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

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to re-evaluate the use of bisphenol A (BPA) in plastic materials and articles intended to come into contact with food, giving particular attention to the exposure of infants.

Uses in food contact materials

BPA is present in certain food contact materials because it is used in the production of polycarbonate and epoxy-phenolic resins. Polycarbonate (PC) is a plastic widely used in articles such as infant feeding bottles, tableware (plates, mugs, jugs, beakers), microwave ovenware, storage containers, returnable water and milk bottles, and refillable water containers. PC is also used for water pipes. Epoxy-phenolic resins are used as an internal protective lining for food and beverage cans and as a coating on metal lids for glass jars and bottles. Epoxy-phenolic resins are also used as a surface-coating on residential drinking water storage tanks and vine vats.

Dietary exposure to BPA

Only consumer exposure via the diet has been considered in the present assessment. Conservative dietary exposure assessments on BPA have been made by the Panel for adults, infants and children. The estimates of total dietary exposure are as shown in Table 1.

Table 1. Conservative estimates of total dietary exposure to bisphenol A at different ages

Age of consumer	Food/Beverages consumed	Dietary exposure to BPA based on conservative migration value in microgram/kg bw/day (Figures in parenthesis represent exposure based on typical migration value)
3 month infant	Breast milk only	0.2
3 month infant	Infant formula fed with glass or non-PC bottle	2.3
3 month infant	Infant formula fed with PC bottle	11* (4#)
6 month infant	Infant formula fed with PC bottle and commercial foods/beverages	13* (8.3#)
1.5 year-old child	2 kg commercial foods/beverages	5.3
Adult	3 kg commercial foods/beverages	1.5

* Based on the upper value of 50 microgrammes BPA/litre of infant formula
\# Based on the typical value of 10 microgrammes BPA/litre of infant formula

Assessment of human bisphenol A exposure by biomonitoring of urinary excretion of bisphenol A metabolites in the general population gives an estimated average daily total exposure to BPA of up to 7 microg BPA/adult/day and upper range exposures up to 10 microg BPA/adult/day (0.16 microg BPA/kg bw/day for a 60 kg person) in the USA and 0.04 to 0.08 microg/kg bw/day in Japan (95 % confidence interval). The discrepancy between the levels of exposure estimated through urinary biomarkers and the levels of exposure estimated above by combining food consumption data with BPA concentrations in the diet is likely to be due to the conservative assumptions made.

Previous evaluations

The European Union in 2003 published a comprehensive Risk Assessment Report (RAR) for BPA from all sources in the context of the legislation on existing substances. BPA was also evaluated for use in plastic materials and articles intended to come into contact with foods by the Scientific Committee on Food (SCF) in 2002. The SCF considered that the overall oral No-Observed-Adverse-Effect Level (NOAEL) for BPA, from the experimental animal data then available, was 5 milligrams/kg bw/day. The NOAEL was taken from a comprehensive 3-generation reproduction study in the rat that used dose levels ranging down to 1 microgram/kg bw/day. Applying an uncertainty factor of 500 to the NOAEL, the SCF

derived a temporary Tolerable Daily Intake (TDI) of 0.01 mg BPA/kg bw/day. Since then, a considerable number of new papers have been published in the scientific literature, addressing various aspects of the toxicity of BPA, including its fate in the body and the issue of possible low-dose effects. BPA is known to have weak oestrogenic activity. The present re-evaluation builds on the previous evaluation by the SCF and focuses on the effects of BPA on reproduction and the endocrine (hormonal) system, about which there has been much scientific debate.

Due to the much lower bioavailability of BPA from oral administration compared to that from other routes of exposure and the relevance of the oral route for human exposure from food, effects seen in experimental animals following oral administration were considered the most pertinent data for the risk assessment.

Toxicokinetics and toxicodynamics

The Panel noted that new comparative data on toxicokinetics of BPA show that there are major species differences between rodents and humans in the way that BPA is handled in the body. For example, there are major differences in disposition of BPA-glucuronide due to different pathways of elimination from the liver in rodents and primates. This has important implications for the relevance of observations on the effects of BPA in sensitive strains of rodents, including low-dose effects, for human health risk assessment.

In humans and other primates, BPA given orally is rapidly transformed to BPA-glucuronide during first pass metabolism in the gut wall and the liver. The BPA-glucuronide formed, which is devoid of endocrine activity, is rapidly excreted in the urine, with an elimination half-life of less than 6 hours. Thus, there is very low oral bioavailability of the parent substance, BPA, in humans and other primates. Due to this rapid biotransformation and excretion and plasma protein binding in humans, peak BPA-concentrations after dietary exposures to BPA available for receptor binding are predicted to be very low even in worst case exposure scenarios. In rats, orally administered BPA also predominantly undergoes glucuronidation, but the BPA-glucuronide formed is excreted from the liver into the gut in the bile. In the gut, BPA-glucuronide is then cleaved into BPA and glucuronic acid and BPA is reabsorbed as such into the blood stream. This enterohepatic recirculation results in slow elimination of BPA in rodents. The Panel noted 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. The Panel also 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.

Toxicity studies

In reviewing the earlier and the recently published studies on BPA, the Panel noted that some studies indicated differences in behaviour or reproductive parameters between control and treated animals at dose levels lower than the previously accepted overall NOAEL of 5 mg/kg bw/day. However, the Panel had considerable reservations both about the biological significance of the reported observations and the robustness of the studies.

The effects of BPA reported in some studies at low doses in sensitive animal systems were small changes in organ weight or changes in tissue architecture, increased or decreased receptor expression, changes in hormone concentrations in plasma or tissues, small changes in the time required to attain puberty landmarks, and behavioural effects. The Panel noted that the changes observed were often not sustained through adulthood. The biological consequences of many of the changes in the affected animals are unknown and some, such as small increases in prostate weight, are not considered as precursors of pathological change. While some of the changes may be indicative of biomarkers of effect in very sensitive species and strains, in the light of present knowledge, they cannot be readily interpreted as adverse effects.

The Panel also noted that in some studies reporting low-dose effects, only a single dose level was investigated, or there was absence of a dose-response relationship where several dose levels had been used. Many studies also used only small numbers of animals per dose group. There are also a number of other potential confounding factors in these types of study that may contribute to the lack of consistency in the database and this is discussed in more detail in the opinion.

For studies to be used for risk assessment purposes it is important that adequate numbers of animals are tested to control for individual variability of responses and that an adequate range of doses is tested to show a dose-response relationship. With regard to the claimed non-monotonic dose-responses for BPA, the Panel notes that toxicologists are familiar with U-shaped and inverted U-shaped dose-response curves for hormonal activities, but the presence of a response at one dose level only does not necessarily indicate a causal relationship between the administration of a substance and an observed change. To demonstrate U-shaped dose responses in a robust way, it is necessary to have reasonably spaced dose intervals, usually of less than 10-fold, and not steps of 1000-fold as in some recent studies.

The Panel also noted that the results of the studies reporting low-dose effects are in contrast to the results of other studies using comprehensive protocols developed for testing both structure and reproductive function in parents and offspring and performed following internationally recognised guidelines with regard to study design and animal model selection. A two-generation reproductive toxicity study has recently been reported in an oestrogen-sensitive strain of mice administered a wide range of BPA doses in the diet. BPA administration in the low-dose range did not result in changes in reproductive organs or performance and gave an overall NOAEL of 5 mg/kg bw/day, with liver toxicity as the most sensitive endpoint. The positive control substance, 17ß-oestradiol, resulted in reproductive and developmental toxicity.

Moreover, a number of other studies applying low doses of BPA were also unable to demonstrate low-dose effects on reproduction or development. Thus, the literature remains inconsistent with regard to strain and species sensitivity to low-dose effects of BPA.

Conclusions

The Panel considered that low-dose effects of BPA in rodents have not been demonstrated in a robust and reproducible way, such that they could be used as pivotal studies for risk assessment. Moreover, the species differences in toxicokinetics, whereby BPA as parent compound is less bioavailable in humans than in rodents, raise considerable doubts about the relevance of any low-dose observations in rodents for humans. The likely high sensitivity of

the mouse to oestrogens raises further doubts about the value of that particular species as a model for risk assessment of BPA in humans.

For these reasons, the Panel concluded that the overall NOAEL of 5 mg/kg bw/day, based on the results of a comprehensive three-generation study in the rat, identified in the SCF evaluation of 2002 is still valid, and in the Panel's view is further supported by the NOAEL of 5 mg/kg bw/day in a recent two-generation reproductive toxicity study of BPA in mice. The NOAEL derived from the multigeneration study in rats was used by the SCF to derive a temporary TDI of 0.01 mg/kg bw, applying a 500-fold uncertainty factor, comprising 10 for interspecies differences, 10 for interindividual differences and 5 for uncertainties in the database on reproductive and developmental toxicity.

The available studies cover the majority of endpoints considered relevant for assessment of reproductive effects and other toxicities and do not indicate the presence of effects on reproduction or development at doses lower than 50 mg/kg bw/day. The lowest NOAEL of 5 mg/kg bw/day derived in the recent two-generation reproductive toxicity study in mice is based on liver effects. Toxic effects of repeated administration of BPA on the liver in mice have also been observed in previous studies with a LOAEL of 120 mg/kg bw/day, suggesting that liver toxicity is at least as sensitive an endpoint for BPA as reproductive and developmental effects. The NOAEL for liver toxicity in mice is identical to the derived NOAEL for reproductive toxicity of bisphenol A in rats used in the EU RAR, which is based on effects on adult and offspring body weight gain.

The Panel's conclusions are based on the now available, extensive database on repeated-dose toxicity, reproductive and developmental toxicity of BPA in rodents and on the comparison of toxicokinetics in primates, including humans, and rodents. The Panel concluded that the new studies provide a basis for revising the uncertainty factors that were used by the SCF to derive the temporary TDI of 0.01 mg/kg bw in 2002. In particular, the Panel now considers that the database concerning reproduction and development has been considerably strengthened and that the additional uncertainty factor of 5, introduced by the SCF in 2002 for the uncertainties in the database on reproduction and development, is no longer required. The Panel also concluded, in view of the well described species differences in toxicokinetics, showing a low level of free BPA in humans compared with rats, that a default uncertainty factor of 100 applied to the overall NOAEL from the rodent studies can be considered as conservative. The Panel therefore established a full TDI of 0.05 mg BPA/kg bw, derived by applying a 100-fold uncertainty factor to the overall NOAEL of 5 mg/kg bw/day.

Dietary exposure assessments on BPA have been made by the Panel for adults, infants and children. The estimates of potential dietary exposure to BPA in infants took account of breast feeding, feeding formula using PC bottles and consumption of commercial foods and beverages. The resulting exposure assessments ranged from 0.2 microg/kg bw/day in 3–month-old breastfed infants up to 13 microg/kg bw/day in 6-12–month-old infants. These estimates were based on conservative migration values of BPA and the 95th percentiles of consumption. The estimates of potential dietary exposure in young children and adults were respectively 5.3 and 1.5 microg/kg bw/day based on conservative migration values of BPA and conservative estimates of consumption of commercial foods and beverages. The Panel noted that the conservative estimates of exposure were less than 30% of the TDI in all population groups considered. These exposure estimates include BPA migration into canned foods and into food in contact with PC table ware or storage receptacles. On the other hand, they do not include either potential migration of BPA from receptacles into food during

microwave heating or potential migration of BPA into drinking water due to the use of PC and of epoxy-phenolic resins in water pipes and in water storage tanks. Information on potential migration of BPA from these sources would be useful.

KEYWORDS

Bisphenol A, BPA, 2,2-bis(4-hydroxyphenyl)propane, food contact materials, CAS No. 00080-05-7.

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BACKGROUND

The substance 2,2-bis(4-hydroxyphenyl)propane, CAS Number 80-05-7, more commonly known as bisphenol A (BPA), is used as a monomer in the manufacture of polycarbonates and epoxy resins. Polycarbonates and epoxy resins are used in food contact material for a variety of purposes.

BPA was last evaluated in 2002 by the Scientific Committee for Food (SCF) for use in plastic materials and articles intended to come into contact with foodstuffs. The SCF considered the overall oral NOAEL for BPA to be 5 mg/kg bw/day. This NOAEL was based on the results of a comprehensive three-generation study in the rat, in which the lowest dose showing effects was 50 mg BPA/kg bw/day. The pivotal effects were significant reductions in adult body weight and pup body and organ weights. This NOAEL was considered the appropriate one on which to base the TDI and an uncertainty factor of 500 was considered appropriate. Using this uncertainty factor (10 for interspecies differences, 10 for inter-individual differences and 5 for remaining uncertainties in the database for BPA), a temporary TDI of 0.01 mg BPA/kg bw/day was derived.

In the present opinion, BPA has been re-evaluated in the light of the new information published within the last 4 years. Some of the studies available as abstracts only in 2002 have now been published; a large number of new publications addressing different aspects of the toxicity and toxicokinetics of BPA and also the issue of low-dose effects, have become available and are now included in the evaluation.

TERMS OF REFERENCE

The Commission asks the European Food Safety Authority (EFSA) to re-evaluate Bisphenol A for use in food contact materials in the light of any new studies that have become available. Particular attention should be given to the exposure of infants to Bisphenol A.

ASSESSMENT

1. Chemistry

Name of the substance:2,2-BIS(4-HYDROXYPHENYL)PROPANE
CAS number:00080-05-7
Synonyms (examples):Bisphenol A
Bis(4-hydroxyphenyl)dimethyl methane,

4,4’-dihydroxydiphenyl propane,

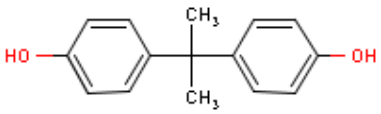
4,4’-dihydroxy-2,2-diphenyl propane,

Diphenylolpropane

4,4’-isopropylidenediphenol

Molecular weight:228.3

Structure:



2. Uses

A comprehensive review of BPA uses is available in the European Union Risk Assessment Report of Bisphenol A (EU 2003). Main uses in food contact materials and other uses are briefly summarised.

2.1 Use in food contact materials

BPA is primarily used as a monomer in the production of polycarbonate (PC) and as a precursor or a starting material for monomers of certain epoxy resins.
PC is a plastic widely used in food contact articles such as infant feeding bottles, tableware (plates, mugs, jugs, beakers), microwave ovenware, storage containers, returnable water and milk bottles (mainly used in Northern Europe) and refillable water containers ('carboys' used in offices, hospitals etc). Epoxy-phenolic resins are used as an internal, protective lining of cans for foods and beverages. Epoxy-phenolic resins are usually formed by reaction of BADGE (Bisphenol A diglycidyl ether) with BPA (Runyon *et al.,* 2002). Epoxy resins are applied to the insides of cans and then heated to high temperature for further reactions and cross-linking. Metal lids for glass jars and bottles are often also coated with an epoxy-phenolic resin. According to industry, BPA itself is not used as a cross-linker in Europe for can coatings (Association of Plastics Manufacturers in Europe, 2006a). On the other hand, phenolic cross-linkers, that contain residual BPA, are used in Europe to some extent for can coatings. There are some applications of BPA as an accelerator in amine-based hardeners for epoxy resins. These hardeners are not used in can coatings, but in corrosion-resistant coatings for tanks, pipelines, flooring, etc.

Minor uses of BPA include phenoplast resins, unsaturated polyester resins, alkyloxylated bisphenol-A, polyols/polyurethanes and modified polyamides for can coatings.

BPA was found to be contained in commercial polyvinyl chloride (PVC) cling films and plastic sheeting bags available on the market in Spain and migration studies suggested it would migrate into food (Lopez-Cervantes and Paseiro-Losada, 2003; Lopez-Cervantes *et al.,* 2003). EFSA therefore asked the Association of Plastics Manufacturers in Europe to investigate the currrent use of BPA in PVC. The former application of BPA in the PVC polymerisation process by some manufacturers in the EU appeared to have ceased (Association of Plastics Manufacturers in Europe, 2006a). Its current use as a stabiliser /

antioxidant in PVC, being added in the form of a formulated stabiliser package or directly during a compounding stage, was also investigated. The only formulation including BPA was found to be used for gaskets for metal bottle caps sold outside the EU (Association of Plastics Manufacturers in Europe, 2006c). Thus, based on this information, no BPA exposure from food contact uses of PVC should be expected in the EU today.

According to some authors, natural or synthetic (silicone) rubber used to produce baby feeding teats and baby soothers (pacifiers) could contain BPA, since plastics are often blended with rubbers to modify their properties (Ozaki & Baba, 2003). In an isolated study by Tan & Mustafa (2003), BPA was observed to leach from baby feeding teats available on the Malaysian market. However, the World Association of Manufacturers of Bottles and Teats was contacted and stated that BPA is not and has never been used in the manufacture of teats (Association of Plastics Manufacturers in Europe, 2006b). Thus, based on this information, no BPA exposure from food contact uses of natural or synthetic rubber should be expected.

The food contact uses considered by the Panel in the following exposure assessment are therefore PC and epoxy-phenolic resins.

2.2 Other uses

A special field of potential use of BPA is in materials in contact with water for human consumption. PC is used for water pipes and epoxy-phenolic resins are widely used as a surface-coating on residential drinking water storage tanks (Bae *et al.*, 2002). The Panel noted that the quality of water for human consumption is subject to Council Directive 98/83/EC (3.11.1998) and that materials in contact with water intended for human consumption are under the mandate of the Construction Product Directive 89/106/EEC (21.12 1988), but considered that these uses should be considered in the assessment of dietary exposure to BPA.

BPA is also used in a variety of other, non-food applications: epoxy resin based paints, wood filler, adhesives, surface coatings, printing inks, carbonless and thermal paper, flame retardants, tyre and brake fluid manufacture, resin-based composites and sealants used in dentistry.

3. Exposure

Consumer exposure to BPA may occur through oral and dermal exposure. Only consumer exposure via the diet has been considered in the present assessment, including exposure via drinking water which could potentially contain BPA due to its presence in pipelines or storage tanks.

Oral exposure occurs mainly via consumption of food and beverages (dietary exposure) or when commodities for children are put in the mouth. To estimate exposure via the diet, data on migration of BPA into foods and food simulants are combined with data on food consumption. The present assessment is aimed at providing conservative estimates of exposure, i.e. at high percentiles of the distribution, by combining high but realistic levels of BPA migration with high levels of consumption of food.

3.1 Studies on migration of BPA in to foods and food simulants

Potential consumer exposure from plastic and coated metal food contact articles can arise under conditions where residual monomer in the polymer matrix becomes available for migration, if breakdown of the polymer occurs and generates additional BPA monomer or when BPA is used in an additive to food contact material.

3.1.1 Migration from coatings of canned products and from lids of glass jars and bottles

The main potential for BPA contamination of canned products is likely to be migration of BPA-monomer from incompletely polymerised epoxy resin coatings and of BPA as residual impurity of BADGE.

A review of studies conducted to measure BPA migration from food and beverage cans has been performed in the EU Risk Assessment Report (RAR) on BPA (EU, 2003). Overall, three studies provided 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 are representative for the sterilisation process. Concentrations of up to about 70-90 microg BPA/kg of can contents were observed using fatty foods or simulants and these results were considered to be representative of realistic worst-case conditions. A value of 100 microg BPA/kg in the contents of a typical food can was used in the EU RAR on BPA. For soft drink/beverage cans, results from the three above-mentioned studies did not indicate BPA concentrations above the detection limit (2 microg BPA/kg) in the can contents. It was assumed that the results of these studies could be generally applied. According to the EU RAR, for alcoholic beverage cans the only detectable levels of BPA were found in *sake*, an alcoholic drink mainly used in Asian countries. As this alcoholic beverage is of little relevance to the EU market, this result was not used in the RAR for the purposes of the risk characterisation. Therefore, no exposure to BPA in canned soft or alcoholic beverages was taken forward for risk characterisation in the EU RAR on BPA.

A limited number of studies have investigated BPA concentrations in samples of canned commercial products and are summarised in Table 2. In both food and beverages, concentrations of BPA above 100 microg/kg were rarely observed. The 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 microg BPA/kg and 65 solid foods with concentrations in the range of 5 to 91 microg BPA/kg. BPA concentrations above 100 microg/kg were not observed in these surveys. In other surveys, the highest observed concentration in canned beverages was 212 microg/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 microg BPA/kg in corned beef and 212 microg BPA/kg in chicken (Imanaka *et al*., 2001). In New Zealand, individual samples of tuna, corned beef and coconut cream reached up to 191 microg 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 microg BPA/L (range 9 to 31 microg /L), 66 microg BPA/L (range 33 to 107 microg/l) and 84 microg BPA/L (range 50 to 134 microg/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 microg/L) were found in all unheated cans. When the heat treatment was performed, the migration was up to 5 microg BPA/L at 80°C and up to 30 microg BPA/L at 100°C.

Table 2. BPA concentrations in canned commercial products according to recent published studies

Reference /Country	Type of product	LOD/LOQ (microg/kg)	Number of products analysed	Percent samples above LOQ	Percent samples above 100 microg/kg	Minimum (microg/kg)	Maximum (microg/kg)	Average concentration of values above LOQ (microg/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	Foods	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 the Panel.

[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.

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.

The Panel 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.

The Panel considered whether to use a migration value of 100 microg 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. The Panel noted that in the 3 surveys conducted in the EU in canned commercial products, BPA in canned beverages was always less than 7 microg BPA/kg and that the average concentration of BPA in solid foods in which it was quantified was up to 40 microg BPA/kg. Although limited, these data were used to develop an exposure scenario considering 10 microg BPA/kg as the value for for canned beverages and 50 microg BPA/kg as the value for canned solid foods. This scenario was used by the Panel to provide a conservative assessment of exposure to BPA through canned products in adults and children consuming a variety of products.

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, a value of 100 microg BPA/kg canned foods and beverages was used.

3.1.2 Migration from polycarbonate tableware and food storage containers

PC articles are reusable and are washed between uses. Depolymerization of PC may occur with release of BPA during the lifetime of these articles (Mountfort et al., 1997; Brede et al., 2003). A review of studies conducted to measure BPA migration from polycarbonate tableware and food storage containers has been performed in the EU RAR on BPA (EU, 2003). No detectable levels were found in the food or drink contents of tableware and the highest reported level of BPA from tableware into food simulants was 5 microg BPA/kg. This migration level was used as the basis for calculating exposure from this source in the EU RAR on BPA. The same assumption was used by the Panel. Recent studies suggest that migration 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.

3.1.3 Migration from polycarbonate infant feeding bottles

Most baby feeding bottles are made from PC. A number of studies have been conducted on migration of BPA from PC baby feeding bottles.

In the SCF opinion on BPA (EC, 2002), the range of migration from PC feeding bottles into water and infant formulae considered was <10 to 20 microg BPA/kg (EC, 2002). Higher migration values obtained under aggressive conditions were not considered as representative of actual use. The data made available to the SCF suggested no significant effect from repeated-use, abrasion, heating, or chemical sterilisation of PC. Exposure in infants was estimated considering a migration level of 10 microg BPA/kg of infant formula and an intake of 0.7 litre of formula per day in infants aged 0-4 months and weighing 4.5 kg, leading to a potential exposure to BPA of 1.6 microg/kg bw/day (EC, 2002).

A comprehensive review of studies conducted to measure BPA migration from PC baby feeding bottles until 2003 has been performed in the EU RAR on BPA (EU, 2003). In a number of these studies BPA concentrations were below the detection limit; however the detection limit was sometimes high. In one study conducted on 163 commercial baby feeding bottles filled with simulant at 50°C and agitated on a horizontal shaker, migration of up to 110 microg/L was observed in 95% ethanol but no migration was detected (detection limit 10 microg/L) in water and 3% acetic acid (Simoneau *et al*., 2000). Hanai (1977) observed BPA migration varying from 3 to 55 microg/L (detection limit 2 microg/L) in bottles filled with boiling water (95°C) and kept overnight at room temperature. Earls *et al*. (2000) detected BPA in 5 out of 12 bottles filled with either 3% acetic acid or boiling water, placed in refrigerator (1-5°C) for 24 hours and heated to approximately 40°C by immersion in boiling water, with BPA concentrations ranging from 20 to 50 microg/L (detection limit 10 microg/L). Since only one measurement was performed for each bottle, these studies did not investigate if some bottles show consistently high levels of migration. Thus in these two studies conducted on commercially available baby feeding bottles under experimental conditions close to realistic conditions of use, concentrations of up to about 50 microg BPA/L were detected in the food simulant contents. This value was used in the EU RAR as the basis for estimating potential exposure from the use of PC feeding bottles for infants.

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 the Panel to assess potential exposure to BPA.

Brede *et al.* (2003) subjected PC 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 microg BPA/L (range of 0.11 to 0.43 microg/L) while the mean level from bottles subjected to simulated repeated use was 8.4 microg BPA/L (ranging from 3.7 to 17 microg/L) after 51 dishwasher cycles and 6.7 microg BPA/L (ranging from 2.5 to 15 microg BPA/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 PC.

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 BPA/cm^2 (ranging from non detectable to 1.34 ng/cm^2) at 80°C. Mean BPA leaching from the used bottles filled with water was 3.37 ng/cm^2 (ranging from 0.11 to 25.51 ng/cm^2) at 80°C. The Panel noted that based on these data and considering a contact area of 172 cm^2 (for a standard feeding bottle filled to the 200 ml mark), BPA leaching would range from non-detectable to 1.15 microg/L in new bottles and from 0.1 to 21.9 microg/L in used bottles.

In a study performed in the UK (CSL, 2004), migration from samples of two different brands of PC 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 microg BPA/L) to 4.5 microg BPA/L in 10% ethanol and from non-detectable (<0.3 microg BPA/L) to 0.7 microg BPA/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, the Panel noted that BPA migrates from PC 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. The Panel 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.

The Panel noted that according to the two migration studies conducted since 2003 under conditions mimicking realistic conditions of use, levels of BPA migration in used PC bottles were respectively up to 22 microg/L (Tan & Mustafa, 2003) and up to 14 microg/L (Brede, 2003). These upper values were lower than the upper value of 50 microg BPA/L identified in the EU RAR (2003). Although based on a limited number of bottles, the studies used in the EU 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 microg BPA/L of infant formulae in used bottles was therefore used by the Panel as the basis to calculate a conservative estimate of exposure in infants under the assumption of daily use of PC bottles leaching high amounts of BPA. Available data were not adequate to assess average migration from PC bottles but a migration value of 10 microg /L was considered to complement this conservative scenario with a more typical situation.

3.1.4 Migration from coatings for wine storage vats

Three migration studies from wine vats coated with epoxy resins were performed by Larroque *et al.* (1989). In one study, migration observed after 4 years from resins applied manually to glass was up to 160 mg of BPA released in wine simulants per kg resin. In another study, migration of 0.7 to 1.8 mg BPA/kg resin was observed after 3 years contact with wine simulants when the resin was applied to an aluminium support and migration of 1 to 13 mg BPA/kg resin after 1 year of contact with the resin applied to glass support. The authors suggested to consider a BPA migration of 100 mg/kg resin with a 1500 l vat lined with 10 kg resin; BPA concentration in the wine would then be 650 microg/L. This value was used in the EU RAR, while it was stated that it was a very worst-case estimate of exposure. The RAR also reports that the use of epoxy-lined wine vats in the EU is limited – the majority of wine is reported to be stored in uncoated stainless steel vats.

The Panel noted that resins were poorly described in the paper by Larroque *et al*. (1989). The highest migrations seemed to be observed when the resins were degraded, with appearance of visible cracks on the surface of the coating. The high migration values observed could therefore be related to the deterioration of resins with time.

The Association of Plastics Manufacturers in Europe (2006a) 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.

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 microg/L. Mean BPA concentrations for all wine samples above the LOQ was 0.58 microg/L. In seven samples, BPA levels ranged from 0.2 to 0.5 microg/L. Only in one sample (stored 10.5 months in a steel vat) was a significantly higher BPA level of 2.1 microg/L found. The Panel noted that eventhough 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 that found in canned beverages (within 10 microg/L). No specific scenario is therefore needed to account for BPA migration into wine.

3.1.5 Possible migration of BPA from other sources

BPA may potentially be present in drinking water for a variety of reasons. It could be present as a result of industrial pollution, due to migration from PVC used for pipes, hoses or lining of steel pipes, or due to migration from epoxy-phenolic resins used as a surface-coating agent in residential drinking water storage tanks and in water heaters in households.

BPA migration into water from 3 different epoxy-phenolic resins has been measured by Bae *et al*. (2002). Migration into water ranged from 0.1 to 1734 microg BPA/m^2 of coatings. The amount of BPA leaching was shown to increase as the water temperature increased. However, the Panel noted that this study used coatings prepared on glass plates under laboratory conditions which are not comparable with practical use and did not describe the curing conditions. Moreover, only the first migration was assessed which may not be appropriate for repeated use articles.

Also Romero *et al*. (2002) studied migration of BPA from epoxy paints on stainless steel test pieces. After 5 days of exposure at 40-45 °C to deionised water using a surface/volume ratio of 1 dm^{-1}, the migration of BPA was 200-300 microg/m^2. The authors did not describe the curing process used. The authors also describe the analysis of a sample taken from a water reservoir. No migration of BPA was detected, but the authors did not describe the length of the time interval between painting of the reservoir and sampling.

When water is chlorinated, which is the most widely used water disinfecting technique in practice, BPA is rapidly oxidised and chlorinated BPA congeners are formed as by-products (Gallard *et al*., 2004). In common chlorinated drinking water (pH≥6.5; [Cl_2]≥0.2 mg/L) the half-life of BPA would be less than 3 h.

More data would be needed to quantify the possible dietary exposure to BPA and chlorinated derivatives of BPA via drinking water and to assess the possible health significance of any chlorinated derivatives.

BPA has been found in recycled paper and paperboard used for food packaging (pizza cardboard, paper bags) and in kitchen towels made from recycled paper probably due to its use in thermal paper and printing inks. BPA concentration was found to be 10 or more times higher than in virgin products: 0.19 to 26 microg BPA/g of paper versus 0.034 to 0.36 microg BPA/g of paper (Ozaki *et al.*, 2004). Further data would be needed to quantify the impact of these sources in terms of BPA exposure in the population.

3.2 Estimates of dietary exposure in different age groups

3.2.1 Potential dietary exposure in infants 0-6 months

The potential dietary exposure in infants 0-6 months needs to be assessed according to their consumption pattern. A variety of situations can occur from babies exclusively fed with infant formulae to babies exclusively breast fed.

The food consumption scenario previously used by the Panel (EFSA, 2006), based on the German DONALD study (Kersting, 1998) is that of a 3 month infant weighing on average 6.1 kg and consuming 174 ml/kg bw/day of infant formula (95th percentile of consumption 1060 ml/day; based on a reconstitution ratio of 135 g/L of liquid formula). This conservative consumption scenario was applied by the Panel in the present opinion.

Based on a high concentration of 50 microg of BPA/L migrating from PC bottles into infant formula, potential dietary exposure from this source of a 3 month infant at the 95th percentile of consumption would be 8.7 microg/kg bw/day. Based on a more typical concentration of 10 microg of BPA/L of infant formula, potential dietary exposure from this source of a 3 month infant at the 95th percentile of consumption would be 1.7 microg/kg bw/day.

The Panel 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 microg BPA/kg. Based on a reconstitution ratio of 135 g/L of liquid formula and on a concentration value of 100 microg BPA/kg, the above mentioned 3 month infant consuming 174 ml/kg bw/day of reconstituted infant formulae would consume 23 g / kg bw of powdered infant formulae, leading to a potential exposure of up to 2.3 microg BPA / kg bw /day. The Panel noted that this potential source of exposure is quantified on the basis of a very limited number of samples from a non-EU market.

Overall, the potential dietary exposure in infants 0-6 months fed from PC bottles with infant formulae previously packed in food cans with epoxy-phenolic coating based on a migration value of 50 microg/L of infant formula would be 11 microg BPA/kg bw/day (8.7 + 2.3 microg BPA/kg bw/day). This is the estimate of dietary exposure in infants fed every day with PC bottles leaching BPA at the highest concentration observed in realistic conditions of use. A more typical scenario, based on a migration value from PC bottles of 10 microg/L of infant formula would lead to a dietary exposure of 4 microg BPA/kg bw/day (1.7 + 2.3 microg BPA/kg bw/day).

The potential dietary exposure in infants fed with infant formulae from glass feeding bottles or plastic feeding bottles not containing BPA would derive from the canned powdered infant formulae. Potential exposure would be up to 2.3 microg BPA/kg bw/ day.

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 microg/L) with values in the range from 0.28 to 0.97 microg/L. The mean value was 0.61 microg/L. Considering the consumption of 174 ml/kg bw of human milk per day in infants exclusively breastfed, the Panel calculated a potential dietary exposure of 0.1 microg BPA/kg bw/day at the mean and 0.2 microg BPA/kg bw/day at the highest BPA concentration observed. The Panel 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.

3.2.2 Potential dietary exposure in infants 6-12 months

The food consumption pattern of infants aged 6-12 months may typically include human milk, a number of beverages fed in feeding bottles (water, fruit juice, milk) and homogenised foods that can be either prepared at household level or purchased as commercial baby food.

The Panel estimated the dietary exposure in infants aged 6-12 months based on the consumption of commercial baby foods and drinks derived from the DONALD study (Kersting *et al.*, 1998) which was used previously in the AFC opinion on semicarbazide

(EFSA, 2005). The 95th percentile of consumption in consumers only was highest at 6 months of age, with a value of 52 g/kg bw (407 g /day for an average body weight of 7.8 kg). Based on a contamination level of 100 microg BPA/kg, the potential dietary exposure from commercial baby foods and drinks would therefore be 5.2 microg BPA/kg bw/day. Considering that infants have a less varied diet than small children or adults, only this conservative estimate of BPA migration was used (see section 3.1.1).

An additional potential source of BPA exposure is PC table ware and receptacles used for the storage of food. Considering the highest reported level of BPA in food simulants (5 microg BPA/kg) and a consumption of 52 g of food/kg bw, this source of dietary exposure is potentially small (0.3 microg BPA/kg bw/day). This source of exposure also applies to infants aged 6-12 months who are fed with food prepared at home.

In this age group an additional potential exposure is through infant formulae. According to the DONALD study (Kersting *et al*., 1998), the highest consumption in this age group on a body weight basis is at 6 months of age, with a value of 118 ml /kg bw derived from 16 g/kg bw of formulae for an average body weight of 7.8 kg. Based on a conservative migration value of 50 microg BPA/L from PC bottles into infant formula, potential exposure through infant formula would be 5.9 microg BPA/kg bw/day. Based on a more typical migration value of 10 microg BPA/L from PC bottles into infant formula, potential exposure through infant formula would be 1.2 microg BPA/kg bw/day. An additional 1.6 microg BPA/kg bw/day may derive from the use of powdered formula packed in cans lined with epoxy-phenolic resins, based on a migration value of 100 microg BPA/kg of powder.

Overall, in the age group 6-12 months, a conservative dietary exposure scenario is that of an infant weighing 7.8 kg fed with commercial baby foods containing BPA at the highest level measured, using PC table ware and also fed with infant formulae in PC bottles leaching BPA at the highest concentration observed in realistic conditions of use. The total intake of BPA under this scenario would be 13 microg BPA/kg bw/day. A more typical scenario just for the infant formula, based on a migration value of 10 microg/L would lead to a total dietary exposure of 8.3 microg BPA/kg bw/day.

3.2.3 Potential dietary exposure in young children

For epoxy-phenolic resin food contact applications (canned food and beverages), a conservative estimate of dietary exposure was obtained, based on values of 50 microg BPA/kg for canned solid food and 10 microg BPA/kg for canned beverages, using an average body weight of 11 kg for a child aged 1.5 years (CEC, 1993) and consumption of 2 kg of canned products (one–third solid foods, two-thirds beverages). This gave a conservative estimate of dietary exposure of 4.4 microg BPA/kg bw.

The Panel used the EU RAR estimate of potential dietary exposure from PC tableware and food storage containers of 10 microg BPA/day, derived from consumption of 2kg food/day containing 5 microg BPA/kg food. This gave a potential exposure of 0.9 microg/kg bw for a child weighing 11 kg.

Overall a conservative estimate of potential BPA exposure in small children consuming a variety of canned products would be 5.3 microg/kg bw/day (4.4 microg/kg bw/day from canned foods and beverages plus 0.9 microg/kg bw/day from PC tableware and food storage containers). This exposure estimate is based on the assumption that all foods and beverages are in the form of canned products and are taken from polycarbonate tableware. It will result

in an overestimate of actual exposure from these sources, although the degree of overestimation is unknown.

3.2.4 Potential dietary exposure in adults

In the present opinion the Panel estimated the potential dietary exposure to BPA based on conservative values for both BPA concentration in foods and beverages and for consumption patterns of canned products. The exposure scenario considered by the Panel was based on the assumption that 1 kg of canned foods and 2 litres of canned beverages are consumed each day by a 60 kg adult, using values of 50 microg BPA/kg for canned solid food and 10 microg BPA/kg for canned beverages. This gave a conservative estimate of potential exposure for adults of 1.2 microg BPA/kg bw/day.

Another potential source of BPA exposure is migration from PC table ware and storage containers. A potential dietary exposure of 15 microg BPA/day, i.e. 0.25 microg BPA/kg bw/day was derived, based on the assumption that there is migration of 5 microg BPA/kg food or beverage.

The overall potential dietary exposure in the adult population would be 1.45 microg BPA/kg bw/day.

3.2.5 Summary of potential dietary exposure estimates

The potential dietary exposure estimated by the Panel for the different scenarios considered for infants, children and adults is presented in Table 3.

Uncertainty in the present dietary exposure assessment is in part related to the absence of data that would allow quantification of exposure to BPA from some sources. In all population groups, potential exposure to BPA present in drinking water due to pipelines or storage tanks could not be quantified. The same is true for possible increased BPA exposure due to the use of containers heated in microwave ovens. On the other hand, conservative assumptions of BPA concentration and of food and beverage consumption were made for all other potential sources of exposure. These have been added up, leading to an overall exposure assessment which is likely to be conservative.

Table 3. Conservative estimates of potential dietary exposure to BPA

Source of exposure	BPA concen-tration in food/beverage (microg/kg or microg/L)	Potential dietary exposure to BPA (microg/kg bw/day)					
		3 month infant [1]			6 month infant [2]	Child aged 1.5 years [3]	Adult [4]
		Breastfed	Fed with glass or non-PC bottle	Fed with PC bottle			
Breast milk	<1	<0.2	-	-			
Migration from PC bottle to milk [5]	50 / 10	-	-	8.7 /1.7	5.9 /1.2	-	
Migration from epoxy resin can to powdered formula	100	-	2.3	2.3	1.6	-	
Migration from epoxy resin can to commercial foods and beverages [6]	100 50/10	-	-	-	5.2	4.4	1.2
Migration from PC table ware	5	-	-	-	0.3	0.9	0.3
TOTAL		**0.2**	**2.3**	**11 / 4 [5]**	**13 / 8,3[5]**	**5.3 [2]**	**1.5 [2]**

(1) Infant weighing 6.1 kg, consuming 174 ml/kg bw of breast milk or of infant formulae reconstituted from 23 g/kg bw of powder.
(2) Infant weighing 7.8 kg, consuming 52 g/kg bw of commercial foods and beverages and 118 ml /kg bw of infant formulae reconstituted from 16 g/kg bw of powder.
(3) Child weighing 11 kg, consuming 2 kg of commercial foods (one third solid foods, two-thirds beverages).
(4) Adult weighing 60 kg, consuming 3 kg of commercial foods (1 kg solid foods, 2 kg beverages).
(5) Two exposure scenarios were considered for BPA exposure from PC bottles in infants. A conservative scenario was based on a concentration value of 50 microg BPA/L of infant formulae to calculate exposure in infants fed every day with PC bottles leaching BPA at the highest concentration observed in realistic conditions of use. Another scenario based on migration value of 10 microg BPA/L was considered in order to estimate exposure in a more typical situation (see paragraph 3.1.3).
(6) Two exposure scenarios were considered for migration from epoxy resin linings of cans: for infants, a value of 100 microg BPA/kg food or beverage; for adults and children, values of 50 microg BPA/kg was used for solid canned foods and 10 microg BPA/L for canned beverages.

3.3 Other sources of exposure

A comprehensive review of occupational exposure to BPA is available in the EU RAR on BPA (EU, 2003). This source of exposure is not considered in the present assessment. Potential exposure may arise from consumer use of epoxy-phenolic resin based paints (0.02 microg BPA/event for inhalation and 3.6 microg BPA/event for dermal exposure), wood filler (9 microg BPA/event), and adhesives (14 microg BPA/event) (EU, 2003). Due to the low level of exposure per event and to the low frequency of such events, these are minor sources of BPA exposure compared with potential dietary exposure. Other uses of BPA, such as in printing inks and thermal paper, may lead to a more frequent dermal exposure. However, according to the EU RAR on BPA, these uses result in negligible potential for consumer exposure in comparison with the other sources considered (EU, 2003).

The main route of exposure from environmental sources is the oral route. Human exposure via the environment based upon typical human consumption and inhalation rates was estimated in the EU RAR on BPA. It was estimated to be 0.0002 microg/kg bw/day at the regional level and 60 microg/kg bw/day at the local level (highest local exposure near a PVC production plant due to the use of BPA as an inhibitor in PVC production) in a 70 kg body weight subject (EU, 2003). The exposure at local level therefore corresponds to 4200 microg BPA/day.

Overall 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 microg/kg bw/day) due to relatively high dietary consumption per unit body weight and the use of PC 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 microg/kg/day for adult males and 0.034–0.059 microg/kg bw/day for adult females. The 95% confidence intervals for high-exposure (95^{th} percentile) were estimated to be 0.037–0.064 microg/kg bw/day for adult males and 0.043–0.075 microg/kg/day for adult females. No details were available to the Panel in relation to the characteristics of the diet of subjects from which urines were collected. The authors 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 PC tableware and PC feeding bottles were substituted with non-PC articles (with less than 6% of feeding bottles currently being made with PC).

Another potential source of exposure to BPA are resin-based composites and sealants used in dentistry. BPA is used to make bisphenol A glycidyl methacrylate (bis-GMA) and bisphenol A dimethacrylate (bis-DMA) which are used as monomers for the manufacture of dental resins. *In situ* polymerisation is performed when the teeth are being filled and unpolymerised material is rapidly released in saliva after curing. Moreover BPA leaching could occur if the resin deteriorates with time since the enzymatic activity of saliva, esterases, extreme pH and saliva storage can hydrolyse bis-GMA (Pulgar et al., 2000).

According to the EU RAR on BPA (EU, 2003), any exposure from dental fissure sealants is likely to be an infrequent acute event. However, the Panel was aware that dental sealing may also be systematically performed as a preventive measure against dental decay in young children.

A review on BPA exposure through dental sealants is available on the website www.bisphenol-a.org/human/dental.html of the American Plastics Council (Arlington, VA). According to this review high BPA concentration values found in saliva by Olea et al. (1996) are questionable and lower values were reported by other authors (Arenholt-Bindslev et al., 1999; Fung et al., 2000). In this review by industry, an exposure assessment was performed based on the single highest value observed in this last study. Based on the assumptions of 931 microg of BPA leached in saliva in the hours immediately following application in a child weighing 25 kg, acute exposure was estimated to be 37 microg BPA /kg bw. The Panel observed that in the Olea et al. (1996) study a non-specific method with low separation selectivity was used and that, since BPA eluted very close to the solvent front, this technique is prone to errors. The Panel therefore did not consider these data in the exposure assessment.

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

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 Annex 1.

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

The Panel also noted that blood levels for BPA in unintentionally exposed human subjects (reported as up to 10 microg BPA/L) are higher than the peak BPA concentrations determined in blood of monkeys (5 nM, app. 1.1 microg BPA/L) after oral administration of a dose of 100 microg BPA/kg bw or in blood of humans given oral doses of 60 – 80 microg BPA/kg bw. In these human subjects, free BPA in plasma was not detected even within a short time after

administration with an LOD of 10 nM (2.3 microg BPA/L.). These intentionally-administered doses are 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 microg BPA/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 microg BPA/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 bisphenol A concentrations indicated by ELISA using instrumental analytics have failed, and consistently indicate a large overestimation of bisphenol A concentrations by ELISA (Inoue *et al*., 2002; Tominaga, *et al.,* 2006; Fukata *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.

Considering the evidence as a whole, the Panel concludes 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 microg BPA/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 microg BPA/L, while samples from a cohort in Germany did not contain detectable concentrations of BPA with a detection limit of 1.1 microg BPA/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 microg/L (95th percentile) (Calafat *et al.*, 2005). Based on a total urine volume of 2 liters excreted over 24 h, available data give an estimate of an average daily dietary exposure of BPA of up to 7 microg BPA/adult and upper range dietary exposures up to 10 microg BPA per adult (0.16 microg BPA/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 microg BPA/kg bw/day for male and 0.043 – 0.075 microg BPA/kg bw/day for female adults (Miyamoto and Kotake, 2006).

The Panel 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. Toxicological evaluation

4.1 Absorption, distribution, biotransformation and excretion of bisphenol A

A number of recent publications have addressed the biotransformation and toxicokinetics of BPA in primates and in pregnant and non-pregnant rodents of different ages. BPA is rapidly absorbed from the gastrointestinal tract, and formation of BPA-glucuronide is the major pathway of BPA biotransformation in primates and in rats. BPA-glucuronide is also the major BPA metabolite formed in rat, mouse and human hepatocytes and the isolated perfused rat liver (Inoue *et al.,* 2005; Pritchett *et al.*, 2002). However, there are major differences in disposition of BPA-glucuronide due to different pathways of elimination from the liver in rodents and primates. Formation of BPA conjugates has to be considered as a deactivation reaction, since both BPA-glucuronide and BPA-sulphate have a much lower hormonal activity as compared to the activity of BPA (Matthews *et al.,* 2001; Shimizu *et al.,* 2002; Snyder *et al.,* 2000; Stowell *et al*., 2006).

4.1.1 Toxicokinetics of BPA in humans and primates

In primates, including humans, orally administered BPA is rapidly absorbed from the gastrointestinal tract and undergoes intensive first-pass metabolism to BPA-glucuronide in the gut wall and in the liver. In adult human subjects (three males and three females), after oral administration of BPA (5 mg/person, 60 to 80 microg/kg bw), BPA-glucuronide was the only metabolite of BPA detected in urine and blood samples. Concentrations of BPA were below the limit of detection both in urine (6 nM) and blood samples (either free or plasma protein bound, 10 nM). BPA-glucuronide was cleared from human blood ($t_{1/2}$ = 3.4 h) and excreted in urine ($t_{1/2}$ = 5.4 h); the ingested doses of BPA were completely recovered in urine as BPA-glucuronide (Volkel *et al.*, 2002) within 42 hours after administration. The rapid elimination of BPA-glucuronide from blood and a rapid urinary excretion of BPA-glucuronide was confirmed in two further studies in human subjects given oral doses of bisphenol A (Tsukioka *et al*., 2003; Volkel *et al.*, 2005).

Consistent with these data in humans, following a single oral dose of 100 microg ^{14}C-BPA/kg bw to cynomolgus monkeys, 79-86% of the administered radioactivity was excreted in urine over 7 days, with highest rates of excretion within the first 7 hours after administration. The faecal excretion of radioactivity over 7 days was minimal. Half-life of excretion of BPA-derived radioactivity from blood in monkeys after oral administration was 10 hours and the predominant plasma and urinary metabolites of BPA were a mono- and a diglucuronide of BPA. The concentrations of unchanged ^{14}C-BPA in blood were at or below the limit of detection (app. 5 nM) (Kurebayashi *et al.*, 2002). A rapid elimination from blood and an extensive first-pass metabolism of orally administered bisphenol A in primates is also

indicated by results of a recent study on the blood toxicokinetics of bisphenol A in chimpanzees and monkeys (Tominaga *et al.,* 2006).

Thus, the results on the toxicokinetics of BPA in 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.

4.1.2 Toxicokinetics of BPA in rodents.

Most of the studies examining the toxicokinetics in rodents used rats as experimental models. In contrast to primates, in adult rats several studies using oral doses of BPA ranging from 20 microg/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 circulation 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. Most of a BPA dose administered by intravenous injection is initially bound to plasma proteins in rats, but is also rapidly taken up into the liver and transformed there to BPA-glucuronide, which is excreted with bile from liver (Kurebayashi *et al.*, 2003). 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 circulation 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).

The toxicokinetics of BPA were also assessed in pregnant and neonatal Sprague-Dawley rats (Domoradzki *et al.*, 2003). A dose of 10 mg BPA/kg bw was administered orally to non-gravid and pregnant rats on gestation days 6, 14, and 17. Additional rats were administered oral doses of 10 mg BPA/kg bw on GD 11, 13, and 16. Tissue distribution, biotransformation, the rates or routes of excretion of BPA, or the plasma concentration-time profiles of BPA-glucuronide were not altered during gestation as compared to non-pregnant rats. No selective affinity of either yolk sac/placenta or embryo/fetus for BPA or BPA metabolites relative to maternal plasma or tissues was observed.

In neonatal rats orally administered 1 or 10 mg BPA/kg bw at postnatal days 4, 7, or 21, BPA was metabolised to BPA-glucuronide at all three ages, although an age dependency in the number and concentration of plasma metabolites was observed, consistent with the ontogeny of glucuronyl transferases. BPA-glucuronide and BPA concentrations in the plasma were higher in neonates than in adults, except at 24 h postdosing, suggesting an immaturity in the development of hepatic excretory function in neonatal rats. 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 beta-glucuronidase activity and an absence of enterohepatic recirculation. A dose-dependent biotransformation of BPA in neonatal rats was also observed.

After the low dose of 1 mg/kg bw, BPA was almost completely metabolised to BPA-glucuronide which represented 94 – 100 % of plasma radioactivity. In plasma of neonatal rats administered 10 mg/kg bw of BPA, a number of additional small radioactive peaks (no structure available and no quantitation performed) were observed, indicative of minor metabolites of BPA in addition to the major peaks representing BPA and BPA-glucuronide. These data indicate that there is sufficient capacity for formation of BPA-glucuronide from BPA after exposure to low doses from early in neonatal life to efficiently metabolise BPA to the non-oestrogenic glucuronide in rats (Domoradzki *et al.*, 2004). At a higher dose of 10 mg/kg bw, glucuronidation of BPA may be saturated and BPA may undergo further biotransformation by other pathways.

After intravenous administration to pregnant rats, BPA was distributed extensively to the placenta and fetus, but the distribution into the amniotic fluid was low. The elimination of BPA from the placenta, fetus, and amniotic fluid paralleled that of the maternal serum during the terminal elimination phase (Shin *et al.*, 2002). After oral administration of ^{14}C-BPA (500 microg/kg bw) to dams or lactating rats, there was limited distribution of BPA-derived radioactivity to the fetus and neonate (Kurebayashi *et al.*, 2005). In summary, these data do not suggest a selective accumulation of BPA in the fetus of rats.

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 microg/kg bw) of ^{3}H-BPA in pregnant CD-1 mice (Zalko *et al.*, 2003) after subcutaneous (sc) injection. BPA was extensively metabolised in CD-1 mice. Identified metabolites included BPA-glucuronide as major metabolite, but also several double conjugates, and conjugated methoxylated derivatives. Fetal radioactivity (more than 4% of the administered radioactivity 24 hours after administration of BPA) was associated with free BPA, BPA-glucuronide, and a disaccharide conjugate. While these data suggest a more intensive biotransformation of BPA in CD-1 mice, which have been reported to be specifically sensitive to low-dose effects of BPA, 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 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 bisphenol A 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(p-hydroyphenyl)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).

Due to the highly efficient biotransformation of BPA in the gut wall and the liver after oral administration, other routes of administration will give a higher bioavailability of free BPA,

producing a potentially different toxicity profile, and they are thus considered not relevant for risk assessement of dietary exposure. Since dietary uptake is the relevant exposure scenario as a basis for this assessment, studies on effects of BPA in animals after oral administration are considered the most pertinent.

4.1.3 Toxicodynamic and toxicokinetic modelling

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 microg BPA/kg bw separated into three meals, peak concentrations of free (not bound to plasma proteins) BPA in blood were predicted as 3 pmol/L in a one year old child and as 3.7 pmol/L in 50 year old adults. Normalised for the oestrogenic activity of endogenous 17-ß-oestradiol, the highest increase in the oestrogenic activity induced by this dose of BPA was calculated to be 0.22% (Filser *et al.*, 2003) for 11-year old boys (lowest circulating 17-β-oestradiol levels).

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

Summary of toxicokinetics

In summary, there are major differences between humans and rodents in the toxicokinetics of BPA. After oral administration, BPA is rapidly absorbed from the gastrointestinal tract, metabolised in the gut wall and the liver to BPA-glucuronide. 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 ($t_{1/2}$ < 6 hours) and plasma protein binding, peak free BPA concentrations in humans after dietary exposure to BPA that are available for receptor binding are very low, even after worst case dietary exposures. In contrast, BPA-glucuronide is eliminated in bile in rodents and undergoes enterohepatic circulation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract. The enterohepatic circulation results in slow excretion and increased systemic availability of free BPA.

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 collected at intervals over 96 h (1 -4 % of applied dose, whereas BPA-glucuronide in urine accounted for 20-40 % of applied dose). In both of the human studies (Volkel *et al.*, 2002; Volkel *et al.*, 2005) and the monkey study

(Kurebayashi *et al.*, 2002), 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 primates with higher AUCs (area under the curve, i.e. concentrations over time) for free BPA in rats.

4.2 Mutagenicity and Carcinogenicity

BPA is not considered to be genotoxic in bacteria and in mammalian cells, and has also not been shown to increase the incidence of tumors in rats exposed for life to high doses of BPA by dietary administration. A number of reviews have concluded that BPA is not genotoxic or carcinogenic (EC, 2002; EU, 2003; Haighton *et al.*, 2002).

The effects of lactational or transplacental exposure to BPA on tumor incidence induced by established carcinogens was assessed in two studies using models for reproductive organ carcinogenicity. Modulating effects of BPA on prostate cancer incidence in male offspring exposed transplacentally and lactationally to BPA were assessed in female F344 rats administered 0, 0.05, 7.5, 30, and 120 mg BPA/kg/day by gavage during pregnancy and the lactation period (Ichihara *et al.*, 2003). When F1 males reached 5 weeks of age, they were given 10 subcutaneous injections of 3,2'-dimethyl-4-aminobiphenyl (DMAB) (21/group) or corn oil (the four groups dosed with BPA, n = 12/group) and were then sacrificed at week 60. There were no effects of BPA-administration on the accessory sex organ weights of male offspring. Transplacental and lactational exposure to BPA also did not affect the incidences of preneoplastic and neoplastic lesions in the accessory sex organs (prostate and seminal vesicle) of F1 rats and did not induce proliferating lesions.

Effects of maternal exposure to BPA on uterine carcinogenesis were studied in offspring of Donryu rats administered BPA (0, 0.006 and 6 mg/kg/day, n = 12, 15 and 19/group respectively) daily by gavage from gestation day 2 to postnatal day 21 (Yoshida *et al.*, 2004). BPA-administration did not exert an influence on uterine development including weight, gland genesis and oestrogen receptor alpha expression, vaginal opening and gonadotropin secretion in the female offspring up to puberty. After maturation, no effects were evident with regard to oestrous cyclicity in female offspring treated with BPA. In addition, the treatment had no effects on age-related morphological changes of the reproductive and endocrine organs and uterine carcinogenesis up to 15 months of age.

To investigate possible effects of BPA-exposure in a thyroid carcinogenesis model, 6-week-old female castrated F344 rats (12/group) were given a single subcutaneous injection of 2 000 mg/kg bw of N-bis(2-hydroxypropyl)nitrosamine. One week after injection, rats were fed with a basal diet; cholesterol pellets containing 0.5 mg 17-beta-oestradiol-3-benzoate (EB); or a diet with 1000 ppm methoxychlor (MXC) or 10,000 ppm BPA for 20 weeks. Furthermore, additional groups were administered 200 ppm sulfadimethoxine (SDM) in the drinking water. Thyroid follicular cell hyperplasias, adenomas and/or carcinomas were induced only in the EB+SDM group and the incidences of non-malignant lesions were significantly increased. No effects on thyroid proliferative lesions or on thyroid-stimulating hormone (TSH) levels were observed in the other groups. The results of this study indicate that EB, but not MXC and BPA, exerts promoting effects on thyroid carcinogenesis in rats (Takagi *et al.*, 2002).

Daily oral doses of BPA (20, 40 and 100 microg/kg bw, in corn oil for 6 – 8 days, or 20 microg/kg bw for 3, 5, or 7 days) were administered to female mice (exact numbers not given) to assess effects of BPA on meiosis, after observing increases in meiotic disturbances in oocytes of control female mice, which were attributed to leaching of BPA from damaged polycabonate cages used for animal housing (Hunt *et al.*, 2003). A controlled application of BPA was reported to show dose and duration of exposure-dependent effects of BPA on meiosis with statistical significance reached after 7 days. However, problems with the statistical evaluation, a possible inappropriate pooling of data, and generally weak effects of BPA do not permit a conclusive evaluation of this study. In addition, the Panel has been informed that the key findings of the study by Hunt *et al*. (2003) were not replicated in a repeat study in mice fed a low phytoestrogen diet. The results obtained did not suggest that BPA caused increased meiotic arrest, aberrant spindles or non-disjunction at low doses (Dr. U. Eichenlaub-Ritter, personal communication).

4.3 Developmental and reproductive toxicity

Reported low-dose effects of BPA in a number of different animal systems and on different reproductive or developmental endpoints and the inability to reproduce these effects in larger and statistically more powerful studies remain at the center of controversy. Publications reporting low-dose effects and the results and design of the studies attempting to confirm low-dose effects of BPA administration available before 2002 were summarised in the SCF evaluation of 2002 (EC, 2002). The comprehensive three-generation study on BPA in the rat, from which the overall NOAEL for reproductive toxicity in the SCF evaluation was derived, has now been published (Tyl *et al.*, 2002); the results from this study were, however, already available for the 2002 evaluation and the results are described in detail there. Moreover, detailed overviews of the results of studies on the reproductive and developmental toxicity of BPA published before 2002 are given by the European Chemicals Bureau RAR on BPA (EU, 2003).

During the time frame from the last evaluation of BPA by the SCF in 2002, further positive and negative results of low-dose BPA administration have been published and the controversy on possible low-dose effects of BPA in sensitive strains of rodents has continued. However, a recent two-generation reproductive toxicity evaluation of BPA in mice performed following a modified OECD-guideline (416) and under Good Laboratory Practice (GLP) did not confirm the presence of low dose effects (Tyl *et al*., 2006) as described below. Recent studies assessing the effects of low doses of BPA using pre- and postnatal administration on reproductive organ development and function published since the last evaluation by the SCF (2002) are also described below.

4.3.1. Reproductive and developmental studies in mice

A two-generation reproductive toxicity study was conducted (Tyl *et al*., 2006), following the current OECD test guideline 416 and performed under GLP. The OECD protocol includes paying special attention to the reproductive organs and attainment of puberty, and in this study the prostate was weighed not only in total but also as ventral and dorsolateral lobes separately. Groups of 6-week-old CD-1 mice (28/sex/group; designated the F0 generation) were exposed to BPA in the diet at 0 (2 groups), 0.018, 0.18, 1.0, 30, 300, or 3500 ppm BPA (estimated daily BPA doses were approximately 0, 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day, respectively) for an 8-week prebreed exposure period, a 2-week mating period, a 20-day gestation period, and a 3-week lactation period. At the weaning of offspring on postnatal day (PND) 21, 28 F1 offspring/sex/group were randomly selected for retention and were

similarly exposed through prebreed, mating, gestation, and lactation, with termination of the study and necropsy of F1 dams and F2 offspring at F2 weaning. In addition, 1 F1 male/litter was randomly selected at weaning to be retained for 3 months with exposure continuing, with necropsy, andrology, and histopathology concurrent with the F1 parental males. A positive control group exposed to dietary 17ß-oestradiol (E2, 0.5 ppm) resulting in E2 intake of app. 0.08 mg/kg bw/day, was included.

F0 and F1 parental males were necropsied at the end of the gestation of their F1 and F2 litters, respectively, with andrologic and histopathologic assessments. F0 and F1 females were assessed for oestrous cyclicity by daily vaginal smears for the last 3 weeks of their prebreed exposure periods and were necropsied at the weaning of their F1 and F2 litters, respectively, on PND 21, with stage of oestrus at demise, organ weights, ovarian primordial follicle counts, and histopathology. F1 and F2 offspring were evaluated for anogenital distance (AGD) at birth and on PND 21. The F1 offspring that were not retained for breeding of the F2 generation were examined for gross external abnormalities, euthanised, and discarded. Retained F1 postweanlings (parental and extra) were evaluated for acquisition of puberty, and these animals continued treatment throughout prebreeding (at least 8 weeks), mating, gestation, and lactation of F2 litters, as described above for the F0 animals (with F1 litters). At the weaning of the F2 pups, up to 3 F2 pups/sex/litter were randomly selected and examined for external and visceral alterations. The remaining F2 offspring were examined for gross external abnormalities, euthanised, and discarded. All weanling and adult necropsies included body weights, gross examination of all cavities and organs, weights of selected organs, and histopathology of selected organs for F0 and F1 adults and F1 and F2 weanlings.

There were no treatment-related effects on reproductive parameters for adults observed at any BPA dose. There were also no effects on mating or fertility, or for gestational, stillbirth, live birth, or survival indices for F0 or F1 parents, and no effects on postimplantation loss, number of pups/litter, or sex ratio (% males)/litter. After doses of 600 mg/kg bw/day, BPA induced systemic toxicity expressed as reduced body weights only in the F1 generation and increased kidney and liver weights in F0 and F1 adult males and females and in retained F1 males. In addition, treatment-related reductions in spleen (males and females) and testes weights were observed in the F1 and F2 weanlings. Treatment-related histopathologic findings in F0 and F1 adults were an increase in the incidences of minimal to mild centrilobular hepatocyte hypertrophy and minimal to mild nephropathy. An increased incidence of mild to minimal hypoplasia of the seminiferous tubules of the testis correlated with the reduction in testes weight in the F1 and F2 male weanlings. The only other developmental effect related to treatment with BPA at this dose in F1 offspring was delayed acquisition of preputial separation (PPS). After doses of 50 mg/kg bw/day of BPA, the only treatment-related effect observed was an increased incidence of centrilobular hepatocyte hypertrophy of minimal to mild severity in adult F0 and F1 males and F1 females. At all the lower BPA-doses (<50 mg/kg bw/day), no treatment-related effects were observed.

For the E2 positive control group, a clear sensitivity of the mouse strain used to oestrogen treatment was demonstrated by findings of reduced gestational index, increased stillbirth index, and reduced live birth index, reduced litter sizes, acceleration of puberty (earlier vaginal opening) in F1 females, delay in PPS in F1 males, increased weights of the uterus plus cervix plus vagina in F0/F1 adults and F1/F2 weanlings, decreased testes and epididymal weights in F1/F2 male weanlings, and reduced AGD in F1/F2 males on PND 21 (but not PND 0). The F1 and F2 weanling males also exhibited an increased incidence of seminiferous tubule hypoplasia of the testis. The F1 and F2 weanling females exhibited increased

incidences (>90%) of vaginal epithelial keratinization and bilateral luminal dilatation of the uterine horns.

The overall NOAEL was 5 mg BPA/kg bw/day based on liver effects. The NOAEL for developmental toxicity was 50 mg BPA/kg bw/day and 600 mg BPA/kg bw/day for reproductive toxicity (Tyl *et al*., 2006).

A study exposed C57BL/6N and ICR mice to BPA orally at doses of 2, 20, or 200 microg/kg bw/day at various developmental stages (adulthood, shortly after weaning, and the embryonic/fetal stage) and did not report BPA exposure-related body changes in weight, in weights of reproductive organs (testes, epididymides, seminal vesicles), cauda epididymal sperm density, or histology of reproductive organs including the ventral prostate in either strain of mouse (Nagao *et al*., 2002). The positive control, 17-beta-oestradiol, induced effects on reproductive organ weights and histological changes in reproductive organs in oestrogen sensitive C57 mice, but not in ICR-mice.

Another study reported effects of oral BPA administration at low doses on prostate development in male mice as assessed by serial dissection and reconstruction and by PCNA-staining in offspring. Pregnant CD-1 mice (n = 5 - 6) were "fed" (dripping BPA dissolved in tocopherol-stripped corn oil into the mouth, exact dose consumed not given) targeted doses of 0.1 microg/kg bw/ day ethinyloestradiol, 0.1 microg/kg/day of diethylstilboestrol, and 10 microg/kg bw/ day BPA on gestation day (GD) 14-18 (Timms *et al.*, 2005). Fetuses were removed by Caesarean section on GD 19, intrauterine positioning was recorded, and one male fetus/litter that developed in utero between a male and a female fetus was selected for assessment of prostate morphology. Both ethinyloestradiol and BPA produced an increase in the number and size of dorsolateral prostate ducts (control, 53 + 6.7; ethinyloestradiol, 64 + 5.1; BPA, 74.1 + 5.1) and an overall increase in prostate duct volume (control, 25,921 + 3661 microm^2; ethinyloestradiol 46,795 + 3688; BPA 49,592 + 4790) in male mouse fetuses. Histochemical staining of sections with antibodies to proliferating cell nuclear antigen and mouse keratin 5 indicated that these increases may be due to a marked increase in proliferation of basal epithelial cells located in the primary ducts. The urethra was malformed in the colliculus region and was significantly constricted where it entered the bladder. High dose diethylstilboestrol (200 microg/kg/day, dosing schedule as above) completely inhibited dorsolateral prostate duct formation. The Panel noted that only a single dose level was used in this study and thus a dose-response for BPA was not assessed and prostate weights (absolute or relative) were not given.

A possible influence of BPA on fertility in Swiss mice was assessed after daily oral doses of 0, 5, 25 and 100 microg BPA/kg bw for 28 days to adult females (n = 15 per dose), which were then mated with sexually mature males (Al-Hiyasat *et al.*, 2004). Exposure to 25 and 100 microg/kg bw BPA resulted in increases in number of resorptions and significant increases in relative uterine weights. Relative ovarian weights were significantly increased at the highest dose of BPA. The Panel noted that a significant reduction in body weight was observed in BPA-treated dams and that there were high variations in uterine weights in the BPA groups. The Panel concluded that the study is not relevant to use for risk assessment.

A number of low-dose studies by other routes of exposure were also reported. Given the efficient first pass metabolism upon oral exposure non-oral exposure routes are considered to be less relevant for the present risk assessment. Nevertheless they are described in detail in Annex 2.

4.3.2 Reproductive organ and developmental studies in rats

A number of publications have also investigated effects of BPA exposure on reproductive and developmental parameters in the rat after exposure in utero during pregnancy, in the neonatal stage, during puberty and as adults using both conventional high-dose protocols and low doses.

The effects of BPA administration on body weight gain, oestrous cyclicity and plasma luteinizing hormone (LH) were assessed in offspring of female Sprague-Dawley rats exposed to BPA in drinking water from day 6 of pregnancy through the period of lactation (Rubin *et al.*, 2001). The Panel notes that doses were stated to be approximately 0.1 mg and 1.2 mg BPA/kg bw/day, 6 dams/dose, but it is likely that there was underestimation of exposure due to an assumed low water consumption. Offspring exposed to BPA in utero (n = 12 – 28 offspring/group, but only six dams treated) exhibited an increase in body weight. In addition, female offspring exposed perinatally to the higher dose of BPA exhibited altered patterns of oestrous cyclicity (changes not defined) and decreased levels of plasma LH in adulthood.

Pregnant female rats were exposed to BPA in drinking water at concentrations of 0, 0.2, 2, 20 and 200 mg/mL (estimated highest daily dose of 1.25 mg/kg bw/day based on a drinking water consumption of 25 mL/day) from gestational day 1 (GD1) to GD22 or 23 through 2 hours after parturition. The male pups were sacrificed 2 hours after birth (GD23) or Caesarean section (GD22). Maternal BPA exposure at the highest dose significantly reduced serum testosterone concentrations in pups two hours after birth, but not after delivery by Caesarean section on GD22 (Tanaka *et al*., 2006).

Another study administered BPA orally at a daily dose of 0.1 or 50 mg/kg bw/day or 0.2 mg/kg bw/day of 17-alpha-ethinyloestradiol (EE2), different vehicles were used for administration of BPA and EE2, treatment from GD 6 to day 21) to pregnant Sprague-Dawley rats and groups were treated sequentially (Schonfelder *et al.*, 2004). Female offspring were sacrificed in oestrus. Uterine morphologic changes as well as oestrogen receptor (ER) alpha and ER beta distribution and expression were measured by immunohistochemistry and Western blot analysis. Morphologic changes in the uterine epithelium of postpubertal offspring during oestrus were observed in the animals exposed to both BPA doses (the thickness of the total epithelium was significantly reduced). ER alpha expression was increased in the 50-mg BPA and EE2-treated group. In contrast, decreased ER beta expression in all BPA- and EE2-treated animals was observed. In addition, apparently from the same animals, morphological changes were observed in the vagina of postpubertal offspring and the full-length ER alpha was not expressed during oestrus in the vagina of female offspring exposed to either dose of BPA when compared to the control group, whereas full length ER alpha expression did not differ from the control group during the diestrus stage (Schonfelder *et al.*, 2002a). Testicular histology was assessed at 9-12 months of age in male offspring apparently from the same animals using the optical dissector for quantifying cell numbers (Wistuba *et al.*, 2003). Spermatogenesis was qualitatively normal in all groups. BPA increased Sertoli cell number per organ but not when expressed as per gram testis. EE2 did not affect cell number per organ but did affect numbers on a per gram testis basis due to a lowered testis weight. Administration of BPA resulted in minor increases in Sertoli cell numbers and had no effect on spermatogenesis. The results described above were taken from experiments where none of the control and BPA treatments were performed at the same time (as disclosed by the main author in NIEHS, 2001). The Panel considered that this casts doubt on the robustness of the observations and makes this study unsuitable for risk assessment.

BPA and nonylphenol (NP) were assessed for effects on endocrine/reproductive systems following transplacental and lactational exposure of rat offspring (Takagi *et al.*, 2004). BPA was administered in the diet at concentrations of 60, 600 and 3000 ppm (no food consumption given, estimated doses of 5, 50 and 250 mg/kg bw/day, 5–6 animals/group) to maternal Sprague-Dawley rats from GD 15 to PND 10. Ethinyloestradiol (EE) at 0.5 ppm (estimated dose 0.04 microg/kg bw/day) was used as positive control. During pregnancy and lactation, including the exposure period, a soy-free rodent diet was provided. Effects on endocrine/reproductive systems were evaluated by examining the anogenital distance, organ weights before puberty (PND 21, five males and five females, at least one male and one female/litter), onset of puberty, oestrous cyclicity, and organ weights and histopathology of adult endocrine organs (at 11 weeks of age, eight males and eight females, at least one male and one female/litter), as well as the volume of the sexually dimorphic nucleus of preoptic area. Both NP and BPA at high doses caused decreases in maternal body weights and retardation of offspring growth, but did not affect endocrine/reproductive endpoints in offspring. EE induced irreversible changes in estrous cyclicity and histopathology of ovaries and uterus of adult females.

Daily administration of BPA (doses of 0, 7.5, 120 mg/kg bw/day) to F344 female rats by gavage during pregnancy (n = 19 - 22/group) and the lactation period (n = 12 resp. 11/group) did not induce morphological abnormalities in the accessory sex organs of male offspring (5 to 10 per dose group examined). However, lowered numbers of sperm in the testis were found after 120 mg BPA/kg bw/day. In the second study, the same protocol with a higher number of male offspring was used, but no reduction in the sperm count was apparent (Yoshino *et al.*, 2002).

The reproductive toxicity of BPA was compared in mice and rats after different treatment schemes (Takahashi and Oishi, 2003). Male Wistar rats, SD rats, CD-1(ICR) mice and C57 mice (n = 8 per group, age of 28 days at beginning of exposure) were administered BPA in the diet at a level of 0 (control) and 0.25% for 8 weeks. Daily BPA doses were approximately 400 mg BPA/kg bw in mice and 210 mg BPA/kg bw in rats. No overt signs of general or reproductive toxicity were observed. Serum testosterone concentrations were not decreased in BPA-fed rats and mice.

The effects of BPA and nonylphenol on pubertal development in juvenile/peripubertal male Sprague-Dawley rats were assessed after exposure from PND 23-52/53 (Tan *et al.*, 2003). Two groups of rats (N = 12) were administered either 100 mg/kg body weight of nonylphenol or BPA by gavage. Another group of rats were administered orally with a mixture of 100 mg/kg body weight of nonylphenol and BPA. Vehicle only (Tween-80 with corn oil, 1:9; v/v) was administered to controls. Observations made included growth, age at preputial separation, thyroid, liver, testis and kidney weight and histology, epididymal and seminal vesicle plus coagulation gland weight. Nonylphenol and BPA caused delay in puberty onset as well as testicular damage; spermatogenesis was affected in most treated rats. BPA also caused the enlargement of the kidney and hydronephrosis. Administration of nonylphenol and BPA as a mixture caused less than additive effects. The Panel noted that only a single dose level was used in this study and thus a dose-response for BPA was not assessed.

Four experiments from the same laboratory assessed a possible reduction of sperm production in sexually mature rats by BPA (Ashby *et al.*, 2003). Daily BPA doses of 0.020 mg/kg bw, 2 mg/kg bw, or 200 mg/kg bw in corn oil (containing 6.5 % ethanol) were administered by gavage to adult Sprague-Dawley rats (n = 10/dose group) over PND 91-97. This experimental

design was applied to studies using different diets (RM3, Purina 5002, and CE2). The studies were terminated when the rats reached the age of 18 weeks. BPA did not induce effects on the reproductive parameters monitored at any dose level. The test protocol was adapted to include restriction of the number of animals per cage, removing bedding from the cages, and changing to the use of glass water bottles. A significant difference in daily sperm production was observed between the control groups of the first and the second study. As the change in diet from RM3 to Purina 5002 was the major difference between those two studies, a repeat of the second study was conducted. This, however, did not confirm the differences. In a fifth study, the sperm parameters for control animals maintained on the three different diets did not show significant differences.

To investigate anti-androgenic effects of BPA, a rodent Hershberger assay in immature male Sprague-Dawley rats was performed (Kim *et al.*, 2002). An androgen agonist, testosterone (0.4 mg/kg bw/day), was administered for 7 consecutive days by subcutaneous (s.c.) injection as a positive control. Additionally, a pure androgen antagonist, flutamide (1, 5, 10 mg/kg bw/dayday, orally) was co-administered with testosterone (0.4 mg/kg bw/day, s.c.). BPA was also administered orally (doses 10, 100, and 1 000 mg/kg bw, daily dosing) with or without testosterone (0.4 mg/kg per day, s.c.) for 7 consecutive days, each treatment group consisted of 10 animals. In the testosterone-treated groups, glans penis, seminal vesicles, ventral prostate, and levator ani plus bulbocavernosus muscles (LABC) weights were significantly increased compared with control. Flutamide dose-dependently inhibited the testosterone-induced re-growth of seminal vesicles, ventral prostate, and LABC, with a significant decrease at flutamide 1.0 mg/kg and above ($P<0.05$). Serum LH levels were also significantly increased (5 mg/kg and above, $P<0.05$), but no changes in serum testosterone levels. BPA did not exhibit androgenic or anti-androgenic activities.

Pregnant Sprague-Dawley (SD) and Alderley Park (Wistar-derived) rats were exposed by gavage during GD 6-21 to BPA (20 microg/kg bw/day, 100 microg/kg bw/day, or 50 mg/kg bw/day) or ethinylestradiol (EE; 200 microg/kg bw/day) (Tinwell *et al.*, 2002). The number of animals used for assessment varied between 23 and 53 from 6 to 7 litters. The sexual development of the derived pups was monitored until termination at PND 90-98. The endpoints evaluated were litter size and weight, anogenital distance at birth, day of vaginal opening, first oestrus and prepuce separation, weights of the liver, seminal vesicles, epididimydes, testes, ventral prostate, uterus, vagina, cervix and ovaries, and daily sperm production. Males were terminated at PND 90 and females at PND 98. The only statistically significant effects observed were a decrease in daily sperm production and an increase in the age of vaginal opening for the Alderley Park rats at the highest dose evaluated (50 mg BPA/kg bw/day). The dose of EE induced maternal toxicity, resulting in difficulties in interpretation.

Effects of genistein (GEN), BPA and p-tert-octylphenol (OCT) on uterine wet weight, thickness of the uterine epithelium, uterine gene expression of clusterin (CLU), and thickness of the vaginal epithelium were compared (Diel *et al.* 2004) in DA/Han (DA), Sprague-Dawley (SD) and Wistar (WIS) rats after repeated oral application. Rats were orally administered 5, 50 and 200 mg BPA/kg bw for 3 consecutive days. The uterotrophic response to treatment with BPA, OCT and GEN was similar in the three strains, and GEN was more potent as compared to OCT and BPA. This was confirmed by analysis of other biological endpoints, despite some differences in the magnitude of their response among strains and to distinct compounds. For instance, the uterus wet weight response to EE treatment indicated lower sensitivity of SD rats than that of DA and WIS rats, but this was not observed for

responses of the uterine or vaginal epithelium. Moreover, blood concentrations were assessed at the time of killing and related to biological responses: plasma levels of total and unconjugated BPA and GEN depended upon the dose administered and varied to some extent within treatment groups and among the three rat strains. There was no correlation between individual compound concentrations analysed 24 h after the last dose and the uterotrophic wet weights in the three strains.

In a series of experiments, effects of BPA on testicular steroidogenesis was assessed after *in utero* exposure of Long-Evans rats to BPA. Dose-response was only partially investigated and histology was not assessed. Doses of 2.4, 10, 100 000 and 200 000 microg BPA/kg bw/day were given by gavage (n = 10 -12/group) in oil from PND 21-35 (Akingbemi *et al.*, 2004). BPA suppressed serum LH (from 0.5 to 0.25 ng/ml) and testosterone (from 2.5 to 1.8 ng/ml) levels at the lowest dose only. BPA also decreased serum 17-beta-oestradiol levels (from 0.3 ng/ml in controls to between 0.21 and 0.28 ng/ml, effect not clearly dose-dependent) in rats exposed to 2.4, 10 and 100 000 microg BPA/kg bw/day. Leydig cells isolated from BPA-treated animals were exposed to BPA (0.01, 0.1, 1, 10. 100, and 1 000 nM) and a decreased testosterone biosynthesis (by 25%) was only reported at a concentration of 0.01 nM BPA; diethylstilboestrol (identical concentrations) reduced testosterone production by approximately 30 % at all concentrations tested. No consistent effects on reproductive parameters were observed in adult rats after perinatal and chronic postnatal exposures to 2.4 microg BPA/kg bw/day. Relative seminal vesicle weights, but not prostate weights, were decreased by BPA. Exposures of pregnant and nursing dams, i.e. from GD 12 to PND 21, decreased testosterone levels in the testicular interstitial fluid in male offspring at adulthood from 420 + 34 ng/mL in controls to 261 + 22 ng/mL in BPA-exposed groups. The Panel noted that data were pooled from two experiments and numbers of dams treated were not given.

Effects of exposure to BPA through the placenta and milk on the reproductive system in male offspring were assessed. Pregnant Sprague-Dawley rats were gavaged with BPA at 0, 4, 40 and 400 mg/kg body weight, from GD 6 through lactation day 20 (Watanabe *et al.*, 2003). Plasma testosterone concentrations in offspring at 9 weeks old were significantly higher in BPA groups as compared with those of the control. Little alteration in testes weight was seen in BPA-exposed offspring. There was no change in plasma concentrations of luteinizing hormone and follicle-stimulating hormone at 9 weeks old. The pathway of E2 (17-beta-oestradiol) formation from testosterone seemed not to be affected by BPA.

The effects of a 90-day dietary administration of BPA and some other hormonally active compounds on an oestrogen-regulated fat depot, on serum TSH, T3, T4, LH, and lipid concentrations were assessed in ovariectomized Sprague-Dawley rats (n = 12 per treatment group) (Seidlova-Wuttke *et al.*, 2005). Daily BPA doses were reported as 33 and 333 microg/kg bw, but are not entirely clear from the description of the study protocol. According to the manuscript, BPA-administration increased the paratibial fat depot and decreased serum leptin concentrations (effects are described as “negligible”), but did not influence serum triglycerides, LDL, HDL, cholesterol, TSH, T4, T3 and LH.

A number of low-dose studies by other routes of exposure were also reported. Given the efficient first pass metabolism upon oral exposure non oral exposure routes are considered to be less relevant for the present risk assessment. Nevertheless they are described in detail in Annex 2.

4.4 Gene expression

A number of studies have addressed changes in the expression of several genes after BPA-treatment in several target organs for BPA (Funabashi *et al.*, 2004; Funabashi *et al.*, 2003; Masutomi *et al.*, 2004b; Naciff *et al.*, 2002; Ramos *et al.*, 2003; Seidlova-Wuttke *et al.*, 2004; Thuillier *et al.*, 2003). Some of the studies reported pronounced effects; however, inadequate reporting, absence of positive controls or dose-response, inappropriate routes of administration, and lack of information of the relationship between the changes in gene expression and possible adverse effects do not permit a conclusive evaluation. For example, intracerebellar injection of BPA (given as 3 microL of 10^{-12} to 10^{-14} molar solutions of BPA injected, no doses given and unclear description) stimulated ERK1/2 signaling in rat brain within 6 min after injection (Zsarnovszky *et al.*, 2005).

The uterotrophic activity of BPA (administration by gavage) to immature Wistar-derived rats was evaluated over the dose range from 2 microg to 800 mg/kg bw/day (Ashby and Odum, 2004). Expression levels of three oestrogen responsive uterine genes were determined using real-time RT-PCR - namely, complement component 3, lipocalin 2, and PR. 18S rRNA and RNA polymerase II large subunit acted as control genes. Observations of gene expression were made 4 h and 72 h after the first of three daily oral administrations of BPA. Increases in gene expression were observed over the uterotrophic dose range (approximately 200-800 mg BPA/kg bw/day). Over the dose range 2 microg to 20 mg/kg bw BPA there was no uterotrophic response and no increase in oestrogene responsive uterine gene expression. BPA did not produce reproducible changes in gene expression in the uterus of immature rats at dose levels that were not also uterotrophic.

A well-reported study compared the effects of transplacental exposure to BPA on gene expression in rat testes over a wide dose range with those of ethinyloestradiol and genistein. Dams were dosed from GD 11 to 20 with 0.002, 0.2, 0.5, 50 or 400 mg BPA/kg bw by sc injection and sacrificed two hours after the last dosing. Gene expression in fetal testes and epididymis and histology were assessed and no changes in histology were induced by the doses of BPA applied. Moreover, doses of BPA below 0.5 mg/kg bw/day did not induce gene expression changes (Naciff *et al.*, 2005) and monotonic dose-response curves were observed for all three agents investigated.

4.5 Behavioural effects of BPA administration

Effects of low dose BPA exposures during gestation on several aspects of hormone-dependent behavioural endpoints were investigated by several authors in mice and rats.

Suzuki *et al.* (2003) assessed effects of prenatal and neonatal exposure to BPA in ddY-mice on the enhancement of the dopamine D1 receptor-dependent rewarding effect induced by a psychostimulant methamphetamine. Adult female mice were administered BPA in food at concentrations of 0.002, 0.5 and 2 mg/kg food from mating to weaning. Markedly enhanced hyperlocomotion and its sensitisation induced by methamphetamine were reported after treatment with BPA. Chronic exposure to BPA produced an up-regulation of dopamine D1 receptor function to activate G-protein in the mouse limbic forebrain. Additionally, chronic BPA exposure produced a significant increase in levels of the dopamine D1 receptor mRNA in the whole brain. In contrast, no change in protein levels of methamphetamine-targeted proteins, dopamine transporter or the type 2 vesicle monoamine transporter in the brain was

observed by prenatal and neonatal exposure to BPA. The Panel noted however, that food consumption data were not reported.

The effects of exposure of female CD-1 mice to BPA during fetal life and/or in adulthood during the last part of pregnancy on subsequent maternal behaviour was assessed (Palanza *et al.*, 2002). Pregnant females were orally adminstered (dripping into the mouth) daily doses of corn oil (n = 14, controls) or targeted doses of 10 microg BPA/kg body weight during GD 14-18 (n = 9). As adults, the prenatally treated female offspring were time-mated and again fed either corn oil (controls, n = 51) or the same doses of BPA on GD 14-18 (n = 31). Maternal behaviour was observed on PND 2-15 and reflex responses were examined in the offspring. Dams exposed to BPA either as fetuses or in adulthood spent less time nursing their pups and more time out of the nest compared with the control group. Females exposed to BPA both as fetuses and in adulthood did not significantly differ from controls. No alterations in postnatal reflex development were observed in the offspring of the females exposed to BPA.

The effect of fetal exposure to BPA on aggressive behaviour and hormonal changes in mice was assessed in male offspring (Kawai *et al.*, 2003). On GD 11-17, female CD-1 mice (10/group) were "fed" (dripping BPA dissolved in oil into the mouth, exact dose consumed not given) BPA in corn oil at doses of 0, 2 or 20 microg/kg bw/day. Aggression rating and blood sampling of the offspring were done at 8, 12, and 16 weeks of age. Aggression scores increased significantly ($p < 0.01$) at 8 weeks of age in male mice exposed to BPA at both the 2 and 20 microg/kg bw/day doses compared with a control group, but no difference was found after 12 weeks. Relative testis weight (per gram of body weight) was significantly lower at 8 and 12 weeks in mice (n = 8 – 14) treated with 2 microg/kg bw/day than in controls ($p < 0.05$) and was significantly lower at 12 weeks in mice treated with 20 microg/kg bw/day than in controls ($p < 0.01$). The Panel noted that the changes in relative testes weight were small (ranging from approximately 6.2 to 7.8 mg/g) and not dose-related; serum testosterone concentration in treated mice was not significantly different from that in controls.

The effect of prenatal and neonatal exposure to BPA on functional changes in dopamine DS3 receptors was investigated in mice after oral administration of BPA in the diet (0 and 2000 mg/kg feed, equivalent to an approximate dose of more then 250 mg/kg/day to dams from mating to weaning (Mizuo *et al.* 2004). Prenatal and neonatal exposure to BPA resulted in the attenuation of dopamine D3 receptor-mediated G-protein activation by 7-OH-DPAT in the mouse limbic forebrain. This treatment also caused a significant decrease in the B(max) value of PD128907, a dopamine D3 receptor ligand, in this area. No change in dopamine D3 receptor mRNA expression in the limbic forebrain and lower midbrain was observed by prenatal and neonatal exposure to BPA. The Panel noted that only a single dose level was used and thus a dose-response for BPA was not assessed. Furthermore, information on the high-dose treatment on other parameters indicative of reproductive toxicity and data on food consumption were absent.

Effects of perinatal maternal exposure to BPA (oral gavage of 4, 40, and 400 mg BPA/kg bw/day in olive oil from GD 10 to PND 20) on the behaviour of offspring in F344 rats was investigated (Negishi *et al.*, 2003). A positive control was not included into the study design. Perinatal BPA exposure caused a dose-dependent reduction in body weight gain in male and female offspring. Spontaneous activity analyses revealed that BPA elongated immobile time during the dark phase in female offspring. At 4 weeks of age, male offspring exposed to BPA at 40 and 400 mg/kg bw/day performed avoidance responses at a significantly higher rate in the shuttlebox avoidance test. At 8 weeks of age, however, male offspring only at 4 mg/kg

bw/day showed significantly lower responses. In the open-field behaviour test at 8 weeks of age, male offspring exposed to BPA at 4 mg/kg bw/day showed a higher percent of grooming than the control male offspring. The same author (Negishi *et al.*, 2004) also assessed behavioural alterations in the male offspring after exposure to BPA (0.1 mg/kg bw/day orally in corn oil) and nonylphenol (NP; 0.1 mg/kg bw/day and 10 mg/kg bw/day orally) administered daily from GD 3 to PND 20) to female F344 rats, again without a positive control. Neither BPA nor NP exposure affected behavioural characteristics in an open-field test (8 weeks of age), in a measurement of spontaneous motor activity (12 weeks of age), or in an elevated plus-maze test (14 weeks of age). A passive avoidance test (13 weeks of age) showed that both BPA- and NP-treated offspring tended to delay entry into a dark compartment. An active avoidance test at 15 weeks of age revealed that BPA-treated offspring showed significantly fewer avoidance responses and low-dose NP-treated offspring exhibited slightly fewer avoidance responses. Furthermore, BPA-treated offspring showed a significant increase in the number of failures to avoid electrical unconditioned stimuli within 5-sec electrical shock presentation compared with the control offspring. In a monoamine-disruption test using 5 mg/kg (intraperitoneal) tranylcypromine (Tcy), a monoamine oxidase inhibitor, both BPA-treated and low-dose NP-treated offspring at 22-24 weeks of age failed to show a significant increment in locomotion in response to Tcy.

The long-term effects of perinatal exposure to BPA on later behaviour in adult Sprague-Dawley rats of both sexes has been studied. BPA or vehicle was administered orally to dams from mating to weaning, at a daily oral dose (40 microg/kg bw/day from mating to weaning, by feeding oil solution into the mouth, n = 9 pregnant animals) (Adriani *et al.*, 2003). The offspring of both sexes (one/litter) were tested at adolescence (PND 35-45) for novelty preference (experiment 1). After a 3-day familiarisation to one side of a two-chamber apparatus, on day 4 rats were allowed to freely explore the whole apparatus. BPA-exposed females spent significantly less time than did controls in exploration of the novel side (i.e., increased neophobia), whereas no effect was found in the male group. At adulthood, the same animals were food-deprived and tested for profiles of impulsive behaviour in operant chambers provided with two nose-poking holes (delivering either five or one food pellet). After the establishment of a baseline preference for the large reinforcer, a delay was introduced before the delivery of the five food pellets, which was progressively increased each day (10, 20, 30, 45, 60, 80, 100 sec). All animals exhibited a progressive shift toward the immediate but smaller reinforcer. A reduced level of impulsive behaviour (i.e., a shift to the right in the intolerance-delay curve) was evidenced in BPA-treated rats. The frequency of inadequate responding (during the length of the delay) also provided a measure of restless behaviour. The profile of BPA-treated males was feminised, strongly resembling that of control females. Animals were then tested (experiment 3) for the response to an amphetamine challenge (1 mg/kg bw, sc). The drug-induced increment activity was significantly less marked in BPA-treated male rats compared with controls.

The effect of BPA (0.1 and 1 mg/L in drinking water, approximate doses of 30 and 300 microg/kg bw/day, from conception to PND 21) on the sexual differentiation of open-field behaviour and the sexually dimorphic nuclei in the brain in the offspring of Wistar rats exposed during the fetal and suckling periods was assessed (Kubo *et al.*, 2003). BPA abolished the sex differences of the open-field behaviour and inverted the sex differences in the locus ceruleus (LC) volume, without affecting the reproductive system. Diethylstilboestrol affected the open-field behaviour, LC volume and reproductive system, while resveratrol affected the LC volume and the reproductive system.

Agonistic and sexual behavior of adult female and male Sprague-Dawley rats was assessed after administering one dose level of BPA (40 microg/kg bw/day, "feeding" by pipette into the mouth) to mothers from mating to weaning (Farabollini *et al.*, 2002) or 400 microg/kg bw/day BPA from GD 14 to PND 6 (Dessi-Fulgheri *et al.*, 2002). An intruder test revealed an increase in defensive behaviour due to the low dose of BPA in males, but not in females. Male sexual orientation toward a stimulus female was not affected by BPA, whereas the sexual activity test revealed a slight impairment of sexual performance due to BPA in terms of latency and frequency of intromissions. In females, BPA produced a small increase in sexual motivation and receptive behaviour. A reduction of mating, social interest and immature behaviour was observed after the higher dose.

In summary, BPA at low doses given during gestation and/or lactation is reported to cause effects on some of the behavioural endpoints assessed. Overall, however, there were no consistent treatment-related effects in the behavioural endpoints and apparently contradictory observations were published. For example, neophobia was found as an effect in one study (Adriani *et al.*, 2003) in females and not in males. In other studies (Negishi *et al.*, 2003), no effect was found in the open field (which should show an effect if neophobia is present) in male offspring, and BPA was also reported to abolish and invert the sexual differentiation in the open field (Kubo *et al.*, 2003). Moreover, the Panel noted the absence of positive controls, use of test paradigms which are not widely used, lack of assessment of dose-response in several studies, partial lack of information on blinding of investigators to status of animals, and insufficient information on food consumption in some studies. The neurobehavioural database reveals that there are no consistent adverse effects of perinatal exposures to doses of BPA below 50 mg/kg/day. The reported influence of low doses of BPA on the sex difference in morphometric measurements of the locus coeruleus should be considered as a preliminary finding that needs to be repeated in a larger study, with the litter as the experimental unit, blinded evaluation and comparison to historical control data.

4.6 Hormonal activities and other effects of bisphenol A *in vitro*

The weak oestrogenicity of BPA has been known for a long time. Some new results regarding oestrogen receptor subtype confirmed previous observations of a higher affinity of BPA to the oestrogen receptor beta as compared to the oestrogen receptor alpha. The relative binding affinity of BPA to the oestrogen receptor alpha is 3 to 4 orders of magnitude below that of the reference compound 17-beta-oestradiol. The affinity of BPA to the oestrogen receptor beta is approximately 40-fold lower than that of 17-beta-oestradiol (Seidlova-Wuttke *et al.*, 2005).

BPA has also been shown to have a weak antagonism to thyroid hormone receptors with affinities four to five orders of magnitude below that of triiodothyronine (Moriyama *et al.*, 2002). BPA also may weakly interfere with different steps in androgen receptor function *in vitro* (Lee *et al.*, 2003). However, BPA showed no androgenic/antiandrogenic effects in rats *in vivo* (Kim *et al.*, 2002) and reduced steroidogenesis only at the lowest of three doses given to rats *in vivo*.

Several studies have assessed effects of BPA on calcium homeostasis in different cell types in culture. BPA (in nanomolar to picomolar concentrations) influenced calcium homeostasis, sometimes giving inverted U-shape dose-response curves. However, the relevance of these *in-*

vitro observations in specific cellular systems for the assessment of adverse effects of BPA *in vivo* is unclear (Alonso-Magdalena *et al.*, 2006; Quesada *et al.*, 2002; Walsh *et al.*, 2005; Wozniak *et al.*, 2005).

DISCUSSION

The Panel considered that, given the efficient first pass metabolism upon oral exposure, studies using non-oral exposure routes are less relevant for the present dietary risk assessment.

There is general agreement that high doses of chemicals with hormonal activities may have effects on human reproduction and may cause reproductive toxicity (Witorsch, 2002a). BPA has been shown to have oestrogenic activity, both in *in vitro* test systems and *in vivo* in rodent uterotrophic assays. However, the oestrogenic potency of BPA relative to that of oestradiol has been shown to be weak. Non-monotonic dose-response curves have been observed for potent oestrogens such as diethylstilboestrol in the low-dose range (0.01 to 0.1 microg/kg bw/day) in some studies (Newbold *et al.*, 2004), but not in others (Lobenkofer *et al.*, 2004). The issues of possible effects of low doses of chemicals with weak endocrine activities on sensitive species of rodents and the implications of these possible effects for human health risk assessment is still debated. A number of issues require consideration when evaluating low dose effects of BPA in rodents for human health risk assessment.

Robustness and reproducibility of low dose effects

Low-dose effects on specific biological endpoints have been reported in some studies, but were not replicated in others. A number of papers have been published reporting BPA-induced behavioural or reproductive toxic effects at low doses, but with either only one dose level being investigated, or with absence of any dose-response relationship where several dose levels have been used. Many studies also used only small numbers of animals/dose group. For studies to be used for risk assessment purposes adequate numbers of animals are required to take into account individual variability of responses, and an adequate range of doses needs to be included to demonstrate dose-response relationship. With regard to the claimed non-monotonic dose response for BPA, the presence of a response at one only dose level does not necessarily indicate a causal relationship between the administration of BPA and that effect. To demonstrate U-shaped dose–responses, it is necessary to have reasonably closely spaced dose intervals.

The results of the studies reporting low-dose effects are in contrast to the results of studies using protocols developed for reproductive toxicity studies designed according to internationally recognised guidelines and performed in compliance with Good Laboratory Practice (GLP). None of these studies, including a recent two-generation reproductive toxicity study using a mouse strain with demonstrated sensitivity to reproductive toxicity of low doses of 17ß-estradiol under the study conditions, showed evidence of low-dose effects of BPA in rodents (down to 0.003 mg/kg bw/day by oral exposure). Moreover, a number of other studies applying low doses of BPA were also unable to demonstrate low-dose effects of BPA. It has been argued that failure of the GLP-studies with larger numbers of animals to obtain evidence of low-dose effects of BPA may be due to a high phytoestrogen content of the diet used in these studies, absence of positive controls, and/or the use of oestrogen-insensitive animal models (such as the Sprague-Dawley rat) (vom Saal *et al.*, 2005). However, some

publications have reported low-dose effects of BPA or oestrogens in the Sprague-Dawley rat strain or other "less oestrogen-sensitive" strains of rodents (Adriani *et al.*, 2003; Ashby, 2003; Honma *et al.*, 2002; Putz *et al.*, 2001a; Putz *et al.*, 2001b; Rubin *et al.*, 2001; Schonfelder *et al.*, 2002a; Schonfelder *et al.*, 2004). It has also been noted that the content of phytoestrogens in the diets used in the studies with different outcomes is not widely different (Ashby *et al.*, 2003; Degen *et al.*, 2002; Owens and Chaney, 2005) and effects on prostate tissue architecture were reported in CD-1 mice kept on a soy-based diet and exposed to low doses of BPA in utero (Timms *et al.*, 2005), suggesting that the dietary content of phytoestrogens in such studies is probably of very limited relevance.

There are several possible confounders, which may modify the outcome of a study addressing possible low-dose effects of BPA, and failure to control for these confounders may also have contributed to the inconsistent database. The possible confounders include animal model and strain used, study design, animal handling and housing, failure to use concurrent controls and positive controls, biological variability, energy content of diet, statistical evaluation of data, and intrauterine positioning (Ashby *et al.*, 2004; Elswick *et al.*, 2001; Elswick *et al.*, 2000; Everitt and Foster, 2004; Markaverich *et al.*, 2002; Masutomi *et al.*, 2004a; Milman *et al.*, 2002; Naciff *et al.*, 2004; Odum *et al.*, 2004; Thigpen *et al.*, 2003; vom Saal *et al.*, 2005).

Possible health significance of the changes reported after low-dose administration of BPA

The effects of BPA reported in highly sensitive animal systems represent small changes in organ weight or changes in tissue architecture, increased or decreased receptor expression, changes in hormone concentrations in plasma or tissues, small changes in the time required to attain puberty landmarks and behavioral effects. Moreover, the changes observed are often not sustained through adulthood. The pathophysiological consequences of the changes in the affected animals are unknown and some of the changes such as the small increases in prostate weights reported in some studies after BPA exposure *in utero*, are not considered as precursors of pathological changes (Milman *et al.,* 2002). As has been stated elsewhere (UK Committee on Toxicology, 2001), "*the effects (of low dose BPA administration) on reproductive organs may represent very sensitive intermediate biomarkers that indicate the need for further studies, but cannot be defined as "adverse". Overall the Committee concluded that it is not appropriate, at this time, to base human health risk assessment on these effects"*.

Toxicokinetics

The process of extrapolation of animal data to humans must also consider species differences in toxicokinetics and toxicodynamics: the available information on species differences between rodents and humans has implications for the extrapolation of results in rodents to humans in the risk assessment of BPA.

New data on the biotransformation and toxicokinetics in rodents and humans have demonstrated differences between the fate of BPA in humans and rodents. In humans and other primates, BPA given orally is rapidly transformed to BPA glucuronide during first pass metabolism in the gut wall and the liver. The BPA-glucuronide formed, which is devoid of endocrine activity, is rapidly excreted in urine resulting in a terminal elimination half-life of BPA of less than 6 hours, and in low oral bioavailability of free BPA in primates. In rats, orally administered BPA also predominantly undergoes glucuronidation, but the bisphenol A glucuronide formed is excreted from liver with bile into the gut. Enterohepatic circulation of

BPA due to cleavage of BPA-glucuronide in the gut and reabsorption of BPA from the intestine results in a slow elimination of BPA in rodents. Moreover, in urine of rats dosed orally with BPA, part of the dose was excreted as free BPA, which was below the limit of detection in all urine and blood samples in humans administered BPA. Since free BPA found in urine is translocated to urine from blood in the kidney, these observations and the much more rapid elimination of BPA in primates indicate major species differences in the blood levels and AUCs (area under the curve, i.e. concentrations over time) of free BPA between rodents and primates with much higher AUCs for free BPA in rats.

In mice BPA glucuronidation is also a major pathway of elimination, but in this species oxidation products of BPA have also been identified after low-dose subcutaneous administration, suggesting possible formation of metabolites with higher oestrogenic potency.

Toxicodynamics

The majority of low dose effects of BPA on gestational parameters have been reported in the mouse. Major species differences in the physiology of gestation between humans and mice exist however. These include: i) the role of the corpus luteum and its hormonal control in pregnancy; ii) sources and pathways of sex steroid production during gestation; and iii) the specific types of oestrogens secreted and the levels within the fetus attained throughout gestation. The much higher levels of oestradiol, oestrone and oestriol sustained by the human fetus as compared to the mouse fetus suggest a much lower sensitivity of humans as compared to rats or mice to possible additive effects of weak oestrogens such as BPA (Witorsch, 2002b).

CONCLUSIONS

The original observations of the weak oestrogenic activity of BPA have triggered a large number of toxicological studies. The present toxicology assessment focused on reproductive and endocrine system-related effects of BPA since these endpoints have been the center of controversy. Due to the relevance of the oral route for human exposures from food, effects seen in animals after oral dosing have been considered the most pertinent for the risk assessment.

The literature remains inconsistent with regard to strain and species sensitivity to low-dose effects on development and reproduction of BPA. The Panel considers that while low-dose effects and non-monotonic dose-response curves may be theoretically possible (Conolly and Lutz, 2004), low dose effects of BPA in rodents have not been demonstrated with the sufficient certainty to serve as pivotal studies for risk assessment. The more recent observations of species differences in toxicokinetics of BPA between primates, including humans, and rodents, and in particular the low bioavailability of BPA (free systemic BPA) in primates, further weaken the relevance of observations of low-dose effects of BPA in sensitive strains of rodents for human health risk assessment.

In reviewing the recently published studies on BPA the Panel concluded that while some oral studies did report differences between controls and treated animals at lower dose levels than the currently accepted overall No-Observed-Adverse-Effect Level, none of these effects were sufficiently well demonstrated to be used as pivotal effects for the risk assessment and to justify a revision of the TDI. Therefore, the Panel concluded that the NOAEL of 5 mg/kg bw/day, based on the results of a comprehensive three-generation study in rats and established in the SCF evaluation of 2002, remains valid and in the Panel's view is further supported by the NOAEL of 5 mg BPA/kg bw/day, based on liver effects, established in a recent two-generation reproductive toxicity study in mice. The NOAEL derived from the multi-generation study in rats was used by the SCF to derive a temporary TDI of 0.01 mg/kg bw, applying a 500-fold uncertainty factor (comprising 10 for interspecies differences, 10 for interindividual differences and 5 for the uncertainties in the database on reproductive and developmental toxicity).

The Panel's conclusions on BPA are based on the now available, extensive database on repeated-dose toxicity, reproductive and developmental toxicity of BPA in rodents and on the comparison of toxicokinetics in primates, including humans, and rodents. The Panel concluded that the new studies provide a basis for revising the uncertainty factors that were used by the SCF to derive the temporary TDI of 0.01 mg/kg bw in 2002. In particular, the Panel now considers that the database concerning reproduction and development has been considerably strengthened and that the additional uncertainty factor of 5, introduced by the SCF in 2002 for the uncertainties in the database on reproduction and development, is no longer required. The Panel also concluded, in view of the well described species differences in toxicokinetics, showing a low level of free BPA in humans compared with rats, that a default uncertainty factor of 100 applied to the overall NOAEL from the rodent studies can be considered as conservative. The Panel therefore established a full TDI of 0.05 mg BPA/kg bw, derived by applying a 100-fold uncertainty factor to the overall NOAEL of 5 mg/kg bw/day.

Dietary exposure assessments on BPA have been made by the Panel for adults, infants and children. The estimates of potential dietary exposure to BPA in infants took account of breast feeding, feeding formula using PC bottles and consumption of commercial foods and

beverages. The resulting exposure assessments ranged from 0.2 microg/kg bw/day in 3-month-old breastfed infants up to 13 microg/kg bw/day in 6-12–month-old infants. These estimates were based on conservative migration values of BPA and the 95th percentiles of consumption. The estimates of potential dietary exposure in young children and adults were respectively 5.3 and 1.5 microg/kg bw/day, based on conservative migration values of BPA and conservative estimates of consumption of commercial foods and beverages. The Panel noted that the conservative estimates of exposure were less than 30% of the TDI in all population groups considered. These exposure estimates include BPA migration into canned foods and into food in contact with PC table ware or storage receptacles. On the other hand, they do not include either potential migration of BPA from receptacles into food during microwave heating or potential migration of BPA into drinking water due to the use of PC and of epoxy-phenolic resins in water pipes and in water storage tanks. Information on potential migration of BPA from these sources would be useful.

The Panel noted that exposure assessed from urinary excretion, measured in groups of subjects from the general population in the USA, Japan and Korea, indicate overall average daily BPA exposures in adults of up to 10 microg BPA per adult (0.16 microg BPA/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 0.04 to 0.08 microg BPA/kg bw/day for adults. The discrepancy between the levels of exposure estimated through urinary biomarkers and the levels of exposure estimated above by combining food consumption data with BPA concentrations in the diet is likely to be due to the conservative assumptions made.

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ANNEX 1. REPORTED CONCENTRATIONS OF BPA IN PLASMA OR URINE OF HUMAN SUBJECTS

Table 1. Reported plasma or blood concentrations of bisphenol A in human subjects without known specific exposures to BPA

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

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

Table 2. Reported urine concentrations of bisphenol A in human subjects without known specific exposures to BPA

Reference and sampling region	Analytical method sample workup	No. of samples analyzed	Concentration ranges reported	Comments
(Arakawa *et al.* 2004) Japan	GC/MS/MS, glucuronidase treatment, solvent extraction followed by solid phase extraction	Samples from 5 health adults, on 5 consecutive days, in addition, 24h urine samples from 36 male subjects	< 0.58 to 13 microg BPA/day (median of 1.3 microg/day) in five subjects observed over 5 days; < 0.21 microg BPA/day to 14 microg BPA/day for the 36 other subjects (median of 1.2 microg BPA/day	Detection limit 0.38 microg BPA/L of urine, no information on contamination of solvents or leaching of BPA
(Matsumoto *et al.* 2003) Japan	HPLC with fluorescence detection, glucuronidase treatment, solvent extraction	Morning spot urine from 46 male and 4 female students,	Up to 30 microg BPA/g creatinine /app. 18 microg BPA/L, 39 % of samples collected in 1999 were below LOD of 1,7 microg BPA/g creatinine (1 microg BPA/L)	Single trace method, no information on contamination
(Hanaoka *et al.* 2002) Japan	HPLC with electrochemical detection, glucuronidase treatment followed by protein precipitation,	Spot urine samples from 42 individuals without intentional BPA-exposure, 42 males exposed to BADGE	BPA > 1 micromol/mol creatinine in controls (< app. 1.2 microg BPA/L), no further details	Single trace method, no information on contamination.
(Kim *et al.* 2003) Korea	HPLC with fluorescence detection, separate assessments with and without glucuronidase treatment, solvent extraction;	Spot urine samples collected from 15 healthy men and 15 healthy women	BPA from 0.28 to 2.36 microg/L (mean 0.58 + 0.14) in males and 0.068 – 1.65 microg/L (mean 0.56 + 0.1) in females; BPA-glucuronide from 0.16 to 11.67 microg/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 microg/L (mean 0.49 + 0.27) in males and < LOD to 3.4 (mean 1.2 + 0.32) in females	Single trace method, no information on contamination,
(Ye *et al.* 2005) United States	LC/MS/MS with column switching, glucuronidase treatment,	30 spot urine samples from adults	Mean conc. of 3.5 microg BPA/L, (95 percentile of 11.5 microg BPA/L)	LOD of 0.4 microg/L, reagent blank gives response of app. 0.1 microg BPA/L
(Yang *et al.* 2003) Korea	HPLC with fluorescence detection; glucuronidase treatment, solvent extraction	Morning spot urine from 34 adult males and 39 adult females,	Geometric mean of 8.91 + 8.32 microg BPA/L	LOD of 0.34 microg/L, no background
(Kawaguchi *et al.* 2005) Japan	GC/MS with EI ionization, thermal desorption; glucuronidase treatment, sorptive extraction after derivatisation	Urine samples from 5 health subjects, no further information	Range from < LOD to 5.41 microg BPA/L	LOD 0.1 microg/L, reagent background not detailed

(Mao *et al.* 2004) China	HPLC after fluorophore derivatisation with p-nitrobenzoyl chloride; acid hydrolysis to cleave conjugates followed by solid phase extraction	10 healthy male and 10 healthy female subjects, no information on urine collection	Range from < LOD to 3.95 mg BPA/L, mean 1.22 + 1.38 mg BPA/L;	LOD 2.7 microg BPA/L, but poorly resolved peak for BPA, peak assignment on retention time only, very high concentrations of endogenous hormones indicted by assay suggest systematic error in evaluation
(Kuklenyik *et al.* 2003) United States	GC/MS with chemical ionization and electrophore derivatisation, negative ion detection; glucuronidase treatment and extractive derivatisation	30 urine samples from individuals painting houses and 6 unexposed individuals used as controls	Quantitative evaluation of BPA levels in urine of unexposed controls not detailed, based on graphic presentation estimated as below 2 microg/L	LOD of 0.1 microg BPA/L, no information on BPA-contamination of blanks
(Calafat *et al.* 2005) United States	GC/MS with chemical ionization and electrophore derivatisation, negative ion detection; glucuronidase treatment and extractive derivatisation	Spot urine samples from 394 adults in the US, collected at different times of the day	Geometric mean of 1.21 microg BPA/L for urban and of 1.56 microg BPA/L for rural residents	LOD of 0.1 microg BPA/L; no information on BPA-contamination of blanks
(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 microg BPA/L; BPA-glucuronide detected in all samples with concentrations from 0.2 to 19.1 microg BPA-gluc/L (median of 1.2 micro/L)	Background of 0.26 microg BPA-glucuronide/L
(Volkel *et al.* 2005) Germany	LC/MS/MS with and without glucuronidase treatment	Randomly collected urine samples from 7 males and 12 females without known BPA-exposure	All samples below LOD of 1.14 microg BPA/L	LOD 1.14 microg BPA/L, background below LOD after method adjustment
(Ye *et al.* 2005) United States	LC/MS/MS with column switching, separate analysis for free BPA without enzymatic hydrolysis, after glucuronidase and after sulfatase treatment	Randomly collected urine samples from 30 adult individuals without known BPA-exposure	Means for free BPA were < LOD, for BPA-glucuronide 3.1 microg/L, BPA-sulphate 0.5 microg/L	LOD 0.3 microg BPA/L
(Fukuta *et al.* 2006) Japan	HPLC with electrochemical detection for total and free BPA; BPA-glucuronide concentrations in some samples confirmed by LC/MS-MS	Randomly collected urine samples from 21 male and 31 female subjects, age 22 – 51 years	2 samples showed free BPA (0.24 and 0.35 microg/L); mean of total BPA was 1.92 + 1.99 microg/L ELISA kits gave total BPA concentrations of 15.9 + 9.9; 16.7 + 19.5; and 18.6 + 23.7 microg/L	LOD 0.5 microg BPA/L for HPLC

ANNEX 2. DESCRIPTION OF RECENT LOW DOSE STUDIES BY NON-ORAL ROUTES OF EXPOSURE

Given the efficient first pass metabolism following oral exposure, non-oral exposure routes are considered to be less relevant for the present risk assessment. Nevertheless, recent low-dose studies by non-oral routes have been reviewed by the Panel and are described here for completeness.

Developmental and reproductive toxicity

Reproductive and developmental studies in mice

Inconsistent low-dose effects after non-oral administration of BPA were also reported. One study compared the effects of genistein (GEN), resveratrol (RES), zearalenone (ZEA), BPA and diethylstilboestrol (DES) on reproductive and mammary gland development in female CD-1 mice (Nikaido *et al.*, 2004). Beginning on GD 15, pregnant mice (n = 6) were administered 0.5 or 10 mg/kg/day GEN, RES, ZEA or BPA, and 0.5 or 10 microg/kg/day of DES by daily subcutaneous injection for four consecutive days. Vaginal opening was monitored, 6 animals per group of offspring were autopsied at 4, 8, 12 and 16 weeks of age and oestrous cyclicity was monitored from 9 to 11 weeks of age. Maternal exposure to BPA did not accelerate puberty onset or modify the oestrous cycle. Mammary gland differentiation was accelerated in mice after BPA at 4 weeks of age, According to the publication, mice treated with GEN, RES, BPA or DES spent more time in diestrus; but BPA did not induce statistically significant changes. The Panel noted that a statistical evaluation of the BPA effects on the mammary gland was not performed. Moreover, a publication from the same author apparently using the same protocol reported absence of BPA-effects on mammary gland and estrous cycle when given as 4 daily subcutaneous injections (dose of 10 mg BPA/kg bw/day) to female CD-1 mice beginning at 15 days of age. (Nikaido *et al.*, 2005).

In a series of publications, the effects of perinatal exposure to BPA (25 and 250 ng BPA/kg bw/day, but reported as 25 and 250 microg/kg bw/day), administered sc by Alzet mini-pumps from day 9 of pregnancy for 14 days through postnatal day 4, n = 6 -10 per group) on the peripubertal development of the mammary gland, the genital tract and on brain sexual differentiation was investigated in CD-1 mice (Markey *et al.*, 2001, 2005; Munoz-de-Toro *et al.*, 2005, Rubin *et al.*, 2006). BPA exposure enhanced the mammary gland sensitivity to oestradiol in ovariectomized CD-1 mice. In their intact 30-day-old littermates, the area and numbers of terminal end buds relative to the gland ductal area increased while their apoptotic activity decreased. There was a positive correlation between ductal length and the age at first proestrus. The age at first proestrus was reduced by BPA. A significant increase of progesterone receptor-positive ductal epithelial cells localised in clusters was also reported in BPA-treated animals. Lateral branching was significantly enhanced at 4 months of age in mice exposed to 25 ng BPA /kg bw/day. A decreased wet weight of the vagina, small increases in the incorporation of bromodeoxyuridine into the DNA of endometrial gland epithelial cells, and increased expression of oestrogen receptor-alpha (ERalpha) and progesterone receptor in the luminal epithelium of the endometrium and subepithelial stroma were reported in BPA-exposed animals. Changes were in the range of 20 to 40 % of control values, but positive controls and controls without Alzet pumps were not included and the selection of animals for assessment was based on oestrous cyclicity. Apparently, results on other parameters determined in this animal study were reported separately. The panel noted

absence of dose-response for many changes reported or the evaluation of samples for only one dose level

Low-dose effects of BPA on sexual maturation and reproduction of offspring were also investigated (Honma *et al.*, 2002) after sc injection of BPA (2 and 20 microg/kg), diethylstilboestrol (DES; 0.02, 0.2, and 2 microg/kg), or oil vehicle once per day from GD 11-17 into dams (10 mated mice/group). For both female and male offspring (F1), body weights were measured on postnatal day (PND) 0 (the day of birth), 11, 22, and 60, and anogenital distance was measured on PNDs 22 and 60. Pups were weaned at PND 22 and males were caged separately from females. Vaginal smears were taken daily beginning the day of vaginal opening for 30 days. The age at vaginal opening was significantly earlier (reduced from 27.3 in controls to 26.2 days) in females exposed to 20 microg/kg BPA in utero and all DES-exposed animals (25 days at the 2 microg/kg bw dose). Body weight at vaginal opening was lower than controls (23.5 g) in all exposed females (21.5 in both BPA-groups). The first vaginal oestrus was earlier (from app. 27.8 to 27.0 days) in females exposed to 20 microg/kg BPA and all DES groups. From PND 90 to 120, gestationally exposed F1 female mice were mated with unexposed males. Total numbers of pups and sex ratio in F1 mice exposed to BPA or DES, and those of their offspring (F2) were not different from controls in all treatment groups.

BPA given at higher doses also influenced some parameters indicative of pre- and postnatal development of the reproductive organs in female ICR-mice. BPA or diethylstilboestrol (DES) were given by sc injections to pregnant mice (number not given) during GD 10-18 (Suzuki *et al.*, 2002). Some offspring (2 or 3 pups per litter) treated prenatally with 10 and 100 mgBPA/kg bw or 0.67 and 67 microg DES/kg bw were ovariectomized at 30 days and sacrificed at 40 days of age. Vaginal smears were examined in the remaining offspring, then these offspring were mated with normal males. Prenatal exposure to 10 mg BPA/kg bw reduced the number of mice with corpora lutea (4 %) compared to controls (7 %) at 30 days, but more than 80% of mice from either prenatally exposed BPA group were fertile at 90 days. Mice exposed prenatally to maternal doses of 67 microg DES/kg bw were sterile and showed ovary-independent vaginal and uterine epithelial stratification. Mice exposed prenatally to BPA did not show ovary-independent vaginal and uterine changes. The number of offspring and litter sex ratio from mice exposed prenatally to BPA (10 or 100 mg/kg bw) or 0.67 microg DES/kg bw were not different compared to controls. In a postnatal treatment group, female mice were given sc injections of BPA (15 or 150 microg/pup) or DES (0.3 or 3 microg/pup) for 5 days from the day of birth, then some mice were ovariectomized at 30 days and examined at 40 and 90 days. In the remaining mice, vaginal smears were examined from 61 to 90 days and ovarian histology was evaluated at 90 days. Mice exposed postnatally to 150 microg BPA (approximate dose of 30 mg/kg bw) exhibited ovary-independent vaginal epithelial stratification. Postnatal DES (0.3 and 3 microg/pup) treatment also induced ovary-independent vaginal stratification. Polyovular follicles having more than one oocyte in a follicle were induced by postnatal injections of BPA (150 microg/pup) or DES (0.3 or 3 microg/pup) at 30 days.

Effects of non-oral BPA administration on sperm quality in mice were also investigated in several studies. BPA administered at daily doses of 0.5 and 50 microg BPA/day/animal (app. dose 0.3 and 30 mg/kg bw, subcutaneous injection) during the first 5 days after birth caused a decrease in the percentage of moving sperm (from app. 50 % in controls to 25 % in the high dose BPA group), and an increase in the incidence of malformed sperm was reported in the

epididymides of mice at 10 weeks of age (Aikawa *et al.*, 2004). No marked changes in the testicular histology of BPA-treated mice were reported. Effects of 50 microg of BPA were ameliorated by the concurrent administration of 100 IU of retinol acetate. Neonatal treatment with 0.5 microg of BPA for 5 days resulted in an increase in the incidence of malformed sperm, whereas the BPA effect became more severe in mice nursed by mothers fed a vitamin A-deficient diet before and after parturition.

BPA (0, 20 or 200 microg/kg bw/day, n = 12/group) was administered sc to adult ICR mice and Wistar rats for 6 days to assess effects on spermiogenesis (Toyama *et al.*, 2004). Effects assessment on the testes by electron and light microscopy revealed a number of abnormalities in the spermatids and effects on the ectoplasmic specialization between the Sertoli cell and spermatids. Rats and mice responded similarly to BPA. The ectoplasmic specialization between adjoining Sertoli cells, or blood-testis barrier, was not affected. Animals kept for an additional two months after cessation of the administration were shown to be fertile and the testes showed normal histology, indicating that the effects were transitory.

The same authors (Toyama and Yuasa, 2004) assessed effects of BPA exposure in newborn animals (subcutaneous injection of 0.1, 1, 5 and 10 microg/pup in ICR mice, n = 3 - 4 per treatment group, approximately 0.6 mg/kg bw to 66 mg/kg bw; and 1, 10, 100 and 600 microg/pup, approximately 0.2 mg/kg bw to 120 mg/kg bw in Wistar rats, n = 3 - 4 per treatment group) on spermiogenesis later in life. Testes were examined by light and electron microscopy at 15 weeks of age. 17-beta-oestradiol (E2) or beta-oestradiol-3-benzoate (E2B) were used as positive controls. BPA, E2, and E2B had similar effects on testes. When treated animals reached puberty and spermiogenesis began, the first sign of the effects was detected in the steps 2-3 spermatids and the acrosomal granule and nucleus were deformed. Ectoplasmic specialization between the Sertoli cell and spermatids was also affected. Fully matured animals did not show effects in the testes, and the animals were fertile.

Several studies have assessed the effects of BPA on the presence of hormone receptors and their expression in mouse tissues. In summary, these studies reported increased expression of several nuclear receptors (Imanishi *et al.*, 2003; Nishizawa *et al.*, 2003; Takao *et al.*, 2003) in reproductive organs in mice after low-dose treatment with BPA; however, the evaluation of these studies is hampered by inadequate reporting of exposure conditions.

Reproductive and developmental studies in rats

Subcutaneous injection of BPA to Wistar rats at a dose of 200 mg/kg bw/day for 4 weeks (n=5, age 28 days at start of dosing) significantly decreased the body weight, testis, epididymis, prostate and seminal vesicle weights, and the testicular daily sperm production. Intraperitoneal injection of BPA at a dose of 20 mg/kg bw/day for 4 weeks (n=5, age 28 days at start of dosing) decreased the prostate weight, but not the testis or epididymis weights, and decreased serum testosterone.

Neonatal male Sprague-Dawley rats were given subcutaneous injection of BPA (targeted doses were 0.002, 0.011, 0.056, 0.277 and 97 mg/kg bw/day) or 17beta-estradiol (E2) at a daily dose of 0.9 mg/kg bw/day from PND 0 to PND 9. Groups of animals were sacrificed at PND10, 35 and 150. Preputial separation, copulatory rate, fertility rate, sperm analysis, serum testosterone levels, and gene expression in the testis were assessed. Males in the E2 group showed a decrease in testis weight and alterations of estrogen-mediated gene expression in the testis on PND 10, and by PND 150 incomplete preputial separation, decreases in the

copulatory rate, testicular and accessory organ weights and number of sperm. Males in all BPA groups showed normal reproductive parameters. (Kato *et al.*, 2006).

Pregnant Wistar rats were exposed to BPA (25 µg/kg body weight-bw-/day) or to vehicle by implated osmotic pumps from GD 8 to GD 23. Female offspring were sacrificed at postnatal day (PND) 30, 50, 110 or 180. On PND 50 a group of rats received a single dose of NMU (25 mg/kg) and were sacrificed at either PND 110 or 180. At puberty, animals exposed to BPA showed an increased proliferation/apoptosis ratio both in the epithelial and stromal compartments. During adulthood, BPA exposed animals showed an increased number of hyperplastic ducts and augmented stromal nuclear density. Moreover, the stroma associated with hyperplastic ducts showed signs of desmoplasia and contained an increased number of mast cells, suggesting a heightened risk of neoplastic transformation. Administration of a subcarcinogenic dose of NMU to animals exposed prenatally to BPA increased the percentage of hyperplastic ducts and induced the development of neoplastic lesions. (Durando *et al.*, 2006). The Panel noted that mammary gland development studies in rodents may not represent useful models for humans due to significant differences in the hormonal milieu.
Effects of BPA on reproductive organs of female offspring were investigated after administration of BPA (0.25, 1, or 4 mg/pup, highest dose estimated as app. 200 mg BPA/kg bw, corn oil as vehicle, n = 5 per dose group) given subcutaneously during the neonatal period from post-natal days (PND) 0 to 9 (Kato *et al.*, 2003). Daily doses of 10 microg 17-beta-oestradiol (E2) with the same application scheme were used as positive controls. Animals ovariectomized at 80 days were given subcutaneous injections of 1 microg/kg E2 for 3 days from PND 94 to 96. Clefts in the clitoris, early vaginal opening, irregular estrous cycles, a decrease in the area occupied by the corpora lutea (CL) in the ovary, and multiple cystic follicles in the ovary were found in the animals treated neonatally with doses above 1 mg BPA/pup. Uterine fluid weight measured after E2 treatment on PND 94-96 was less than controls. In addition to these abnormalities, unusual body weight gains, persistent vaginal cornification, and lack of CL were observed in females treated neonatally with the high dose of 4 mg BPA/pup. The ovary weight on PND 80 and uterine fluid weight measured after E2 treatment on PND 94-96 were less than controls for the 4 mg BPA group. Neonatal treatment with 10 microg E2 induced similar effects as found in the 4 mg BPA group.

The effect of neonatal administration of BPA on reproductive functions in Sprague-Dawley rats was assessed after subcutaneous application of BPA (daily doses were 0, 2, 11, 56, 277, and 9 700 microg/kg bw) once daily from PND 0 to PND 9 (eight males/dose group). Reproductive functions were assessed in groups of males sacrificed at PND 10, 35, and 150 and 17-beta-oestradiol (0.9 mg/kg, sc, same treatment scheme as used for BPA). Administration of BPA did not affect preputial separation, copulatory rate, fertility rate sperm counts, serum testosterone levels, or gene expression in testes, while estradiol induced effects on most of the parameters assessed (Kato *et al.*, 2006).

The effect of neonatal treatment with BPA (app. 50 mg/kg sc, daily on PND 2 to 12, n = 3 to 6/group) on final Leydig cell number per testis in Wistar rats was evaluated (Sharpe *et al.*, 2003). Leydig (3-beta-hydroxysteroid dehydrogenase immunopositive) cell development and function (plasma testosterone levels) were studied through puberty into adulthood. Treatment with the positive control GnRHa impaired testis growth, Leydig cell (nuclear) volume per testis and testosterone levels during puberty, when compared with controls, but final Leydig cell volume/number in adulthood was comparable with controls. As adult testis weight was reduced by 45% in GnRHa-treated rats, the percentage Leydig cell volume per testis was

approximately double ($p < 0.01$) that in controls, and also at day 35. Testosterone levels in adulthood in GnRHa-treated rats were lower ($p < 0.01$) than in controls but were within the lower end of the normal range. Treatment with DES caused a dose-dependent suppression of testes growth, Leydig cell (nuclear) volume per testis and testosterone levels up to day 35. Although by adulthood, Leydig cell volume/number per testis was comparable with controls in DES-treated rats, testosterone levels remained reduced. Neonatal treatment with either BPA or OP had little consistent effect on any of the parameters studied except that both treatments significantly elevated testosterone levels on day 18, as did treatment with DES.

Other endpoints

In adult mice given single subcutaneous doses of 10 microg BPA/kg bw of either E2 or BPA induced a rapid decrease in glycemia that correlated with a rise of plasma insulin. Longer exposures to E2 and BPA induce an increase in pancreatic beta-cell insulin content in an oestrogen-receptor-dependent manner (Alonso-Magdalena et al., 2006). The Panel noted that effects suggesting an interference of BPA with pancreatic function has not been reported in any of the repeated-dose studies on BPA using wide-dose ranges of BPA and oral administration.

ABBREVIATIONS

PD128907	Specific dopamine D3 receptor
7-OH-DPAT	7-hydroxy-N,N-di-n-propyl-2-aminotetralin
AFC	Scientific Panel on Food Additive, Flavourings, Processing Aids and Materials in Contact with Food
AUC	area under the curve
B(max)	Concentration resulting in maximum receptor binding
BADGE	bisphenol A diglycidyl ether
bis-DMA	bisphenol A dimethacrylate
bis-GMA	bisphenol A glycidyl methacrylate
BPA	bisphenol A
bw	Body weight
CAS No.	Chemical Abstract Service Number
CE2	Specific rat diet
CEC	Commission of the European Communities
CL	corpora lutea
CLU	clusterin
CSL	Central Science Laboratory
D	Dalton
D1	Dopamine receptor D1
DA	DA/Han
DES	diethylstilboestrol
DMAB	3,2'-dimethyl-4-aminobiphenyl
E2	17-beta-oestradiol
E2B	beta-oestradiol-3-benzoate
EB	17-beta-oestradiol-3-benzoate
EC	European Commission
EE	Ethinyloestradiol
EE2	17-alpha-ethinyloestradiol
EEC	European Economic Community
EFSA	European Food Safety Authority
ER	oestrogen receptor
ERalpha	oestrogen receptor-alpha
ERK1/2	Extracellular Signal Regulated Mitogen-Activated Kinase 1
EU	European Union
F1	1 generation of offspring
FCM	Food Contact Material
FSA	Food Safety Authority
GD	gestational day
GEN	genistein

GLP	Good Laboratory Practice
GnRHa	Gonadotropin-Releasing Hormone antagonist
h	hour
HDL	High density lipoprotein
LABC	levator ani plus bulbocavernosus muscles
LC	locus ceruleus
LDL	Low density lipoprotein
LH	luteinizing hormone
LOD	Limit of detection
LOQ	Limit of quantitation
microg	microgram
mL	millilitre
mRNA	Messenger Ribonucleic Acid
MW	molecular weight
MXC	methoxychlor
n	number
n.a.	not available
n.r.	not reported
NMU	Nitrosomethyl urea
NOAEL	No-Observed-Adverse-Effect Level
NP	nonylphenol
OCT	p-tert-octylphenol
OECD	Organisation for Economic Co-operation and Development
OP	ocytlphenol
PBPK model	physiologically based pharmacokinetic model
PC; non-PC	polycarbonate; non-polycarbonate
PND	postnatal day
ppm	parts per million
PPS	preputial separation
PVC	polyvinyl chloride
RAR	Risk Assessment Report
RES	resveratrol
RM3	specific rat diet
RT-PCR	reverse transcription-polymerase chain reaction
s.c.	subcutaneous
SCF	Scientific Committee on Food
SD	Sprague-Dawley
SDM	sulfadimethoxine
T3	triiodothyronine
T4	thyroxine

Tcy	tranylcypromine
TDI	Tolerable Daily Intake
TSH	thyroid-stimulating hormone
UK	United Kingdom
USA	United States of America
v/v	Volume/volume
WIS	Wistar
ZEA	zearalenone