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Bisphenol A and baby bottles: challenges and perspectives

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Table of Contents

Executive summary

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane) is a chemical used primarily in the manufacture of polycarbonate plastic, epoxy resins and as a non-polymer additive to other plastics. Because of the extensive use of BPA in the manufacture of consumer products, such as polycarbonate baby bottles, epoxy-resin can liners, food containers and utensils, dental sealants, protective coatings, flame-retardants, and water supply pipes, there is a widespread human exposure to BPA.

Several risk assessment studies have been performed over the last 10 years by different regulatory bodies in Europe, USA, Japan and Canada. These risk assessments were mainly based on studies performed in compliance with regulatory guidelines and good laboratory practice (GLP), using oral administration, large groups of animals and several dose groups. In these studies, effects were seen at dose levels from 50 mg/kg bw/day. As those levels are well above estimated exposure levels of humans (0.07-12 µg/kg bw/day), most risk assessors and managers considered the margin of safety sufficient. On the other hand, a large number of research studies in the scientific literature described effects at dose levels below those most risk assessments indicated as safe. Whereas the reliability and relevance of these studies for risk assessment purposes were questioned because of methodological limitations (small numbers of animals, fewer or single dose groups, non-oral routes of administration), this has raised a very strong debate. Following the precautionary principle, Denmark has decided in March 2010 to provisionally ban all BPA-containing food containers intended for children under 3 years old. This decision followed a similar ban imposed recently in Canada and in some US-States. In May 2010, the National Assembly of France voted in favour of a ban of BPA in baby feeding bottles.

Against this background, the present report gives an overview of the results from the various risk assessment studies carried out so far, taking into account also information from research studies, and highlighting the main areas of uncertainty.

On the exposure side, it must be noted that there are relatively few studies of BPA release in actual food products, including infant milk formula. Instead, many studies have been carried out on the release of BPA from polycarbonate containers into food simulants (e.g. water, aqueous acetic acid and ethanol solutions, olive oil). However, not all these studies can be taken as conclusive. For example, it has been found that a higher pH value leads to increased release of BPA, but there is still uncertainty concerning the effect of minerals in water, especially due to water hardness. The effect of bottle aging due e.g. to household treatment is also difficult to estimate, as studies comparing releases from new and used bottles were not based on the same production lot.

Concerning the results from human health effect assessments, while there is general consensus concerning data on acute and local effects and genotoxicity of BPA, there is not yet agreement as regards reproductive and developmental toxicity (specifically neurodevelopment) and carcinogenicity. There is also some debate on the toxicokinetics of BPA which is still not fully resolved. Since studies in this field are based on animal experiments, data on absorption, distribution, metabolism and excretion are of utmost importance in order to understand the transferability of the experimental results to humans. It has been demonstrated that after oral intake BPA is metabolised rapidly mainly into BPA-glucuronide, which is water-soluble and can be excreted via the urine, making BPA no longer available for biological activity within the body. This deactivation of BPA is more effective in humans than in rodents and consequently humans should be exposed internally to lower circulating levels of BPA and thus would be expected to be less sensitive to BPA effects than rodents. However, currently there is a discussion whether there may be different kinetic pathways for specific populations (e.g. newborns). Some recent studies reported the detection of free BPA in blood and urine in animals and humans, leading to speculation on whether BPA might accumulate in the body and/or whether other, non-food sources may contribute to human exposure. There are also indications that transfer of BPA may take place from the mother to the foetus, which in turn may imply an exposure in pre-natal life following the mother's intake of BPA. However, the reliability of these findings should be confirmed by both sound analytical data on the amount of free BPA in human plasma and urine, and a better understanding of toxicokinetics in animals and humans.

The biggest challenge in the risk assessment of BPA concerns the systemic toxicological endpoints, namely reproductive and (neuro)developmental toxicity and carcinogenicity, especially in relation to potential endocrine disrupting properties since BPA has been shown to have weak binding affinity to oestrogen receptors.

Regulatory risk assessment studies have used studies carried out according to international guidelines and in compliance with good laboratory practice (GLP) to derive a no-adverse-effect level (NOAEL). Several research studies, however (often not using the oral route of administration) reported effects at dose levels much lower than these NOAELs. More precisely, they suggested a non-monotonic dose response curve, meaning that effects observed at very low doses would disappear at intermediate doses and new, different effects would appear again at higher dose levels - those that were used for determining the NOAEL in the regulatory risk assessments. Guideline studies in compliance with GLP could however not confirm these low dose effects.

Other experimental findings in research studies have suggested an increased susceptibility to pre-cancerous changes following administration of BPA via maternal subcutaneous injection in rats, in contradiction to studies carried out according to international guidelines in which orally administered BPA was found not to be carcinogenic.

As regards the effects of BPA on neural development, there are many research studies published that have suggested that BPA treatment during development

can cause alterations in brain development and behaviour, again at very low exposure levels. Conversely, a study finalised in September 2009 and carried out in compliance with international guidelines did not find any evidence of BPA as a developmental neurotoxicant.

Finally, some epidemiological studies have linked potential health effects such as diabetes, obesity, liver enzymes abnormalities, and cardiovascular diseases with the exposure to BPA. In this case, interpretation is made difficult by the fact that some confounding factors might not have been accounted for.

In conclusion, most of the current uncertainties regarding human health risks of BPA derive from diverging opinions on the reliability of studies carried out with different methodologies, which result in contradictory interpretations of the data collected to date. Whereas following guideline criteria and good laboratory practices is normally considered a point of strength in risk assessment, some researchers believe that these criteria should not be used to discount findings in other studies which do not conform to these strict criteria.

In principle, this controversy might be solved via a new series of globally agreed toxicological studies based on harmonized test programmes to be discussed preliminarily in an international context, also inviting those who are critical about the existing risk assessments to participate in the discussion. Carrying out such tests under the supervision of an international panel of independent experts would be a further possibility to facilitate acceptance of the results and agreement on the conclusions. A significant step in this direction is represented by the decision of the U.S. National Institute for Environmental Health and Safety (NIEHS) in 2009 to specifically call upon cooperation between academic laboratories and governmental bodies when launching a grants programme for new research on BPA. Fostering this kind of collaboration offers in principle the possibility to get the best scientific contribution out of both players; as such, it is a positive reference for possible future action at international level.

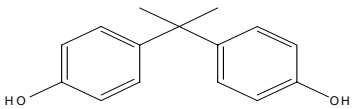
Finally, and considering also that some BPA-containing products (particularly polycarbonate baby bottles) are already being banned in some countries and/or are being progressively and voluntarily phased out by the industry, the assessment of BPA-free substitute materials especially for potentially sensitive subpopulations may also soon become a priority for the risk assessors.

1 Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, CAS No 80-05-7, commonly known as BPA) is a chemical produced in large volumes worldwide and has a large market diffusion in many consumer products. BPA is used mainly as a monomer in the production of polycarbonate plastics (PC) and as a precursor of epoxy resins. Polycarbonate plastics are used in food and drink containers, the resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. In addition, BPA is used in the production of other types of resins, flame-retardants, and in the processing of polyvinyl chloride plastic and in the manufacturing of thermal paper. Some polymers used in dental sealant and tooth coatings contain BPA as listed in reports of the European Commission's former European Chemicals Bureau (ECB, 2003, 2008).

Scientists believe that consumer exposure occurs primarily via food in contact with BPA containing materials, such as polycarbonate infant feeding bottles and tableware, plastic food containers and food and beverage cans lined with epoxy resins. The highest estimated daily intake of BPA in the general population in relation to body weight is assumed to be for infants, and small children, via their food where it has been in contact with polycarbonate baby bottles and baby food cans lined with epoxy resins.

Figure 1: Chemical formula of Bisphenol A (2,2-bis(4-hydroxyphenyl)propane)



BPA has received considerable attention in recent years due to widespread sources for human exposure, and its potential harmful effect on humans. BPA is described as an endocrine disruptor (hormone like substance), since it has the ability to bind to the nuclear oestrogen receptor and exert weak oestrogenic effects. One of the main concerns is neurodevelopmental toxicity, based on the concept that the developing brain of a human foetus or infant is inherently more susceptible to injury from toxic agents than that of an adult. It is suggested that metabolic disorders and carcinogenicity are other biological effects that might be related to BPA exposure.

There are a large number of studies published in the scientific literature describing the results of laboratory animal studies. These include studies of traditional designs (following internationally agreed guidelines and in compliance with Good Laboratory Practice) carried out to assess the toxicity of BPA, as well as a wide variety of research studies examining possible effects following exposure to low doses of BPA during critical periods of development. This might result in adverse health outcomes later in life due to its oestrogenic or other biological properties (Sekizawa, 2008). Many of these latter research

studies are difficult to interpret with regard to how they contribute to the weight-of-evidence for human health risks, as some of them have technical or design shortcomings such as the use of smaller number of animals, fewer and single dose groups and often including non-oral routes of exposure. Their reports often do not provide sufficient experimental details to permit an assessment of technical adequacy. These “low dose effect" studies suggest a higher in vivo potency for BPA than would be predicted based on binding to the oestrogen receptor (i.e. 10,000 to 100,000 less potent than oestrogen) and claim that effects are seen at very low and very high doses but disappear at intermediate doses, suggesting an inverted U-shaped dose-response curve (hormesis).

In addition, there is an ongoing discussion on differences in the metabolism and clearance of BPA between laboratory animals and humans. This is of special importance, as only the free BPA is a weak oestrogenic compound, but not the corresponding bound (glucuronide) metabolite.

In humans, following oral exposure, BPA is transformed to the BPA monoglucuronide metabolite in a very efficient first pass effect in the gut and liver, whereas rodents suffer from prolonged exposure to free BPA due to enterohepatic circulation. It has been argued that BPA may be de-conjugated in several tissues and may not be rapidly cleared from the body of human embryos, which are exposed through the placenta, and of newborns, which may therefore be a particularly sensitive subpopulation. As regards the latter, however, the European Food Safety Authority (EFSA) has issued in 2008 an opinion stating that newborns can also rapidly eliminate BPA (EFSA, 2008).

Several risk assessments of BPA were performed over the last 10 years by different regulatory bodies and expert groups in Europe, Canada, USA and Japan. The hazard assessment was mainly based on experiments conducted in accordance with international guidelines and Good Laboratory Practices, using oral administration, a large number of animals and a wide range of doses. Based on these data the EFSA (2008), ECB (2008), Food and Drug Administration of the United States of America (US-FDA; 2008) and the Japanese National Institute of Advanced Industrial Science and Technology (AIST, 2007) did not see a concern at current exposure conditions for humans.

In contrast, concern for low dose effects were expressed by experts of the Chapel Hill meeting on “Bisphenol A: An Examination of the Relevance of Ecological, In vitro and Laboratory Animal Studies for Assessing Risks to Human Health” (Vom Saal *et al*., 2007), the US National Toxicology Program (Chapin *et al*., 2008), the Scientific Committee of the US FDA (2008), some Member States of the European Union, Canada (2008). The concern mainly expressed is in relation to neurobehavioral effects at low doses of BPA. Therefore, some countries currently consider or have already enacted a ban for use of BPA especially in containers for baby food and baby bottles, like Canada, France, Denmark and some of the US States.

The uncertainty in the understanding of the mechanism of action for BPA toxicity, the toxicokinetics, the discussion of the shape of the dose-response

curve and any species, sex and/or inter-individual differences make the risk assessment of BPA however extremely difficult.

Presently many organisations are again looking into the potential health risks of BPA. The US-FDA's National Center for Toxicological Research is currently carrying out in-depth studies in co-operation with the National Toxicology Program (NTP), to answer key questions and clarify uncertainties. In the interim, the US-FDA is taking precautionary steps to reduce human exposure to BPA in the food supply by e.g. encouraging industry to stop producing BPA containing baby bottles and infant feeding cups for the US market, facilitating the development of alternatives to BPA and supporting efforts to replace BPA or minimize BPA levels in food can linings.

In January 2010, the French Food Safety Agency (AFSSA, 2010), concluded an opinion with the following recommendations: "The significance of warning signals observed in the *in-vitro* and *in-vivo* studies at doses lower than the NOAELs of 5 mg/kg bw/day in terms of health safety should be determined. In the meantime, and taking into account the fact that these signals for humans is uncertain, the relevance of increasing the safety factor of the TDI should be debated and the other sources to bisphenol A than food contact materials should be investigated."

In the face of a potential phasing out of polycarbonate baby bottles, plastic substitutes have appeared on the market in the USA and now also in Europe. Such materials include for example polyether sulphone (PES), polypropylene (PP), pure silicone, polyamide and a new co-polyester polymer.

With an EFSA opinion on BPA due in spring 2010, and an international expert panel put together jointly by the World Health Organisation and Food and Agriculture Organisation (WHO/FAO) to analyse the issue at world level in October 2010[1] an in-depth analysis of these controversies is not within the scope of our report.

However, the situation is urging legislators to seek up-to date knowledge to support rapid and informed decisions. In this context, our report is aimed at providing the reader with an overview of the BPA issue, by presenting results from different regulatory risk assessment studies conducted globally, and the underlying data and methodology that have contributed to the results. In addition, relevant recently published studies that may have an impact on the conclusions and related areas of uncertainty at the base of the current concerns are analysed and presented. Finally, some recommendations are given for further research.

[1] Joint FAO/WHO Expert meeting to review toxicological and health aspects of Bisphenol A, Canada, October 2010, available at: http://www.who.int/foodsafety/chem/chemicals/Call_for_experts.pdf

2 Applications of BPA and possible pathways of exposure

2.1 Use of BPA

It is estimated that more than 1 million tonnes of BPA is used on a yearly base in the EU (ECB, 2008). Approximately 90% of BPA is used as monomer in the preparation of polycarbonate plastics (ca. 70%) and epoxy resins (ca. 20%), whereas the remaining is used in the production of other polymers, or as an additive in manufacturing (tyres, thermal paper, brake fluids).

Polycarbonates produced from BPA generally have good optical clarity, impact resistance and ductility at room temperature and below. This makes them ideally suited to a wide range of end applications in consumer products. For example, polycarbonate is used in the following contexts:

- compact discs manufacture;
- solid and multi wall sheet in glazing applications and film;
- food contact containers, e.g. returnable milk and water bottles (e.g. used in water cooler machines) and baby bottles;
- medical devices;
- polycarbonate blends for diverse technical applications;
- modified high heat resistant co-polycarbonates of BPA used mainly in the automotive and electric/electronics industry.

Epoxy resins are used e.g. as protective coatings; structural composites; electrical laminates; electrical applications; adhesives.

2.2 Human Exposure to BPA

Individuals working in the manufacture of BPA containing products can be exposed to BPA at the workplace. The general population can be exposed to BPA via consumer products and via the environment.

As regards consumer products, the main source of exposure is epoxy resin coatings inside food and beverage containers and polycarbonate tableware and bottles, such as those used for infant formula milk. More specifically, exposure to BPA can arise under conditions where residual monomer in the polymer migrates into food and beverages, or where the polymer itself hydrolyses, thereby releasing BPA. Consumption of the food or beverage from containers that underwent such conditions would then result in ingestion of BPA.

Other, relatively minor sources of consumer exposure to BPA are dental fissure sealants, epoxy-based surface coatings, adhesives, printing inks and thermal paper. Inhalation e.g. from potential contamination of the environment and dermal exposure is considered negligible, at least for the normal consumers.

As our report focuses mainly on BPA in relation with polycarbonate baby bottles, all the other potential consumer exposure and occupational exposure will not be discussed further. It is however worth noting that, because there are indications of BPA transfer from the mother to the foetus, also exposure of

pregnant women from products not specifically meant for infants use may be of relevance as regards BPA health effects in pre-natal life.

2.3 Release of BPA from polycarbonate

BPA can leach from polycarbonate into liquid foods because of two different processes: diffusion of residual BPA present in polycarbonate after the manufacturing process, and hydrolysis of the polymer catalysed by hydroxide (OH^-) in contact with aqueous food and simulants (Ehlert *et al.,* 2008; Mercea, 2009). For dry foods diffusion is the only relevant process.

Release of BPA from polycarbonate containers into food depends on the contact time, temperature, and type of food. Food simulants are often used in release studies to represent the different types of food e.g. 50% of ethanol in water is the food simulant for milk, and 3% of acetic acid in water is the simulant for fruit juice.

Reviewing the scientific literature and recent information from some EU National Reference Laboratories on food contact materials (confidential) shows that there are relatively few studies on the release of BPA into the real food matrix so far. BPA was not detected in fruit juice (ECB, 2003), milk formula (EU Member State data, 2009; Mountfort *et al*., 1997) or soup (Japan, 1998) that had been in contact with polycarbonate products. It must be mentioned that results for low levels of release depend on the limit of detection of BPA in food matrices (0.01-0.03 mg/kg).

Instead, evidence of BPA release comes from the much larger number of studies that were conducted using food simulants and tap water. BPA may release substantially into oil based food simulants up to 1.5 mg/l (Biles *et al*., 1997; ECB, 2003; Howe and Borodinsky, 1998; Japan, 1998; Wong *et al*., 2005).

Biles *et al*. (1997) showed that the release of BPA increases with the level of ethanol in aqueous solution in the range of 8-50% from 0.87 mg/l to 5.9 mg/l at 65°C after 10 days. Less extreme time-temperature conditions resulted in lower release (ECB, 2003 and 2008; EU Member State official control data, 2008-2009; Howe and Borodinsky, 1998; Japan, 1998; Kawamura *et al*., 1998; Kubwabo *et al*., 2009; VWA, 2008; Wong *et al*., 2005).

Most literature studies report a release of BPA into 3% acetic acid below the limit of detection (ECB, 2003; EU Member State official control data, 2009; Howe and Borodinsky, 1998; Japan, 1998; Maragou *et al*., 2008; Sim and Jianhua, 2008; VWA, 2005, 2008) and some above (D'Autuno *et al*., 2001; ECB, 2003).

The release of BPA from polycarbonate into deionised water and tap water was studied in detail by many research groups. The highest concentration reported is 1 mg/l at 65°C for 10 days (Biles *et al.*, 1997).

Very few studies analyse release at more than two temperatures. Biedermann-Brem and Grob, (2009) systematically studied the effect of temperature on the release of BPA into tap water and boiled tap water of the same water supply by

heating in a microwave for 5 min. The concentration of BPA in tap water increased from <0.0001 mg/l at 50°C to 0.0006 mg/l at boiling temperature whereas the concentration of BPA in boiled tap water having a pH of about 9.5 increased from <0.002 mg/l at 50°C to 0.033 mg/l at boiling temperature.

A higher pH clearly increases the release of BPA (Biedermann-Brem *et al*., 2008), independent from other physical considerations such as exposure time, temperature and heating mode (microwave/thermal oven). This has special relevance if one considers that some food preparation processes may cause an increase of the pH above 8, which is normally the highest value for food. An example is boiling tap water in a pan or microwave for several minutes during which carbon dioxide can be released, consequently increasing the pH.

Biedermann-Brem and Grob (2009) and Mercea (2009) also indicate that the mineral content may also influence release. However, they failed to show clear evidence that the effect was really caused by the mineral composition and not by the pH.

Some scientists studied the difference of release of BPA by new and used baby bottles and came to contradictory conclusions (Le *et al*. 2008; Mercea, 2009; Tan and Mustafa, 2003). Furthermore, the authors did not report whether the used bottles were of the same production lot as the new ones, so their experiments cannot be taken as conclusive on whether the release of BPA would decrease or increase with age of the bottle.

Repeated testing with the same bottles clearly demonstrated that the release decreased or at least remained constant during successive release experiments with water (Ehlert *et al.*, 2008; EU Member State research, 2009; Kawamura *et al.*, 1998; Lázaro Martínez *et al.*, 2009; Maragou *et al.*, 2008; Maia *et al.*, 2009; Sun *et al.*, 2000; Yoshida *et al.*, 2003). The same observation was made with 10% aqueous ethanol solutions (Biles *et al*., 1997; ECB, 2008; EU Member State research, 2009). One EU Member State laboratory (2009) observed an increase of the release of BPA into 3% acetic acid during successive experiments at 100°C for 30 min.

Washing the bottles also induces chemical ageing of the internal surface, but experimental results on this topic are not homogeneous. Both Biedermann *et al*. (2008) and Mercea (2009) observed that the bottles released lower amounts of BPA into aqueous food simulants after washing. In contrast, Brede *et al*. (2003) observed an increase. To explain the discrepancies, it has been speculated that the pH of the detergent solution could be crucial, but the rinsing after washing before drying the bottle may also have an effect. Manual brushing for cleaning the bottles does not raise the release of BPA (Maragou *et al*., 2008).

Conclusions on conditions affecting release of BPA

- **Release of BPA from polycarbonate into aqueous liquids is caused by diffusion and hydrolysis of polycarbonate catalysed by hydroxide;**

- **Main parameters affecting the release of BPA are contact time, temperature and pH of the food simulant, all having a positive correlation**
- **Dissolution of scale during boiling of water in the polycarbonate bottle may raise the concentration of BPA due to the rise of the pH;**
- **Brushing of the bottle does not seem to raise the release of BPA;**
- **The effect of aging is difficult to estimate, as studies suggesting this effect were not based on experiments with new and used bottles of the same production lot;**
- **Residual alkaline detergent remaining on the surface of the baby bottle after dishwashing may increase the release of BPA;**
- **Some food preparation processes cause an increase of the pH above the normal pH of food;**
- **The possible effect of the mineral composition of the aqueous food simulant on the release of BPA is not clear to date;**
- **Release data derived from studies using harmonised analytical protocols and performed under realistic conditions (treatment of baby bottles, preparation of infant food) are necessary.**

2.4 Exposure Estimates

When babies are not breast-fed they will get infant formula preparations that are mostly served in a polycarbonate bottle. In any case, also breast-fed infants may receive additional food or expressed milk via the bottle.

The SCF (2002) established realistic worst-case exposure to BPA based on migration data into food. The SCF noted that their conclusions were based on a draft version of the EU Risk Assessment Report (ECB, 2003). This worst case exposure was set to be 0.0016 mg/kg bw/day for newborns and infants of 0-4 month and 0.0008 mg/kg bw/day for infants of 6-12 month, respectively and was based on a concentration of 0.01 mg/kg food, a consumption of 0.7 l/day and a body weight of 4.5 and 8.8 kg, respectively. The exposure data also showed that infants and young children may be more exposed to BPA than adults.

EFSA (2006) made a reassessment of the exposure to BPA. Exposure of infants in the age of 0-6 month is divided into three groups. Breast-fed infants are estimated to be exposed to 0.0002 mg/kg bw/day, infants fed with a non-polycarbonate containing bottle to 0.0023 mg/kg bw/day and infants fed with polycarbonate bottles to 0.011 mg/kg bw/day. In the latter two cases, the exposure includes migration of BPA from the epoxy-resin can lining into powdered milk formula, Exposure of infants in the age of 6-12 month is estimated to be 0.013 mg/kg bw/day.

The EU Risk Assessment Report (ECB, 2008) considered exposure of 0-6 month-old infants to BPA only from polycarbonate baby bottle release, because powdered infant milk formula is not packaged in epoxy resin-coated cans in the

EU. The exposure of 1-2 month and 4-6 month old babies is estimated to be 0.008 mg/kg bw/day and 0.007 mg/kg bw/day, respectively. Infants in the age of 6-12 month are considered to be exposed only to BPA released from epoxy-resin canned food, of which the exposure is estimated to be 0.004 mg/kg bw/day.

In 2008, the US Food and Drug Administration (FDA) published a draft assessment of BPA (US FDA, 2008). The FDA made an inventory of formula consumption of newborns up to 1-year old infants, specifying consumption for each month. A distinction was made between liquid and powdered formula. Exposure to BPA from cans containing powdered formula was not considered because infant formula cans do not contain epoxy resins[2]. The average concentration of BPA in liquid formula in epoxy-lined cans is about 0.0025 mg/kg. Summing up exposure from epoxy lined cans and polycarbonate bottles,the total exposure of 0-2 month old infants was estimated to be about 0.002 mg/kg bw/day, whereas for older infants the exposure was estimated to be in the range of 0.0002-0.0006 mg/kg bw/day. It should be mentioned that liquid infant food preparations packaged in cans are not available on the European market.

Canada also prepared an assessment of BPA in 2008 (Environment Canada and Health Canada, 2008). The age groups of 0-1 months, 2-3 months, 4-7 months, 8-12 months and 12-18 months were considered. For each age group average and maximum exposure concentrations and food consumptions were established. The assessment included exposure to liquid infant formula, filling polycarbonate baby bottles with room temperature water and boiling water.

It must be noted that the EU (reports from SCF, ECB, EFSA) estimates in general higher exposure values of BPA than USA and Canada. A study carried out in Japan and reported by Chapin *et al.* (2008) found even lower total exposure estimates of BPA for babies than those calculated by the US FDA. The major factor contributing to the difference between the EU and USA exposure assessment is the estimation of the concentrations of BPA that is released into food. The EU and USA exposure assessments are in better agreement on estimation of body weight and food consumption.

Most risk assessments attempt to define the reasonably foreseeable use conditions leading to the highest exposures to define a realistic, yet worst case exposure scenario. However, some risk assessment reports do not give a justification for why they consider the selected exposure scenarios for the specific applications of BPA to represent realistic worst cases.

Official control laboratories in the EU test polycarbonate baby bottles for compliance at 70°C for 2 h (EU, 1982). However, an official control laboratory may also test at 40°C for 10 days if parents may have the habit to store the remaining of milk feeding for a next feeding. These test conditions in legislation are based on the assumption that substances migrate by diffusion only. The test conditions may therefore not reflect the realistic worst-case conditions for

[2] The use of epoxy resin-lined cans for powdered formula would be expensive and not necessary since powdered formula is not heat sterilised in the same way as liquid formula.

polycarbonate, since they do not account for releases owing to hydrolysis from presence of hydroxide.

Realistic worst-case exposure scenario's for infants from milk or infant formula preparations are necessary in order to calculate the highest likely exposure and for compliance testing of baby bottles against the specific migration limit of BPA of 0.6 mg/kg. The present specific migration limit is based on the average body weight of adults of 60 kg, the consumption 1 kg of food and a TDI of 0.01 mg/kg bw (EU, 2002). Although EFSA advised a 5-fold higher TDI (EFSA, 2006), the SML was not raised correspondingly, as a measure of precaution towards infants. Since scientific literature shows that infants are the most exposed sub-group of the population, the question may arise whether the principle of calculating the SML based on an adult body weight should be reconsidered.

Conclusions on exposure to BPA

- **Risk assessment reports should look more into the specific conditions that can influence BPA release (e.g. contact time and temperature) when defining worst-case realistic exposure scenarios.**
- **The Specific Migration Limit of BPA for food contact materials is based on exposure of adults. It is recommended to consider whether the SML should not be related to the most exposed part of the population, i.e. infants.**

3 Human Health Effects Assessment

During the last 10 years regulatory bodies have undertaken hazard and risk assessments of BPA Scientific Committee on Food (SCF; 2002), European Chemicals Bureau (ECB; 2003, 2008); European Food Safety Authority (EFSA; 2006); US Food and Drug Administration (FDA; 2008), Environment Canada and Health Canada (2008), and the Japanese National Institute of Advanced Industrial Science and Technology (AIST; 2007). Hazard assessment data are also published extensively in the scientific literature.

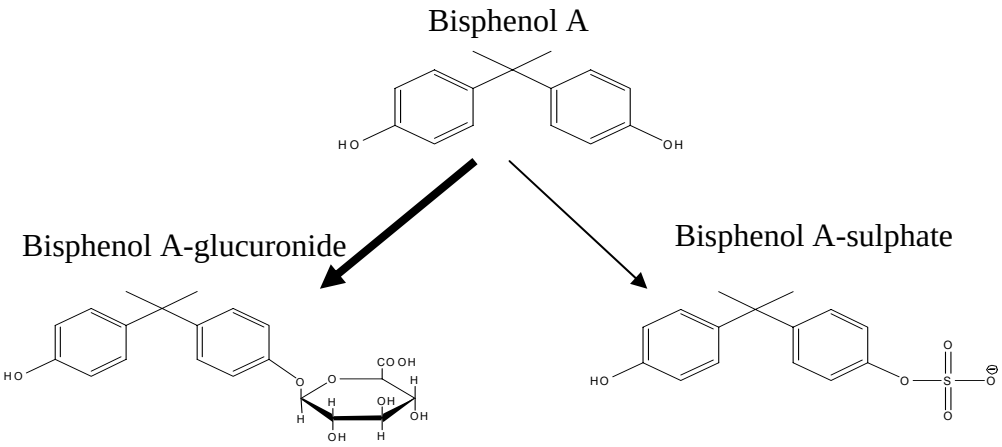
Data reported indicate that BPA is of *low acute toxicity* by all routes of exposure relevant to human health. BPA has been classified as irritating to the respiratory system and for the potential to cause serious damage to the eyes. BPA is also considered capable of eliciting skin sensitisation responses in humans and is classified for possible risk of impaired fertility. Acute and local effects induced by BPA are not disputed and will therefore not be further discussed within this reference report.

However, for toxicological endpoints that require repeated and systemic exposure, such as reproductive and developmental toxicity, and carcinogenicity, there are quite a lot of controversial studies, which have triggered the current concern for human exposure to BPA. These endpoints, together with the toxicokinetics of BPA are reviewed and discussed within this report and special focus will be given to oral exposure as this is seen as the most relevant exposure route for the general population due to migration of BPA from food contact materials into food.

3.1 Toxicokinetics and metabolism

In humans and rodents, orally administered BPA is rapidly and efficiently (>95% of dose) absorbed from the gastrointestinal tract (Kurebayashi *et al.,* 2002; Völkel *et al.,* 2002; Pottenger *et al.,* 2000) and undergoes extensive first-pass metabolism in the gut wall (Inoue *et al.,* 2003) and in the liver (Inoue *et al.,* 2001; Pritchet *et al.,* 2002). During this first-pass metabolism, the major metabolic pathway involves conjugation of BPA to BPA glucuronide. In addition to BPA glucuronidation, sulphation of BPA was suggested as a minor pathway of BPA metabolism in rats and humans (Ye *et al.,* 2006). The metabolisation of BPA is shown in Figure 2 (Dekant & Völkel, 2008).

Figure 2 Biotransformation of BPA in humans and rodents to BPA glucuronide and BPA-sulphate



The conjugation of BPA is considered to be a deactivation reaction, since both BPA glucuronide and BPA sulphate are devoid of oestrogenic (endocrine) activity (Matthews *et al.,* 2001; Shimizu *et al.,* 2002). Therefore due to the rapid biotransformation and excretion and plasma protein binding, peak concentrations of free BPA that are available for oestrogen receptor binding after oral exposure, are very low in humans. In contrast to humans, BPA glucuronide is eliminated in bile in rodents and undergoes entero-hepatic recirculation after cleavage to BPA and glucuronic acid by glucuronidase in the intestinal tract (Pottenger *et al.,* 2000; Snyder *et al.*, 2000).

An oral dosing study in rats found that the tissue concentrations of BPA-derived radioactivity were highest in the liver, kidney and carcass and lowest in the brain and testes, and there were no large differences between adult and neonatal animals (Domoradzki *et al.,* 2003, 2004). In pregnant rats very low levels of BPA were found to be distributed to the foetus following a relatively high oral dose (10 mg/kg bw/day) of BPA (Domoradzki *et al.,* 2003). In this study, no selective affinity for either yolk sac/placenta or embryo/foetus of BPA or BPA metabolites relative to maternal plasma or tissues was observed. The distribution of BPA to the foetus (or placenta) did not alter the overall pharmacokinetic fate of BPA in pregnant rats, compared to non-pregnant rats. However, there are also data indicating that repeated maternal exposure in rats and humans could lead to an accumulation of foetal circulating levels of free BPA and corresponding elevated in utero exposure (Ikezuki *et al.,* 2002; Welshons *et al.,* 2006).

There might be age dependent kinetic differences between special subpopulations. Studies in rats (e.g. Domoradzki *et al.,* 2004) showed that in newborns the glucuronidation pathway is more susceptible to saturation than in adults which suggests a higher internal exposure to free BPA. EFSA (2008) concluded that in humans early-life immaturity in glucuronidation capacity is likely to be compensated by presence of sulpho-transferases which would result in a efficient detoxification of BPA even in newborns.

Due to the high water solubility, BPA glucuronide formed during first-pass metabolism is rapidly cleared from human blood by the kidneys and excreted in the urine with terminal half-lives of around 5 hours after oral administration. Three independent studies in humans (Tsukioka *et al.*, 2003; Völkel *et al.,* 2002, 2005) covering a dose range of about 0.3 to 80 µg/kg bw showed that the applied doses of BPA were completely recovered in urine as BPA glucuronide within 42 hours and more than 90% of the dose applied was recovered in urine within the first 6 h after administration. No free BPA was detected and no gender differences in the kinetics of BPA-glucuronide in plasma and urine were reported. There was no indication of a potential for bioaccumulation of BPA in the body (Dekant & Völkel, 2008).

There is a major species difference between humans and rodents in the elimination of BPA glucuronide from liver. In contrast to humans, BPA undergoes enterohepatic circulation in rats (Kurebayashi *et al.,* 2002, 2003) which results in slow excretion ($t_{1/2}$ = 15-22 hours) and increased systemic availability of free BPA in rodents. After glucuronidation of BPA in the liver, BPA glucuronide is eliminated with the bile, but cleaved by glucuronidase in intestinal bacteria and re-absorbed from intestine. Thus, urinary excretion of BPA and its metabolites accounts for only 10-40% of the applied dose and the major route of elimination in the rat is via the faeces (50-83%), in part as parent BPA.

Urinary excretion in rats seems also to be sex-dependent, with females excreting approximately twice as much (24-28%) as males (14-16%). Since BPA has a high oral bioavailability in the rat, the free BPA found in the faeces is more likely to be derived from BPA glucuronide excreted in the bile and hydrolysed to free BPA in the gastrointestinal tract rather than representing unabsorbed BPA which might have passed along the gastrointestinal tract into the faeces unchanged. The rate of biliary excretion tends to be higher in males than females. Most of the urinary radioactivity was found to be in the form of BPA-glucuronide (82%) with free BPA and BPA sulphate making minor contributions (14% and 4% respectively). Excretion seemed not be affected by pregnancy at three different stages of gestation (Domoradzki *et al.,* 2004). Data from a few studies suggest limited excretion of BPA in human breast milk (Vandenberg *et al.,* 2007).

Recent data described below however reported the detection of free BPA concentrations in human and rodent blood and urine samples and suggest that there might be higher human exposure to free BPA as postulated in the abovementioned studies.

It was suggested that deconjugation at local tissue sites by the enzymes β-glucuronidase and arylsulfatase C can reactivate glucuronated and sulphonated BPA into free BPA (Ginsberg *et al.,* 2009). The enzyme β-glucuronidase does not only exist in the intestines but also throughout the body, including the placenta and foetal liver and may therefore be relevant for exposure of the foetus to free BPA. Arylsulfatase C develops early in life and may deconjugate BPA sulphate, an important BPA conjugate in newborns.

A re-analysis of urinary biomonitoring results from 1,469 adult human participants performed in the context of the National Health and Nutrition

Examination Survey (Stahlhut *et al.*, 2009) suggested a longer half-life of BPA in the human body than expected from the existing data. It was found that BPA levels did not decline rapidly with fasting time and they therefore suggest either substantial non-food exposure or accumulation of BPA in body tissues such as fat, or both.

Information on internal exposure to BPA is of importance because toxic effects are related to blood concentrations rather than to external exposure and this information would also allow the detection of species differences in metabolism. Free BPA was detected in human plasma and urine in several studies, sometimes at relatively high doses up to 60 ng/ml plasma (Lee *et al.,* 2008), however, a number of confounders which may influence the test results have also been reported, e.g. large overestimation by using semi-quantitative analytical methods based on enzyme linked immunosorbent assays (ELISA), or a potential contamination of reagents or other laboratory ware with BPA due to storage or processing (Dekant & Völkel, 2008).

Toxicokinetic models have been used to simulate the BPA kinetics in humans and to determine blood concentrations. A recent study has used two approaches: simple kinetic principles were applied to calculate steady state plasma concentration and a physiologically-based model was used to simulate the blood concentration-time profile in several age groups exploring the influence of not yet fully developed metabolic capacity on the blood concentrations in the newborn (Mielke & Gundert Remy*,* 2009). Both approaches gave concordant results, are in very good agreement with experimental results (Völkel *et al.*, 2002) and agree with results obtained with a different physiologically-based model (Edginton & Ritter*,* 2009). These model simulations show BPA blood concentrations several orders of magnitude lower that most measurements reported in the literature and the authors claim that high blood concentrations of BPA are not plausible and are not in conformity with normal human physiology. Due to a not yet fully developed glucuronidation activity in the newborn and not full compensation by the unimpaired sulphation pathway, the newborn is predicted to have three times greater blood concentration than the adult at the same external exposure level.

Conclusions on toxicokinetics and metabolism

- **BPA is considered to be rapidly conjugated into BPA glucoronide and BPA sulphate and consequently eliminated from the human body due to the water solubility of these metabolites;**
- **Internal exposure to free BPA available for biological activity within the body is therefore expected to be very low;**
- **Recent data from measurements of un-conjugated (free) BPA in human blood and urine however suggest higher internal exposure of humans to free BPA;**
- **Newborns are expected to be exposed to higher internal BPA values due to immature glucuronidation activity.**

- **These discrepancies warrant further investigations for better understanding of toxicokinetics, species and inter-individual differences, possible other sources of exposure to BPA and potential confounders impacting on the results.**

3.2 Mode of Action – endocrine disruption

The US Environmental Protection Agency (US-EPA) has defined an endocrine disrupting chemical (EDC) as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes. This definition is not limited to the oestrogen system. Rather, endocrine disruption encompasses effects on other endocrine systems including effects mediated by androgens, thyroid hormone, prolactin, and insulin, among others (Wetherill et al., 2007).

BPA has been considered a weak environmental oestrogen, based on traditional bioassays, as it binds to the oestrogen receptors, alpha and beta (Gould *et al.*, 1998; Kuiper *et al.*, 1998; Pennie *et al.*, 1998) with an affinity being about 10.000 to 100.000-fold weaker than that of 17β-oestradiol (natural hormone).

Some investigators have reported that exposure to low doses of BPA has produced disruptive effects in androgen or oestrogen responsive tissues, within the immune system, the thyroid, and the developing nervous system (Richter *et al.,* 2007; Vandenberg *et al.,* 2007; Wetherill *et al.,* 2007). They also reported that BPA can stimulate the aforementioned cellular responses at very low concentrations through genomic (nuclear oestrogen receptor) or non-genomic (membrane-associated or intracellular transduction) mechanisms, with effects on cellular function at very low doses (Wetherill *et al.,* 2007). Furthermore, it has been suggested that some of the effects of BPA may be mediated through the cell surface oestrogen receptor (GPR30), rendering BPA equipotent with 17β-oestradiol and diethylstilbestrol (Alonso-Magdalena *et al.,* 2005). BPA has also been shown to disrupt the normal activity of the oestrogen nuclear hormone receptors in a diverse set of target tissues like the pancreas (Adachi *et al.,* 2005).

It should be noted that humans (including newborns and infants) can be exposed to many substances with potential endocrine disrupting activity. Many food products may contain such substances, many of them being from natural sources. A prominent example being soy-based products as they contain isoflavones such as the phyto-oestrogens genistein and daidzein. As soy based infant food becomes increasingly popular (especially in cases where an allergy against milk occurs) the impact of BPA as an endocrine disrupting chemical needs to be put into perspective. Another important aspect, which is difficult to investigate, is the effect of mixtures of similarly acting substances on human health.

3.3 Repeated dose toxicity of BPA

The EU risk assessment report on BPA (ECB, 2008) based its results for deriving a NOAEL for systemic effects on two key toxicological studies

performed in mice: a 13 week range finding study for a two-generation study (Tyl *et al.*, 2005) and the subsequent two-generation trial (Tyl *et al*., 2008).

Effects on bodyweight gain, liver and kidney have been observed and a NOAEL of 50 mg/kg/day has been identified from the two-generation study in mice (Tyl *et al*., 2008). This NOAEL was taken forward to the risk characterisation. The effects seen at 5 mg/kg, such as changes in body and organ weight (liver), were not considered treatment related, as they were not consistent over generations or did not show a dose response relationship.

Risk assessment performed by EFSA in 2006 was based on an overall NOAEL (for repeated exposure) of 5 mg/kg bw/day derived from a three-generation study (Tyl *et al.*, 2002) supported by the same NOAEL from the two generation study by Tyl *et al*. (2008) for deriving a tolerable daily intake (EFSA, 2006).

In conclusion, EFSA used a lower NOAEL than the EU risk assessors based on slightly different interpretation of the same data.

Metabolic disorders and heart diseases

Recent publications of epidemiological studies and *in-vivo* and *in-vitro* studies suggest that human exposure to BPA is related to metabolic syndrome, heart disease, and liver toxicity. Metabolic syndrome, sometimes referred to as insulin resistance syndrome or syndrome X, combines various disorders including type 2 diabetes, dyslipidemia, obesity and high blood pressure (Newbold *et al.,* 2009a; Elobeid *et al.,* 2008).

An epidemiological survey reported significant correlations between increased BPA concentrations in urine and the prevalence of type 2 diabetes, cardiovascular diseases, and liver enzymes abnormalities (Lang *et al.,* 2008). This study has been corroborated by other reports in humans, which highlighted the association of BPA with medical disorders, such as diabetes, liver enzyme abnormalities and polycystic ovarian syndrome (Takeuchi *et al.,* 2004). Other cross sectional studies have been conducted in 2009 showing association between BPA exposure and oxidative stress, which might contribute to insulin resistance (Hong *et al.,* 2009). These reported findings require further investigation in order to establish the link to BPA exposure.

In-vitro and animal *in-vivo* studies have supported the abovementioned epidemiological findings though it must be mentioned that they were usually not compliant with Good Laboratory Practice (GLP), often using a low number of animals, testing only one or a few doses, and with a non-oral route of exposure.

Several animal studies showed that (over) stimulation of the oestrogen receptor alpha in pancreatic ß –cells by oestradiol or BPA produced an excessive insulin signalling in the liver, endothelium and in fat, thus potentially leading to obesity, glucose intolerance, and dyslipedimia (Ropero *et al.,* 2008; Nadal *et al.,* 2009; Alonso-Magdalena *et al.,* 2006, 2008).

Another study demonstrated that levels of BPA in rodents induced adverse effects on the brain, reproductive system, and metabolic processes, such as

insulin homeostasis and liver enzymes and suggested an increased vulnerability to BPA exposure during development (Richter *et al.,* 2007).

Recently French researchers have also shown that peri-natal exposure to BPA in rats affects intestinal function (Braniste *et al*., 2009).

Conclusions on repeated dose toxicity

- **The lowest overall NOAEL of 5 mg/kg bw/day for risk assessment has been determined for liver effects;**
- **Epidemiological studies suggest correlations between BPA exposure and heart diseases, liver toxicity and metabolic syndrome (diabetes and obesity); In vitro and in vivo studies corroborate the findings, though both, epidemiological and laboratory studies have limitations;**
- **Consequently, further exploration of the cause-effect relationship between BPA exposure and metabolic and/or cardiovascular diseases would require longitudinal epidemiological studies of larger populations.**

3.4 Genotoxicity of BPA

BPA appears to have demonstrated aneugenic potential in *in-vitro* tests in the absence of metabolic activation, however it did not demonstrate genotoxic potential in *in-vivo* studies. Risk assessments of e.g. the ECB (2003, 2008), EFSA (2006), Environment Canada and Health Canada (2008) did not consider genotoxicity as a toxicological endpoint of concern.

3.5 Carcinogenicity

The EU risk assessment report (ECB, 2008) concluded, that BPA has not shown any significant carcinogenic activity in two standardised oral cancer bioassays in rat and mice (NTP, 1982). More recent studies have investigated potential promoting effects of prenatal and/or neonatal exposure of rats to BPA on carcinogenesis induced by established carcinogens/initiators in specific organs (prostrate, uterus, thyroid, lungs, liver, thymus, oesophagus, liver and mammary gland), but the related studies were characterised by serious methodological limitations (ECB, 2008).

The risk assessment of Environment Canada and Health Canada (2008) recognised the interpretation from several studies that prenatal and/or neonatal exposure to BPA in rats at low doses during foetal development may increase susceptibility to neoplastic transformation in the prostate and mammary gland in adult rats (Ho *et al.,* 2006; Prins *et al.,* 2007; Murray *et al.,* 2007; Durando *et al.*, 2007; Markey *et al.,* 2003, 2005; Muñoz-de-Toro *et al.,* 2005; Newbold *et al.,* 2007; Keri *et al.,* 2007). However they also concluded that the limited evidence was insufficient to demonstrate that early BPA exposure, acting independently, could lead to neoplastic events. In the above mentioned studies as well as in more recent studies (Vandenberg *et al.,* 2008; Jenkins *et al.,* 2009) BPA was

usually administered via osmotic pumps implanted into pregnant dams or via maternal subcutaneous injection. In contrast to the results of these studies also the absence of carcinogenic effects following exposure during this sensitive period has been reported (Ichihara *et al.,* 2003).

The US FDA in its report of 2008 concluded that all of the studies highlighted in recent assessments, though interesting with regard to potential modes of action or target organs for BPA, are difficult to interpret with regard to long-term effects (chronic exposure) and oral safety assessment for regulatory purposes. This holds particularly true as available pharmacokinetic (PK) data indicate that routes of exposure for BPA are critical to any carcinogenic outcome.

The FDA further concluded that reported studies on BPA had several inconsistencies and inadequacies, such as non-oral routes of administration, limited endpoints, lack of proper histopathological evaluations and in-appropriateness of models used, including a lack of continuous exposure as would occur in the human population. Furthermore, findings from oral studies are limited in their interpretation and applicability in a risk assessment context.

Conclusions on carcinogenicity

- **BPA was not found to be carcinogenic in standard carcinogenicity studies;**
- **Concern has been expressed for increased susceptibility to precancerous changes following *in-utero* or neonatal exposure to BPA in rats;**
- **More information is necessary to understand the early life exposure to BPA in the process of carcinogenesis, in particular via routes most relevant to human exposure (i.e. oral).**

3.6 Reproductive toxicity of BPA

Reproductive toxicity of BPA is an issue of key importance for risk assessment. It has been shown that BPA can have an endocrine modulating activity in a number of *in-vitro* and *in-vivo* screening assays.

There is a significant number of studies on the reproductive toxicity of BPA in experimental animals published, many of them reporting effects at very low doses. The effects seen include change in prostate growth and development, mammary gland organisation, sexually dimorphic behaviour, onset of oestrus cyclicity, early puberty, body weight, genital malformations and others (reviewed in Richter *et al.*, 2007, Wetherill *et al.,* 2007; NTP, 2007; Willhite *et al.,* 2008).

It should be noted that the studies showing effects on fertility or development at very low dose levels are essentially screening tests and many of them employ experimental protocols, which have not undergone any international validation. These tests have used smaller numbers of animals and fewer or single dose groups than regulatory guideline studies. In addition, many *in-vivo* tests have used non-oral routes of exposure, which is important to consider for a substance like BPA, which shows its activity only as the parent compound, but

not in the conjugated form to which it is rapidly transformed following oral exposure. These above mentioned studies showed effects at doses that are lower than proposed safe levels (e.g. TDI) or sometimes even lower than measured exposure levels of humans. Several authors of these research studies suggest that the effects seen at low doses (µg/kg bw range) are not seen at intermediate dose levels (low mg/kg bw range) and are therefore not detected in traditional risk assessment studies. They propose a non-monotonic dose-response curve (inverted U-shape) for BPA effects on reproductive and developmental effects.

These studies have been reviewed extensively within several regulatory risk assessments but were considered of restricted relevance. The EU risk assessment (ECB, 2003) mainly considered as key studies three OECD test guideline-compliant and comprehensive studies: a two-generation study in the rat (Chemical Compound Safety Research Institute, 2000), a multi-generation study in the rat (Tyl *et al.,* 2002) and a continuous breeding study in the mouse (NTP, 1985).

Based on these studies it was concluded in 2001 that there was no convincing evidence that BPA is a developmental toxicant and a provisional NOAEL of 50 mg/kg bw/day for developmental effects, derived from the rat multi-generation study (Tyl *et al.,* 2002) was suggested for the risk characterisation.

To address the remaining uncertainties surrounding the potential for BPA to produce adverse effects on the development of the male reproductive tract at low doses (0.002-0.05 mg/kg bw/day) in mice, it was concluded that further research was needed. The EU regulators agreed on the conduction of a new 2 generation study, based on OECD Test Guideline 416, with modifications to further strengthen the validity of the results (second vehicle control group, a positive control group). The study also included additional assessments and extending histopathological examinations, and covered a total of 6 different exposure levels from very low (0.003 mg/kg bw/day) to relatively high (600 mg/kg bw/day) doses of BPA, where effects can be expected. A steering group (chaired by a representative of the European Commission's ECB and including experts from several EU Member States) was set up to supervise the study design and interpretation of the results. Within the context of the EU risk assessment (2008) this study was considered as the gold-standard on the reproductive toxicity of BPA to supersede all other preceding publications investigating the same standard reproductive and developmental endpoints.

The results of this two-generation study in mice (Tyl *et al.,* 2008) indicated no evidence of general toxicity at BPA exposure levels below 50 mg/kg/day, Nevertheless some limited effects such as slightly increased duration of gestation, reduced pup bodyweight during lactation, and a slight increase in the incidence of undescended testes, seminiferous tubule hypoplasia and delayed acquisition of preputial separation were observed in offspring at weaning at the highest exposure level of 600 mg/kg bw/day, where also general toxicity was seen.

In contrast to BPA exposure, the positive control group exposed to a natural hormone (17β-oestradiol) presented evidence of reproductive toxicity from

exposure levels of 0.01 mg/kg bw/day, indicating the sensitivity of CD-1 mice to oestrogens and the relatively limited oestrogenic activity of BPA in comparison.

Moreover, there was no indication that the male reproductive tract was affected by exposure to low doses of BPA, thus, the overall study NOAEL for both general and reproductive toxicity was set at 50 mg/kg bw/day.

In 2008, Environment Canada and Health Canada and US FDA established also a NOAEL of 50 mg/kg bw/day for reproductive effects. However, Environment Canada and Health Canada recognised that the diverging results in response to BPA exposure at low doses might arise from differences in a number of experimental variables such as species and strain differences, the tissues and/or endpoint(s) assessed, and variability in feed with respect to amounts of oestrogenic contaminants. In addition, inappropriate use or lack of positive controls as well as effects that present a non-monotonic dose-response curve needed to be taken into consideration for further assessment (vom Saal *et al.,* 2005; Richter *et al.*, 2007).

Additionally, the dosing period or time of exposure with respect to critical developmental windows were considered as important aspects, particularly when assessing alterations resulting from peri-natal exposures. Furthermore, the nature of the effects were such that it was difficult to characterise the degree to which they would be considered potentially adverse and, hence, form the basis of a human health risk assessment.

Since the finalisation and publication of the EU risk assessment report on BPA in 2008 more publications have become available investigating effects of BPA on fertility and development (e.g. Newbold *et al.*, 2009; Salian *et al.,* 2009a,b,c; Monje *et al.,* 2009; Bouskine *et al.,* 2009; Vandenberg *et al.,* 2007, 2008; Murray *et al.,* 2007; Wadia *et al.,* 2007).

Most of these studies have focused on exposure during postulated sensitive and vulnerable windows for development, such as the peri-natal or postnatal period, but have used small numbers of animals which were mainly exposed to BPA via subcutaneous implanted pumps (peri-natal) or subcutaneous injection (neonatal exposure). While informative, this new information needs to be seen in the context of the evaluation of similar earlier studies, showing similar low-dose effects (i.e. non-relevant routes of exposure from a risk assessment context, low statistical power, non-standard methods, etc).

In contrast to the above mentioned studies, one recent study (Ryan *et al.,* 2010) showed no effects on female Long Evans rat offspring exposed *in-utero* and during lactation via maternal oral exposure to BPA (2, 20 and 200 µg/kg bw/day). The control substance oestradiol (50 µg/kg bw/day) however induced effects such as increased anogenital distance and reduced pup body weight, acceleration of the age at vaginal opening, reduced F1 fertility and F2 litter sizes and malformations of the external genitalia (5 µg/kg bw/day) in a dose dependent manner. Oestradiol-exposed F1 females also showed indications of defeminisation of the central nervous system. This study also found no significant evidence for a non-monotonic (U-shaped) dose response curve of oestradiol.

Conclusions on reproductive toxicity

- **Some research studies have indicated a non-monotonic dose response curve with effects on reproduction and development at very low dose levels (µg/kg bw-range) disappearing at intermediate levels, with different effects appearing at higher mg/kg-bw doses;**
- **NOAELs from such studies would be at several orders of magnitude lower than the TDI and even lower than measured exposure levels in humans;**
- **However these studies have methodological limitations and were often not using the (relevant) oral route of administration;**
- **Guideline studies in compliance with GLP, using the oral route of exposure, did not confirm the above mentioned low dose effects of Bisphenol A on reproduction and development.**

3.7 Neurodevelopmental toxicity of BPA

Neural development is a complex process beginning in early embryonic development and continuing through different life stages. Precise functioning of the nervous system is critical for all mental, sensory and motor activities, and regulates homeostasis through interaction with the endocrine system (Clancy *et al.,* 2001).

Several studies in rodents have investigated neurotoxic endpoints and suggested that BPA treatment during development can cause alterations in brain development and behaviour at doses that are relevant to human exposure.

The EU risk assessment report from 2008 (ECB, 2008) included an extended review of all information available at that time on effects on neurological development following prenatal and perinatal exposure to BPA. From the large number of studies (>30), many developmental neurotoxicity endpoints were evaluated: locomotory and exploratory activity; grooming, cognitive, emotional, social, sexual and maternal behaviour; behavioural response to pharmacological challenge; brain morphology, immunohistochemistry, and receptor/gene expression. The impact of these studies on the hazard assessment for BPA was assessed using a weight of evidence approach that focused on the reliability and consistency of the evidence.

This evaluation revealed limitations in the design and reporting in all of the available studies including small group size, inappropriate statistical analysis, brief reporting of methods and results, lack of compliance with GLP or use of one BPA dose level. The overall assessment drew attention to a low level of confidence in the reliability of the studies and a lack of consistency in the results, such that no firm conclusions could be drawn.

Some European countries (Denmark, Sweden, Norway) did not agree with this conclusion and stated that some of the studies in the developmental neurotoxicity database were sufficiently reliable for regulatory use (Negishi *et al.*, 2004; Carr *et al.*, 2003; Ryan *et al.,* 2006; Adriani *et al.,* 2003). These

countries therefore suggested either to use the available, but limited data for risk assessment or to perform further investigation of developmental neurotoxicity. The final expert panel report on the reproductive and developmental toxicity of BPA performed by US NTP, reached a similar conclusion in November 2007.

The opinion of EFSA (2006) was in line with the ECB risk assessment report (2008), concluding that the neurobehavioral database revealed that there are no consistent adverse effects of peri-natal exposure to doses of BPA below 50 mg/kg bw/day.

Environment Canada and Health Canada stated that although the overall rigour of the neurobehavioral dataset was considered limited, the uncertainties associated with the results from neurodevelopmental and behavioural testing warranted concern with respect to human health, in particular during certain sensitive life stages.

The US National Toxicology Program (NTP) expressed some concern for adverse effects of BPA on the brain and behaviour based upon "limited evidence" of effects of low doses in rodent studies (NTP, 2008).

In order to respond to the remaining uncertainties of regulatory bodies in the EU and the USA with regard to possible neurodevelopmental effects, the American Chemistry Council (Stump et al., 2010) performed a further study. The objective of this study was to determine the potential of BPA to induce functional and/or morphological effects to the nervous system of offspring following oral exposure of the mother during pregnancy and lactation.

The study was conducted in accordance with OECD Test Guideline 426 and US EPA Guideline 870.6300 and was in conformance with GLP. The NOAEL for systemic effects was determined to be about 6 mg/kg bw/day and 13 mg/kg bw/day during gestation and lactation, respectively, corresponding to 75 mg BPA/kg feed. Effects such as reductions in mean body weight and body weight gain in dams and offspring could only be observed at high dietary concentrations of 750 and 2250 mg BPA/kg feed.

There was no evidence of developmental neurotoxicity at any dietary concentration and the NOAEL for developmental neurotoxicity of BPA was 164 mg/kg bw/day and 410 mg/kg bw/day during gestation and lactation, respectively, which was at the highest dietary concentration tested (2250 mg BPA/kg feed). Again, to put this into perspective, this high concentration is about 100.000 times higher than concentrations in infant food that may be derived from polycarbonate bottle release.

There was no evidence of non-monotonic exposure-response curves for any parameter and based on the conditions of this study, there was no evidence that BPA is a developmental neurotoxicant in rats.

In relation to the study described above the French Food Safety Agency (AFSSA) gave also its opinion in early 2010. AFSSA questioned whether the OECD Test Guideline 426 would be entirely suitable for characterising subtle effects on the nervous system, such as may be observed with endocrine disruptors and BPA in particular.

Another recent study showed that peri-natal and neonatal exposure to low doses of BPA (2.2 and 200 µg/kg bw/day) did not alter the expression of well characterised sexually dimorphic behaviours or alter the age of puberty or reproductive function in the female Long Evans rat offspring in contrast to oestradiol (Ryan *et al.,* 2010). A previous publication of the same research group (Howdeshell *et al.,* 2008) also did not reveal any effects on male rats, using the same treatment.

A recent epidemiological study suggested that pre-natal BPA exposure may be associated with externalising behaviours (e.g. aggression and hyperactivity) in two-year-old children, especially among female children (Braun *et al.,* 2009).

Conclusions on neurodevelopmental toxicity

- **Neural development has become the toxicological endpoint raising most concern for regulatory bodies ;**
- **A recent guideline study found no evidence that BPA is a developmental neurotoxicant.**
- **Many research studies report neurodevelopmental effects at very low dose levels, however the effects seen are manifold and not consistent.**
- **Despite a low confidence in the reliability of these studies, some regulators applied a precautionary approach and concluded that BPA may constitute a danger to human health.**
- **For a better understanding of the potential risk, all available information should be critically reviewed and evaluated in terms of validity and reliability of the test system and for their relevance for effects on the behaviour and cognitive development of humans at relevant exposure levels and exposure routes.**

4 Risk Characterisation

Risk assessment of BPA has been performed by different regulatory bodies based on available information on exposure to BPA and the data from toxicity studies (reviewed by Beronius *et al.,* 2009).

Depending on the different intended purposes of the evaluation, conclusions relating to risk are expressed differently. For example the purpose of the SCF and EFSA was to propose a TDI and to conclude whether or not exposure via food is sufficiently below this TDI. In contrast, in the assessments by the ECB, the National Institute of Advanced Industrial Science and Technology in Japan (AIST), Environment Canada and Health Canada and US FDA a *Margin of Safety* (MOS) or *Margin of Exposure* (MOE) was determined.

4.1 Selection of exposure values

The estimated exposure levels for the general population in most of the investigated risk assessments varied between 0.07 and 14 µg/kg bw/day. These exposure estimates were based on studies or assumptions regarding food intake and BPA concentrations in certain food commodities.

Estimated exposure levels based on urinary concentration were discussed but not included in most of the risk characterisations. Some studies however based the calculation of the total exposure on urinary concentrations (AIST, 2007; NTP-CERHR 2008). The Japanese risk assessment used also a probabilistic approach to exposure assessment (AIST, 2007). The use of maximum and/or average values for BPA concentrations in food and food intake varied between the different assessments. Estimates were generally considered to represent worst-case exposure scenarios.

In all of the assessments, it was concluded that, based on estimations derived from food consumption and concentration data, infants and/or young children have the highest exposure to BPA compared to the general population. This was explained by an estimated relatively high dietary intake via polycarbonate baby bottles and tableware as well as canned foods. Children also consume a large amount of food per kg body weight compared to adults.

The Chapel Hill experts (a group of experts that met in 2006 to examine the potential relationship between BPA exposure and negative trends in human health; vom Saal *et al.,* 2007) estimated an oral intake of BPA to be orders of magnitude higher than used in former risk assessment studies, i.e. 1500 µg/kg bw/day. They based this relatively high value on reported human blood concentrations of BPA and physiologically-based pharmacokinetic (PBPK) modelling. These experts discussed that the high BPA blood concentrations could indicate that there are other important sources of exposure than food intake.

4.2 Selection of dose descriptors for hazard assessment

Many studies have investigated toxic effects of BPA in animals and identified target organs. The effects of most concern have been those related to the

hormonal-like activity of BPA and potentially related effects on physical, neurological and behavioural development.

From the huge number of studies investigating the toxic effects of BPA, the two-generation study in mice (Tyl *et al.,* 2008) and the three-generation study in rats (Tyl *et al.,* 2002) were identified as critical studies for risk characterisation.

As already mentioned in the human health effects assessment section above, these studies used a large number of animals, investigated a wide range of doses and were conducted according to GLP and based on enhanced OECD guidelines specifically designed to pick up low dose effects. Regulatory bodies have generally 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.

Different expert groups have however interpreted the data from the same studies differently to establish the critical effect and the NOAEL for BPA to be used in the risk characterisation. The ECB (2003, 2008) established a NOAEL of 50 mg/kg bw/day (Tyl *et al.,* 2002, 2008) for systemic and reproductive effects to be carried forward to the risk characterisation. The effects seen at that dose such as changes in body and organ weight were not considered to be treatment related, as they were not consistent over generations or did not show a dose response relationship.

EFSA used an overall NOAEL of 5 mg/kg bw/day for systemic and reproductive effects in adult mice derived from the three-generation study (Tyl *et al.,* 2002) supported by the same NOAEL from the two-generation study (Tyl *et al.,* 2008), taking the abovementioned effects at 50 mg/kg bw to be treatment-related.

Japanese, American and Canadian risk assessors established a NOAEL at 5 mg/kg bw/day for systemic effects and an additional NOAEL for reproductive effects at 50 mg/kg bw/day. However, NTP (2008) and Environment Canada and Health Canada (2008) expressed some uncertainty about the NOAEL as there was also limited evidence for other effects, mainly on the development of the brain and behaviour, at lower doses in other studies.

At the Chapel Hill expert meeting (vom Saal *et al.*, 2007), the experts argued that the studies reporting effects of BPA exposure at low doses should be considered relevant for human health risk assessment and that a NOAEL has not been clearly established.

4.3 Assessment factors

The established NOAELs from toxicity studies are used in the risk assessment in different ways. In 2006, EFSA determined a tolerable daily intake (TDI) of 50 µg/kg bw/day by dividing the NOAEL of 5 mg/kg bw/day through a default assessment factor of 100 (10 for inter-species differences by 10 for intra-individual differences).

ECB (2003, 2008), US FDA (2008), AIST (2007) and Environment Canada and Health Canada (2008) calculated a Margin of Safety (MOS) and Margin of Exposure (MOE) respectively by dividing the NOAEL through the estimated

human exposure. The MOS or MOE are considered sufficient, if they are higher than a reference MOS or MOE. The MOS or MOE is an overall assessment factor, including the factors for interspecies and inter-individual and possible other factors. Usually the default overall assessment factor is 100 (10 for inter-species and 10 for inter-individual differences) but can be higher, if other factors for duration or uncertainty in the database are considered or lower, if the available data for example on toxicokinetics justifies lower assessment factors.

The assessment factors used for deriving the TDI (i.e. 100) or for the reference MOS should be considered conservative, as with an interspecies factor of 10, humans would be considered 10 times more sensitive than rodents. The faster metabolism of BPA in the human body in comparison to rodents would in principle allow reducing the interspecies factor, however this was not applied. The inter-individual variation of 10 is usually considered sufficient to cover all of the general population, including potentially more sensitive sub-population such as infants.

However, as discussed above in previous chapters, doubts were raised concerning the correct interpretation of the toxicokinetics data and it has been argued that BPA may also not be rapidly cleared from the body of human embryos and foetuses, which are exposed through the placenta, nor by newborns. The latter may therefore be a particularly vulnerable subpopulation.

Internal exposure simulations for BPA calculated, for the same external exposure, 3-11 fold higher blood concentrations of free BPA in newborns than in adults, based on assumed reduced glucuronidation activity in newborns.

Bio-monitoring data has shown that higher levels of un-conjugated BPA were found in human serum and urine than would be predicted from human toxicokinetics data.

These results appear to contradict the fast metabolism theory suggesting that humans would be less susceptible to BPA than laboratory animals. Other studies and opinions however are questioning the plausibility and reliability of such statements. The uncertainty around the toxicokinetics warrants further investigations and a re-consideration of whether the inter-individual factor of 10 can be considered sufficient to cover also potentially more vulnerable subpopulations such as unborn and infants.

4.4 Results of the risk characterisations

When based on the same dose descriptors (NOAELs), all risk assessment studies came to the same conclusion, .

The assessments by the ECB in 2003 and 2008, EFSA in 2006 and 2008, US FDA in 2008 and AIST in 2007 concluded that there is no risk to the general population based on the current knowledge.

Environment Canada and Health Canada in 2008 considered the margins of exposure in relation to the NOAELs of 5 and 50 mg/kg bw/day respectively, as sufficient. However they were concerned about neurodevelopmental and behavioural effects, which were suggested in some studies, though limited

when subjected to a weight of evidence analysis, at doses well below these NOAELs. As a consequence risk management measures have been proposed, e.g. the ban of polycarbonate baby bottles.

The ECB in 2008 concluded that the effects seen at dose levels several orders below the established NOAELs could not be used for risk characterisation because of a low level of confidence in the reliability of the studies and a lack of consistency in the results.

These conclusions are not supported by a number of scientists, which at the Chapel Hill meeting in 2006 concluded, that there is a risk to the entire population at current exposure levels. A recent publication (Beronius *et al.,* 2009) gives a detailed overview on the different health risk assessments of BPA and their conclusions.

Conclusions on risk assessment results

- **Traditional risk assessments were using worst case values for the exposure of the general public to consumer products of 0.07 – 14 µg/kg bw/day;**
- **The lowest overall NOAEL from guideline/GLP studies was determined to be 5 mg/kg bw/day;**
- **The margin of safety between the exposure and effect values were considered sufficient, considering a default assessment factor of 100 (10 for interspecies and inter-individual variation) or higher;**
- **It is discussed whether the assessment factors would be sufficient to cover also potentially susceptible subpopulations (unborn, newborns);**
- **Some research studies applying low doses of BPA suggest effects at several orders of magnitude lower than the guideline/GLP studies and even lower than estimated exposure levels;**
- **Even though results of different risk assessments are similar, the risk management decisions are different. Some countries consider a precautionary approach, based on remaining concerns for low dose effects and have proposed risk management measures (e.g. ban of baby bottles made from polycarbonate).**

5 Conclusions

The risk to humans of BPA exposure has been assessed during the last 10 years by several regulatory authorities, institutions and expert groups in Europe, the United States, Canada and Japan. Some of these risk assessments were carried out by regulatory bodies, whereas others were conducted by government-funded expert groups; in all cases, potential adverse health effects of BPA were identified and evaluated, and human exposure levels were estimated in order to draw conclusions about health risks at current exposure levels (Beronius et al., 2009). Nevertheless, there is so far no agreement upon the risk posed by BPA to human health.

By their very nature, the results of risk assessments depend on how a number of key factors are considered, such as the estimations of exposure levels, the identification of critical studies and NOAELs from these studies, the assessment factors and the significance attributed to reports of low-dose effects. In the case of BPA, the differences in the conclusions regarding human health risks of BPA and the selection of relevant studies for determining a NOAEL are largely due to diverging opinions concerning the reliability and relevance of studies reporting effects at low-doses.

Over the last decade, a large number of studies have reported effects of BPA exposure at doses in the µg/kg bw range, leading scientists and others to question the NOAEL established for regulatory purposes, which is three orders of magnitude higher. However, the studies reporting the low-dose effects and postulating an inverted U-shape dose-response curve (i.e. that effects are seen at very low and very high doses but would disappear at intermediate doses) generally did not comply with internationally accepted and validated test guidelines and GLP principles, such as the OECD guidelines. Because such criteria are normally considered a strength or even a quality criterion for evaluating toxicity data in several of the regulatory risk assessment processes, this yielded differing opinions concerning the reliability and relevance of these studies.

This approach has been criticised by some scientists (e.g. Myers et al. 2009), whose view is that guideline studies in compliance with GLP requirements for public health decisions are insufficient to guarantee scientific reliability and validity, and that such criteria should not be used to select adequate studies. They claim that guideline studies according to GLP standards with BPA that were the basis for decision making (especially Tyl et al. 2008) were using insensitive, out-of-date protocols, insensitive animal strains and would have other conceptual and methodological flaws. In the view of these criticisms, regulatory decisions should be based on studies using the most sensitive assays, and not depend on GLP as a criterion for selecting data. Tyl (2009) repelled the criticism and emphasized the importance for the risk assessment of guideline-compliant studies, because of their statistical power to detect reproducible effects linked to adverse outcomes by using appropriate exposures (routes, doses, durations), validated end points linked to adverse outcomes and appropriate group sizes and numbers. Exploratory research studies, she objected, are usually creative, short term and not tracked to adverse outcomes.

In the present report, the authors have made an overview of the scientific issues at the base of the on-going debate on BPA highlighting remaining uncertainties.

Concerning exposure, one can observe for example that there are few experimental studies on the release of BPA into the real food matrix so far, so one may envisage further release studies to be carried out with harmonized analytical protocols, not only on food simulants but also with infant formula preparations, to allow a better definition of realistic worst-case exposure scenarios.

As regards toxicity testing, the dose-response curve may be investigated further by orally administering a wide range of different doses to laboratory animals, in order to detect low dose and high dose effects. This may be complemented by the analysis of toxicokinetic parameters and particularly plasma and/or urine concentrations. Future studies may also include in-utero exposure followed by a long observation period to explore possible adverse effects later in life.

In parallel, monitoring of plasma and urine levels in humans exposed to different levels of BPA would allow to understand and quantify more precisely the interspecies differences between laboratory animals and humans. Longitudinal epidemiological studies of larger populations may help clarifying the cause-effect relationship between BPA exposure and metabolic and cardiovascular diseases.

Furthermore, the authors of the present report would like to underline that:

- In order to guarantee the acceptance of results of further testing on BPA, globally harmonized test programmes should be agreed preliminarily in an international context, also inviting those who are critical towards the existing risk assessments to participate. Such test programmes, to be carried out in an international context, could be further strengthened by being subject to monitoring and validation by an international panel of independent experts. To raise the quality and reliability of results, proficiency testing campaigns could be carried out prior to the performance of the study. In all these respects, initiatives like the Experts meeting called by WHO/FAO for October 2010 provide an excellent forum for discussion.
- Since BPA-containing products have already being banned by some countries, and because the industry has also started to voluntarily and progressively phase out polycarbonate baby bottles, attention should be paid not only to BPA, but also to the potential risks that may arise from substitute materials used in BPA-free products. Last but not least, looking not only at BPA but more generally at chemicals with endocrine disrupting potential, an emerging question is to what extent the principles traditionally used in risk assessment are appropriate for such substances. For example, whether or not it is correct to assume the existence of a threshold for certain effects, and whether or not the severity of an effect always increases as a result of increasing dose.

In all cases, cooperation and synergies between academia and regulatory bodies in the area of regulatory toxicology should be sought for and promoted,

to get the best scientific contribution out of both players. This requires setting common priorities, but also reaching consensus about the test protocols and the solidity of the investigation methods to be used. A good initiative in this sense was the decision of the U.S. National Institute for Environmental Health and Safety (NIEHS) to launch in 2009 a grants programme specifically addressing key uncertainties on BPA[3]. The laboratories receiving support were brought together to meet with scientists from academia and government, with the aim of reaching consensus on study protocols.

While it is too early to say whether this or other initiatives will eventually bring consensus about the health effects of BPA, the way the programme has called upon academic research laboratories and governmental bodies to cooperate is inspiring, and a very positive reference for similar action at international level.

[3] 28 Oct 2009: NIEHS Awards Recovery Act Funds to Address Bisphenol A Research Gaps, at http://www.niehs.nih.gov/news/releases/2009/bisphenol-research.cfm

Glossary and abbreviations

AIST	National Institute of Advanced Industrial Science and Technology (Japan)
AFSSA	Agence française de sécurité sanitaire des aliments (French Food Safety Agency)
BfR	Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment - Germany)
BPA	Bisphenol A
bw	Bodyweight
CEF Panel	Panel on food contact materials, enzymes, flavourings and processing aids (EFSA)
CERHR	Center for the Evaluation of Risks to Human Reproduction (U.S.)
EC	European Commission
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EDC	Endocrine Disrupting Chemicals
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked ImmunoSorbent Assay
EPA	Environmental Protection Agency (U.S.)
ER	Estrogen receptor
EU	European Union
F1	"first filial" generation consists of all the offspring from the parents
F2	"second filial" generation, comprised of offspring(s) resulting from a cross of the members of F1
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration (U.S.)
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
GPR30	G Protein-coupled Receptor 30
MOE	Margin of Exposure
MOS	Margin of Safety
NHANES	National Health and Nutrition Examination Survey (U.S.)
NIEHS	National Institute of Environmental Health Sciences
NOAEL	No observed adverse effect level
NTP	National Toxicology Program (U.S.)
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances (U.S.)
PBPK	Physiologically-based pharmacokinetics
PC	Polycarbonate
PES	Polyether sulphone
PP	Polypropylene
PVC	Polyvinyl chloride
RAR	Risk Assessment Report
REACH	Registration, Evaluation and Authorisation of Chemicals
SCF	Scientific Committee for Food
SML	Specific Migration Limit (for food contact materials)
TDI	Tolerable Daily Intake
VWA	Voedsel en Waren Autoriteit (Food & Consumer Product Safety Authority – NL)
WHO	World Health Organisation

References

Adachi T, Yasuda K, Mori C, Yoshinaga M, Aoki N, Tsujimoto G, et al., 2005. Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors. Food Chem Toxicol 43(5) 713–7199.

Adriani W, Seta DD, Dessi-Fulgheri F, Farabollini F, Laviola G, 2003. Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. Environ Health Perspect 111 395-401.

AFSSA, 2010. Opinion of the French Food Safety Agency on the critical analysis of the results of a study of the toxicity of bisphenol A on the development of the nervous system together with other recently-published data on its toxic effects. French Food Safety Agency, Request no. 2009-SA-0270,

AIST, 2007. Risk Assessment document Series No 4: Bisphenol A (Eds. Nakanishi J., Miyamoto K., Kawasaki H.). Japanese National Institute of Advanced Industrial Science and Technology,.. Available on-line at : http://unit.aist.go.jp/riss/crm/mainmenu/e_1-10.html.

Alonso-Magdalena P, Laribi O, Ropero AB, Fuentes E, Ripoll C, Soria B, Nadal A, 2005. Low doses of bisphenol A and diethylstilbestrol impair Ca2+ signals in pancreatic alpha-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. Environ Health Perspect 113(8) 969-77.

Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A, 2006. The estrogenic effect of bisphenol A disrupts pancreatic ß-cell function in vivo and induces insulin resistance. Environ Health Perspect 114 106–112.

Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M, Gauthier BR, Nef S, Stefani E, Nadal A, 2008. Pancreatic insulin content regulation by the estrogen receptor ER. PLoS ONE 3:e2069.

Beronius A, Rude C, Hakansson H, Hanberg A, 2009. Risk to all or none?. A comparative analysis of controversies in the health risk assessment of Bisphenol A. Reprod Toxicol doi:10.1016/j.reprotox.2009.11.007.

BfR, 2010. Selected questions and answers on bisphenol A in baby bottles and baby bottle teats. German Federal Risk Assessment Institute. Available from: www.bfr.bund.de/cd/7294

Biedermann-Brem S, Grob K, 2009. Release of bisphenol A from polycarbonate baby bottles: water hardness as the most relevant factor. European Food Research and Technology 228 679-684.

Biedermann-Brem S, Grob K, Fjeldal P, 2008. Release of bisphenol A from polycarbonate baby bottles: mechanisms of formation and investigation of worst case scenarios. European Food Research and Technology 227 1053-1060.

Biles JE, McNeal TP, Begley TH, Hollifield HC, 1997. Determination of Bisphenol-A in Reusable Polycarbonate Food-Contact Plastics and Migration to Food-Simulating Liquids, Journal of Agricultural and Food Chemistry 45 3541-3544.

Bouskine A, Nebout M, Brücker-Davis F, Benahmed M, Fenichel P, 2009. Low Doses of Bisphenol A Promote Human Seminoma Cell Proliferation by Activating PKA and PKG via a Membrane G-Protein–Coupled Estrogen Receptor Environ Health Perspect 117 1053–1058.

Braniste V., Jouault A, Gaultier E., Polizzi A., Buisson-Brenac C., Leveque M., Martin P.G., Theodorou V., Fioramonti J., Houdeau E. Impact of oral Bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. PNAS. Edition of 14-18 December 2009

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Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM,Lanphear BP, 2009. Prenatal Bisphenol A Exposure and Early Childhood Behavior. Environ Health Perspect 117 1945–1952.

Brede C, Fjeldal P, Skjevrak I, Herikstad H, 2003. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Additives and Contaminants 20 684-689.

Carr RL, Bertasi FR, Betancourt AM, Bowers SD, Gandy BS, Ryan PL, Willard ST 2003. Effect of neonatal rat bisphenol A exposure on performance in the Morris water maze. J Tox Environ Health Part A. 66 2077-2088.

R.E. Chapin, J. Adams, K. Boekelheide, L.E. Gray, S.W. Hayward, P.S.J. Lees, B.S. McIntyre, K.M. Portier, T.M. Schnorr, S.G. Selevan, J.G. Vandenbergh, S.R. Woskie 2008. NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. Birth Defects Research (Part B) 83 157–395

Chemical Compound Safety Research Institute (2000). Two-generation reproduction study of bisphenol A in rats. Chemical Compound Safety Research Institute unpublished report - study No: SR-98101.

Clancy B, Darlington RB, Finlay BL 2001. Translating developmental time across mammalian species. Neuroscience 105(1) 7-17.

D'Antuono A, Campo Dall'Orto V, Lo Balbo A, Sobral S, Rezzano I, 2001. Determination of Bisphenol A in Food-Simulating Liquids Using LCED with a Chemically Modified Electrode, Journal of Agricultural and Food Chemistry 49 1098-1101.

Dekant W, Volkel W, 2008. Human exposure to bisphenol A by biomonitoring: methods, results and assessment of envi¬ronmental exposures. Toxicol Appl Pharmacol 228 114–134.

Domoradzki JY, Pottenger LH, Thornton CM, Hansen SC, Card TL, Markham DA, et al., 2003. Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages. Toxicol Sci 76 21–34.

Domoradzki JY, Thornton CM, Pottenger LH, Hansen SC, Card TL, Markham DA, Dryzga MD, Shiotsuka RN, and Waechter JM Jr, 2004. Age and dose dependency of the pharmacokinetics and metabolism of bisphenol A in neonatal sprague-dawley rats following oral administration. Toxicol Sci 77 230-42.

Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, Munoz-de-Toro M, 2007. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environ Health Perspect 115(1) 80-86.

ECB 2003. European Union Risk Assessment Report. 4,4'-isopropylidenediphenol (bisphenol-A), CAS No: 80-05-7. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission Joint Research Centre, 3rd Priority List, Luxembourg: Office for Official Publications of the European Communities, EUR 20843 EN. Available from: http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=ora

ECB 2008. European Union Risk Assessment Report Draft: Updated risk assessment of 4,4'-isopropylidenediphenol (Bisphenol A) (CAS No. 80-05-7; EINECS No. 201-245-8). European Commission, European Chemicals Bureau, Existing Substances. Available from: http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=ora

ECHA (European Chemicals Agency) (2008). REACH Guidance on Information Requirements and Chemicals Safety Assessment. European Chemicals Agency. Available at: http://guidance.echa.europa.eu/.

Edginton A, Ritter L, 2009. Predicting Plasma Concentrations of Bisphenol A in Children Younger Than 2 Years of Age after Typical Feeding Schedules, using a Physiologically Based Toxicokinetic Model. Environ Health Perspect 117 645–652.

EFSA 2006. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A) Question number EFSA-Q-2005-1 00. The European Food Safety Authority Journal, 428:1-75. Available from: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620772817.htm

EFSA 2008. Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC) on toxicokinetics of bisphenol A. The European Food Safety Authority Journal 759:1–10. Available: http://www.efsa.europa.eu/en/scdocs/scdoc/759.htm.

Ehlert K.A., Beumer C.W.E., Groot M.C.E., 2008. Migration of bisphenol A into water from polycarbonate baby bottles during microwave heating. Food Additives and Contaminants 25(7) 904–910.

Elobeid MA, Allison DB, 2008. Putative environmental-endocrine disruptors and obesity: a review. Curr Opin Endocrinol Diabetes Obes 15(5) 403-8. Review.

Environment Canada and Health Canada 2008. Screening Assessment for the Challenge Phenol, 4,4' -(1-methylethylidene)bis- (Bisphenol A).. Available from: http://www.ec.gc.ca/substances/ese/eng/challenge/batch2/batch2_80-05-7.cfm

EU 1982. COUNCIL DIRECTIVE of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs (82/711/EEC). Official Journal of the European Union L297 26-30. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31982L0711:EN:NOT

EU 2002. COMMISSION DIRECTIVE 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs Official Journal of the European Union L220 18-58. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002L0072:EN:NOT

Ginsberg G, Rice D, 2009. Does Rapid Metabolism Ensure Negligible Risk from Bisphenol A? Environ Health Perspect 117 1639–1643.

Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, et al., 1998. Bisphenol A interacts with the estrogen receptor alphain a distinct manner from estradiol. Mol Cell Endocrinol 142(1/2) 203–14.

Ho SM, Tang WY, Belmonte de Frausto J, Prins GS, 2006. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66(11) 5624–32.

Hong YC, Park EY, Park MS, Ko JA, Oh SY, Kim H, Lee KH, Leem JH, Ha EH, 2009. Community level exposure to chemicals and oxidative stress in adult population. Toxicol Lett 184(2) 139-44.

Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE Jr, 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male long evans hooded rat. Toxicol Sci 102(2):371-82.

Howe SR, Borodinsky L, 1998. Potential exposure to bisphenol A from food-contact use of polycarbonate resins. Food Additives and Contaminants 15(3) 370-375.

Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T, 2003. Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. J Toxicol Sci 28(3) 165-171.

Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y, 2002. Determination of bisphenol A concentrations in human bio¬logical fluids reveals significant early prenatal exposure. Hum Reprod 17 2839–2841.

Inoue H, Yuki G, Yokota H, Kato S, 2003. Bisphenol A glucuronidation and absorption in rat intestine. Drug Metab Dispos 31 140–144.

Inoue K, Yamaguchi A, Wasa M, Yoshimura Y, Makino T, Nakazaw H, 2001. Quantitative detection of bisphenol A diglycidyl ether metabolites in human plasma by liquid chromatography-electrospray mass spectrometry. J chromatogr B Biomed Sci Appl 765 121-126.

Japan 1998. An Interim Report of the Japanese Study Group on Health Effects of Endocrine Disrupting Chemicals
http://www.ffcr.or.jp/__492565a0001a8909.nsf/0/5e69e178bbad89414925681d001fa22d?OpenDocument

Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, Lamartiniere C, 2009. Oral Exposure to Bisphenol A Increases Dimethylbenzanthracene-Induced Mammary Cancer in Rats. Environ Health Perspect 117 910–915.

Kawamura Y, Koyano Y, Takeda Y, Yamada T, 1998. Migration of Bisphenol a from Polycarbonate Products. Journal of the Food Hygienic Society of Japan 39(3) 206-212.

Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS, 2007. An evaluation of evidence for the carcinogenic activity of bisphenol A. Reprod Toxicol 24(2) 240-252.

Kubwabo C., Kosarac I., Stewart B., Gauthier B.R., Lalonde K., Lalonde P.J. 2009. Migration of bisphenol A from plastic baby bottles, baby bottle liners and reusable polycarbonate drinking bottles. Food Additives and Contaminants 26(6) 928–937

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA, 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 39(10) 4252-63.

Kurebayashi H., Betsui H., Ohno Y. (2003) Disposition of a low dose of 14C-bisphenol A in male rats and its main biliary excreation as BPA glucuronide. Toxicological Sciences 73(1) 17-25

Kurebayashi H, Harada R, Stewart RK, Numata H, Ohno Y, 2002. Disposition of a low dose of bisphenol A in male and female cynomolgus monkeys. Toxicol Sci 68 32–42.

Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, Melzer D, 2008. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300:1303–1310.

Lázaro Martínez J.M., Leal Denis M.F., Denaday L.R., Campo Dall' Orto V. 2009. Development and characterization of a new polyampholyte–surfactant complex applied to the solid phase extraction of bisphenol-A. Talanta 80 789–796

Le HH, Carlson EM, Chua JP, Belcher SM, 2008. Bisphenol A is released from polycarbonate drinking bottles and mimics the neu¬rotoxic actions of estrogen in developing cerebellar neurons. Toxi¬col Lett 176 149–156.

Lee YJ, Ryu HY, Kim HK, Min CS, Lee JH, Kim E, Nam BH, Park JH, Jung JY, Jang DD, Park EY, Lee KH, Ma JY, Won HS, Im MW, Leem JH, Hong YC, Yoon HS, 2008. Maternal and fetal exposure to bisphenol A in Korea. Reprod Toxicol 25(4) 413-9.

Maia J., Cruz J.M., Sendón R., Bustos J., Sanchez J.J., Paseiro P. 2009. Effect of detergents in the release of bisphenol A from polycarbonate baby bottles. Food Research International 42 1410–1414

Maragou NC, Makri A, Lampi EN, Thomaidis NS, Koupparis MA, 2008. Migration of bisphenol A from polycarbonate baby bottles under real use conditions, Food Additives and Contaminants 25(3) 373–383.

Markey CM, Coombs MA, Sonnenschein C, Soto AM, 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. Evol Dev 5(1) 67-75.

Markey CM, Wadia PR, Rubin BS, Sonnenschein C, Soto AM, 2005. Long-term effects of fetal exposure to low doses of the xe¬noestrogen bisphenol-A in the female mouse genital tract. Biol Reprod 72 1344–1351.

Matthews JB, Twomey K, Zacharewski TR, 2001. In vitro and in vivo interactions of bisphenol A and its metabolite A glucuronide, with estrogen receptors alpha and beta. Chem Res Toxicol 14 149-157.

Mercea P, 2009. Physicochemical processes involved in migration of bisphenol A from polycarbonate. Journal of Applied Polymer Science 112 579–593.

Mielke H, Gundert-Remy U, 2009. Bisphenol A levels in blood depend on age and exposure. Toxicol Lett. 190(1) 32-40.

Monje L, Varayoud J, Muñoz-de-Toro M, Luque EH, Ramos JG, 2009. Neonatal exposure to bisphenol A alters estrogen-dependent mechanisms governing sexual behavior in the adult female rat. Reproductive Toxicology 28(4) 435-442

Mountfort KA, Kelly J, Jickells SM, Castel L, 1997. Investigations into the potential degradation of polycarbonate baby bottles during sterilization with consequent release of bisphenol A. Food Add Contam 14 737-740.

Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C, Soto AM, 2005. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology 146(9) 4138-47.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM, 2007. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol 23(3) 383-390.

Myers JP, vom Saal FS, Akingbemi BT, Arizono K, Belcher S, Colborn T, Chahoud I, Crain DA, Farabollini F, Guillette LJ, Hassold T, Ho S-M, Hunt PA, Iguchi T,Jobling S, Kanno J, Laufer H, Marcus M, McLachlan JA, Nadal A, Oehlmann J, Olea N, Palanza P, Parmigiani S, Rubin BS, Schonf elder G, Sonnenschein C, Soto AM, Talsness CE, Taylor JA, Vandenberg LN,Vandenbergh JG, Vogel S, Watson CS, Welshons WV, Zoeller RT, 2009. Why public health agencies cannot depend upon ‘Good Laboratory Practices’ as a criterion for selecting data: the case of bisphenol-A. Environ Health Perspect 117 309-315.

Nadal A, Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB, 2009. The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. Mol Cell Endocrinol 304(1-2):63-8.

Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y, 2004. Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by

perinatal exposure to bisphenol A and nonylphenol in male rats. Environ Health Perspect 112 1159-64.

Newbold R, Jefferson WN, Padilla-Banks E, 2009. Prenatal Exposure to Bisphenol A at Environmentally Relevant Doses Adversely Affects the Murine Female Reproductive Tract Later in Life. Environ Health Perspect 117 879–885.

Newbold RR, Jefferson WN, Padilla-Banks E, 2007. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod Toxicol 24(2) 253 - 258.

Newbold RR, Padilla-Banks E, Jefferson WN, 2009a. Environmental estrogens and obesity. Mol Cell Endocrinol 304(1-2) 84-9.

NTP, 1982. Carcinogenesis bioassay of bisphenol A in F344 rats and B6C3F1 mice (feed study). No. 215. Research Triangle Park (NC): National Toxicology Porgram (US).

NTP, 1985. Bisphenol A: Reproductive and fertility assessment in CD-1 mice when administered in the feed. National Toxicology Program Report NTP-85-192; Order No. PB86-103207 (NTIS) 1-346.

NTP-CERHR, 2007. Expert panel report on the reproductive and developmental toxicity of bisphenol A. http://cerhr.niehs.nih.gov/chemicals/bisphenol/BPAFinalEPVF112607.pdf

NTP-CERHR, 2008. Monograph on the potential human reproductive and developmental effects of bisphenol A. http://cerhr.niehs. nih.gov/chemicals/bisphenol/bisphenol.pdf

Pennie WD, Aldridge TC, Brooks AN, 1998. Differential activation by xenoestrogens of ER alpha and ER beta when linked to different response elements. J Endocrinol 158(3) R11-4.

Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM, 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicol Sci 54 3–18.

Prichet JJ, Kuester RK, Sipes IG, 2002. Metabolism of bisphenol A in primary cultured hepatocytes from mice, rats, and h umans. Drug Metab Dispos 30 1180-1188.

Prins GS, Birch L, Tang WY, Ho SM, 2007. Developmental estrogen exposures predispose to prostate carcinogenesis with aging. Reprod Toxicol 23(3) 374-82.

Richter C, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS, 2007. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol 24 199–224.

Ropero AB, Alonso-Magdalena P, García-García E, Ripoll C, Fuentes E, Nadal A, 2008. Bisphenol-A disruption of the endocrine pancreas and blood glucose homeostasis. Int J Androl 31(2) 194-200.

Ryan BC, Hotchkiss AK, Crofton KM, Gray LE Jr, 2010 In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. Toxicol Sci. 2010 114(1) 133-48.

Ryan BC, Vandenbergh JG, 2006. Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. Hormones and Behav. 50 85-93.

Salian S, Doshi T, Vanage G, 2009. Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. Toxicology 265 56–67.

Salian S, Doshi T, Vanage G, 2009a. Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A. Life Sciences 85 11–18.

Salian S, Doshi T, Vanage G, 2009bc. Perinatal exposure of rats to Bisphenol A affects the

fertility of male offspring. Life Sciences 85 742-752.

Sekizawa J, 2008. Low-dose effects of bisphenol A: a serious threat to human health? J Toxicol Sci 33(4) 389-403.

SCF 2002. European Commission—Health & Consumer Protection Directorate-General. Opinion of the Scientific Committee on Food on Bisphenol A. European Commission—Health & Consumer Protection Directorate-General, SCF/CS/PM/3936 Final2002. Available online at: http://ec.europa.eu/food/fs/sc/scf/out128_en.pdf.

Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y, Ozawa S, 2002 Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicol In Vitro 16 549-556.

Sim J., Jianhua L. 2008. Chemical test for baby bottles sample, TÜV Sud PSB Singapore, Report S08CHM03672-JS of 23 Jul. 2008 www.takaso.com/img/BPA.pdf

Snyeder RW, Maness SC, Gaido KW, Welsch F, Sumner SC, Fennell TR, 2000. Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol 168 225-234.

Stahlhut RW, Welshons WV, Swan SH, 2009. Bisphenol A data in NHANES suggest longer than expected half-life, substantial non-food exposure, or both. Environ Health Perspect 117 784–789.

Stump DG, Beck MJ, Radovski A, Garman, RH, Freshwater LL, Sheets LP, Marty MS, Waechter JM Jr, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Chappelle AH, Hantges SG, 2010. Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. Toxicol Sci 115(1) 167-82.

Sun Y, Wada M, Al-Dirbashi O, Kuroda N, Nakazawa H, Nakashima K, 2000. High-performance liquid chromatography with peroxyoxalate chemiluminescence detection of bisphenol A migrated from polycarbonate baby bottles using 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride as a label. J Chrom B Biomed Sci Appl 749 49-56.

Takeuchi T, Tsutsumi O, Nakamura N, Ikezuki Y, Takai Y, Yano T, et al., 2004. Gender difference in serum bisphenol A lev¬els may be caused by liver UDP-glucuronosyltransferase activity in rats. Biochem Biophys Res Commun 325 549–554.

Tan B, Mustafa A, 2003. Leaching of bisphenol A from new and old babies' bottles and new babies' teats, Asia Pacific Journal Public Health 15 118–123.

Tsukioka T, Brock J, Graiser S, Nguyen J, Nakazawa H, Makino T, 2003. Determination of trace amounts of bisphenol A in urine by negative-ion chemical-ionization-gas chromatography/mass spectrometry. Anal Sci. 19(1) 151-3.

Tyl RW, Myers CB, Marr MC. 2005. Thirteen-week range-finding study for the two-generation reproductive toxicity evaluation of Bisphenol A (BPA; CAS No. 80-05-7) administered in the feed to CD-1 (Swiss) mice. Final report to the American Plastics Council, dated August 10, 2005; unpublished

Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter Jr JM, 2008. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol Sci 104 362–384.

Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, SeelyJC, Joiner RL, Butula JH, Dimond SS, Cagen SZ, Shitsuka RN, Stropp GD, Waechter JM, 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci 68 121–146.

Tyl RW. 2009. Basic Exploratory Research versus Guideline-Compliant Studies Used for Hazard Evaluation and Risk Assessment: Bisphenol A as a Case Study. Environ Health Perspect 117 1644-1651.

US FDA 2008. Draft assessment of bisphenol A for use in food contact applications. United States Food and Drug Administration. Available from: http://www.fda.gov/ohrms/dockets/AC/08/briefing/2008-0038b1_01_02_FDA%20BPA%20Draft%20Assessment.pdf

Vandenberg L, Maffini M, Schaeberle C, Angelo A. Ucci, Sonnenschein C, Rubin B, Soto A, 2008. Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. Repr Tox 26 210–219.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV, 2007. Human exposure to bisphenol A (BPA). Reprod Toxicol 24 139–177.

Völkel W, Bittner N, Dekant W, 2005. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by HLPC-MS/MS. Drug Metab Dispos.

Völkel W, Colnot T, Csanady GA, Filser JG, Dekant W, 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem Res Toxicol 15 1281–1 287.

vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette LJ, Hauser R, Heindel JJ, Ho S-M, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N, Prins GS, Richter CA, Rubin BS, Sonnenschein C, Soto AM, Talsness CE, Vandenbergh JG, Vandenberg LN, Walser-Kuntz DR, Watson CS, Welshons WV, Wetherill YB, Zoeller RT, 2007. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol 24 131–138.
vom Saal FS, Myers JP 2008. Bisphenol A and Risk of Metabolic Disorders, *JAMA.* 2008;300(11):1353-1355

vom Saal FS, Richter CA, Ruhlen RR, Nagel SC, Timms BG, Welshons WV, 2005. The importance of appropriate controls, animal feed, and animal models in interpreting results from low-dose studies of bisphenol A 7. Birth Defects Res A Clin Mol Teratol 73(3) 140-145.

VWA 2005. Migration of bisphenol A and plasticizers from plastic feeding utensils for babies, Dutch Food and Consumer Product Safety Authority, report no. ND05o410. Available online at: http://www.vwa.nl/portal/page?_pageid=119,1639827&_dad=portal&_schema=PORTAL&p_file_id=10413

VWA 2008. Bisphenol A in baby bottles (in Dutch), Dutch Food and Consumer Product Safety Authority, project no. ND082217. Available online at: http://www.vwa.nl/portal/page?_pageid=119,1639827&_dad=portal&_schema=PORTAL&p_file_id=31842

Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM, 2007. Perinatal Bisphenol A Exposure Increases Estrogen Sensitivity of the Mammary Gland in Diverse Mouse Strains Environ Health Perspect 115 592–598.

Welshons WV, Nagel SC, vom Saal FS. 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology. 147(6 Suppl) S56-69.

Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM, 2007. In vitro molecular mechanisms of bisphenol A action. Reprod

Toxicol. 24(2) 178-98.

WIL Research Laboratories, LLC; 2009. Dietary Developmental Neurotoxicity Study of Bisphenol A in Rats. unpublished study for which the full study report is available

Willhite CC, Ball GL, McLellan CJ, 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. J Toxicol Environ Health B Crit Rev 11(2):69-146. Review.

Wong KO, Leo LW, Seah LH, 2005. Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles. Food Additives and Contaminants 22(3) 280-288

Ye X, Kuklenyik Z, Needham LL, Calafat AM, 2006. Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry. J Chromatogr B. 831(0) 110-115

Yoshida H, Harada H, Nohta H, Yamaguchi M, 2003. Liquid chromatographic determination of bisphenols based on intramolecular excimer-forming fluorescence derivatization. Analytica Chimica Acta 488 211–221.

Zalko D., Soto, A.M., Dolo, L., Dorio, C., Rathahao, E. Debrauwer L., Faure, R.Cravedi J.P, , 2003. Biotransformations of bisphenol A in a mammalian model: Answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environmental Health Perspectives* 111 (3), pp. 309-319

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Abstract

Despite the fact that for more than a decade many toxicological studies were carried out world wide, there is not yet a full understanding of the impact of Bisphenol A (BPA) on human health. As BPA may migrate into infant formula preparations from polycarbonate baby bottles, there is a special concern about its possible effect on the development of infants and young children. The potential endocrine disrupting properties of BPA trigger especially this discussion. Several risk assessment studies have been performed; nevertheless, there is still not yet a full agreement between all the risk assessors and the issue of BPA continues to generate discussion and is at the centre of political debate.

Most of the debate arise from diverging opinions concerning the reliability and relevance of studies reporting effects at low doses, often carried out in university laboratories, without following international guideline criteria or good laboratory practices. However, some researchers believe that these criteria should not be used to select best available information. In principle, this controversy might be solved via a new series of globally agreed toxicological studies, possibly to be carried out under the supervision of a panel of independent experts, with the participation of both academic research laboratories and regulatory bodies. To raise the quality and reliability of results, it may be agreed to carry out proficiency testing campaigns prior to the performance of the study.

This report provides an overview of the scientific issues which are at the base on the on-going discussions on BPA, by summarising the risk assessment activities carried out so far, having taken into account the latest scientific information available, and considering future challenges, such as the lack of information on some BPA-free plastics which may be used as substitutes for polycarbonate.

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