (\pm SD) serum BPA levels for the cases were significantly higher than controls (2.59 ± 5.23 vs. 0.77 ± 0.38 ng/ml). However, median BPA levels in the two groups were similar (0.71 vs. 0.705), so whether there was a genuine difference between the groups (as claimed by the author) is questionable. The large SD for cases and similar median values for cases and controls indicates that a small number of women with very high BPA levels were responsible for the higher group mean levels. A comparison of BPA levels within the cases showed that the higher levels of BPA were associated with ANA-positive status (10 cases) but not with hyperthyroidism (8 cases), luteal phase defect (9 cases), hyperprolactinaemia (5 cases) or aPL-positive status (6 cases). Thirty-five cases achieved pregnancies subsequent to the study, of which 17 miscarried again. The mean serum BPA levels for those who miscarried again was higher, though not statistically significantly, than those who did not (4.39 ± 8.08 vs. 1.22 ± 1.07 ng/ml), although the median values for the two groups were similar (0.71 vs. 0.91 ng/ml).

There are a number of limitations to this study (as pointed out by Berkowitz, 2006). Group sizes were relatively small, so the subjects may not be representative of the populations from which they are drawn. The cases and controls were not comparable in terms of occupation and may not have been comparable in terms of the presence of potential confounding factors. The BPA levels for the cases were measured at a time-frame that was not relevant to the induction of their miscarriages. The reliability of the reported BPA measurements is

uncertain because the ELISA method is not optimal for BPA in serum due to cross-reactivity and other problems (Fukata *et al.* 2006, discussed in section 4.1.1.3.2). A parametric statistical test (Welch's test) was used to compare mean BPA levels for the cases and controls, but the distribution of results indicate that use of a non-parametric would have been appropriate. Overall, given the significant limitations in the design and conduct of this study and lack of a convincing difference in serum BPA levels between the cases and controls, no conclusions can be drawn regarding an association between BPA exposure and recurrent miscarriage.

4.1.2.9.3 Impact of new information and summary of reproductive toxicity

The new 2-generation study in mice (Tyl *et al.* 2007) provides a comprehensive, definitive, investigation of the effects of BPA on reproduction at exposure levels spanning the low ($\mu\text{g}/\text{kg}$ bw/day) to high (mg/kg bw/day) ranges. This study has shown that BPA causes adverse effect on pregnancy and the offspring, observed as a slightly increased duration of gestation, reduced pup bodyweight during lactation, a slight increase in the incidence of undescended testes at weaning, seminiferous tubule hypoplasia in offspring at weaning, and delayed acquisition of preputial separation, at 600 $\text{mg}/\text{kg}/\text{day}$, an exposure level that also caused mild parental toxicity. Fertility was not affected by BPA exposure, which resolves the previous uncertainty regarding the the NOAEL for fertility in mice. A study NOAEL for reproductive toxicity of 50 $\text{mg}/\text{kg}/\text{day}$ has been identified. As there was no evidence of an adverse effect on the development of the male reproductive tract at $\mu\text{g}/\text{kg}$ bw/day doses of BPA, the study resolves the uncertainties surrounding the potential to produce adverse effects on development at low doses. Thus, a NOAEL of 50 $\text{mg}/\text{kg}/\text{day}$ for reproductive toxicity, which was a provisional position in the original risk assessment report, should be used in the risk assessment.

No conclusions could be drawn from the new developmental toxicity studies⁴ or from a human study investigating the possible association between recurrent miscarriage and BPA exposure. Therefore, these studies do not influence the conclusions of the original risk assessment report.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Toxicokinetics: The toxicokinetics of BPA have been well studied in rats both *in vivo* and *in vitro*, and have been investigated to a lesser extent in mice and cynomolgus monkeys. Two studies have investigated the toxicokinetics and fate of an oral dose of labelled BPA in human volunteers.

In the species studied (rats, mice, monkeys, humans), the available evidence suggests that following oral administration, BPA is rapidly and extensively absorbed from the gastrointestinal tract. Analysis of plasma AUC values suggests that the extent of absorption from the GI tract is up to 86% in rats and up to 85% in monkeys. The only relevant human

⁴ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

studies suggest that, on the basis of the recovery of labelled BPA-glucuronide from the urine, a relatively low dose of BPA (54-88 µg/kg) was completely absorbed after oral dosing.

An *in vitro* dermal absorption study using human skin found limited absorption of BPA at millimolar concentrations; the extent of absorption was in the region of 10% of the applied dose.

There are no data on the toxicokinetics of BPA following inhalation exposure. However, on the basis of the observed absolute organ weight changes in a repeat inhalation study and the high partition coefficient, it would be prudent to assume that absorption via the inhalation route can occur, but the data do not allow a quantitative estimation of absorption to be made. Furthermore, because first-pass metabolism would not take place following exposure by this route, or by the dermal route, the systemic bioavailability is likely to be substantially greater for these routes than is associated with the oral route.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

The available data indicate that BPA is subject to extensive first-pass metabolism following absorption from the gastrointestinal tract.

In all species studied, the major metabolic pathway involves conjugation of BPA to BPA-glucuronide. Studies conducted in rats suggest that in neonates the glucuronidation pathway is more susceptible to saturation than in adults indicating an age-dependent increase in metabolic capacity. *In vitro* studies with microsomal preparations also suggest species differences, with the rank order for the metabolic clearance rate per unit weight of tissue being mice > rats > humans. When the total clearance rates for the whole liver were calculated, the rank order was reversed (humans > rats > mice).

In addition to the glucuronidation pathway, *in vivo* and *in vitro* studies suggest that in the rat, BPA may be subject to limited oxidation to bisphenol O-quinone by cytochrome P450, and also to conjugation to BPA-sulphate and 5-hydroxy-BPA.

A study in pregnant mice given subcutaneous doses of BPA also found that glucuronidation was the major pathway for the metabolism of BPA, although dehydrated, sulphated and methoxylated conjugates of BPA were also produced. Some minor metabolites were double conjugates, such as a double conjugate of BPA with glucuronide and N-acetyl galactosamine which was found in the intestine, placenta, amniotic fluid and foetal tissue. A study in cynomolgus monkeys showed that BPA-glucuronide was the major metabolite, although there was evidence for production of a minor metabolite, possibly BPA-sulphate or 5-hydroxy-BPA. Studies conducted in humans provide evidence for the glucuronidation of BPA in man; some studies also found evidence for the sulphation of BPA.

Most studies investigating the distribution of BPA measured tissue radioactivity levels after giving labelled BPA to experimental animals. An oral dosing study in rats found that the tissue concentrations of BPA-derived-radioactivity were highest in the liver, kidney and carcass, and lowest in the brain and testes, and there were no large differences between adult and neonatal animals. A number of studies in rats suggest that BPA metabolites and especially free BPA have a limited distribution to the embryo/foetal or placental compartments following oral administration. No selective affinity of either yolk sac/placenta or embryo/foetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed in a recent study in rats after oral dosing. However, maternal and embryo/foetal exposure to free BPA did occur, but systemic levels were found to be low due to extensive first-pass metabolism.

Regarding the distribution of free, unconjugated BPA to tissues after oral dosing, since free BPA is removed rapidly from the blood after absorption by first pass metabolism, it has been suggested that in rodents the availability of free BPA to extrahepatic tissues is likely to be limited following oral exposure. In adult rats it has been estimated that no more than 5-10% of the administered dose of free BPA is available to the tissues, although this figure may be higher in neonates. In humans, the systemic availability of free BPA is very low as enterohepatic recirculation of BPA does not occur.

In summary, there are differences between humans and rodents in the distribution of BPA. After oral administration, BPA is rapidly metabolised in the gut wall and the liver to BPA-glucuronide. This metabolite is devoid of endocrine activity. In humans, the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion. Due to the rapid biotransformation and excretion ($t_{1/2} = 5$ hours) and plasma protein binding, peak free BPA concentrations in humans after oral exposure that are available for estrogen receptor binding are very low. In contrast, BPA glucuronide is eliminated in bile in rodents and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic recirculation results in slow excretion ($t_{1/2} = 15-22$ hours) and increased systemic availability of free BPA in rodents.

This conclusion is supported by the observation that in urine of rats dosed orally with BPA, a part of the dose was excreted as free BPA in urine (1-4 % of applied dose, whereas BPA-glucuronide in urine accounted for 20-40 % of applied dose). In both of the human studies and the monkey study free BPA was below the limit of detection in all urine and blood samples (equivalent to a ratio of free BPA to BPA-glucuronide of < 0.5 %). Since free BPA found in urine is translocated from blood to urine in the kidney, these observations of higher free BPA levels in urine of rats compared with primates further support the existence of species differences in blood levels of free BPA between rodents and humans with higher AUCs for free BPA in rats.

The major route of elimination in the rat is via the faeces. The available data indicate that the percentage of the administered dose recovered in the faeces is in the range 50% to 83%. Urinary excretion is of secondary importance in the rat, with 13% to 42% of the administered dose being recovered in the urine. Over 7 days post-dosing approximately 80% and 70% of the administered dose was eliminated in the faeces in males and females, respectively. Elimination was rapid; the majority of the dose was excreted by 72 hours post-dosing. A sex difference was also observed for urinary elimination, with females excreting approximately twice as much radioactivity (24-28%) than males (14-16%). A study in female SD rats found that excretion was not affected by pregnancy at 3 different stages of gestation. Data from a number of studies suggest limited excretion of BPA in the milk. However, the data do not allow a reliable quantitative determination to be made.

Following oral administration to rats, a high proportion of the administered dose (45-66%) was rapidly excreted in the bile in the form of BPA-glucuronide, with the rate of biliary excretion tending to be higher in males than females. Most of the faecal radioactivity was found to be in the form of free BPA. Since BPA has a high oral bioavailability in the rat, the free BPA found in the faeces is more likely to be derived from BPA-glucuronide excreted in the bile and hydrolysed to free BPA in the gastrointestinal tract rather than representing unabsorbed BPA which might have passed along the gastrointestinal tract into the faeces unchanged. Most of the urinary radioactivity was found to be in the form of BPA-glucuronide (82%) with free BPA and BPA-sulphate making minor contributions (14% and 4% respectively).

In contrast to the findings in rodents, in cynomolgus monkeys given BPA orally most of the administered dose (82–85%) was recovered in the urine, with only 2-3% of the dose being recovered in the faeces. In two studies in human volunteers given a low dose of BPA orally, the administered dose was completely recovered in the urine as BPA-glucuronide. No free BPA was detected and no gender differences in the kinetics of BPA-glucuronide in plasma and urine were reported.

Acute toxicity: No useful information is available on the effects of single exposure to BPA in humans. Oral LD₅₀ values beyond 2,000 mg/kg are indicated in the rat and mouse, and dermal LD₅₀ values above 2,000 mg/kg are evident in the rabbit. Few details exist of the toxic signs observed or of target organs. For inhalation, a 6-hour exposure to 170 mg/m³ (the highest attainable concentration) produced no deaths in rats; slight and transient slight nasal tract epithelial damage was observed. These data indicate that BPA is of low acute toxicity by all routes of exposure relevant to human health.

Irritation: Limited human anecdotal information of uncertain reliability is available from written industry correspondence suggesting that workers handling BPA have in the past experienced skin, eye and respiratory tract irritation. It cannot be determined whether the reported skin reactions were related to skin sensitisation or irritation. However, a recent well conducted animal study clearly shows that BPA is not a skin irritant. A recent well conducted animal study shows that BPA is an eye irritant; effects persisted until the end of the study (day 28 postinstillation) in 1 of 3 rabbits. Overall, taking into account the animal and human evidence, BPA has the potential to cause serious damage to the eyes.

Slight and transient nasal tract epithelial damage was observed in rats exposed to BPA dust at 170 mg/m³ for 6 hours. Slight local inflammatory effects in the upper respiratory tract were observed in rats exposed to 50 mg/m³ and 150 mg/m³ of BPA in 2 and 13 week repeat inhalation studies, but were not observed at 10 mg/m³ in the same studies. Increased duration of exposure did not increase the severity of the response at 50 and 150 mg/m³. Taken together with anecdotal human evidence, these data suggest BPA has a limited respiratory irritation potential.

Skin sensitisation: With respect to skin sensitisation, there are several reports of patients with dermatitis responding to BPA in patch tests. However, it is unclear whether BPA or related epoxy resins were the underlying cause of the hypersensitive state. Anecdotal information indicates skin inflammation in workers handling BPA, although given the uncertain reliability of this information no conclusions can be drawn from it. In animals, the available studies are negative, but the test reports lack detail and no reliable justifications were given for the choice of concentrations used. In the study using the highest challenge concentration, 50% in a guinea pig closed-patch test, a sensitisation rate of 12.5% was obtained. Based on the findings from the most robust study, it appears that BPA may possess a skin sensitisation potential, albeit a limited one.

A recent LLNA study has shown that BPA does not possess skin sensitisation potential. However, in this study the concentration of BPA was not maximised. Therefore, there remains some uncertainty as to whether high concentrations (> 30%) of BPA can still exert skin sensitising activity. Similarly, a recent photo-LLNA has shown that BPA does not possess skin photo-sensitisation potential. However, again, in this study the concentration of BPA employed was not maximised. Although there are sporadic reports showing that BPA in the presence of UV light can elicit skin responses in humans, comprehensive medical surveillance data obtained from BPA manufacture plants has shown that no cases of skin

sensitisation have been identified among approximately 875 employees examined for several years. Due to the nature of these data, although it can be concluded that the risk of skin sensitisation is low under the exposure conditions experienced by these workers, a potential skin sensitisation hazard cannot be completely excluded.

Overall the new information shows that BPA does not possess a skin sensitisation potential under the exposure conditions tested. However, it cannot be excluded that high concentrations (> 30%) of BPA may exert skin sensitising activity and, in the presence of UV light, also photo-reactivity.

Respiratory sensitisation: There are no data from which to evaluate the potential of BPA to be a respiratory sensitiser. However, based on the lack of reports of cases of respiratory sensitisation, there are no grounds for concern for this endpoint.

Repeated dose toxicity: No useful information on the effects of repeated exposure to BPA in humans is available. Experimental studies are available in rats, mice and dogs.

In rat inhalation studies, the principal effect of repeated exposure was the same as observed following a single exposure: slight upper respiratory tract epithelium inflammation. Very slight to slight inflammation and hyperplasia of the olfactory epithelium were observed in rats following exposure to 50 mg/m³ (6 hours/day, 5 days/week for 13 weeks). There was no significant increase in the severity of these effects on the olfactory epithelium in animals exposed to 150 mg/m³. A NOAEL of 10 mg/m³ was identified in rats in this 13-week study.

Oral studies in rats and mice have shown that the repeated dose toxicity of BPA involve effects on bodyweight gain, liver and kidney. A NOAEL of 50 mg/kg/day has been identified in multigeneration study in rats and a recent 2-generation study in mice for these effects. This NOAEL rather than the original LOAEL of 120 mg/kg/day for liver effects from the published report is taken forward to the risk characterisation.

There are no animal data available for repeated dermal exposure.

Overall, therefore, the inhalation NOAEL of 10 mg/m³ for local effects on the respiratory tract and the oral NOAEL of 50 mg/kg/day for systemic effects are taken forward to the risk characterisation of repeated dose toxicity.

Mutagenicity: No human data regarding mutagenicity are available. However, BPA appears to have demonstrated aneugenic potential *in vitro*, positive results being observed without metabolic activation in a micronucleus test in Chinese hamster V79 cells and in a non-conventional aneuploidy assay in cultured Syrian hamster embryo cells. Additionally, in cell-free and cellular systems there is information that shows BPA disrupts microtubule formation. BPA has been shown to produce adduct spots in a post-labelling assay with isolated DNA and a peroxidase activation system, but it does not appear to produce either gene mutations or structural chromosome aberrations in bacteria, fungi or mammalian cells *in vitro*. However, some deficiencies in the conduct of these studies have been noted and the negative results cannot be taken as entirely conclusive. BPA does not appear to be aneugenic *in vivo*, since a recently conducted, standard mouse bone marrow micronucleus test has given a negative result. BPA was negative in a briefly reported dominant lethal study in rats but, given the limited details provided, this is not regarded as an adequate negative result. The only other data in somatic cells *in vivo* are from a ³²P-postlabelling assay, which showed that BPA is capable of producing DNA adduct spots in rat liver following oral administration. These adduct spots were not characterised fully.

Considering all of the available genotoxicity data, and the absence of significant tumour findings in animal carcinogenicity studies, it does not appear that BPA has significant mutagenic potential *in vivo*. Any aneugenic potential of BPA seems to be limited to *in vitro* test systems and is not of concern. The relevance of the finding that BPA can produce rat hepatic DNA adduct spots in a postlabelling assay is not entirely clear. However, given the absence of positive results for gene mutation and clastogenicity in cultured mammalian cell tests, it seems unlikely that these are of concern for human health.

New information on the mutagenicity of BPA has shown that BPA produces an increase in congression failure, a misalignment of chromosomes during the metaphase stages of meiosis II in oocytes of female mice. However, in view of several methodological weaknesses and flaws identified in these new data along with the reporting inadequacies, and taking into account the known mutagenicity and toxicological profile of BPA, these results cannot in themselves be taken as conclusive evidence of an effect of BPA on germ cell meiosis. Furthermore, these findings have not been confirmed in more recent publications. Therefore, the original conclusion from the published assessment that BPA has no significant mutagenic potential *in vivo*, is still valid.

Carcinogenicity: There are no human data contributing to the assessment of whether or not BPA is carcinogenic. In animals, BPA has not shown any significant carcinogenic activity in two standard oral cancer bioassays in rats and mice. No inhalation or dermal carcinogenicity studies are available, although in repeat exposure inhalation toxicity studies, BPA did not exhibit properties that raise concern for potential carcinogenicity. Only minimal inflammation was seen in the upper respiratory tract at 50 mg/m³ in a 13 week study and the severity did not increase up to concentrations close to the maximum attainable concentration in the experimental system used, 150 mg/m³.

New information on the potential carcinogenic and/or promoting effects of BPA in prenatal and neonatal rat models indicates that BPA does not possess significant carcinogenic potential and does not exert promoting activity on the carcinogenesis induced by established carcinogens/initiators in specific organs. Taking into account all of the animal data available the evidence suggests that BPA does not have carcinogenic potential.

Reproductive toxicity: BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays. The potency of this activity in these assays generally ranged from 3 to 5 orders of magnitude less than that of oestradiol. No significant oestrogenic activity has been observed with BPA glucuronide *in vitro*. It should be noted that these studies investigating endocrine modulating activity are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. However, the first phase of the validation of the uterotrophic assay in OECD indicates that this model is robust and reproducible across laboratories. Whilst this assay can be used to identify oestrogenic activity and can be an early screening test, its use for risk characterisation purposes is still a matter for discussion. In addition, many of the available *in vivo* studies have used parenteral routes of exposure, the relevance of which are uncertain with respect to relevant routes of human exposure.

The effects of BPA on fertility and reproductive performance have been investigated in four good quality, comprehensive, studies: a 2-generation study in the rat, a multigeneration study in the rat, a continuous breeding study in the mouse and a 2-generation study in the mouse. Although no effect on fertility was seen in the rat 2-generation study, low-dose levels were employed (0.2-200 µg/kg/day). In the multigeneration study, an effect on fertility (reduction

in litter size) was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity (a reduction in body weight gain (>13%) in both sexes and renal tubule degeneration in females only), it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. In the light of this uncertainty, and given that an adverse effect on fertility has been seen in the mouse continuous breeding study, it is prudent to assume that BPA may be having a direct effect on fertility in this study. No effects on fertility were seen at 50 mg/kg/day and below in the multigeneration study. In the mouse 2-generation study, using dose levels of 0.003-600 mg/kg/day, no effects on fertility, reproductive organ weights and histopathology or sperm production were observed. However, the continuous breeding study in the mouse provided some evidence of an adverse effect on fertility. In the F₀ generation, no effects on fertility were seen at 300 mg/kg/day, but at dose levels of approximately 600 mg/kg/day and above, reductions in the numbers of litters produced, litter size and numbers of live pups per litter were observed in each of the 4-5 litters produced. These effects were observed in the absence of significant parental toxicity. In contrast, no adverse effects on fertility were observed in the single litter tested at each dose level from the F₁ generation. A statistically significant and dose-related decrease in epididymal weight was seen at all doses in the F₁ generation. However, the significance of this finding is uncertain given that there was no effect on fertility in this generation, and where an adverse effect on fertility was seen (in the F₀ generation) there was no effect on epididymal weight. Furthermore, there were no effects on epididymal weight in the mouse two generation study. Overall, a NOAEL of 50 mg/kg/day can be identified for effects on fertility based on the rat multi-generation study.

No evidence that BPA is a developmental toxicant was observed in standard developmental toxicity studies in rats and mice. In rats, a maternal LOAEL and foetal NOAEL of 160 and 640 mg/kg/day, respectively, were identified. In mice, maternal and foetal NOAELs were 250 and 1,000 mg/kg/day, respectively. In a rat multigeneration study, a statistically significant decrease in mean pup body weight gain, with concomitant delays in the acquisition of developmental landmarks (vaginal patency and preputial separation) was observed at 500 mg/kg on post-natal days 7-21 in males and females of all generations (F₁-F₃). These decreases in pup body weight gain and delays in development were seen in the presence of maternal toxicity. No maternal toxicity and no treatment-related effects were reported in the offspring of animals exposed to 50 mg/kg. Similarly, effects on F₁ (but not F₂) pup bodyweight gain were observed in the mouse two generation study at 600 mg/kg/day, a dose level that also caused mild parental toxicity. Additionally, there was an increase in the incidence of undescended testes and seminiferous tubule hypoplasia in F₁ and F₂ offspring at weaning at 600 mg/kg/day, although similar effects were not seen in adult F₁ males. No adverse effects were seen at 50 mg/kg/day and below in the mouse study.

Several additional studies have focused on the potential of BPA to affect male reproductive tract development in rats and mice. Conflicting results have been reported in these studies, in both species. In mice, adverse effects on male reproductive tract development (an increase in prostate weight in two studies and a reduction in epididymis weight in one study) have been reported at low dose levels, in the range 2-50 µg/kg. However, these results have not been reproducible in two other studies, one of which included additional dose levels, and using larger group sizes compared with those used in either of the two studies showing effects. Also, no effects on male reproductive tract development were observed in a recent mouse two generation study, which was conducted specifically to help resolve the uncertainties surrounding the potential for BPA to affect development at low doses. Giving most weight to the negative 'gold standard' mouse two generation study, and taking account of the fact that there were no functional changes in reproductive parameters or reproductive organ

development at low dose levels in the rat multigeneration and two generation studies, it is concluded that BPA does not have an adverse effect on male reproductive tract development at low dose levels.

The effect of prenatal and perinatal exposure to BPA on neurological development has been investigated in a large number of recent studies. Many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. Although a number of these studies claimed to have detected a BPA-related effect on development, no firm conclusions could be drawn about developmental neurotoxicity because of a low level of confidence in the reliability of the studies and a lack of consistency in the results.⁵

Also, no conclusions could be drawn from a human study investigating the possible association between recurrent miscarriage and BPA exposure.

Thus, a NOAEL of 50 mg/kg/day can be identified for developmental toxicity, taken from the rat multigeneration study and the mouse two-generation study.

Summary: Overall, the hazardous properties of BPA have been evaluated in animals to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure and reproductive toxicity have been identified. No dose response information is available for eye irritation. A NOAEL of 10 mg/m³ has been identified for repeated dose toxicity to the respiratory tract. A NOAEL of 50 mg/kg has been identified for systemic effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg has been established in multi- and two-generation studies for effects on fertility and development.

For the purposes of risk characterisation, absorption via the oral and inhalation routes will be assumed to be 100%; dermal absorption will be taken to be 10%.

To conduct the risk characterisation for workers and consumers, it is necessary to compare human exposure for the inhalation/dermal route with oral N(L)OAELs from repeated dose animal studies, because of the absence of significant inhalation/dermal toxicity data. A direct comparison between exposure and systemic effects (with the exception of effects on the liver) must take account of the first pass effect, which has been shown to limit systemic bioavailability by the oral route (see toxicokinetic section). To compensate for this limited oral bioavailability (shown to be around 5-10% of the administered dose in rodents – see toxicokinetic section), the oral animal N(L)OAELs have been reduced by a factor of 10 for the comparison of inhalation or dermal exposure and systemic effects. For effects on the

⁵ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use: Negishi 2004, Carr 2003, Ryan and Vandenberg 2006 and Adriani 2003. The reliability of these studies is judged to be adequate because the behavioural testing has been conducted according to acceptable methods, the group sizes are quite close or equal to those recommended in the OECD TG 426, and the litter has been used as the statistical unit. The effects found in these studies indicate that there is a possible risk for developmental neurotoxicity of BPA at very low exposure levels (0.1-0.25 mg/kg/d). These effects cannot be dismissed based on the other unreliable studies in the DNT database. The above mentioned countries would therefore prefer one of two possible conclusions: 1) the available, but limited data are used for the risk assessment or 2) there is a need for further information (the countries certainly evaluate the database as sufficient to justify a concern warranting further investigation of developmental neurotoxicity), similarly to the proposed conclusion in the final expert panel report on the reproductive and developmental toxicity of BPA performed by NTP, US in November 2007.

liver, adjustment for the first-pass effect is inappropriate as these effects are not related to the systemic availability of free unconjugated BPA, but to the dose of BPA delivered to the liver, which is approximately the same for the oral route (only 10% higher) and the inhalation and dermal routes.

The toxicological starting points for the risk characterisation of BPA have been derived from animal studies. Therefore, to conduct the risk characterisation, extrapolation from animals to humans needs to be performed. New kinetic information in humans and primates has shown that at comparable exposure levels the blood concentrations of free BPA in humans are much lower than those in rodents, indicating that there are important quantitative differences in the fate of BPA between humans and rodents. Following absorption, BPA is metabolised in the liver to BPA-glucuronide, which is devoid of endocrine activity. In humans, the glucuronide is released from the liver into the systemic circulation and cleared by urinary excretion. Due to the rapid biotransformation and excretion ($t_{1/2} = 5$ hours) and plasma protein binding, peak free BPA concentrations in humans that are available for estrogen receptor binding are very low. In contrast, in rats, BPA glucuronide is eliminated in bile and undergoes enterohepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic recirculation results in slow excretion ($t_{1/2} = 15-22$ hours) and increased systemic availability of free BPA in rats. Free BPA has been shown both in vitro and in vivo to have biological (endocrine) activity. Its glucuronide is devoid of endocrine activity. Therefore, it appears reasonable to assume that free BPA is the toxic entity responsible for the different systemic effects (repeat dose effects and reproductive effects) of BPA.

It is proposed that in view of this evidence, for systemic effects (except effects on the liver), the default factor of 2.5 for remaining (kinetic and dynamic) interspecies differences (draft TGD, 2005) is reduced to 1. A reduction of the allometric scaling factor does not seem appropriate, as the observed species differences in the elimination and fate of BPA are not related to the basal metabolic rate. Although a precise quantification of this factor is not possible because no information is available on potential dynamic interspecies differences, a comparison of the elimination half-life in humans and rats indicates that rats would be 3-4 times more sensitive than humans to the effects of BPA, and hence humans would be 0.25-0.3 times more sensitive than rats, on the basis of kinetic differences. For effects on the liver, the rat-human differences in the systemic availability of free unconjugated BPA are unimportant, and hence, for these effects a reduced interspecies factor for remaining differences is not appropriate.

It should also be noted that, although some of the toxicological starting points derive from the mouse for which no oral kinetic data are available, the kinetics of BPA in mice are such that the mouse-human interspecies differences are likely to be even more pronounced than those between rats and humans. In vitro data and in vivo mouse data by the subcutaneous route of administration indicate that, while glucuronidation of BPA seems to be the major pathway of BPA biotransformation in rats, in mice, oxidation products of BPA have been identified after low-dose administration, suggesting possible formation of metabolites with higher oestrogenic potency. It should also be noted that there are major species differences between the mouse and the human, both in the physiology of gestation and in their toxicodynamic sensitivity to oestrogens, the mouse being particularly sensitive to oestrogens, which could predispose that species to sensitivity to weak oestrogens such as BPA. Therefore, the likely high sensitivity of the mouse to oestrogens has to be considered when using that particular species as a model for the risk assessment of BPA in humans.

4.1.3.2 Workers

The health effects of concern for BPA relevant to workers are eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure, and reproductive toxicity. There are no concerns for acute toxicity, skin irritation, respiratory sensitisation, mutagenicity and carcinogenicity and hence conclusion (ii) is drawn for these endpoints.

In order to carry out the risk characterisation for workers, the following assumptions have been made; the body weight of the average worker is 70 kg and the worker inhales 10 m³ air per working day.

Eye irritation

No clinical signs of eye irritation were reported in an acute inhalation study in rats following exposure to 170 mg/m³ BPA for 6 hours. However, anecdotal human evidence, albeit limited, suggests that eye irritation can occur following occupational exposure to BPA. A recent well conducted animal study showed that irritation was observed following instillation of BPA into rabbit eyes. However, provided good occupational hygiene practices are in operation, eye irritation is unlikely to be expressed. Conclusion (ii) is proposed for all scenarios.

Respiratory tract irritation

Anecdotal human evidence suggests that respiratory irritation has been reported in workers, though there are no quantitative details. In an acute inhalation study, slight and transient nasal tract epithelium damage was observed in rats following exposure to 170 mg/m³ (the only concentration tested and the highest attainable concentration) for 6 hours. A NOAEL for these effects was not identified in this study. However, a NOAEL of 10 mg/m³ was identified in rats from a 13-week repeated exposure study. Overall, it is considered that BPA may have limited respiratory tract irritation potential that should be considered for risk characterisation in particular in relation to short-term peak exposures. The Margins of Safety (MOS) between the NOAEL of 10 mg/m³ and the worst case inhalation short-term exposure for each occupational scenario are shown in Table 4.21. The MOS values are evaluated by comparison with the minimal MOS (12.5). In Table 4.21a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.21 Margins of Safety for respiratory tract irritation

Scenario	Worst-case inhalation short-term exposure (mg/m ³)	MOS for respiratory tract irritation based on NOAEL of 10 mg/m ³	Conclusion
Manufacture of BPA	6	1.7	(ii)
Manufacture of PC	0.5	20	(ii)
Manufacture of articles from PC	negligible	Very high	(ii)
Manufacture of epoxy resin	4	2.5	(ii)
Powder coating manufacture	0.3	33	(ii)
Powder coating use	negligible	Very high	(ii)
Thermal paper manufacture	< 4	> 2.5	(ii)

Tin plating manufacture	negligible	Very high	(ii)
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Table 4.21a Assessment factors applied for the calculation of the minimal MOS for respiratory tract irritation in workers from rat data

Uncertainty	Assessment factor
Interspecies differences ^a	2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	12.5

^a for local effects the allometric scaling factor is not applicable

For all scenarios except manufacture of BPA and manufacture of epoxy resins, the MOS values are greater than the minimal MOS of 12.5. Therefore, these MOS are sufficient to conclude that respiratory tract irritation from exposure to short-term peaks will not occur, and conclusion (ii) is drawn. For manufacture of BPA and manufacture of epoxy resins, the MOS values of 1.7 and 2.5 respectively are lower than the minimal MOS of 12.5. This would normally lead to concern. However, considering that the MOSs were calculated comparing a NOAEL derived from a 13-week study and exposures of 6 h/day with 15 minutes peaks, and that the effects at the LOAEL were minor, conclusion (ii) is reached. These conclusions are consistent with the recent IOELV (Indicative Occupational Exposure Limit Value) of 10 mg/m³ (8h-TWA) established by SCOEL (Scientific Committee on Occupational Exposure Limits) for BPA and the lack of a STEL value (SCOEL SUM 113, May 2004).

Skin sensitisation

Overall, while the data do not exclude a skin sensitising activity of BPA at high concentrations (> 30%), there is no evidence that this is a concern for workers in current BPA manufacturing plants (such workers are believed to represent the group most likely to be exposed to BPA dust). Consequently, repeated skin contact with high concentrations of BPA may result in dermatitic responses. In order to avoid this, skin exposure with high concentrations of BPA should be controlled for all exposure scenarios. Therefore, conclusion (iii) is drawn.

Repeated dose toxicity

Local effects on the respiratory tract

The principal effect following repeated exposure to BPA was slight local inflammatory effects in the upper respiratory tract. A NOAEL of 10 mg/m³ was identified in rats for respiratory irritation from a 13-week study. It is particularly noted that the effects seen at 50 mg/m³ were slight and that an increase in the exposure concentration to 150 mg/m³ produced only a slightly greater response, indicating a shallow dose-response curve. Furthermore, extending the duration of exposure from 2 weeks to 13 weeks at these exposure concentrations had only marginal effects. The MOSs between this NOAEL of 10 mg/m³ and the worst case inhalation 8h-TWA exposures for each occupational scenario are shown in

Table 4.22. The MOS values are evaluated by comparison with the minimal MOS (12.5). In Table 4.22a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.22 Margins of Safety for repeated dose local effects on the respiratory tract

Scenario	Worst-case inhalation 8h-TWA exposure (mg/m ³)	MOS for repeated dose local effects on the respiratory tract based on NOAEL of 10 mg/m ³	Conclusion
Manufacture of BPA	3	3.3	(ii)
Manufacture of PC	0.001	10,000	(ii)
Manufacture of articles from PC	0.001	10,000	(ii)
Manufacture of epoxy resin	0.7	15	(ii)
Powder coating manufacture	0.01	1,000	(ii)
Powder coating use	0.5	20	(ii)
Thermal paper manufacture	0.1	100	(ii)
Tin plating manufacture	0.05	200	(ii)

Table 4.22a Assessment factors applied for the calculation of the minimal MOS for repeated dose local effects on the respiratory tract in workers from rat data

Uncertainty	Assessment factor
Interspecies differences ^a	2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	12.5

^a for local effects the allometric scaling factor is not applicable

For all scenarios except manufacture of BPA, the MOS values are greater than the minimal MOS of 12.5. Therefore, for these scenarios there is no concern and conclusion (ii) is drawn. For the manufacture of BPA the MOS value of 3.3 is lower than the minimal MOS of 12.5. This would normally lead to concern. However, it is considered sufficient (and thus, conclusion (ii) applies) for the following reasons:

- only minor effects were observed at the LOAEL and there is a shallow dose response curve;
- rats are obligate nasal breathers, therefore the amount deposited in nasal turbinates in humans would be less than in rats for comparable exposures;
- The lack of a lifetime inhalation study is not considered a concern as extending the duration of exposure from 2 weeks to 13 weeks had only marginal effects.

These conclusions are consistent with the recent IOELV (Indicative Occupational Exposure Limit Value) of 10 mg/m³ (8h-TWA) established by SCOEL (Scientific Committee on Occupational Exposure Limits) for BPA (SCOEL SUM 113, May 2004).

Systemic effects

Oral studies in rats and mice have shown that the repeated dose toxicity of BPA involves effects on bodyweight gain, liver and kidney. An oral NOAEL of 50 mg/kg/day has been identified in a recent 2-generation study in mice for these effects (occurring at the next dose level of 600 mg/kg/day).

Effects on bodyweight gain and kidney

The MOSs between the NOAEL of 50 mg/kg (equivalent to an internal extrapolated NAEL of 5 mg/kg to account for first pass effect following oral exposure) for effects on bodyweight and kidney and the worst case body burdens arising from inhalation and dermal exposure for each scenario are shown in Table 4.23. The MOS values are evaluated by comparison with the minimal MOS (<35). In Table 4.23a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.23 Margins of safety for repeated dose toxicity (systemic effects on bodyweight and kidney)

Scenario	RWC inhalation 8h-TWA exposure (mg.m ⁻³)	RWC inhalation body burden* (mg.kg ⁻¹ .day ⁻¹)	RWC dermal exposure [#] (mg.day ⁻¹)	RWC dermal body burden+ (mg.kg ⁻¹ .day ⁻¹)	RWC combined body burden (mg.day ⁻¹)	MOS for repeated dose systemic effects on bodyweight and kidney based on NAEL of 5 mg.kg ⁻¹ .day ⁻¹	Conclusion
Manufacture of BPA	3	0.43	42	0.06	0.49	10	(iii)
Manufacture of PC	1x10 ⁻³	1x10 ⁻⁴	0.04	6x10 ⁻⁵	1.6x10 ⁻⁴	3x10 ⁴	(ii)
Manufacture of articles from PC	1x10 ⁻³	1x10 ⁻⁴	0.04	6x10 ⁻⁵	1.6x10 ⁻⁴	3x10 ⁴	(ii)
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3	4	(iii)
Powder coating manufacture	0.01	1x10 ⁻³	2	3x10 ⁻³	4x10 ⁻³	1,250	(ii)
Powder coating use	0.5	0.07	2.3	3x10 ⁻³	0.07	71	(ii)
Thermal paper manufacture	0.1	0.01	42	0.06	0.07	71	(ii)
Manufacture of tin plating	0.05	7x10 ⁻³	42	0.06	0.07	71	(ii)

additive							
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* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m³ air inhaled per working day.

Taking into account area exposed

+ Assuming 10% absorption by the dermal route

Table 4.23a Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects on bodyweight and kidney in workers from mouse data

Uncertainty	Assessment factor
Interspecies differences ^a	7 x 1
Intraspecies differences	5
Differences between experimental conditions and exposure ^b	1
Dose response / Type of critical effect ^c	<1
Confidence of the database	1
Overall	<35

^a Allometric scaling factor for the mouse x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1)

^b Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. In a mouse 2-year study a LOAEL of 120 mg/kg/day was identified for minor effects on body weight gain.

^c As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day and the effects at the LOAEL are rather minor, an AF lower than 1 is considered appropriate.

For all scenarios except manufacture of BPA and manufacture of epoxy resins the MOS values are greater than the minimal MOS (<35). These MOSs are considered sufficient to conclude that adverse repeated dose systemic effects will not occur in these scenarios. Therefore conclusion (ii) is drawn for these scenarios. For manufacture of BPA and manufacture of epoxy resins, MOS values of 10 and 4 have been calculated. These MOSs are significantly lower than the minimal MOS (<35). There is concern that adverse repeated dose systemic effects on bodyweight and kidney might occur in these scenarios, and thus, conclusion (iii) is drawn.

Effects on the liver

The MOSs between the NOAEL of 50 mg/kg for effects on the liver (for these effects, adjustment for the first-pass effect is inappropriate as liver effects are not related to the systemic availability of free unconjugated BPA, but to the dose of BPA delivered to the liver, which is approximately the same for the oral route and the inhalation and dermal routes) and the worst case body burdens arising from inhalation and dermal exposure for each scenario are shown in Table 4.24. The MOS values are evaluated by comparison with the minimal MOS (<87.5). In Table 4.24a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.24 Margins of safety for repeated dose toxicity (systemic effects on the liver)

Scenario	RWC inhalation 8h-TWA	RWC inhalation body	RWC dermal exposure [#]	RWC dermal body	RWC combined body	MOS for repeated dose	Conclusion
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	exposure (mg.m ⁻³)	burden* (mg.kg ⁻¹ .day ⁻¹)	(mg.day ⁻¹)	burden+ (mg.kg ⁻¹ .day ⁻¹)	burden (mg.day ⁻¹)	systemic effects on the liver based on NOAEL of 50 mg.kg ⁻¹ .day ⁻¹	
Manufacture of BPA	3	0.43	42	0.06	0.49	102	(ii)
Manufacture of PC	1x10 ⁻³	1x10 ⁻⁴	0.04	6x10 ⁻⁵	1.6x10 ⁻⁴	3x10 ⁵	(ii)
Manufacture of articles from PC	1x10 ⁻³	1x10 ⁻⁴	0.04	6x10 ⁻⁵	1.6x10 ⁻⁴	3x10 ⁵	(ii)
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3	40	(iii)
Powder coating manufacture	0.01	1x10 ⁻³	2	3x10 ⁻³	4x10 ⁻³	12,500	(ii)
Powder coating use	0.5	0.07	2.3	3x10 ⁻³	0.07	710	(ii)
Thermal paper manufacture	0.1	0.01	42	0.06	0.07	710	(ii)
Manufacture of tin plating additive	0.05	7x10 ⁻³	42	0.06	0.07	710	(ii)

* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m³ air inhaled per working day.

Taking into account area exposed

+ Assuming 10% absorption by the dermal route

Table 4.24a Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects on the liver in workers from mouse data

Uncertainty	Assessment factor
Interspecies differences ^a	7 x 2.5
Intraspecies differences	5
Differences between experimental conditions and exposure ^b	1
Dose response / Type of critical effect ^c	<1
Confidence of the database	1
Overall	<87.5

^a Allometric scaling factor for the mouse x default factor for remaining uncertainties (for effects on the liver, the rodent-human differences in the systemic availability of free unconjugated BPA are unimportant, and hence, for these effects a reduced interspecies factor for remaining differences is not appropriate.)

^b Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. A NOAEL of 50 mg/kg bw/d was identified in parental generations in subchronic reproductive toxicity studies in the mouse. The LOAEL was 600 mg/kg bw/d. In a chronic study with mice some liver effects were observed at a dose level of 120 mg/kg bw/d, but without an increase in severity at 650 mg/kg bw/d. Therefore it is judged that an additional factor to extrapolate the subchronic NOAEL to chronic exposure is not necessary.

^c As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day, an AF lower than 1 is considered appropriate.

For all scenarios except manufacture of epoxy resins the MOS values are greater than the minimal MOS (<87.5). These MOSs are considered sufficient to conclude that adverse repeated dose systemic effects on the liver will not occur in these scenarios. Therefore conclusion (ii) is drawn for these scenarios. For manufacture of epoxy resins, an MOS of 40 has been calculated. This MOS is lower than the minimal MOS (<87.5). There is concern that adverse repeated dose systemic effects on the liver might occur in these scenarios, and thus, conclusion (iii) is drawn.

Toxicity to reproduction

An overall NOAEL of 50 mg/kg has been established in a multigeneration study in rats and a two generation study in mice for effects on fertility and development occurring at the next doses level of 500-600 mg/kg⁶. The MOSs between this NOAEL of 50 mg/kg (equivalent to an internal extrapolated NAEL of 5 mg/kg to account for first pass effect following oral exposure) and the worst case body burdens arising from inhalation and dermal exposure for each scenario are shown in Table 4.25. The MOS values are evaluated by comparison with the minimal MOS (20). In Table 4.25a the assessment factors used to establish the minimal MOS are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.25 Margins of safety for reproductive toxicity (effects on fertility and development)

Scenario	RWC inhalation 8h-TWA exposure (mg.m ⁻³)	RWC inhalation body burden* (mg.kg ⁻¹ .day ⁻¹)	RWC dermal exposure# (mg.day ⁻¹)	RWC dermal body burden+ (mg.kg ⁻¹ .day ⁻¹)	RWC combined body burden (mg.day ⁻¹)	MOS for reproductive toxicity based on NAEL of 5 mg.kg ⁻¹ .day ⁻¹	Conclusion
Manufacture of BPA	3	0.43	42	0.06	0.49	10	(iii)
Manufacture of PC	1x10 ⁻³	1x10 ⁻⁴	0.04	6x10 ⁻⁵	1.6x10 ⁻⁴	3x10 ⁴	(ii)
Manufacture of articles from PC	1x10 ⁻³	1x10 ⁻⁴	0.04	6x10 ⁻⁵	1.6x10 ⁻⁴	3x10 ⁴	(ii)
Manufacture of epoxy resins	0.7	0.1	840	1.2	1.3	4	(iii)
Powder coating manufacture	0.01	1x10 ⁻³	2	3x10 ⁻³	4x10 ⁻³	1,250	(ii)
Powder coating use	0.5	0.07	2.3	3x10 ⁻³	0.07	71	(ii)
Thermal	0.1	0.01	42	0.06	0.07	71	(ii)

⁶ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

paper manufacture							
Manufacture of tin plating additive	0.05	7x10 ⁻³	42	0.06	0.07	71	(ii)

* Assuming 100% absorption by inhalation, 70 kg body weight, 10 m³ air inhaled per working day.

Taking into account area exposed

+ Assuming 10% absorption by the dermal route

Table 4.25a Assessment factors applied for the calculation of the minimal MOS for reproductive effects in workers from mouse and rat data

Uncertainty	Assessment factor
Interspecies differences ^a	4 x 1
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	20

^a Allometric scaling factor for the rat x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1). The same NOAEL of 50 mg/kg/day has been identified from mouse and rat data; however, since the available information (see section 4.1.3.1) shows that the rat is a better model for humans than the mouse, the allometric scaling of the rat is considered more appropriate than that for the mouse.

For all scenarios except manufacture of BPA and manufacture of epoxy resins the MOS values are greater than the minimal MOS (20). These MOSs are considered sufficient to conclude that adverse reproductive effects (effects on fertility and development) will not occur in these scenarios. Therefore conclusion (ii) is drawn for these scenarios. For manufacture of BPA and manufacture of epoxy resins, MOS values of 10 and 4 have been calculated. These MOSs are significantly lower than the minimal MOS (20). There is concern that adverse reproductive effects might occur in these scenarios, and thus, conclusion (iii) is drawn.

4.1.3.2.1 Summary of risk characterisation for workers

The risk characterisation for workers leads to conclusion (iii) for repeated dose systemic effects and for reproductive toxicity during the manufacture of BPA and the manufacture of epoxy resins. In addition, conclusion (iii) is reached in relation to skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact with high concentrations of BPA.

4.1.3.3 Consumers

The health effects of concern for BPA relevant to the consumer are eye irritation, respiratory tract irritation, skin sensitisation, local effects on the respiratory tract and systemic effects on body weight, liver and kidney following repeated exposure, and reproductive toxicity (effects on fertility and development, including developmental neurotoxicity). There are no concerns

for acute toxicity, skin irritation, respiratory sensitisation, mutagenicity and carcinogenicity and hence conclusion (ii) is drawn for these endpoints.

The relevant routes of exposure for the consumer are inhalation, oral and dermal.

Eye irritation, respiratory tract irritation and repeated dose local effects on the respiratory tract

The potential for eye and respiratory tract irritation and for repeated dose local effects on the respiratory tract could arise as a result of consumer use of paints and varnishes containing BPA. In these applications, the concentration of BPA in the product is $\leq 0.0004\%$. At these concentrations, there is no concern that the irritating properties of BPA will be expressed and therefore there are no concerns for these endpoints and conclusion (ii) is reached.

Skin sensitisation

Dermal exposure to BPA, leading to potential concerns for skin sensitisation, can result from the consumer use of paints, varnishes, wood fillers and adhesives which contain BPA. In these applications, the concentration of BPA in the product is $\leq 0.0004\%$. At these concentrations, there is no concern that the sensitising properties of BPA will be expressed and therefore there are no concerns for this endpoint and conclusion (ii) is reached.

Repeated dose systemic effects and reproductive toxicity

Oral exposure

Potential concerns for repeat dose toxicity (systemic effects on body weight, liver and kidney) and for reproductive effects arise from those consumer exposure scenarios which involve repeated exposure to BPA. Scenarios for which exposures are single, relatively rare events (application of paints and varnish, use of wood fillers, exposures immediately following dental treatment) are not relevant in relation to concerns for these endpoints, and thus will not be considered further.

The sources of consumer exposure which could result in repeated exposure to BPA are food contact applications (infant feeding bottles; polycarbonate tableware; wine from epoxy-resin lined vats; canned food and canned beverages). In addition, the use of adhesives will be considered in relation to these endpoints, since although it is generally unlikely to be a daily event, some consumers may have relatively frequent use. With the exception of adhesives use, each of these scenarios results in oral exposure only; use of adhesives results in dermal exposure only.

Some of these sources will result in exposure to adult and/or infant or child consumers. Table 4.26 gives calculations of body burdens for adult and/or infant and child consumers from sources involving oral exposure and MOSs for repeat dose toxicity (effects on bodyweight, kidney and liver) and reproductive effects (effects on fertility and development, including developmental neurotoxicity)⁷. Body burdens have been calculated using the following assumptions: oral absorption is 100%; an adult consumer weighs 60 kg; a young child (1.5-4.5 years) weighs 11 kg; a 1-2 month baby weighs 4.5 kg; a 4-6 month baby weighs 7 kg, an

⁷ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

infant (6-12 months) weighs 8.7 kg. Bodyweight values for adults and young children are based on UK data (HMSO, 1990, 1992, 1995).

The MOS values for repeated dose effects on bodyweight and kidney are evaluated by comparison with the minimal MOS of <70, and the MOS values for repeated dose effects on the liver are evaluated by comparison with the minimal MOS of <175. The MOS values for reproductive toxicity (effects on fertility and development) are evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.23c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.26 Calculated body burdens from oral exposure and MOSs for repeat dose toxicity (effects on bodyweight, kidney and liver) and reproductive effects (effects on fertility and development)

Source of exposure	Daily ingestion of BPA (mg/day)	Estimated body burden (mg/kg/day)	MOS		Conclusion	
			Repeated dose toxicity (effects on bodyweight, kidney and liver) ¹	Reproductive toxicity (effects on fertility and on development) ²	Repeated dose toxicity (effects on bodyweight, kidney, and liver)	Reproductive toxicity (effects on fertility and development)
Infant feeding bottles (1-2 month baby)	0.035	0.008	6250	6250	(ii)	(ii)
Infant feeding bottles (4-6 month baby)	0.050	0.007	7 100	7 100	(ii)	(ii)
Canned food and beverages (infant 6-12 months)	0.0375	0.0043	11 628	11 628	(ii)	(ii)
Canned food and beverages (young child 1.5-4.5 years)	0.100	0.009	5 555	5 555	(ii)	(ii)
Canned food (adult)	0.050	8×10 ⁻⁴	62 500	62 500	(ii)	(ii)
Canned beverages (adult)	0.020	3×10 ⁻⁴	170 000	170 000	(ii)	(ii)
Wine (adult)	0.010	1.7×10 ⁻⁴	300 000	300 000		
Canned food and beverages including wine (adult)	0.070	0.00125	42 000	42 000	(ii)	(ii)
Polycarbonate tableware (young child, 1.5-4.5 years)	0.010	9×10 ⁻⁴	55 000	55 000	(ii)	(ii)
Polycarbonate tableware (adult)	0.015	2.5×10 ⁻⁴	20 000	20 000	(ii)	(ii)
Canned food and beverages + polycarbonate tableware (young child, 1.5-4.5 years)	0.110	0.01	5 000	5 000	(ii)	(ii)
Canned food and beverages + polycarbonate tableware (adult)	0.085	0.00150	33 333	33 333	(ii)	(ii)

¹ Based on NOAEL of 50 mg/kg for effects on body weight, kidney and liver from 2-gen study in the mouse

² Based on NOAEL of 50 mg/kg from 2-gen study in the mouse

Table 4.26a Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects (effects on bodyweight and kidney) in consumers and humans exposed indirectly via the environment from mouse data

Uncertainty	Assessment factor
Interspecies differences ^a	7 x 1
Intraspecies differences	10
Differences between experimental conditions and exposure ^b	1
Dose response / Type of critical effect ^c	<1
Confidence of the database	1
Overall	<70

^a Allometric scaling factor for the mouse x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1)

^b Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. In a mouse 2-year study a LOAEL of 120 mg/kg/day was identified for minor effects on body weight gain.

^c As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day and the effects at the LOAEL are rather minor, an AF lower than 1 is considered appropriate.

Table 4.26b Assessment factors applied for the calculation of the minimal MOS for repeated dose systemic effects (effects on the liver) in consumers and humans exposed indirectly via the environment from mouse data

Uncertainty	Assessment factor
Interspecies differences ^a	7 x 2.5
Intraspecies differences	10
Differences between experimental conditions and exposure ^b	1
Dose response / Type of critical effect ^c	<1
Confidence of the database	1
Overall	<175

^a Allometric scaling factor for the mouse x default factor for remaining uncertainties (for effects on the liver, the rodent-human differences in the systemic availability of free unconjugated BPA are unimportant, and hence, for these effects a reduced interspecies factor for remaining differences is not appropriate.)

^b Although the experimental conditions involved subchronic rather than chronic exposure, the evidence suggests that the severity of the effects does not increase when duration of exposure increases from 90 days to 2 years. A NOAEL of 50 mg/kg bw/d was identified in parental generations in subchronic reproductive toxicity studies in the mouse. The LOAEL was 600 mg/kg bw/d. In a chronic study with mice some liver effects were observed at a dose level of 120 mg/kg bw/d, but without an increase in severity at 650 mg/kg bw/d. Therefore it is judged that an additional factor to extrapolate the subchronic NOAEL to chronic exposure is not necessary.

^c As there is more than one order of magnitude between the LOAEL of 600 mg/kg/day and the NOAEL of 50 mg/kg/day, an AF lower than 1 is considered appropriate.

Table 4.26c Assessment factors applied for the calculation of the minimal MOS for reproductive effects (effects on fertility and development, including developmental neurotoxicity) in consumers and humans exposed indirectly via the environment from mouse and rat data

Uncertainty	Assessment factor
Interspecies differences ^a	4 x 1
Intraspecies differences	10

Uncertainty	Assessment factor
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect	1
Confidence of the database	1
Overall	40

^a Allometric scaling factor for the rat x factor for remaining uncertainties (justification for a value of 1 provided in section 4.1.3.1). The same NOAEL of 50 mg/kg/day has been identified from mouse and rat data; however, since the kinetic information shows that the rat is a better model for humans than the mouse, the allometric scaling of the rat is considered more appropriate than that for the mouse.

The margins between exposures and the NOAELs are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects (effects on fertility and development) respectively, for all the exposure scenarios in Table 4.23. These margins are considered not to give rise to concern and therefore conclusion (ii) is drawn. These conclusions are in agreement with those of the EFSA (2006) evaluation of BPA. EFSA (2006) stated that the conservative estimates of exposure in all population groups considered were well below the Tolerable Daily Intake (TDI) established by EFSA at 0.05 mg/kg bw/day.

Dermal exposure

In relation to potential exposure arising from the use of adhesives, exposure occurs only as a result of dermal contact. Based on the available information for dermal absorption, the contribution to total body burden arising from dermal exposure is calculated on the basis of 10% uptake. In order to compensate for first pass metabolism, the oral NOAEL of 50 mg/kg/day for repeated dose effects on bodyweight and kidney and reproductive toxicity (effects on fertility and development)⁸ has been adjusted by a factor of 10 for comparison with the dermal exposure estimates. For the repeated dose effects on the liver, adjustment of the oral NOAEL of 50 mg/kg/day for the first-pass effect is inappropriate as liver effects are not related to the systemic availability of free unconjugated BPA, but to the dose of BPA delivered to the liver, which is approximately the same for the oral and dermal routes of exposure. The estimated exposures arising from the use of adhesives for an adult consumer and the resultant MOSs for repeated dose effects on bodyweight and kidney and for reproductive toxicity are shown in Table 4.27. The estimated exposures arising from the use of adhesives for an adult consumer and the resultant MOS for repeated dose effects on the liver are shown in Table 4.28. The MOS value for repeated dose effects on bodyweight and kidney is evaluated by comparison with the minimal MOS of <70 and the MOS value for repeated dose effects on the liver is evaluated by comparison with the minimal MOS of <175. The MOS value for reproductive toxicity is evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

⁸ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

Table 4.27 Calculated body burdens and MOSs for repeat dose toxicity (effects on bodyweight and kidney) and reproductive effects (effects on fertility and development) as a result of use of adhesives (dermal exposure)

Exposure to BPA per event (mg)	Estimated body burden (mg/kg/day)	MOS		Conclusion	
		Repeated dose toxicity (effects on bodyweight and kidney) ¹	Reproductive toxicity (effects on fertility and on development) ²	Repeated dose toxicity (effects on bodyweight and kidney)	Reproductive toxicity (effects on fertility and on development)
0.014	2.3×10 ⁻⁴	22 000	22 000	(ii)	(ii)

¹ NAEL = 5 mg/kg/day (allowing for first pass metabolism)

² NAEL = 5 mg/kg/day (allowing for first pass metabolism)

Table 4.28 Calculated body burdens and MOSs for repeat dose toxicity (effects on the liver) as a result of use of adhesives (dermal exposure)

Exposure to BPA per event (mg)	Estimated body burden (mg/kg/day)	MOS for repeated dose toxicity (effects on the liver) ¹	Conclusion
0.014	2.3×10 ⁻⁴	220,000	(ii)

¹ NOAEL = 50 mg/kg/day

The margins between exposure and the NOAELs are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, for repeated dose effects on the liver and for reproductive effects (effects on fertility and on development), respectively. These margins are considered not to give rise to concern and therefore conclusion (ii) is drawn for this scenario.

4.1.3.3.1 Summary of risk characterisation for consumers

The risk characterisation for consumers leads to conclusion (ii) for all the endpoints as consumer exposure is very low.

4.1.3.4 Humans exposed via the environment

The key health effects relevant to humans exposed via the environment are reproductive toxicity (effects on fertility and on development)⁹ and systemic effects following repeated exposure. Irritation, sensitisation and local effects on the respiratory tract are of low concern where exposure is dissipated throughout the environment and hence conclusion (ii) is reached

⁹ Denmark, Sweden and Norway do not agree with this conclusion. These countries find that some of the studies in the DNT database are sufficiently reliable for regulatory use. Please see the comments from these countries regarding the acceptability of the developmental neurotoxicity database at page 120.

for these endpoints. Furthermore, there are no concerns for acute toxicity, skin irritation, respiratory sensitisation, mutagenicity and carcinogenicity and hence conclusion (ii) is drawn for these endpoints.

4.1.3.4.1 Regional exposure

In Table 4.14, the total daily human exposure to BPA via the environment is estimated to be 9.1×10^{-6} mg/kg/day for regional sources. Inhalation makes no contribution to this exposure estimate as it all comes from oral sources. Comparisons of this intake estimate with the oral NOAEL of 50 mg/kg/day for repeated dose toxicity (effects on bodyweight, kidney and liver) and for reproductive toxicity (effects on fertility and development) respectively to derive MOSs are shown in the table below (table 4.29). The MOS value for repeated dose effects on bodyweight and kidney is evaluated by comparison with the minimal MOS of <70 and the MOS value for repeated dose effects on the liver is evaluated by comparison with the minimal MOS of <175. The MOS value for reproductive toxicity is evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.29 Risk characterisation for repeated dose toxicity (effects on bodyweight, kidney and liver) and reproductive toxicity (effects on fertility and development, including developmental neurotoxicity) following exposure via the environment (regional sources)

Exposure		Effects (systemic)			
		Reproductive effects NOAEL 50 mg/kg/day		Repeated dose toxicity NOAEL 50 mg/kg/day	
Source	Value (mg/kg/day)	MOS	Conclusion	MOS	Conclusion
Regional	9.1×10^{-6}	5×10^6	(ii)	5×10^6	(ii)

Given the low levels of exposure for the regional scenario and the very large margins of safety, which are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects, respectively, these exposures are considered not to be of concern and hence conclusion (ii) is reached for regional sources.

4.1.3.4.2 Local exposure

For all local scenarios except BPA manufacture, inhalation makes no contribution to the exposure estimates as it all comes from oral sources. For BPA manufacture, inhalation makes a more significant contribution (0.3%), but still the oral route contributes the most (99.7%). The human health systemic effects of concern include reproductive toxicity (effects on fertility and development) and repeated dose toxicity (effects on bodyweight, kidney and liver). The highest local exposure is in the locality of plants producing BPA. Exposure is estimated to be 0.041 mg/kg/day. Comparisons of this intake estimate with the oral NOAEL of 50 mg/kg/day for reproductive toxicity and repeated dose effects respectively to derive MOSs are shown in the table below (Table 4.30). The MOS value for repeated dose effects on bodyweight and kidney is evaluated by comparison with the minimal MOS of <70 and the

MOS value for repeated dose effects on the liver is evaluated by comparison with the minimal MOS of <175. The MOS value for reproductive toxicity is evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.30 Risk characterisation for repeated dose toxicity (effects on bodyweight, kidney and liver) and reproductive toxicity (effects on fertility and development) following exposure via the environment (local sources)

Exposure		Effects (systemic)			
		Reproductive effects NOAEL 50 mg/kg/day		Repeated dose toxicity NOAEL 50 mg/kg/day	
Source	Value (mg/kg/day)	MOS	Conclusion	MOS	Conclusion
Local (BPA production)	0.041	1220	(ii)	1220	(ii)

Given the very large margins of safety, which are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects, respectively, these exposures are considered not to be of concern and hence conclusion (ii) is reached for local sources.

4.1.3.5 Combined exposure

The worst case combined exposure would be for someone exposed via the environment near to a BPA production plant, and who is also exposed via food contact materials (oral exposure from canned food and canned beverages and from polycarbonate tableware and storage containers) as described in section 4.1.1.2.

The maximum combined exposure from these sources is 1.45×10^{-3} and 0.043 mg/kg/day for the regional and local scenarios respectively (see section 4.1.1.4.2).

Comparisons of these intake estimates with the oral NOAEL of 50 mg/kg/day for reproductive toxicity and repeated dose effects respectively to derive MOSs are shown in the table below (Table 4.31). The MOS values for repeated dose effects on bodyweight and kidney are evaluated by comparison with the minimal MOS of <70 and the MOS values for repeated dose effects on the liver are evaluated by comparison with the minimal MOS of <175. The MOS values for reproductive toxicity are evaluated by comparison with the minimal MOS of 40. In Table 4.26a, 4.26b and 4.26c the assessment factors used to establish the minimal MOS values are given in accordance to the draft version of the TGD (2005). There is concern when the MOS is lower than the minimal MOS.

Table 4.31 Risk characterisation for repeated dose toxicity (effects on bodyweight, kidney and liver) and reproductive toxicity (effects on fertility and development) for combined exposure scenarios

Exposure		Effects (systemic)	
		Reproductive effects NOAEL 50 mg/kg/day	Repeated dose toxicity NOAEL 50 mg/kg/day

Source	Value (mg/kg/day)	MOS	Conclusion	MOS	Conclusion
Regional	1.45 x10 ⁻³	35 000	(ii)	35 000	(ii)
Local (BPA production)	0.043	1163	(ii)	1163	(ii)

Given the very large margins of safety, which are significantly greater than the minimal MOS values of <70, <175 and 40 for repeated dose effects on bodyweight and kidney, repeated dose effects on the liver and for reproductive effects, respectively, these exposures are considered not to be of concern and hence conclusion (ii) is reached for combined exposure scenarios.

4.2 HUMAN HEALTH (PHYSICOCHEMICAL PROPERTIES)

The physicochemical properties of BPA are available from the literature although the exact values for end points such as vapour pressure can be difficult to verify. There will be slight variation in values quoted by manufacturers according to the nature of the material they produce. Given the low vapour pressure at normal temperatures, lack of flammability and the general stability, the risks arising from the physicochemical properties are small. In common with many organic materials, the finely powdered material is a significant dust explosion hazard (Grossel, 1988). However, this appears to be well known within the manufacturing industry and it is considered that there are adequate controls in place for this risk. Given the controls used during manufacture and use, the risk from this is small. Overall, the risk from physicochemical properties is low and conclusion (ii) is drawn.

5 RESULTS

5.1 RISKS TO HUMAN HEALTH

5.1.1 Human health (toxicity)

The key health effects of exposure to BPA are eye irritation, respiratory tract irritation, skin sensitisation, repeated dose local effects to the respiratory tract, systemic effects following repeated exposure and reproductive toxicity (effects on fertility and on development). No dose response information is available for eye irritation. A NOAEL of 10 mg/m³ has been identified for repeated dose toxicity to the respiratory tract. A NOAEL of 50 mg/kg has been identified for systemic effects following repeated exposure. In relation to reproductive toxicity, a NOAEL of 50 mg/kg has been established in a multigeneration study in rats and a two generation study in mice for effects on fertility and development.

5.1.1.1 Workers

The risk characterisation for workers leads to conclusion (iii) for repeated dose systemic effects and for reproductive toxicity during the manufacture of BPA and the manufacture of epoxy resins. In addition, conclusion (iii) is reached in relation to skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact.

Results

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied should be taken into account.

This applies to the manufacture of BPA and the manufacture of epoxy resins, in relation to concerns for repeated dose systemic effects and reproductive toxicity. In addition, there are concerns for skin sensitisation in all occupational exposure scenarios where there is the potential for skin contact with high concentrations of BPA.

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

This conclusion applies in relation to repeated dose systemic effects and reproductive toxicity for workers in the industry sectors of the manufacture of polycarbonate, manufacture of articles from polycarbonate, powder coatings manufacture and use, thermal paper manufacture and manufacture of tin plating additive. This conclusion also applies in relation to eye and respiratory tract irritation and repeated dose local effects in the respiratory tract for all scenarios.

5.1.1.2 Consumers

For eye and respiratory tract irritation, for repeated dose local effects on the respiratory tract and for skin sensitisation, exposure is very low and it is concluded that there is no concern for

these endpoints. For systemic effects following repeated exposure and for reproductive toxicity, conclusion (ii) is reached for all exposure scenarios.

Results

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

This applies to all consumer exposure scenarios in relation to eye and respiratory tract irritation, skin sensitisation, repeated dose local effects on the respiratory tract, systemic effects following repeated exposure and reproductive toxicity.

5.1.1.3 Humans exposed via the environment

The key health effects relevant to humans exposed via the environment are reproductive toxicity (effects on fertility and on development) and systemic effects following repeated exposure. Irritation, sensitisation and local effects on the respiratory tract are of low concern where exposure is dissipated throughout the environment.

Given the low levels of exposure and the large margins of safety for both the regional and local exposure scenarios, there are no concerns for repeated dose toxicity and reproductive toxicity and hence conclusion (ii) is reached.

Results

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

This applies to both regional and local exposure scenarios in relation to repeated dose systemic effects and reproductive toxicity.

5.1.1.4 Combined exposure

The worst case combined exposure would be for someone exposed via the environment near to a BPA production plant, and who is also exposed via food contact materials (oral exposure from canned food and canned beverages and from polycarbonate tableware and storage containers).

Given the very large margins of safety, there are no concerns for repeated dose toxicity and reproductive toxicity and hence conclusion (ii) is reached.

Results

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

This applies in relation to repeated dose systemic effects and reproductive toxicity.

5.1.2 Human health (risks from physico-chemical properties)

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

There are no significant risks from physico-chemical properties.

6 REFERENCES

- Adriani, W., Seta, D. D., Dessi-Fulgheri, F., Farabollini, F., and Laviola, G. (2003). Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. *Environ Health Perspect* 111, 395-401.
- Aikawa, H., Koyama, S., Matsuda, M., Nakahashi, K., Akazome, Y., and Mori, T. (2004). Relief effect of vitamin A on the decreased motility of sperm and the increased incidence of malformed sperm in mice exposed neonatally to bisphenol A. *Cell Tissue Res* 315, 119-24.
- Akingbemi, B. T., Sottas, C. M., Koulova, A. I., Klinefelter, G. R., and Hardy, M. P. (2004). Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology* 145, 592-603.
- Al-Hiyasat, A. S., Darmani, H., and Elbetieha, A. M. (2004). Leached components from dental composites and their effects on fertility of female mice. *Eur J Oral Sci* 112, 267-72.
- Aloisi, A.M., Seta, D.D., Rendo, C., Ceccarelli, I., Scaramuzzino, A., and Farabollini, F. (2002) Exposure to the estrogenic pollutant bisphenol A affects pain behavior induced by subcutaneous formalin injection in male and female rats. *Brain Res.* 937:1-7
- Alonso-Magdalena, P., Morimoto, S., E., Ripoll, C., Fuentes, E., and Nadal, A. (2006). The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect* 114, 106-112.
- Arakawa, C, Fujimaki, K., Yoshinaga, J., Imai, H., Serizawa, S., and Shiraishi, H. (2004). Daily urinary excretion of bisphenol A. *Environ. Health Prev. Med.* 9, 22-26.
- Arenholt-Bindslev D., V. Breinholt, G. Schmalz, and A. Preiss, 1998, "Time-related bisphenol A content and estrogenic activity in saliva samples collected in relation to placement of dental fissures", *Journal of Dental Research*, 77(B): 692 (abstract 481). Cited by www.bisphenol-A.org/human/dental.html
- Ashby, J. (2003). Endocrine disruption occurring at doses lower than those predicted by classical chemical toxicity evaluations: The case bisphenol A. *Pure Appl. Chem.* 75, 2167-2179.
- Ashby, J., and Odum, J. (2004). Gene expression changes in the immature rat uterus: effects of uterotrophic and sub-uterotrophic doses of bisphenol A. *Toxicol Sci* 82, 458-67.
- Ashby, J., Tinwell, H., Lefevre, P. A., Joiner, R., and Haseman, J. (2003). The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisphenol A between postnatal days 91-97. *Toxicol Sci* 74, 129-38.
- Ashby, J., Tinwell, H., Odum, J., and Lefevre, P. (2004). Natural variability and the influence of concurrent control values on the detection and interpretation of low-dose or weak endocrine toxicities. *Environ Health Perspect* 112, 847-53.

Association of Plastics Manufacturers in Europe (2006). Answer to EFSA request for further information on uses of bisphenol A. D. Thomas, 23 January 2006.

Attia, M.S., Adler, I.D., Eichenlaub-Ritter, U, Ranaldi, R., and Pacchierotti, F. (2004). Aneuploidy studies in mouse germ cells with bisphenol A (abstract PW6022). In: 34th Annual Meeting of the European Environmental Mutagen Society, Maastrich, Netherlands.

Bae, B., Jeong, J. H., and Lee, S. J. (2002). The quantification and characterization of endocrine disruptor bisphenol-A leaching from epoxy resin. *Water Sci Technol* 46, 381-7.

Berkowitz G. (2006). Letters to the Editor; Limitations of a case-control study on bisphenol A (BPA) serum levels and recurrent miscarriage. *Hum Reprod* 21, 565-566.

Braunrath, R., Podlipna, D., Padlesak, S., and Cichna-Markl, M. (2005). Determination of bisphenol a in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection. *J Agric Food Chem* 53, 8911-7.

Brede, C., Fjeldal, P., Skjevraak, I., and Herikstad, H. (2003). Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. *Food Addit Contam* 20, 684-9.

Brenn-Struckhofova Z. & Cichna-Markl M. (2006). Determination of bisphenol A in wine by sol-gel immunoaffinity chromatography, HPLC and fluorescence detection. *Food Addit Contam*, 23, 1227-35.

Calafat, A. M., Kuklennyik, Z., Reidy, J. A., Caudill, S. P., Ekong, J., and Needham, L. L. (2005). Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ Health Perspect* 113, 391-5.

Carr, R.L., Bertasi, F.R., Betancourt, A.M., Bowers, S.D., Gandy, B.S., Ryan, P.L., and Willard, S.T. (2003) Effect of neonatal rat bisphenol A exposure on performance in the Morris water maze. *J Tox Environ Health Part A*. 66, 2077-2088.

Cassidy, A., Bingham, S., and Setchell, K. (1995). Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. *Br J Nutr* 74, 587-601.

Cassidy, A., Bingham, S., and Setchell, K. D. (1994). Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr* 60, 333-40.

CEC (1993). Commission of the European Communities. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food. Thirty first series. Luxembourg: Office for official Publications of the European Communities.

Ceccarelli, I., Della Seta, D., Fiorenzani, P., Farabollini, F., and Aloisi, A.M. (2007). Estrogenic chemicals at puberty change ER α in the hypothalamus of male and female rats. *Neurotoxicol Teratol*; 29, 108-15

CERHR (2007). NTP-CERHR report on the reproductive and developmental toxicity of bisphenol A. Interim draft. April 2007. Report available from <http://cerhr.niehs.nih.gov>.

Cohen, S. M., Meek, M. E., Klaunig, J. E., Patton, D. E., and Fenner-Crisp, P. A. (2003). The human relevance of information on carcinogenic modes of action: overview. *Crit Rev Toxicol* 33, 581-9.

Conolly, R. B., and Lutz, W. K. (2004). Nonmonotonic dose-response relationships: mechanistic basis, kinetic modeling, and implications for risk assessment. *Toxicol Sci* 77, 151-7.

Csanady, G. A., Oberste-Frielinghaus, H. R., Semder, B., Baur, C., Schneider, K. T., and Filser, J. G. (2002). Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein. *Arch Toxicol* 76, 299-305.

CSL (2004). A study of the migration of bisphenol A from polycarbonate feeding bottles into food simulants. Central Science Laboratory Test Report L6BB-1008 for the Boots Group; <http://www.boots-plc.com/environment/library/250.pdf>.

Daston, G. P., Cook, J. C., and Kavlock, R. J. (2003). Uncertainties for endocrine disrupters: our view on progress. *Toxicol Sci* 74, 245-52.

Degen, G. H., Janning, P., Diel, P., and Bolt, H. M. (2002). Estrogenic isoflavones in rodent diets. *Toxicol Lett* 128, 145-57.

Delclos, K. B., Bucci, T. J., Lomax, L. G., Latendresse, J. R., Warbritton, A., Weis, C. C., and Newbold, R. R. (2001). Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. *Reprod Toxicol* 15, 647 -663.

Della Seta, D., Minder, I., Belloni, V., Aloisi, A.M., Dessi-Fulgheri, F., and Farabollini, F. (2006). Pubertal exposure to estrogenic chemicals affects behaviour in juvenile and adult male rats. *Horm Behav* 50, 301-307

Dessi-Fulgheri, F., Porrini, S., and Farabollini, F. (2002). Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. *Environ Health Perspect* 110 Suppl 3, 403-7.

Diel, P., Schmidt, S., Vollmer, G., Janning, P., Upmeier, A., Michna, H., Bolt, H. M., and Degen, G. H. (2004). Comparative responses of three rat strains (DA/Han, Sprague-Dawley and Wistar) to treatment with environmental estrogens. *Arch Toxicol* 78, 183-93.

Dionisi, G., and Oldring, P. K. (2002). Estimates of per capita exposure to substances migrating from canned foods and beverages. *Food Addit Contam* 19, 891-903.

Domoradzki, J. Y., Pottenger, L. H., Thornton, C. M., Hansen, S. C., Card, T. L., Markham, D. A., Dryzga, M. D., Shiotsuka, R. N., and Waechter, J. M., Jr. (2003). Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages. *Toxicol Sci* 76, 21-34.

Domoradzki, J. Y., Thornton, C. M., Pottenger, L. H., Hansen, S. C., Card, T. L., Markham, D. A., Dryzga, M. D., Shiotsuka, R. N., and Waechter, J. M., Jr. (2004). Age and dose dependency of the pharmacokinetics and metabolism of bisphenol A in neonatal sprague-dawley rats following oral administration. *Toxicol Sci* 77, 230-42.

Durando, M., Kass, L., Piva, J., Sonnenschein, C., Soto, A. M., Luque, E. H., and Muñoz-de-Toro, M. (2007). Prenatal Bisphenol A Exposure Induces Preneoplastic Lesions in the Mammary Gland in Wistar Rats *Environ Health Perspect* 115(1), 80-86.

Earls AO, Clay CA and Braybrook JH (2000). Preliminary Investigation into the Migration of bisphenol-A from Commercially-Available Polycarbonate Baby Feeding Bottles. LGC Technical Report LGC/DTI/2000/005.

EC SCF (2002). European Commission (2002) Final opinion of the Scientific Committee on Food on Bisphenol A (Expressed on 17 April 2002) SCF/CS/PM/3936. http://ec.europa.eu/food/fs/sc/scf/out128_en.pdf

EFSA (2006) Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A). Question number EFSA-Q-2005-100. Adopted on 29 November 2006. *The EFSA Journal* (2006) 428, 1 - 75.

Elswick, B. A., Miller, F. J., and Welsch, F. (2001). Comments to the editor concerning the paper entitled "Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals" by C. Gupta. *Exp Biol Med (Maywood)* 226, 74-5; discussion 76-7.

Elswick, B. A., Welsch, F., and Janszen, D. B. (2000). Effect of different sampling designs on outcome of endocrine disruptor studies. *Reprod Toxicol* 14, 359-67.

Ema, M., Fujii, S., Furukawa, M., Kiguchi, M., Ikka, T., and Harazono, A. (2001) Rat two-generation reproductive toxicity study of bisphenol A. *Reprod Toxicol* 15, 505-23

EU (2003). *European Union Risk Assessment Report. Bisphenol A, CAS No: 80-05-7*. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 3rd Priority List, Luxembourg: Office for Official Publications of the European Communities.

Everitt, J. I., and Foster, P. M. (2004). Laboratory Animal Science Issues in the Design and Conduct of Studies with Endocrine-active Compounds. *Ilar J* 45, 417-24.

Facciolo, R.M., Alo, R., Madeo, M., Canonaco, M., and Dessi-Fulgheri, F. (2002) Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst-2. *Environ Health Perspect*. 110 (Suppl 3), 397-402.

Facciolo, R.M., Madeo, M., Alo, R., Canonaco, M., and Dessi-Fulgheri, F. (2005). Neurobiological effects of bisphenol A may be mediated by somatostatin subtype-3 receptors in some regions of the developing rat brain. *Toxicol Sci*. 88, 477-484.

Farabollini, F., Porrini, S., and Dessi-Fulgheri, F. (1999) Perinatal exposure to the estrogenic pollutant bisphenol A affects behaviour in male and female rats. *Pharmacol Biochem Behav* 64, 687-994

Farabollini, F., Porrini, S., Della Seta, D., Bianchi, F., and Dessi-Fulgheri, F. (2002). Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. *Environ Health Perspect* 110 Suppl 3, 409-14.

Filser, J. G., Csanady, G. A., and Faller, T. (2003). Tissue burden and resulting effectiveness of bisphenol A and daidzein in humans in dependence of age. *Umweltforschungsplan des Bundesministers für Umwelt, Naturschutz und Reaktorsicherheit, UBA-FB Report No. 21602001/07*.

Festing, M.F.W. (2006) Design and statistical methods in studies using animal models of development. *ILAR J* 42, 5-14

Foster, W. G., Hughes, C. L., Chan, S., and Platt, L. (2002). Human developmental exposure to endocrine active compounds. *Environmental toxicology and Pharmacology* 12, 75-81.

FSA (2001). Food Standards Agency. Survey of Bisphenols in Canned Foods. Food Surveillance Information Sheet. Number 13/01. April 2001. Food Standards Agency, UK. Available on http://www.foodstandards.gov.uk/food_surv.htm.

Fujimoto, T., Kubo, K., and Aou, S. (2006) Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats. *Brain Res* 1068, 49-55.

Fukata, H., Miyagawa, H., Yamazaki, N., and Mori, C. (2006). Comparison of Elisa- and LCMS-based methodologies for the exposure assessment of bisphenol A. *Toxicology Mechanisms and Methods* 16, 427-430.

Funabashi, T., Kawaguchi, M., Furuta, M., Fukushima, A., and Kimura, F. (2004). Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. *Psychoneuroendocrinology* 29, 475-85.

Funabashi, T., Sano, A., Mitsushima, D., and Kimura, F. (2003). Bisphenol A increases progesterone receptor immunoreactivity in the hypothalamus in a dose-dependent manner and affects sexual behaviour in adult ovariectomized rats. *J Neuroendocrinol* 15, 134-40.

Fung, E. Y., Ewoldsen, N. O., St Germain, H. A., Jr., Marx, D. B., Miaw, C. L., Siew, C., Chou, H. N., Gruninger, S. E., and Meyer, D. M. (2000). Pharmacokinetics of bisphenol A released from a dental sealant. *J Am Dent Assoc* 131, 51-8.

Gallard H, Leclercq A., Croué J.P. (2004) Chlorination of bisphenol A: kinetics and byproducts formation. *Chemosphere* 56, 465-473.

Goodman, J.E., McConnell, E.E., Sipes, I.G., Witorsch, R.J., et al (2006). An updated weight of the evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. *Critical Reviews in Toxicology* 35, 387-457.

Goodson, A., Robin, H., Summerfield, W., and Cooper, I. (2004). Migration of bisphenol A from can coatings--effects of damage, storage conditions and heating. *Food Addit Contam* 21, 1015-26.

Goodson, A., Summerfield, W., and Cooper, I. (2002). Survey of bisphenol A and bisphenol F in canned foods. *Food Addit Contam* 19, 796-802.

Gray, G.M., Cohen, J.T., Cunha, G, et al (2004). Weight of evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. *Human and Ecological Risk Assessment* 10, 875-921.

Guo, T. L., Germolec, D. R., Musgrove, D. L., Delclos, K. B., Newbold, R. R., Weis, C., and White, K. L., Jr. (2005). Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F1 generations of Sprague Dawley rats following developmental and adult exposures. *Toxicology* 211, 207-19.

Haighton, L. A., Hlywka, J. J., Doull, J., Kroes, R., Lynch, B. S., and Munro, I. C. (2002). An evaluation of the possible carcinogenicity of bisphenol A to humans. *Regul Toxicol Pharmacol* 35, 238-54.

Hanai, Y. (1997). Bisphenol-A Eluted from Nursing Bottles. Unpublished Data. Environmental Science Research Center, Yokohama National University.

Hanaoka, T., Kawamura, N., Hara, K., and Tsugane, S. (2002). Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidylether and mixed organic solvents. *Occup Environ Med* 59, 625-8.

Harvey, P. W., and Johnson, I. (2002). Approaches to the assessment of toxicity data with endpoints related to endocrine disruption. *J Appl Toxicol* 22, 241-7.

HMSO (1995). The Toddlers Survey. Gregory, J.R., Collins, D.L., Davies, P.S.W., Hughes, J.M., and Clarke, P.C. National Diet and Nutrition Survey; Children Aged 1.5- 4.5 years. 1: Report of the Diet and Nutrition Survey.

Ho, S-M., Tang, W-Y., de Frausto, J. B., and Prins, G. S. (2006). Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res* 66(11), 5624-5632.

Homey, B., von Schilling C., Blumel J., Schuppe H-C., Ruzicka T., Ahr H-J., Lehmann P., and Vohr, H-W. (1998). An integrated model for the differentiation of chemical-induced allergic and irritant skin reactions (IMDS). *Toxicol and Appl Pharmacol* 153, 83-94.

Honma, S., Suzuki, A., Buchanan, D. L., Katsu, Y., Watanabe, H., and Iguchi, T. (2002). Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod Toxicol* 16, 117-22.

Honma, T., Miyagawa, M., Suda, M., Wang, R. S., Kobayashi, K., Sekiguchi, S. (2003) Effects of perinatal exposure to bisphenol A on brain neurotransmitters in female rat offspring.. 44(3):510-524

Horie, M., Yoshida, T., Ishii, R., Kobayashi, S., and Nakazawa, H. (1999). Determination of bisphenol A in canned drinks by LC/MS. *Bunseki Kagaku*, 48, 579-588.

Hunt, P. A., Koehler, K. E., Susiarjo, M., Hodges, C. A., Ilagan, A., Voigt, R. C., Thomas, S., Thomas, B. F., and Hassold, T. J. (2003). Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Curr Biol* 13, 546-53.

Ichihara, T., Yoshino, H., Imai, N., Tsutsumi, T., Kawabe, M., Tamano, S., Inaguma, S., Suzuki, S., and Shirai, T. (2003). Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. *J Toxicol Sci* 28, 165-71.

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., and Taketani, Y. (2002). Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum Reprod* 17, 2839-41.

Imanaka, M., Sasaki, K., Nemoto, S., Ueda, E., Murakami, E., Miyata, D., and Tonogai, Y. (2001). [Determination of bisphenol A in foods using GC/MS]. *Shokuhin Eiseigaku Zasshi* 42, 71-8.

Imanishi, S., Manabe, N., Nishizawa, H., Morita, M., Sugimoto, M., Iwahori, M., and Miyamoto, H. (2003). Effects of oral exposure of bisphenol A on mRNA expression of nuclear receptors in murine placentae assessed by DNA microarray. *J Reprod Dev* 49, 329-36.

Inoue, H., Tsuruta, A., Kudo, S., Ishii, T., Fukushima, Y., Iwano, H., Yokota, H., and Kato, S. (2005). Bisphenol a glucuronidation and excretion in liver of pregnant and nonpregnant female rats. *Drug Metab Dispos* 33, 55-9.

Inoue, K., Murayama, S., Takeba, K., Yoshimura, Y., and Nakazawa, H. (2003). Contamination of xenoestrogens bisphenol A and F in honey: safety assessment and analytical method of these compounds in honey. *Journal of Food Composition and analysis* 16, 497-506.

Inoue, K., Wada, M., Higuchi, T., Oshio, S., Umeda, T., Yoshimura, Y., and Nakazawa, H. (2002). Application of liquid chromatography-mass spectrometry to the quantification of bisphenol A in human semen. *J Chromatogr B Analyt Technol Biomed Life Sci.* 773, 97-102.

Inoue, K., Yamaguchi, A., Wada, M., Yoshimura, Y., Makino, T., and Nakazawa, H. (2001). Quantitative detection of bisphenol A and bisphenol A diglycidyl ether metabolites in human plasma by liquid chromatography-electrospray mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765, 121-6.

Jaeg, J. P., Perdu, E., Dolo, L., Debrauwer, L., Cravedi, J. P., and Zalko, D. (2004). Characterization of new bisphenol A metabolites produced by CD1 mice liver microsomes and S9 fractions. *J Agric Food Chem* 52, 4935-42.

Jefferson, W. N., Couse, J. F., Padilla-Banks, E., Korach, K. S., and Newbold, R. R. (2002). Neonatal exposure to genistein induces estrogen receptor (ER)alpha expression and multiocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions. *Biol Reprod* 67, 1285-96.

Jordakova, I., Dobias, J., Voldrich, M., and Poustka, J. (2003). Determination of bisphenol A, bisphenol F, bisphenol A diglycidyl ether and bisphenol F diglycidyl ether migrated from food cans using gas chromatography-mass spectrometry. *Czech Journal of Food Sciences* 21, 85-90.

Kang, J. H., Kito, K., and Kondo, F. (2003). Factors influencing the migration of bisphenol A from cans. *J Food Prot* 66, 1444-7.

Kang, J. H., and Kondo, F. (2002). Bisphenol A migration from cans containing coffee and caffeine. *Food Addit Contam* 19, 886-90.

Kang, J. H., and Kondo, F. (2003). Determination of bisphenol A in milk and dairy products by high-performance liquid chromatography with fluorescence detection. *J Food Prot* 66, 1439-43.

Kato, H., Ota, T., Furuhashi, T., Ohta, Y., and Iguchi, T. (2003). Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. *Reprod Toxicol* 17, 283-8.

Kato, H., Furuhashi, T., Tanaka, M., Katsu, Y., Watanabe, H., Ohta, Y., and Iguchi, T. (2006). Effects of bisphenol A given neonatally on reproductive functions of male rats. *Reproductive Toxicology* 22, 20-29.

Kawaguchi, M., Sakui, N., Okanouchi, N., Ito, R., Saito, K., Izumi, S., Makino, T., and Nakazawa, H. (2005). Stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography--mass spectrometry for measurement of phenolic xenoestrogens in human urine samples. *J Chromatogr B Analyt Technol Biomed Life Sci* 820, 49-57.

Kawai, K., Nozaki, T., Nishikata, H., Aou, S., Takii, M., and Kubo, C. (2003). Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. *Environ Health Perspect* 111, 175-8.

Kawai, K., Murakami, S., Senba, E., Yamanaka, T., Fujiwara, Y., Arimura, C., Nozaki, T., Takii, M., and Kubo, C. (2007) Changes in estrogen receptors alpha and beta expression in the brain of mice exposed prenatally to bisphenol A. *Regul Toxicol Pharmacol* 47, 166-70.

Kersting, M., Alexy, U., Sichert-Hellert, W., Manz, F. and Schoch, G. (1998). Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. *J Pediatr Gastroenterol Nutr* 27, 547-552.

Kim, H. S., Han, S. Y., Kim, T. S., Kwack, S. J., Lee, R. D., Kim, I. Y., Seok, J. H., Lee, B.M., Yoo, S. D., and Park, K. L. (2002). No androgenic/anti-androgenic effects of bisphenol-A in Hershberger assay using immature castrated rats. *Toxicol Lett* 135, 111-123.

Kim, Y. H., Kim, C. S., Park, S., Han, S. Y., Pyo, M. Y., and Yang, M. (2003). Gender differences in the levels of bisphenol A metabolites in urine. *Biochem Biophys Res Commun* 312, 441-8.

Klein, K. O. (1998). Isoflavones, soy-based infant formulas, and relevance to endocrine function. *Nutr Rev* 56, 193-204.

Kubo, K., Arai, O., Ogata, R., Omura, M., Hori, T., and Aou, S. (2001) Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. *Neurosci Lett* 304, 73-6

Kubo, K., Arai, O., Omura, M., Watanabe, R., Ogata, R., and Aou, S. (2003). Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci Res* 45, 345-56.

Kuklennyik, Z., Ekong, J., Cutchins, C. D., Needham, L. L., and Calafat, A. M. (2003). Simultaneous measurement of urinary bisphenol A and alkylphenols by automated solid-phase extractive derivatization gas chromatography/mass spectrometry. *Anal Chem* 75, 6820-5.

Kuo, H. W., and Ding, W. H. (2004). Trace determination of bisphenol A and phytoestrogens in infant formula powders by gas chromatography-mass spectrometry. *J Chromatogr A* 1027, 67-74.

Kurebayashi, H., Betsui, H., and Ohno, Y. (2003). Disposition of a low dose of ¹⁴Cbisphenol-A in male rats and its main biliary excretion as BPA glucuronide. *Toxicol Sci* 73, 17-25.

Kurebayashi, H., Harada, R., Stewart, R. K., Numata, H., and Ohno, Y. (2002). Disposition of a low dose of bisphenol a in male and female cynomolgus monkeys. *Toxicol Sci* 68, 32-42.

Kurebayashi, H., Nagatsuka, S., Nemoto, H., Noguchi, H., and Ohno, Y. (2005). Disposition of low doses of ¹⁴C-bisphenol A in male, female, pregnant, fetal, and neonatal rats. *Arch Toxicol* 79, 243-52.

Kwon, S., Stedman, D.B., Elswick, B.A., Cattley, R.C., and Welsch, F. (2000) Pubertal development and reproductive functions of CrI:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. *Toxicol. Sci.* 55, 399-406.

Laviola, G., Gioiosa, L., Adriani, W., Palanza, P. (2005) d-Amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors. *Brain Res Bull* 65, 235-240.

Lee, H. J., Chattopadhyay, S., Gong, E. Y., Ahn, R. S., and Lee, K. (2003). Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicol Sci* 75, 40-6.

Lobenhofer, E.K., Cui, X., Bennett, L., Cable, P.L., Merrick, B.A., Churchill, G.A., and Afshari, C.A. (2004). Exploration of low-dose estrogen effects: identification of No Observed Transcriptional Effect Level (NOTEL). *Toxicol Pathol* 32, 482-92.

Lopez-Cervantes, J., and Paseiro-Losada, P. (2003). Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. *Food Addit Contam* 20, 596-606.

Lopez-Cervantes, J., Sanchez-Machado, D. I., Pastorelli, S., Rijk, R., and Paseiro-Losada, P. (2003). Evaluating the migration of ingredients from active packaging and development of dedicated methods: a study of two iron-based oxygen absorbers. *Food Addit Contam* 20, 291-9.

MAFF (1998). Joint Food Safety and Standards Group. Food Surveillance Information Sheet - Number 167, November 1998.

Mao, L., Sun, C., Zhang, H., Li, Y., and Wu, D. (2004). Determination of environmental estrogens in human urine by high performance liquid chromatography after fluorescent derivatization with p-nitrobenzoyl chloride. *Anal. Chim. Acta* 522, 241-246.

Markaverich, B., Mani, S., Alejandro, M. A., Mitchell, A., Markaverich, D., Brown, T., Velez-Trippe, C., Murchison, C., O'Malley, B., and Faith, R. (2002). A novel endocrine-disrupting agent in corn with mitogenic activity in human breast and prostatic cancer cells. *Environ Health Perspect* 110, 169-77.

Markey, C. M., Luque, E. H., Munoz De Toro, M., Sonnenschein, C., and Soto, A. M. (2001). In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol Reprod* 65, 1215-23.

Markey, C. M., Wadia, P. R., Rubin, B. S., Sonnenschein, C., and Soto, A. M. (2005). Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract. *Biol Reprod* 72, 1344-51.

Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., and Hirose, M. (2004a). Dietary influence on the impact of ethinylestradiol-induced alterations in the endocrine/reproductive system with perinatal maternal exposure. *Reprod Toxicol* 18, 23-33.

Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Lee, K. Y., and Hirose, M. (2004b). Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals. *Arch Toxicol* 78, 232-40.

Matsumoto, A., Kunugita, N., Kitagawa, K., Isse, T., Oyama, T., Foureman, G. L., Morita, M., and Kawamoto, T. (2003). Bisphenol A levels in human urine. *Environ Health Perspect* 111, 101-4.

Matthews, J. B., Twomey, K., and Zacharewski, T. R. (2001). In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chem Res Toxicol* 14, 149-57.

Melnick, R., Lucier, G., Wolfe, M., Hall, R., Stancel, G., Prins, G., Gallo, M., Reuhl, K., Ho, S. M., Brown, T., Moore, J., Leakey, J., Haseman, J., and Kohn, M. (2002). Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. *Environ Health Perspect* 110, 427-31.

Miyamoto, K. and Kotake, M. (2006, in press) Estimation of Daily Bisphenol A Intake of Japanese Individuals with Emphasis on Uncertainty and Variability. accepted for publication in Environmental Sciences (Journal of the Japan Society of Endocrine Disruptors Research)

Milman, H. A., Bosland, M. C., Walden, P. D., and Heinze, J. E. (2002). Evaluation of the adequacy of published studies of low-dose effects of bisphenol A on the rodent prostate for use in human risk assessment. *Regul Toxicol Pharmacol* 35, 338-46.

Miyamoto, K., and Kotake, M. (2006). Estimation of daily bisphenol a intake of Japanese individuals with emphasis on uncertainty and variability. *Environ Sci* 13, 15-29.

Mizuo, K., Narita, M., Miyagawa, K., Narita, M., Okuno, E., and Suzuki, T. (2004a). Prenatal and neonatal exposure to bisphenol-A affects the morphine-induced rewarding effect and hyperlocomotion in mice. *Neurosci Lett.* 356(2), 95-8.

Mizuo, K., Narita, M., Yoshida, T., and Suzuki, T. (2004b). Functional changes in dopamine D3 receptors by prenatal and neonatal exposure to an endocrine disruptor bisphenol-A in mice. *Addict Biol* 9, 19-25.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., and Nakao, K. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J Clin Endocrinol Metab* 87, 5185-90.

Mountfort, K. A., Kelly, J., Jickells, S. M., and Castle, L. (1997). Investigations into the potential degradation of polycarbonate baby bottles during sterilisation with consequent release of bisphenol A. *Food Additives & Contaminants*, 14, 737-740.

Munguia-Lopez, E. M., Peralta, E., Gonzalez-Leon, A., Vargas-Requena, C., and Soto-Valdez, H. (2002). Migration of bisphenol A (BPA) from epoxy can coatings to jalapeno peppers and an acid food simulant. *J Agric Food Chem* 50, 7299-302.

Munguia-Lopez, E. M., and Soto-Valdez, H. (2001). Effect of heat processing and storage time on migration of bisphenol A (BPA) and bisphenol A-diglycidyl ether (BADGE) to aqueous food simulant from Mexican can coatings. *J Agric Food Chem* 49, 3666-71.

Munoz-de-Toro, M., Markey, C., Wadia, P. R., Luque, E. H., Rubin, B. S., Sonnenschein, C., and Soto, A. M. (2005). Perinatal exposure to Bisphenol A alters peripubertal mammary gland development in mice. *Endocrinology*.

Murray, T. J., Maffini, M. V., Ucci, A. A., Sonnenschein, C., and SotoA. M. (2007). Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reproductive Toxicology* 23(3), 383-390.

Naciff, J. M., Hess, K. A., Overmann, G. J., Torontali, S. M., Carr, G. J., Tiesman, J. P., Foertsch, L. M., Richardson, B. D., Martinez, J. E., and Daston, G. P. (2005). Gene Expression Changes Induced in the Testis by Transplacental Exposure to High and Low Doses of 17{alpha}-Ethinyl Estradiol, Genistein or Bisphenol A. *Toxicol Sci*.

Naciff, J. M., Jump, M. L., Torontali, S. M., Carr, G. J., Tiesman, J. P., Overmann, G. J., and Daston, G. P. (2002). Gene expression profile induced by 17alpha-ethinyl estradiol, bisphenol A, and genistein in the developing female reproductive system of the rat. *Toxicol Sci* 68, 184-99.

Naciff, J. M., Overmann, G. J., Torontali, S. M., Carr, G. J., Tiesman, J. P., and Daston, G. P. (2004). Impact of the phytoestrogen content of laboratory animal feed on the gene expression profile of the reproductive system in the immature female rat. *Environ Health Perspect* 112, 1519-26.

Nagao, T., Saito, Y., Usumi, K., Yoshimura, S., and Ono, H. (2002). Low-dose bisphenol A does not affect reproductive organs in estrogen-sensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage. *Reprod Toxicol* 16, 123-130.

Nagao, T., Wada, K., Kuwagata, M., Nakagomi, M., Watanabe, C., Yoshimura, S., Saito, Y., Usumi, K., and Kanno, J. (2004). Intrauterine position and postnatal growth in Sprague-Dawley rats and ICR mice. *Reprod Toxicol* 18, 109-20.

Nagel, S. C., vom Saal, F. S., Thayer, K. A., Dhar, M. G., Boechler, M., and Welshons, W. V. (1997). Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ Health Perspect* 105, 70-6.

Nagel, S. C., vom Saal, F. S., and Welshons, W. V. (1999). Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. *J Steroid Biochem Mol Biol* 69, 343-57.

Nakamura, K., Itoh, K., Yaoi, T., Fujiwara, Y., Sugimoto, T., and Fushiki, S. (2006). Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of bisphenol A. *J Neurosci Res* 84, 1197-1205.

Narita, M., Miyagawa, K., Mizuo, K., Yoshida, T., and Suzuki, T. (2006). Prenatal and neonatal exposure to low-dose of bisphenol-A enhance the morphine-induced hyperlocomotion and rewarding effect. *Neurosci Lett* 402, 249-252.

Narita, M., Miyagawa, K., Mizuo, K., Yoshida, T., and Suzuki, T. (2007) Changes in central dopaminergic systems and morphine reward by prenatal and neonatal exposure to bisphenol-A in mice: evidence for the importance of exposure period. *Addict Biol* 12, 167-72

Negishi, T., Kawasaki, K., Suzaki, S., Maeda, H., Ishii, Y., Kyuwa, S., Kuroda, Y., and Yoshikawa, Y. (2004). Behavioral alterations in response to fear-provoking stimuli and tranylcypramine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. *Environ Health Perspect* 112, 1159-64.

Negishi, T., Kawasaki, K., Takatori, a., Ishii, y., Kyuwa, S., Kuroda, Y., and Yoshikawa, Y. (2003). Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats. *Environmental Toxicology and Pharmacology* 14, 99-108.

Nerin, C., Fernandez, C., Domeno, C., and Salafranca, J. (2003). Determination of potential migrants in polycarbonate containers used for microwave ovens by high-performance liquid chromatography with ultraviolet and fluorescence detection. *J Agric Food Chem* 51, 5647-53.

Newbold, R. R., Jefferson, W. N., Padilla-Banks, E., (2007) Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reprod Toxicol* 24, 253-258

NIEHS (2001). National Toxicology Program's report of the Endocrine Disruptors Low-Dose Peer Review. Page A-58. August 2001. NTP, NIEHS, Research Triangle Park, NC, USA. Available at <http://ntp.niehs.nih.gov/ntp/htdocs/liason/LowDosePeerFinalRpt.pdf>.

Nikaido, Y., Yoshizawa, K., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., and Tsubura, A. (2004). Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. *Reprod Toxicol* 18, 803-11.

Nikaido, Y., Danbara, N., Tsujita-Kyutoku, M., Yuri, T., Uehara, N., and Tsubura, A. (2005). Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. *In Vivo* 19, 487-494.

Nishizawa, H., Manabe, N., Morita, M., Sugimoto, M., Imanishi, S., and Miyamoto, H. (2003). Effects of in utero exposure to bisphenol A on expression of RARalpha and RXRalpha mRNAs in murine embryos. *J Reprod Dev* 49, 539-45.

Odum, J., Tinwell, H., Tobin, G., and Ashby, J. (2004). Cumulative dietary energy intake determines the onset of puberty in female rats. *Environ Health Perspect* 112, 1472-80.

Ohkuma, H., Abe, K., Ito, M., Kokado, A., Kambegawa, A., and Maeda, M. (2002). Development of a highly sensitive enzyme-linked immunosorbent assay for bisphenol A in serum. *Analyst* 127, 93-7.

Olea, N., Pulgar, R., Perez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A. M., and Sonnenschein, C. (1996). Estrogenicity of resin-based composites and sealants used in dentistry. *Environ Health Perspect* 104, 298-305.

Ouchi, K., and Watanabe, S. (2002). Measurement of bisphenol A in human urine using liquid chromatography with multi-channel coulometric electrochemical detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 780, 365-70.

Owens, J. W., and Chaney, J. G. (2005). Weighing the results of differing 'low dose' studies of the mouse prostate by Nagel, Cagen, and Ashby: Quantification of experimental power and statistical results. *Regul Toxicol Pharmacol* 43, 194-202.

Ozaki, A., and Baba, T. (2003). Alkylphenol and bisphenol A levels in rubber products. *Food Addit Contam* 20, 92-8.

Ozaki, A., Yamaguchi, Y., Fujita, T., Kuroda, K., and Endo, G. (2004). Chemical analysis and genotoxicological safety assessment of paper and paperboard used for food packaging. *Food Chem Toxicol* 42, 1323-37.

Pacchierotti, F., Ranaldi, R., Eichenlaub-Ritter, U., Attia, S., and Adler I.-D. (2007). Evaluation of aneugenic effects of bisphenol A in somatic and germ cells of the mouse. Submitted for publication.

Palanza, P. L., Howdeshell, K. L., Parmigiani, S., and vom Saal, F. S. (2002). Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environ Health Perspect* 110 Suppl 3, 415-22.

Patisaul, H.B., Fortino, A.E., and Polston, E.K. (2006) Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. *Neurotox Teratol.* 28, 111-118

Patisaul, H.B., Fortino, A.E., and Polston, E.K. (2007) Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A. *NeuroToxicol* 28, 1-12

PlasticsEurope (2007). Answer to HSE request for further information on medical surveillance data in BPA manufacture. M. Burcher, 26 October 2007.

Porrini, S., Belloni, V., Della Seta, D., Farabollini, F., Giannelli, G., and Dessi-Fulgheri, F. (2005). Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats. *Brain Res Bull* 65, 261-6.

Pottenger, L. H., Domoradzki, J. Y., Markham, D. A., Hansen, S. C., Cagen, S. Z., and Waechter, J. M., Jr. (2000). The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicol Sci* 54, 3-18.

Pritchett, J. J., Kuester, R. K., and Sipes, I. G. (2002). Metabolism of bisphenol a in primary cultured hepatocytes from mice, rats, and humans. *Drug Metab Dispos* 30, 1180-5.

Pulgar, R., Olea-Serrano, M. F., Novillo-Fertrell, A., Rivas, A., Pazos, P., Pedraza, V., Navajas, J. M., and Olea, N. (2000). Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. *Environ Health Perspect* 108, 21-7.

Putz, O., Schwartz, C. B., Kim, S., LeBlanc, G. A., Cooper, R. L., and Prins, G. S. (2001a). Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: I. Effects on the prostate gland. *Biol Reprod* 65, 1496-505.

Putz, O., Schwartz, C. B., LeBlanc, G. A., Cooper, R. L., and Prins, G. S. (2001b). Neonatal low- and high-dose exposure to estradiol benzoate in the male rat: II. Effects on male puberty and the reproductive tract. *Biol Reprod* 65, 1506-17.

Quesada, I., Fuentes, E., Viso-Leon, M. C., Soria, B., Ripoll, C., and Nadal, A. (2002). Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *Faseb J* 16, 1671-3.

Ramos, J. G., Varayoud, J., Kass, L., Rodriguez, H., Costabel, L., Munoz-De-Toro, M., and Luque, E. H. (2003). Bisphenol a induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. *Endocrinology* 144, 3206-15.

Rivas, A., Lacroix, M., Olea-Serrano, F., Laios, I., Leclercq, G., and Olea, N. (2002). Estrogenic effect of a series of bisphenol analogues on gene and protein expression in MCF-7 breast cancer cells. *J Steroid Biochem Mol Biol* 82, 45-53.

Rodriguez-Mozaz, S., López de Alda, M.J. and Barceló, D. (2004). Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction-liquid chromatography-mass spectrometry. *J. Chromatogr. A*, 1045, 85-92.

Romero J., Ventura F., Gomez M. (2002) Characterisation of point samples used in drinking water reservoirs: Identification of endocrine disruptor compounds. *Journal of Chromatographic Science* 40, 191-197.

Rubin, B. S., Murray, M. K., Damassa, D. A., King, J. C., and Soto, A. M. (2001). Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environ Health Perspect* 109, 675-80.

Rubin, B. S., Lenkowski, J. R., Schaeberle, C. M., Vandenberg, L. N., Ronsheim, P. M., and Soto, A. M. (2006). Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology* 147, 3681-3691.

Runyon, J., Noti, A., Grob, K., Biedermann, M., and Dudler, V. (2002). Isolation of the < 1000 Dalton Migrants from Food Packaging Materials by Size Exclusion Chromatography (SEC). *Mitt. Lebensm. Hyg.* 93 93, 57-72.

Ryan, B.C., and Vandenberg, J.G. (2006). Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. *Hormones and Behav.* 50, 85-93

Safe, S. H. (2000). Endocrine disruptors and human health--is there a problem? An update. *Environ Health Perspect* 108, 487-93.

Sajiki, J., Takahashi, K., and Yonekubo, J. (1999). Sensitive method for the determination of bisphenol-A in serum using two systems of high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl.* 736, 255-261.

Sajiki, J. (2001). Determination of bisphenol A in blood using high-performance liquid chromatography-electrochemical detection with solid-phase extraction. *J Chromatogr B Biomed Sci Appl.* 755, 9-15.

Sakamoto, H., Yokota, H., Kibe, R., Sayama, Y., and Yuasa, A. (2002). Excretion of bisphenol A-glucuronide into the small intestine and deconjugation in the cecum of the rat. *Biochim Biophys Acta* 1573, 171-6.

Schonfelder, G., Flick, B., Mayr, E., Talsness, C., Paul, M., and Chahoud, I. (2002a). In utero exposure to low doses of bisphenol A lead to long-term deleterious effects in the vagina. *Neoplasia* 4, 98-102.

Schonfelder, G., Friedrich, K., Paul, M., and Chahoud, I. (2004). Developmental effects of prenatal exposure to bisphenol a on the uterus of rat offspring. *Neoplasia* 6, 584-94.

Schonfelder, G., Wittfoht, W., Hopp, H., Talsness, C. E., Paul, M., and Chahoud, I. (2002b). Parent bisphenol A accumulation in the human maternal-fetal-placental unit. *Environ Health Perspect* 110, A703-7.

Schuler, M., P. Muehlbauer, P. Guzzie and D.A. Eastmond (1999) Noscapine hydrochloride disrupts the mitotic spindle in mammalian cells and induces aneuploidy as well as polyploidy in cultured human lymphocytes. *Mutagenesis* 14, 51-6.

Seidlova-Wuttke, D., Jarry, H., Christoffel, J., Rimoldi, G., and Wuttke, W. (2005). Effects of bisphenol-A (BPA), dibutylphtalate (DBP), benzophenone-2 (BP2), procymidone (Proc), and linurone (Lin) on fat tissue, a variety of hormones and metabolic parameters: A 3 months comparison with effects of estradiol (E2) in ovariectomized (ovx) rats. *Toxicology*, in press.

Seidlova-Wuttke, D., Jarry, H., and Wuttke, W. (2004). Pure estrogenic effect of benzophenone-2 (BP2) but not of bisphenol A (BPA) and dibutylphtalate (DBP) in uterus, vagina and bone. *Toxicology* 205, 103-12.

Shao, B., Han, H., Hu, J., Zhao, J., Wu, G., Xue, Y., Ma, Y., and Zhang, S. (2005). Determination of alkylphenol and bisphenol A in beverages using liquid chromatography/electrospray ionization tandem mass spectrometry. *Analytica Chimica Acta* 530, 245.

Sharpe, R. M., Rivas, A., Walker, M., McKinnell, C., and Fisher, J. S. (2003). Effect of neonatal treatment of rats with potent or weak (environmental) oestrogens, or with a GnRH antagonist, on Leydig cell development and function through puberty into adulthood. *Int J Androl* 26, 26-36.

Shimizu, M., Ohta, K., Matsumoto, Y., Fukuoka, M., Ohno, Y., and Ozawa, S. (2002). Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. *Toxicol In Vitro* 16, 549-56.

Shin, B. S., Yoo, S. D., Cho, C. Y., Jung, J. H., Lee, B. M., Kim, J. H., Lee, K. C., Han, S. Y., Kim, H. S., and Park, K. L. (2002). Maternal-fetal disposition of bisphenol A in pregnant Sprague-Dawley rats. *J Toxicol Environ Health A* 65, 395-406.

Simoneau C, Roeder G and Anklam E (2000). Migration of bisphenol-A from baby bottles: effect of experimental conditions and European survey. 2nd International Symposium on Food Packaging: Ensuring the Safety and Quality of Foods (ILSI conference), Vienna, Austria, 8-10 November 2000.

Snyder, R. W., Maness, S. C., Gaido, K. W., Welsch, F., Sumner, S. C., and Fennell, T. R. (2000). Metabolism and disposition of bisphenol A in female rats. *Toxicol Appl Pharmacol* 168, 225-34.

Sun, Y., Irie, M., Kishikawa, N., Wada, M., Kuroda, N., and Nakashima, K. (2004). Determination of bisphenol A in human breast milk by HPLC with column-switching and fluorescence detection. *Biomed Chromatogr* 18, 501-7.

Stowell, C. L., Barvian, K. K., Young, P. C., Bigsby, R. M., Verdugo, D. E., Bertozzi, C. R., and Widlanski, T. S. (2006). A role for sulfation-desulfation in the uptake of bisphenol a into breast tumor cells. *Chem Biol* 13, 891-897.

Sugiura-Ogasawara M., Ozaki Y., Sonta S. I., Makino T., and Suzumori K. (2005) Exposure to bisphenol A is associated with recurrent miscarriage. *Hum Reprod* 20, 2325–2329.

Sugiura-Ogasawara M (2006) Letters to the Editor; Limitations of a case–control study on bisphenol A (BPA) serum levels and recurrent miscarriage *Hum Reprod* 21, 566-567.

Susiarjo, M., Hassold, T.J., Freeman, E., and Hunt, P. (2007). Bisphenol A exposure in utero disrupts early oogenesis in the mouse. *PLOS Genetics* 3(1), 1-8.

Suzuki, A., Sugihara, A., Uchida, K., Sato, T., Ohta, Y., Katsu, Y., Watanabe, H., and Iguchi, T. (2002). Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. *Reprod Toxicol* 16, 107-16.

Suzuki, M., Aoyama, T., Ohno, H., Nakashima, S., Iwama, M., and Mitani, K. (2004). Migration of bisphenol A from polyvinyl chloride products. *Kankyo Kagaku* 14, 375-379 (only abstract available in English).

Suzuki, T., Mizuo, K., Nakazawa, H., Funae, Y., Fushiki, S., Fukushima, S., Shirai, T., and Narita, M. (2003). Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: enhancement of the methamphetamine induced abuse state. *Neuroscience* 117, 639-44.

Takagi, H., Mitsumori, K., Onodera, H., Nasu, M., Tamura, T., Yasuhara, K., Takegawa, K., and Hirose, M. (2002). Improvement of a two-stage carcinogenesis model to detect modifying effects of endocrine disrupting chemicals on thyroid carcinogenesis in rats. *Cancer Lett* 178, 1-9.

Takagi, H., Shibutani, M., Masutomi, N., Uneyama, C., Takahashi, N., Mitsumori, K., and Hirose, M. (2004). Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life. *Arch Toxicol* 78, 97-105.

Takahashi, O., and Oishi, S. (2003). Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice. *Food Chem Toxicol* 41, 1035-44.

Takao, T., Nanamiya, W., Nazarloo, H. P., Matsumoto, R., Asaba, K., and Hashimoto, K. (2003). Exposure to the environmental estrogen bisphenol A differentially modulated estrogen receptor-alpha and -beta immunoreactivity and mRNA in male mouse testis. *Life Sci* 72, 1159-69.

Takao, Y., Lee, H. C., Kohra, S., and Arizono, K. (2002). Release of bisphenol A from food can lining upon heating. *Journal of Health Science* 48, 331-334.

Takashima, Y., Tsutsumi, M., Sasaki, Y., et al. (2001) Lack of effects of bisphenol A in maternal rats or treatment on response to their offspring to N-nitrosobis(2-hydroxypropyl)amine. *Journal of Toxicologic Pathology* 14, 87-98.

Takeuchi, T., and Tsutsumi, O. (2002). Serum bisphenol a concentrations showed gender differences, possibly linked to androgen levels. *Biochem Biophys Res Commun* 291, 76-78.

- Takeuchi, T., Tsutsumi, O., Ikezuki, Y., Takai, Y., and Taketani, Y. (2004). Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. *Endocr J* 51, 165-9.
- Tan, B. L., Kassim, N. M., and Mohd, M. A. (2003). Assessment of pubertal development in juvenile male rats after sub-acute exposure to bisphenol A and nonylphenol. *Toxicol Lett* 143, 261-70.
- Tan, B. L., and Mustafa, A. M. (2003). Leaching of bisphenol A from new and old babies' bottles, and new babies' feeding teats. *Asia Pac J Public Health* 15, 118-23.
- Tanaka, M., Nakaya, S., Katayama, M., Leffers, H., Nozawa, S., Nakazawa, R., Iwamoto, T., and Kobayashi, S. (2006). Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth. *Hum Exp Toxicol* 25, 369-373.
- Tando, S., Itoh, K., Yaoi, T., Ikeda, J., Fujiwara, Y., and Fushiki, S. (2007). Effects of pre- and neonatal exposure to bisphenol A on murine brain development. *Brain and Develop* 29, 352-356
- Teeguarden, J. G., and Barton, H. A. (2004). Computational modeling of serum binding proteins and clearance in extrapolations across life stages and species for endocrine active compounds. *Risk Anal* 24, 751-70.
- Teeguarden, J. G., Waechter, J. M., Jr., Clewell, H. J., 3rd, Covington, T. R., and Barton, H. A. (2005). Evaluation of Oral and Intravenous Route Pharmacokinetics, Plasma Protein Binding, and Uterine Tissue Dose Metrics of Bisphenol A: A Physiologically Based Pharmacokinetic Approach. *Toxicol Sci* 85, 823-838.
- Thayer, K. A., Ruhlen, R. L., Howdeshell, K. L., Buchanan, D. L., Cooke, P. S., Preziosi, D., Welshons, W. V., Haseman, J., and vom Saal, F. S. (2001). Altered prostate growth and daily sperm production in male mice exposed prenatally to subclinical doses of 17alphaethinyl oestradiol. *Hum Reprod* 16, 988-96.
- Thigpen, J. E., Haseman, J. K., Saunders, H. E., Setchell, K. D., Grant, M. G., and Forsythe, D. B. (2003). Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. *Comp Med* 53, 607-15.
- Thomson, B. M., Cressey, P. J., and Shaw, I. C. (2003). Dietary exposure to xenoestrogens in New Zealand. *J Environ Monit* 5, 229-35.
- Thomson, B. M., and Grounds, P. R. (2005). Bisphenol A in canned foods in New Zealand: an exposure assessment. *Food Addit Contam* 22, 65-72.
- Thuillier, R., Wang, Y., and Culty, M. (2003). Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors alpha and beta in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. *Biol Reprod* 68, 867-80.

Tinwell, H., Haseman, J., Lefevre, P. A., Wallis, N., and Ashby, J. (2002). Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. *Toxicol Sci* 68, 339-48.

Toyama, Y., Suzuki-Toyota, F., Maekawa, M., Ito, C., and Toshimori, K. (2004). Adverse effects of bisphenol A to spermiogenesis in mice and rats. *Arch Histol Cytol* 67, 373-81.

Tominaga, T., Negeshi, T., Hirroka, H., Miyachi, A., Inoue, A., Hayasaka I, and Yoshikawa, Y. (2006). Toxicokinetics of bisphenol A in rats, moeys and chimpanzees by the LCMS/ MS method. *Toxicology* 226, 208-17.

Toyama, Y., and Yuasa, S. (2004). Effects of neonatal administration of 17beta-estradiol, beta-estradiol 3-benzoate, or bisphenol A on mouse and rat spermatogenesis. *Reprod Toxicol* 19, 181-8.

Tsukioka, T., Brock, J., Graiser, S., Nguyen, J., Nakazawa, H., and Makino, T. (2003). Determination of trace amounts of bisphenol A in urine by negative-ion chemicalionization-gas-chromatography/mass spectrometry. *Anal. Sci.* 19, 151-3.

Tyl, R. W., Myers, C. B., Marr, M. C., Thomas, B. F., Keimowitz, A. R., Brine, D. R., Veselica, M. M., Fail, P. A., Chang, T. Y., Seely, J. C., Joiner, R. L., Butala, J. H., Dimond, S. S., Cagen, S. Z., Shiotsuka, R. N., Stropp, G. D., and Waechter, J. M. (2002). Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. *Toxicol Sci* 68, 121-46.

Tyl, R.W., Myers, C.B., Marr, M.C. (2004a) Rangefinding study for the two-generation reproductive toxicity evaluation of 17 β -estradiol (E2, CAS no. 50-28-2) administered in the feen to CD-1 $\text{\textcircled{R}}$ Swiss mice (modified OECD 416). *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

Tyl, R.W., Myers, C.B., Marr, M.C. (2004b) Two-generation reproductive toxicity evaluation of 17 β -estradiol (E2, CAS no. 50-28-2) administered in the feen to CD-1 $\text{\textcircled{R}}$ Swiss mice (modified OECD 416). *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

Tyl, R.W., Myers, C.B., Marr, M.C. (2005) Thirteen-week rangefinding study for the two-generation reproductive toxicity evaluation of bisphenol A (BPA; CAS 80-05-7) administered in the feed to CD-1 $\text{\textcircled{R}}$ (Swiss) mice. *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

Tyl, R.W., Myers, C.B., Marr, M.C., Sloan, C.S., Castillo, N.P., Veselica, M.M., Seely, J.C., Dimond, S.S., Van Millar, J.P., Stropp, G.D., and Waechter, J.M. (2006). Two-generation study of dietary 17 β -estradiol (E2) in CD-1 $\text{\textcircled{R}}$ Swiss mice. *The Toxicologist* 90 (1-suppl.), 253 (Abstract No. 1242)

Tyl, R. W., Myers, C. B., and Marr, M. C. (2007). Draft Final Report: Two-generation reproductive toxicity evaluation of Bisphenol A (BPA; CAS No. 80-05-7) administered in the feed to CD-1 $\text{\textcircled{R}}$ Swiss mice (modified OECD 416). *RTI International Center for life Sciences and Toxicology, Research Triangle Park, NC, USA.*

UK Committee on Toxicity (2001). TOX/MIN/2002/02. Minutes of the meeting held on 13 March 2001. Available at: <http://www.food.gov.uk/multimedia/pdfs/cot08.pdf> .

Vidaeff, A. C., and Sever, L. E. (2005). In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence. *Reprod Toxicol* 20, 5-20.

Vohr H.W. (2002). Bisphenol A: local lymph node assay in mice (LLNA/IMDS). Unpublished report. Bayer AG, Department of Toxicology, Friedrich-Ebert-Str., D-42096, Wuppertal. Report No AT00155.

Vohr H.W. (2003). Bisphenol A: study of photoreactive potential in mice (LLNA/IMDS). Unpublished report. Bayer AG, Department of Toxicology, Friedrich-Ebert-Str., D-42096, Wuppertal. Report No AT00413.

Volkel, W., Bittner, N., and Dekant, W. (2005). Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by HPLC-MS/MS. *Drug Metab Dispos.*

Volkel, W., Colnot, T., Csanady, G. A., Filser, J. G., and Dekant, W. (2002). Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol* 15, 1281-7.

vom Saal, F.S., Cooke, P., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani, S., and Welshons, W.V. (1998). A physiologically based approach to the study of bisphenol-A and other oestrogenic chemicals on the size of reproductive organs, daily sperm production, and behaviour. *Toxicol. Indust. Health* 14, 239-260.

Walsh, D. E., Dockery, P., and Doolan, C. M. (2005). Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [Ca²⁺]_i in human breast cancer cells. *Mol Cell Endocrinol* 230, 23-30.

Watanabe, S., Wang, R. S., Miyagawa, M., Kobayashi, K., Suda, M., Sekiguchi, S., and Honma, T. (2003). Imbalance of testosterone level in male offspring of rats perinatally exposed to bisphenol A. *Ind Health* 41, 338-41.

Wenzel, A., Muller, J. and Ternes, T. (2003). Study on endocrine disruptors in drinking water. Fraunhofer Institute, Schmallenberg, Germany. Report ENV.D.1/ETU/2000/0083.

Wistuba, J., Brinkworth, M. H., Schlatt, S., Chahoud, I., and Nieschlag, E. (2003). Intrauterine bisphenol A exposure leads to stimulatory effects on Sertoli cell number in rats. *Environ Res* 91, 95-103.

Witorsch, R. J. (2002a). Endocrine disruptors: can biological effects and environmental risks be predicted? *Regul Toxicol Pharmacol* 36, 118-30.

Witorsch, R. J. (2002b). Low-dose in utero effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. *Food Chem Toxicol* 40, 905-912.

Wong, K. O., Leo, L. W., and Seah, H. L. (2005). Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles. *Food Additives and Contaminants*, 3.

Wozniak, A. L., Bulayeva, N. N., and Watson, C. S. (2005). Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca²⁺ fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ Health Perspect* 113, 431-439.

Yamada, H., Furuta, I., Kato, E. H., Kataoka, S., Usuki, Y., Kobashi, G., Sata, F., Kishi, R., and Fujimoto, S. (2002). Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. *Reprod Toxicol* 16, 735-9.

Yang, M., Kim, S. Y., Lee, S. M., Chang, S. S., Kawamoto, T., Jang, J. Y., and Ahn, Y. O. (2003). Biological monitoring of bisphenol a in a Korean population. *Arch Environ Contam Toxicol* 44, 546-51.

Ye, X., Kuklennyik, Z., Needham, L. L., and Calafat, A. M. (2005). Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal Chem* 77, 5407-13.

Yellayi, S., Naaz, A., Szewczykowski, M. A., Sato, T., Woods, J. A., Chang, J., Segre, M., Allred, C. D., Helferich, W. G., and Cooke, P. S. (2002). The phytoestrogen genistein induces thymic and immune changes: a human health concern? *Proc Natl Acad Sci U S A* 99, 7616-21.

Yoshida, M., Shimomoto, T., Katashima, S., Watanabe, G., Taya, K., and Maekawa, A. (2004). Maternal exposure to low doses of bisphenol A has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. *J Reprod Dev* 50, 349-360.

Yoshida, T., Horie, M., Hoshino, Y., and Nakazawa, H. (2001). Determination of bisphenol A in canned vegetables and fruit by high performance liquid chromatography. *Food Addit Contam* 18, 69-75.

Yoshihara, S., Mizutare, T., Makishima, M., Suzuki, N., Fujimoto, N., Igarashi, K., and Ohta, S. (2004). Potent estrogenic metabolites of bisphenol A and bisphenol B formed by rat liver S9 fraction: their structures and estrogenic potency. *Toxicol Sci* 78, 50-59.

Yoshino, H., Ichihara, T., Kawabe, M., Imai, N., Hagiwara, A., Asamoto, M., and Shirai, T. (2002). Lack of significant alteration in the prostate or testis of F344 rat offspring after transplacental and lactational exposure to bisphenol A. *J Toxicol Sci* 27, 433-439.

Zalko, D., Soto, A. M., Dolo, L., Dorio, C., Rathahao, E., Debrauwer, L., Faure, R., and Cravedi, J. P. (2003). Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environ Health Perspect* 111, 309-19.

Zsarnovszky, A., Le, H. H., Wang, H. S., and Belcher, S. M. (2005). Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex:

potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. *Endocrinology* 146, 5388-96.