EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

Scientific Committee on Food

SCF/CS/PM/3936 Final
3 May 2002

Opinion of the Scientific Committee on Food on Bisphenol A

(Expressed on 17 April 2002)

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http://europa.eu.int/comm/food/fs/sc/scf/index_en.html

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Terms of reference

The Committee is asked to re-evaluate the hazards to human health arising from the migration of bisphenol A present in certain plastic materials and articles intended to come into contact with foodstuffs.

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, as an antioxidant in PVC plastics and as an inhibitor of end polymerisation in PVC. Polycarbonates are used in food contact materials such as returnable beverage bottles, infant feeding bottles, tableware (plates and mugs) and storage containers. Epoxy resins are used in protective linings for food and beverage cans and vats.

BPA was first evaluated in 1986 by the Scientific Committee for Food (SCF) for use in plastic materials and articles intended to come into contact with foodstuffs. At that time, the Committee allocated a Tolerable Daily Intake (TDI) of 0.05 mg/kg bw [1]. This was based on 90-day and long-term oral studies in rats and mice, in which BPA was given via the diet. These showed an overall no-observed-adverse-effect level (NOAEL) of 25 mg/kg bw/day for effects on body weight, taken from the 90-day rat study, to which was applied an uncertainty factor of 500 because of the incomplete database.

The substance has now been re-evaluated in the light of new information, much of which has been published in the last 5 years. For the majority of studies reviewed below, full published papers were available. Much of the data has also been reviewed elsewhere [2-5]. In a few instances, information was available from unpublished study reports. Some of the most recent information is available only from abstracts of conference proceedings. While it is not the usual practice of this Committee to include summary information published only in abstracts in a hazard assessment, descriptions of their findings have been included on this occasion for completeness and relevant results have been considered, but abstract reports have not been used as a basis to derive a numerical TDI.

Estimates of intake

The published studies on migration of BPA from materials and articles used in contact with foodstuffs, have been summarised and discussed elsewhere [5]. A large number of studies have been conducted, although many have used food simulants as model foods

because of the difficulties of measuring low concentrations of BPA in foodstuffs. However, modern methods of analysis are now capable of making these measurements reliably, and recent experimental and survey data have been published. The numerous individual studies cited in the recent EU review [5] will not be listed here, but the following are exemplars of this literature from which the Committee has made realistic worst-case estimates of exposure.

Coatings for beverage and food cans

BPA is used as a starting substance to make epoxy-phenolic resins as the internal lacquer for food and beverage cans. In none of the several studies in which canned alcoholic or soft cold drinks were investigated was any BPA detected [5]. Migration into foods processed and stored in cans is also generally low.

In a study designed to simulate or exaggerate the most severe conditions of use, the simulants 10% ethanol or 95% ethanol were used to represent aqueous and fatty foodstuffs respectively, and high temperature treatment, up to 120^{o}C for 2 hours, was used to simulate pasteurisation of can contents. Levels of BPA in the simulants ranged from non-detectable up to 94 μg/kg [5].

A recent UK Food Standards Agency survey [5,6] has analysed samples of canned vegetables, beverages, fish in aqueous media, soup, desserts, infant formulae, fruit, pasta and meat products, purchased from different retail outlets. The limit of detection in foods/beverages was 2 μg/kg and the limit of quantification was 7 μg/kg. BPA was detected in 36 of the 62 samples of foods at levels up to 70 μg/kg, results which are similar to those reported in other studies on migration into actual foods. Values of 350-420 μg/kg were found in one sample (a meat product). No BPA was detected in beverages or infant formulae. Based on the mean contaminant levels (around 20 μg/kg) found in this survey, the intake of BPA for the 97.5th percentile consumer of canned foods (eating 1.05 kg/day for adults and 0.38 kg/day for infants) was estimated to be 0.37 μg/kg bw/day and 0.85 μg/kg bw/day for 60 kg adults and 8.8 kg infants respectively.

Polycarbonate bottles and other articles

BPA is widely used as a monomer for polycarbonate plastics. Polycarbonates are used to make baby feeding bottles, water bottles, jugs, beakers and microwave ovenware. Many migration studies have been conducted world-wide. There is no significant effect from repeated-use, abrasion, heating, or chemical sterilisation of these plastic articles. The general findings are that migration is low or not detectable, typically in the range <10 to 20 μg/kg for water, fruit juices and infant formulae [5]. In tests using simulants, the migration of BPA can be higher than into foodstuffs. In a European-wide survey [5], migration from bottles into food simulants was not detectable at <3 μg/kg. Applying aggressive extraction conditions using 95% ethanol with shaking at 60ºC for 24 hours, the migration was generally not detectable (<10 μg/kg) and only a few of the 163 bottles tested released BPA at over 20 μg/kg; the highest release was 110 μg/kg. However these

extraction conditions are considered unrepresentative of actual use, since no migration was detected when simulants representative of the normal bottle contents (water or 3% acetic acid) were used. Taking a more realistic worst-case migration level of 10 μg/kg and the highest ratio of food intake to body weight for infants (a 4.5 kg infant consuming 0.7 litre of formula each day), an intake of 1.6 μg/kg bw/day can be estimated.

A number of studies on polycarbonate tableware and food storage containers have shown some migration up to 5 μg/kg into food simulants, but no detectable migration into actual foods or beverages [5].

Coatings for wine storage vats

This food contact application of epoxy-phenolic resins gave the highest intake estimate in the recent assessment [5], at 500 μg/day for adults. Thus, it is a potentially important source of exposure to consider. However, this worst-case estimate can be improved. In 3 series of tests, resins were coated at 100 μm thickness onto inert substrates, cured, and then tested with wine simulants. In series 1 the resin was coated onto glass and cured as recommended by the manufacturer. Migration after 4 years was 30 to 160 mg BPA released per kg resin. In series 2, where the quantity of hardener used to cure the resin was varied to be more or less than the recommended amount, a lower level of 1 to 13 mg BPA per kg resin was released into the simulants after 1 year of contact. In series 3 the resin was prepared and cured as in series 1 but it was applied to an aluminium support rather than to glass; the release of bisphenol A was in the range 0.7 to 1.8 mg per kg resin after 3 years contact with simulants. It was noted by the authors that the resin was applied manually onto the glass support in series 1 and 2, and that after up to 4 years of exposure to the simulants the resin showed signs of physical degradation. It became dull and broken (fractured) with only poor to medium adhesion. By contrast, the resin was applied mechanically to the support in series 3 and no such problems of resin fracture were recorded. Consequently, it is considered that the results from series 1 are unrepresentative because they were not reproduced in series 2 and 3 and the test specimens were technically unsuitable for the long duration of contact when applied manually to a glass support. Taking a release value of 10 mg BPA per kg resin after 1-3 years of contact, and calculating that 1.4 g resin would be used to coat the inside of a 1,500 litre vat with the same coating thickness of 100 μm as studied in the laboratory experiments, then a worst-case estimate of BPA migration into wine is 9 μg/kg. For a wine consumption of 0.75 litre/day the estimated intake of BPA for a 60 kg adult would be 0.11 μg/kg bw/day.

Overall estimates of intake of BPA

The table below summarises the intake estimates for different age groups. The Committee considers these to be realistic worst-case estimates. They are based on acceptable data from studies of migration into actual foods and beverages under normal conditions of processing, storage and use, apart from the estimates for wine, for which only tests with simulants were available. It should be noted that these estimates of intake are around an order of magnitude lower than those adopted in the other recent review [5],

in which worst-case estimates based mostly on highest values observed in foods or food simulants in any study were used and a consumption of 2kg of canned food per day was assumed for young children.

Consumer group	Type of food	Amount consumed/day	Concentration BPA (µg/kg)	Intake estimate (µg/kg bw/day)
Infant 0-4 mo 4.5 kg	Formula	0.7 litre	10	1.6
Infant 6-12 mo 8.8 kg	Formula	0.7 litre	10	0.8
Infant 6-12 mo 8.8 kg	Canned food	0.38 kg	c.20	0.85
Child 4-6 years 18 kg	Canned food	1.05 kg	c.20	1.2
Adult 60 kg	Canned food	1.05 kg	c.20	0.37
Adult 60 kg	Wine	0.75 litre	9	0.11

Summary of toxicity data

Absorption, distribution, metabolism and excretion

The toxicokinetics of BPA has only been studied to any extent in the rat [5,7-12]. Absorption from the gastrointestinal tract is rapid, but systemic bioavailability of the parent compound and its metabolites via this route is lower than following subcutaneous (sc) or intraperitoneal (ip) routes of administration. The proportion of parent compound found in the blood at its maximum concentration (Cmax) following oral administration is only 2-8% of the administered dose, compared with 27-51% following ip injection or 65-76% following sc injection. Following oral administration, up to 83% of the dose is excreted in the faeces within 72h, of which 86-93% is excreted as parent compound. The remainder is excreted in the urine, largely as BPA glucuronide. Very low amounts are retained in the tissues (0.03-0.26% after 7 days) following oral administration. BPA undergoes extensive first pass metabolism in the liver, the main metabolite being the monoglucuronide conjugate of BPA. Other unconjugated metabolites have been detected in smaller amounts but have not been identified. BPA can be transferred across the placenta to the rat fetus, but concentrations are much lower than in the mother [13-15]. BPA glucuronide is transferred to pups in rat maternal milk, but again only in very small amounts [10].

Unpublished comparative studies, using *in vitro* liver microsome preparations from rat, mouse and human, have been summarised elsewhere [Sipes 2001, cited in 5]. They indicate that metabolic clearance of BPA by the liver is higher in male mice (3.0 ml/min/mg of protein), comparable in male and female rats and female mice (1.0 -1.9 ml/min/mg), and lower in both sexes of humans (0.3 - 0.9 ml/min/mg) and in rat fetuses on gestation day 19 (0.7 - 0.9 ml/min/mg). Although the metabolic clearance rate is

slower in humans than in rats or mice, because of liver size the capacity of the liver to metabolise BPA was calculated to be higher in humans (8 mmol/hr) and lower in rats (46 -80 μmol/hr) and mice (14 -24 μmol/hr). Studies using primary cultures of hepatocytes indicate that BPA glucuronide is the major metabolite in the rat, mouse and human [Pritchett, 2001, cited in 5]. A single study in human volunteers, described in abstract only, has confirmed there is absorption of BPA from the gastrointestinal tract, with rapid clearance from blood (half-life 3.5h) [12]. BPA has also been reported in an abstract to be detectable in the human placenta [16].

Short-term and long-term oral toxicity studies

BPA is of low acute oral toxicity, with LD_{50} values >2g/kg bw [2-5]. In subacute, subchronic and chronic toxicity studies with BPA in rats, mice and dogs [2-5,17], rats appear to be the most sensitive species, showing reduced weight gain at doses from 50 mg/kg bw/day or more in repeat-dose dietary studies, and at 160 mg/kg bw in gavage studies. The NOAEL for reduced weight gain in rats when BPA was administered via the diet was 25 mg/kg bw/day [17].

In mice the main effect was induction of multinucleated giant cells in the liver, seen at doses of 143 mg/kg bw and above in a chronic study, with no NOAEL established [2-5,17].

Carcinogenicity and genotoxicity

There was no evidence of substance-related carcinogenicity in 2-year rat and mouse bioassays using dietary administration [17]. A similar conclusion was reached in other recent reviews [2-5].

BPA has been tested extensively for genotoxicity, both *in vitro* and *in vivo* [2-5,18]. The majority of *in vitro* studies, which included tests for gene mutations in bacteria, chromosomal aberrations in cultured mammalian cells and gene mutations in cultured mammalian cells, were clearly negative, with a few producing equivocal results that were not replicated in other studies using the same test system. *In vivo* studies, including a mouse micronucleus test conducted to modern standards and a rat dominant lethal study, were negative. Low levels of DNA adduct formation were observed *in vitro* and *in vivo* but the covalent binding index was only 0.01. *In vitro* tests for aneuploidy were positive at doses close to or causing cytotoxicity, but the absence of micronuclei in the *in vivo* mouse micronucleus test provides some reassurance that the aneugenic potential observed *in vitro* is not expressed *in vivo*. Recent extensive reviews [2-5] have all concluded that BPA is non-genotoxic *in vivo*.

Developmental toxicity, reproduction and endocrine activity

In conventional developmental toxicity studies in rats and mice [19-22], BPA was not teratogenic and no effects were seen on embryonic or fetal growth, survival or development, except for resorptions and reduced fetal weight at maternally toxic doses of

1250 mg/kg bw/day (mouse) and 1000 mg/kg bw/day or more (rat), doses that also caused some maternal deaths.

Two one-generation reproduction studies have been conducted in rats with dietary administration of BPA using conventional protocols [23,24]. Two later multigeneration studies in rats, a three-generation dietary study [25] and a two-generation oral gavage study [26], have used enhanced protocols specifically designed to pick up endocrine disrupter effects. In addition to the usual parameters, they included measurement of anogenital distance, acquisition of puberty (preputial separation, vaginal opening), oestrous cyclicity and sperm parameters. In one study [25] nipple retention in males was also assessed. In the other study [26] timing of testis descent and serum hormone levels (FSH, LH, testosterone, oestradiol, TSH, T3 and T4) were also measured. The doses used in these four reproduction studies ranged from 0.2 μg/kg bw/day to 950 mg/kg bw/day. The most recent, comprehensive, three-generation study employed six dose levels ranging from 1 μg/kg bw/day to 500 mg/kg bw/day [25]. There were no effects on fertility or reproduction, except in the three-generation study [25], in which a significant reduction in total and live pups per litter at birth was observed in every generation at the highest dose of 500 mg/kg bw/day, a dose which also caused adult systemic toxicity. The most sensitive effects were on body weight gain. In the three-generation study [25], significant dose-related reductions in adult body weights were seen in all parental generations at 50 and 500 mg/kg bw/day. In the offspring, at 500 mg/kg bw/day in all three generations there were significant reductions in pre-weaning and weaning body weights and in some absolute organ weights at weaning. At 50 mg/kg bw/day there was a significant reduction in body weight at weaning in F3 males and significant reductions in some organ weights at weaning in F1, F2 and F3 males and in F3 females. The NOAEL for any effect in rat reproduction studies was 5 mg/kg bw/day, taken from this recent three generation study.

In a continuous breeding study in mice, adverse effects on fertility together with systemic toxicity were seen at dietary BPA doses of 600 mg/kg bw and above, with a NOAEL of 438 mg/kg bw/day [27]. In a four-generation mouse study, adverse effects on fertility, testis pathology and spermatogenesis have been reported at 2 and 200 μg/kg bw/day from BPA in the drinking water, but these findings have been published in abstract only [28].

BPA is oestrogenic both *in vitro* and *in vivo*. *In vitro* studies utilising various test systems, mainly a human breast cancer cell line, MCF-7 cells, have shown that BPA both binds to and activates oestrogen receptors, including human ERα and ERβ, but that it is a weak oestrogen. BPA has an affinity/activity about 3-5 orders of magnitude weaker than oestradiol-17β, on a molar basis [29-56]. Dose-related interference by BPA with protein binding of endogenous oestradiol to human sex steroid binding globulin has been shown *in vitro*, although the binding affinity constant for BPA was low [122,123]. Clear evidence of *in vivo* oestrogenic activity of BPA comes not only from positive uterotrophic responses (increased uterine wet weight) in immature or mature ovariectomised rodents [52-75], but also from studies showing induction of vaginal cornification and vaginal cell proliferation [76-78], other oestrogenic effects on uterine and vaginal tissue [55,79-85], increases in the length of dioestrus and oestrus in mature

female rats [53], mammary gland proliferation in immature rats and mice [86-88], triggering of implantation in mated hypophysectomised rats [89], occasionally accelerated vaginal opening in immature mice and rats [58,74], increases in uterine progesterone receptors [85,90,91], increases in serum prolactin levels [79] and increases in prolactin-producing cells in the anterior pituitary [65].

Among the *in vivo* studies described above, the uterotrophic assay provides a sensitive test system for detection of oestrogenic activity, with positive results in both rats and mice via oral, ip and sc routes of administration of BPA. However, there are marked differences in effect levels between species, strains and even within strains. The NOAELs for uterotrophic effects following oral administration of BPA range from 40-400 mg/kg bw/day, with the exception of a single rat study reporting a low effect level (25% increase in uterine weight) of 10 mg/kg bw/day with a NOAEL of 1 mg/kg bw/day [36].

Lower effect levels have been observed in the uterotrophic assay with sc or ip administration but the metabolic studies described earlier have shown that systemic bioavailability is considerably greater following sc or ip injection than oral administration. The most likely reason for this is the extensive first pass metabolism of BPA in the liver. This suggests that it is only the parent compound, BPA, which is oestrogenic and that oral exposure is likely to be of lower risk than other routes of exposure. Indeed, the main metabolite of BPA, BPA monoglucuronide, does not compete with oestradiol-17β for binding to ERα and ERβ and, unlike BPA itself, does not induce ERα- and ERβ-mediated gene expression in MCF-7 cells or HEPG2 cells [10,54]. However, it is noted that recent *in vitro* studies have shown the formation of an as yet unidentified oestrogenic metabolite when BPA is incubated in the presence of a rat liver metabolising system [92].

In several studies in which female rats have been exposed in the prenatal and/or early postnatal period [93-95], or continuously in one-, two- or three-generation studies [23-26], to BPA orally at doses ranging from 1 μg/kg bw/day up to 500 mg/kg bw/day, no effects have been found on timing of puberty (vaginal opening and onset of first oestrus), oestrous cyclicity, lordosis behaviour or, with the exception of one study [96] not replicated by others [97-99], on the size of the sexually dimorphic nucleus in the brain.

In the mouse, one group of workers has found oral exposure to BPA at 2.4 μg/kg bw/day on days 11-17 of gestation had no effect on the timing of vaginal opening but increased female weaning weight and significantly reduced the time between vaginal opening and onset of first oestrus, after adjustment for body weight [100]. A similar mouse study by another group, using 2 or 20 μg/kg bw/day on days 11-17 of gestation, confirmed the absence of any effect on timing of vaginal opening but did not examine time of first oestrus and did not find any increase in female weaning weight [101].

Other studies, published in abstract only [102,103], reported a variety of effects on female rat offspring exposed prenatally to BPA on gestation days 6-21, via oral maternal doses of 0.02, 0.1 or 50 mg/kg bw/day (delayed vaginal opening at 0.02 or 0.1 mg/kg bw/day but premature vaginal opening with 50 mg/kg bw/day) and an increase in

prolongation of oestrus by more than 1 day at 0.1 and 50 mg/kg bw/day. These studies are however considered unacceptable for risk assessment since it has emerged that the BPA treatment groups and comparison groups (vehicle controls and oestradiol 17β-treated groups) were each treated sequentially, not simultaneously, over a period of 14 months [104]. Thus there were no concurrent controls and possible treatment-related effects may be confounded by time-related changes.

In juvenile and adult male rats, BPA does not appear to be androgenic or anti-androgenic [52,105], but one study [106] has reported effects on spermatogenesis in mature male rats. In Sprague-Dawley rats exposed to BPA by oral gavage at several doses ranging from 2 ng/kg bw/day to 200 mg/kg bw/day for 6 days at 13 weeks of age, there were significant reductions in daily sperm production and efficiency of sperm production at 18 weeks of age from doses of 20μg/kg bw/day upwards. The effect was consistent and similar in magnitude (25-30% reduction) at all doses between 20 μg/kg bw/day and 200 mg/kg bw/day. Lesser but non-significant reductions were seen at lower doses and 2 μg/kg bw/day could be taken as the no-effect level. In subsequent studies, designed to replicate these findings, no effects on spermatogenesis in the Sprague-Dawley rat were observed (J.Ashby, personal communication; data available for scrutiny).

In most studies in which male rats have been exposed in the prenatal and/or early postnatal period, or continuously in one-, two- or three-generation studies, to BPA orally at doses ranging from 0.2 μg/kg bw/day to 650 mg/kg bw/day, no adverse effects on male development have been found [23-26,107,108]. One study reported reductions in testis weight and sperm production in adulthood following exposure of rats to a single low maternal dose level of 0.1-0.4 mg/kg bw/day during gestation and the preweaning period [109]. However, the same authors subsequently reported unexplained shifts in the same testicular parameters in controls studied at a later date [110], and their original findings could not be reproduced in a similar, but more rigorous study by other workers using several doses from 2 μg/kg bw/day to 1.8 mg/kg bw/day [111]. Another study, published in abstract only [112], reported increases in seminal vesicle and epididymal weights and increases in androgen receptor proteins in male rat offspring exposed via the diet to 2.2 or 23.1 μg/kg bw/day from conception to 70 days of age, but other effects reported in the same study, of reduced body weight, kidney and spleen weights at these low doses, are not congruent with other studies on BPA. In a study published in abstract only, male rats given 100 or 250 μg/kg bw/day orally on days 1-14 postnatally were reported to have reduced sperm content of the testis and epididymis at 44 days of age [113].

In a study in mice in which BPA was given orally on days 11-17 of gestation, 30-35% increases in prostate weight at doses of 2 and 20 μg/kg bw/day and a 19% decrease in sperm efficiency at 20 μg/kg bw/day were reported in the offspring [42,114]. The same laboratory has reported, in abstract only, increased prostate size in another strain of mouse after prenatal exposure to 10 μg/kg bw/day orally [115]. Another laboratory has also confirmed increases in prostate weight and size and shown increased androgen receptor binding activity in the prostate of male offspring after 50 μg/kg bw/day orally on gestation days 16-18 in the mouse [116] and suggested that both oestrogen- and androgen-mediated pathways may be involved in this response [117]. The effects on

prostate development reported above were not observed by two other laboratories conducting similar studies in the mouse, using doses ranging from 0.2 to 200 μg/kg bw/day on gestation days 11-17 [101,118].

Possible effects on rat reproductive organs, including the prostate, of exposure to BPA from gestation day 2 or 11 to postnatal day 20 or 21, at doses ranging from 1 μg/kg bw/day to 320 mg/kg bw/day given via oral gavage or in the drinking water have been examined by one group [94,119,120]. They found occasional small increases in fresh prostate weight in BPA treated groups, but the results were not consistent between experiments.

Another study, published in abstract only [121], reported a variety of effects on male rat offspring exposed prenatally to BPA on gestation days 6-21, via oral maternal doses of 0.1 or 50 mg/kg bw/day (shorter anogenital distance, increased prostate weight, and reduced daily sperm production at 50 mg/kg bw/day, delayed preputial separation at 0.1 and 50 mg/kg bw/day and variable effects on testosterone levels). These studies are however considered unacceptable for risk assessment since it has emerged that the BPA treatment groups and comparison groups (vehicle controls and oestradiol 17β- treated groups) were each treated sequentially, not simultaneously, over a period of 14 months [104]. Thus there were no concurrent controls and possible treatment-related effects may be confounded by time-related changes.

Evaluation and conclusions

As can be seen from the above summary of the toxicity data, the more recent studies indicate that reproductive and endocrine-related endpoints are important in the risk assessment of BPA.

The data on toxicokinetics of BPA, showing much lower bioavailability of parent compound following oral administration, compared with sc or ip injection, together with the information that the principle metabolite of BPA, the monoglucuronide conjugate, does not bind to or activate the oestrogen receptor, indicate that human risk assessment for oral exposure to BPA should be based on results generated in oral studies. Effects reported at low oral doses will be considered first.

One laboratory has reported effects on spermatogenesis in the adult rat at oral doses from 20 μg/kg bw/day to 200 mg BPA/kg bw/day [106]. The size of the effect (25-30% reduction in daily sperm production and efficiency of spermatogenesis) and the flat nature of the dose-response are compatible with the lack of any functional consequence for fertility in that species, recorded in other studies [23-26]. However, the Committee noted that another laboratory has failed to confirm these findings using the same strain of rat (J.Ashby, personal communication) and that no effects were observed on a more extensive set of sperm parameters in adult males in the comprehensive three-generation study [25], conducted on the same strain of rat, apart from sporadic effects, inconsistent across the generations, at the top dose of 500 mg/kg bw/day. The Committee therefore

concluded, on the present weight of evidence, that the reported findings on juvenile/adult sperm parameters from one laboratory should not be considered a pivotal effect in risk assessment of BPA.

In developmental studies, the mouse appears to be more sensitive than the rat with observations of subtle effects in some studies on male offspring (increased prostate size and weight) [42,114-116] and in one study on female offspring (shorter time between vaginal opening and first oestrus) [100] at oral doses in the range of 2-50 μg BPA/kg bw/day during gestation. However, these effects have not been observed in other studies in mice [101,118]. The implications of these conflicting observations for human risk assessment are difficult to assess at present. The findings in these low-dose studies are therefore considered insufficiently robust for derivation of a TDI, but remain as current uncertainties in the database.

At first sight, the occurrence of effects at such low doses may seem unlikely given the rapid, first-pass metabolism that is seen in rats and humans following oral dosing. However, chemicals that bind to receptors can do so very quickly after systemic absorption and then exert prolonged downstream effects, even though all traces of them have disappeared from the systemic circulation. Alternatively, BPA may be operating via several different mechanisms involving not only interaction with oestrogen receptors but also possibly androgen receptors, or by production of a minor but potent oestrogenic metabolite. There is preliminary evidence for all these possibilities (see earlier). It is also possible that the oral toxicokinetics and/or toxicodynamics of BPA in the mouse may differ from other species. The *in vivo* toxicokinetics of BPA in the mouse are as yet unknown. On the toxicodynamic side, it appears from other published data that the pregnant mouse is extremely sensitive to oestrogens, compared with rats or humans [124].

The marked differences in observed responses to BPA between different laboratories using very similar study designs also complicate human risk assessment. Housing, diet, species, strain and sub-strain (sources of animals) may all conceivably influence study outcomes [104] and it is notable that with BPA, the Sprague-Dawley rat appears to be the most sensitive strain for oestrogenic effects in the uterotrophic assay but seems less sensitive in relation to the various reproductive parameters assessed in multigeneration studies. Similarly, while the mouse is less sensitive than the rat to uterotrophic effects, it may be more sensitive with respect to subtle alterations in reproductive development. This lack of consistency across different, but biologically related endpoints within a species/strain has been noted with other endocrine disrupters, such as the chlorotriazines [125-128].

The rat uterotrophic assays yielded a wide range of NOAELs and it is noted that the lowest effect level, reported in one study, was 10 mg/kg bw/day with a NOAEL of 1 mg/kg bw/day. These assays are clearly important in establishing the oestrogenic activity of BPA *in vivo*. However, it is unclear whether small responses in the uterotrophic assay should be considered adverse given the variability in the assay and the normal cyclical changes in uterine weight, though larger responses clearly would be. The Committee

concluded that the uterotrophic assay results cannot be considered quantitatively robust, given the variability in testing protocols and in results obtained on BPA from laboratory to laboratory, and that they should not be used to derive a TDI.

The Committee has therefore concluded that, on the present weight of evidence, the TDI should be based on robust data from oral studies covering a wide range of doses and which have included thorough investigation of relevant reproductive and endocrine-related parameters. The overall oral NOAEL for BPA from such studies is considered to be 5 mg/kg bw/day, based on the results from the recent, comprehensive three-generation study in the rat, in which the lowest dose at which effects were seen was 50 mg/kg bw/day. The pivotal effects were significant reductions in adult body weight and pup body and organ weights. The effect on adult body weight was considered to be the pivotal effect by the Committee when setting the TDI based on the 90-day study in 1986 [1]. Although a NOAEL of 25 mg/kg bw applies for this effect in adults, only the recent multigeneration study has examined pup body and organ weights and so the NOAEL from that study is now considered the most appropriate one on which to base the TDI. An uncertainty factor of 500 on the NOAEL is considered appropriate, comprising 10 for interspecies differences, 10 for inter-individual differences and 5 for the uncertainties described above that remain in the database. Applying a 500-fold uncertainty factor to the overall NOAEL of 5 mg/kg bw/day gives a temporary TDI of 0.01 mg/kg bw. The Committee recommends that the t-TDI be reviewed once significant new data are available. The Committee also notes that realistic worst-case estimates of consumer exposure via foodstuffs, ranging from 0.00048 mg/kg bw/day for adults to 0.0016 mg/kg bw/day for infants, are below this t-TDI of 0.01 mg/kg bw.

Needs for further research

Further research is needed to resolve the uncertainties surrounding the findings in the mouse, both with respect to dose and significance of the reported effects for humans. Comparative oral toxicokinetic data in humans and mice, together with a consideration of possible toxicodynamic differences between species in sensitivity to oestrogenic substances during pregnancy, are needed in order to ascertain whether the mouse is an appropriate model for humans. It is understood that a new reproduction study in the mouse will be undertaken in the context of an evaluation of BPA under the EU Existing Substances Regulations [5,129] and that further work on toxicokinetics in neonatal and pregnant rats, metabolism in human hepatocytes and comparative physiologically-based pharmacokinetic (PBPK) modelling is underway [129]. The reproduction study should add further to our knowledge about effects in the mouse but, as mentioned above, it is likely that other studies will be needed to address the question of the potential significance for humans of the effects reported by some workers in the mouse.

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