## SCIENTIFIC OPINION

# Scientific Opinion on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A[1]

**EFSA Panel on food contact materials, enzymes, flavourings and processing aids (CEF)**[2, 3]

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## SUMMARY

Bisphenol A [2,2-bis(4-hydroxphenyl)propane, CAS Number 80-05-7] (BPA) is used as a monomer in the manufacture of polycarbonates and epoxy resins, as an antioxidant in PVC plastics and as an inhibitor of end polymerisation in PVC. Polycarbonates are used in food contact plastics such as reusable beverage bottles, infant feeding bottles, tableware (plates and mugs) and storage containers, whereas epoxy resins are used in protective linings for food and beverage cans and vats.

Small amounts of BPA can potentially leach out from food containers into foodstuffs and beverages and therefore be ingested. BPA is permitted for use in food contact plastics in the European Union with a specific migration limit of 0.6 mg/kg food[4].

In 2006, EFSA set the TDI for BPA at 0.05 mg BPA/kg body weight (b.w.)/day. This is based on the No-Observed-Adverse-Effect-Level (NOAEL) of 5 mg/kg b.w./day that has been identified in two multi-generation reproductive toxicity studies in rodents, where the critical effects were changes in body and organ weights in adult and offspring rats and liver effects in adult mice, respectively (EFSA, 2006). In 2008, EFSA reaffirmed this TDI, concluding that age-dependent toxicokinetics differences of BPA in animals and humans would have no implication for the default uncertainty factor (UF) of 100 and in turn for the TDI.

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[1] On request from the European Commission, Questions No EFSA-Q-2009-00864, EFSA-Q-2010-01023 and EFSA-Q-2010-00709, adopted on 23rd September 2010.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Groups on Bisphenol A: David Bell (resigned on 15 January 2010), Wilfried Bursch, Berit Granum, Ulla Hass (her participation was limited to the evaluation of the Stump study until 26 March 2010), Edel Holene, Trine Husøy, Alberto Mantovani, Wim Mennes, Unni Cecilie Nygaard, Terry Parker, Ulrike Reuter, Emanuela Testai, Rosemary Waring, Detlef Wölfle for the preparatory work on this scientific opinion and EFSA staff: Anna F. Castoldi, Saghir Bashir, Laura Ciccolallo, Cristina Croera, Tomas Oberg, Roberta Pinalli Anne Theobald and Didier Verloo for the support provided to this scientific opinion.

[4] Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intending to come into contact with foodstuffs.

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The present opinion follows the requests of the European Commission (EC) to the Panel on food contact materials, enzymes, flavourings and processing aids (CEF) (I) to evaluate a dietary developmental neurotoxicity study of BPA in rats (Stump, 2009); (II) recent scientific literature (2007-2010) in terms of relevance for the risk assessment of BPA and impact on the current tolerable daily intake (TDI) of 0.05 mg BPA/kg body weight (b.w.)/day; (III) to provide advice on the Danish risk assessment underlying the Danish ban of BPA in food contact materials for infants aged 0-3 years.

In order to provide a global view on the risk assessment of Bisphenol A, the CEF Panel decided to address the three mandates as given above in a single opinion and postponed the adoption of this comprehensive document to 23rd September 2010.

The three different questions raised by the Commission are dealt with in three different parts (PARTS I to III) of the opinion. PART IV presents an overview of the conclusions from PARTS I to III, together with an overall conclusion.

PART I

The GLP compliant study by Stump (2009) was performed according to OECD guideline 426 to address any uncertainty regarding potential neurodevelopmental effects of BPA. BPA was administered daily in the diet at concentrations of 0, 0.15, 1.5, 75, 750, and 2250 mg/kg feed to female Sprague-Dawley rats from gestational day (GD) 0 to postnatal day (PND) 21. The relative estimated intakes (in mg/kg b.w./day) were 0, 0.01, 0.12, 5.85, 56.4 and 164 during gestation and 0, 0.03, 0.25, 13.1, 129 and 410 during lactation. The CEF Panel considers this treatment schedule as relevant to human exposure *in utero* and via either breastfeeding or infant bottle feeding (in this study the estimated exposure of rat pups to BPA is *ca.* 30 times higher than that of bottle-fed infants). Dams were evaluated for general signs of toxicity and offspring were evaluated for general toxicity including developmental landmarks and for neurological effects, including behaviour and brain histopathology.

For systemic toxicity (i.e. decreased body weight and/or body weight gain), a NOAEL of 5.85 mg/kg b.w./day during gestation was identified for both mothers and offspring.

Male and female pups were tested for developmental landmarks and for neurological effects, including behaviour and brain histopathology.

On PND 11 2 and 4 pups from the 750 and 2250 mg/kg feed groups, respectively, underwent popcorn seizures and convulsions. This effect was not reproducible in any other published study/report including a follow-up study by Stump (2009). The Panel concluded that the current NOAEL for BPA (5 mg/kg b.w./day) would be sufficiently low to exclude any concern for this effect.

The study by Stump covers motor activity, learning and memory (spatial behaviour), auditory startle response, brain histopathology and morphology. The study does not cover some specific aspects of learning and memory (i.e. avoidance learning, schedule-controlled behaviour, and impulsiveness), anxiety-related behaviour or sexual dimorphic behaviour, but this does not invalidate the study. No treatment-related changes were observed in motor activity tests, auditory startle response or brain histopathology and morphology.

According to the statistical analysis by the study authors, in the Biel maze swimming test for learning and memory on PND 62 the male offspring from the 0.15 mg/kg feed exposure group showed an increase in the number of errors in Path A trials 1-4, which could be interpreted as a delay in learning. The effect reached statistical significance when compared to concurrent controls. No such an effect was observed in pups studied on PND 22 or in any other exposure group on PND 62. When the swimming trials were conducted in the reverse path (i.e. the path B) no delay in learning was observed and also in the repeat Path A trials 11 and 12, no effect was observed in any exposure group, including the 0.15 mg/kg feed group. Therefore, the authors concluded that there were no changes in learning and memory.

The Panel noted that in the Biel Maze test only the error counts were reported as a measure of learning and memory. The animals should have also been evaluated for effects on "time-to-escape" and checked for long term memory effects. EFSA's Assessment and Methodology Unit (AMU) applied a more appropriate statistical evaluation to the Biel Maze data. It was realised that the data suffer from censoring[5]. Based on the re-analysis the Panel considered that no conclusion can be drawn from this study on the effect of BPA on learning and memory behaviour due to large variability in the data.

Based on the body weight effects on dams and offspring and also taking into account the occurrence of seizures and convulsions in the two highest dose groups, which were not observed at the lower dose levels, the study supports the NOAEL which was derived from multigeneration studies in the past (5 mg/kg b.w./day), leading to a TDI of 0.05 mg/kg b.w./day. However, the Panel considered that this test on learning and memory was inconclusive and is of limited value in the risk assessment of BPA.

Overall, based on the body weight effects on dams and offspring and taking into account the occurrence of seizures and convulsions only at the two highest doses, the study supports the previously identified NOAEL of 5 mg/kg b.w./day. However, the Panel considered that this study was inconclusive and is of limited value in the ongoing risk assessment of BPA.

PART II

The CEF Panel has reviewed toxicological data published between 2007 and July 2010 mainly focusing on toxicokinetic, human and animal toxicity studies. For risk assessment purposes only studies complying with these *inclusion criteria* were considered: full research papers in peer-reviewed journals available in the public domains and reporting original data; all human studies (except for purely biomonitoring studies). For the *in vivo* animal toxicity studies the focus was on low dose oral studies employing several test doses including at least one <5 mg/kg b.w./day and involving developmental exposure.

These studies were further assessed with respect to *quality criteria* (sufficient sample size, adequacy of control procedures, inclusion of positive controls when applicable, assessment of correlation between morphological and functional changes, and consideration of litter or dam as the appropriate statistical unit) in order to assess the validity and/or applicability of the individual findings to human risk assessment.

Studies on toxicokinetics of BPA have demonstrated a significantly lower internal exposure after oral intake as compared to parenteral exposure. This confirms that toxicity studies with oral administration have higher relevance for human risk assessment of BPA in food than studies with parenteral administration. In addition, new findings in non human primates (both adults and newborns) further strengthen the view that BPA is eliminated faster in humans than in rodents. This fast BPA elimination in primates results in substantially lower internal exposure to free BPA in humans as compared to rodents. Even human premature infants can metabolise and excrete BPA efficiently (via glucuronidation and sulfation), as supported by recent human data and data in young monkeys. The use of the standard uncertainty factor (UF) of 10 to take into account interspecies differences is therefore considered quite conservative.

In relation to *in utero* exposure, studies on transplacental transport of BPA and BPA-glucuronide in humans and rodents indicated that although transfer may occur, foetal free BPA levels are highly limited by the efflux pump P-glycoprotein, and placental BPA glucuronidation may also take place. The Panel noted that exposure to total BPA (the major constituent being the glucuronide) through

[5] In statistical terminology censoring occurs when the value of an observation is only partially known. In the Biel water maze test the number of errors made by the rats that did not complete the maze within the 3 minute-limit that was used, was recorded and the time to escape was recorded as 180 seconds. These experiments are "right censored", meaning that the rats could have escaped and made more errors, had they been given sufficient time. Therefore the time to escape and the number of errors recorded will have been underestimated.

lactation is limited to a very low fraction. Therefore, *in utero* exposure and exposure through lactation appear to be limited.

The Panel recognises that inter-individual differences occur in expression of the isoenzymes responsible for the detoxification of BPA. However, even in persons with low expression of these enzymes, the metabolising capacity is still sufficient to eliminate free BPA from blood at the low levels of BPA resulting from consumer exposure. Dietary exposures of adults and infants aged 3-6 months were estimated to be up to 1.5 and 13 µg/kg body weight per day, respectively (EFSA, 2006), based on conservative estimates of food consumption and migration from food contact materials. These exposures are not anticipated to surpass the metabolic capacity for BPA in adults or infants.

Recent epidemiological studies have suggested some statistically significant associations of BPA exposure (urine concentrations) and health effects (coronary heart disease, reproductive disorders) in adults and behavioural changes in young girls. The Panel noted that cross sectional epidemiological studies such as these can demonstrate statistical associations between BPA exposure and the presence (e.g. coronary heart disease) or absence (e.g. cancer, asthma) of health outcomes, but the inherent design of cross sectional studies does not allow establishment of a causal relationship between BPA exposure and health effects (e.g. chronic diseases). In addition, the Panel has identified some limitations in these studies, which raise further questions as to the significance of the reported findings. Therefore, the Panel could not draw any relevant conclusion for risk assessment from these studies.

The animal studies on developmental and reproductive toxicology reporting effects at doses lower than 5 mg/kg b.w./day have severe shortcomings and were considered to be invalid. The Panel considers that the valid studies do not raise concern regarding reproductive and developmental toxicity of BPA at doses lower than 5 mg/kg b.w./day.

Potentially significant biochemical changes, e.g. altered receptor expression in different brain regions (see section 5.3), have been reported. However, in the absence of a correlation with a functional adverse effect, the relevance of these observations for human health cannot be assessed. The impact of BPA on development of sexually dimorphic behaviour was addressed in the study by Ryan et al. (2010a), who observed a male-like reduced saccharin preference and inhibition of lordosis behaviour in female rat offspring from oestrogen-treated but not from BPA-treated dams. In the study reported by Stump et al. (2009) (See Part I) the effects of BPA on learning and memory behaviour were inconclusive due to large variability in the data. Other recent studies have methodological shortcomings. The Panel does not consider the currently available data as convincing evidence of neurobehavioural toxicity of BPA.

The study by Jenkins et al. (2009) is the first oral study on a possible BPA-induced enhancement of sensitivity of the mammary gland to carcinogen-induced breast tumour formation in rat offspring following lactational BPA exposure of pups. Using the same model of dimethylbenzanthracene (DMBA)-induced mammary carcinogenesis but *in utero* BPA exposure, Betancourt et al. (2010b) also reported an enhancement of susceptibility for mammary gland carcinogenesis. In consideration of the shortcomings in the design of both studies, in particular the uncertainty regarding the lactational as well as *in utero* exposure of the offspring to BPA, and of the limitations in reporting the Panel concluded that these results cannot be taken into consideration for derivation of a TDI. However, the Panel noted that at the highest dose level studied there is a shift of the ratio between cell proliferation and apoptosis in favour of cell multiplication in the mammary gland. In view of the mechanistic data obtained upon *in utero* exposure in other studies (see section 5.3) and the implications of an increased cell proliferation/apoptosis ratio in carcinogenesis, the effects reported by Jenkins and Betancourt deserve further consideration.

Modulation of immune system-related parameters is also an emerging field also in BPA research. Several studies have reported changes in cytokines, changes in T-cell populations and other aspects of immune modulation. However, the studies were all suffering from shortcomings in experimental

design and reporting. Therefore, at the moment, these studies cannot be taken into consideration for derivation of a TDI.

*In vitro*- and *in vivo*-studies (not compliant with the selection criteria in section 3) on receptors, hormones, immune system, cell proliferation, apoptosis, proteomic, genomic and epigenetic changes have been presented to compile recent data on potentially relevant endocrine mechanisms of action of BPA. High doses of BPA (>5 mg/kg b.w./day) may have biochemical and molecular effects consistent with those observed with other oestrogenic substances. Effects have been claimed to occur at low levels of BPA exposure, which could be independent of the classical hormone receptors. BPA has only weak binding affinities to these receptors, but these effects may alternatively be induced by cell membrane-triggered signalling pathways via protein kinases. However, in the absence of clear dose response curves and due to the shortcomings in experimental design, a conclusion cannot be reached on the implications of the observed biochemical and molecular changes or establish whether they have any impact on human health. Because of the lack of a common clearly defined mode of action of BPA at low doses, the toxicological relevance of the BPA effects described cannot be evaluated and the results cannot be taken into consideration for derivation of a TDI. While low dose effects of BPA are reported for some biochemical changes the Panel is not aware of any clearly reproducible adverse effect expressed specifically at low BPA doses only.

EFSA has established an internal task force to initiate the development of a common strategy towards endocrine active substances. The Panel is aware of EFSA's ongoing work to monitor trends and developments in the assessment of health risks of endocrine active substances.

PART III

This part deals with the EFSA advice on the Danish risk assessment of BPA. The conclusion of the DTU Food Institute is based upon three major lines of arguments: (i) a degree of uncertainty with regard to the effects on learning ability, since in the study by Stump et al (2009) impaired learning ability was found in male offspring with low dosage of BPA; (ii) doubts on the monotonic ("normal") dose-response for BPA; (iii) some endpoints which have not been considered, namely certain aspects of learning and memory (avoidance learning, schedule-controlled behaviour, impulsiveness), anxiety-related behaviour and gender-specific (i.e., sexually dimorphic) behaviour.

With respect to the learning and memory endpoint of the Stump study, as examined in the Biel Maze test, the Panel concluded that the influence of BPA on learning and memory behaviour cannot be evaluated. Regarding this endpoint, the study is inconclusive and cannot be used for the risk assessment of BPA.

It has been argued that BPA may show a non-monotonic dose-response curve. Low dose effects of BPA have been reported, which might be independent of the effect on the classical hormone receptors (See Part II). However, most of these studies have several shortcomings such as lack of dose responses and limitations in experimental design. The Panel is not aware of any clearly reproducible adverse effect expressed specifically at low BPA doses only.

Altogether, the Panel concluded that the study by Stump et al. (2009) cannot be used for the risk assessment of BPA, because of large variability in the data. Therefore, the study is inconclusive and cannot impact on the risk assessment of BPA. Methodological shortcomings also apply to a number of studies addressing other neurobehavioural endpoints (e.g. learning and memory behaviour, anxiety-related behaviour and gender-specific behaviour), which were considered invalid or inadequate for risk assessment purposes. The Panel does not consider the currently available data sufficiently indicative of neurobehavioural toxicity as an endpoint of concern for BPA.

Overall, based on the comprehensive evaluation of recent human and animal toxicity data, the Panel concluded that no new study could be identified, which would call for a revision of the current TDI of 0.05 mg/kg b.w./day. This TDI is based on the NOAEL of 5 mg/kg b.w./day from a multi-generation

reproductive toxicity study in rats, and the application of an uncertainty factor of 100, which is regarded as conservative based on all information on BPA toxicokinetics.

The Panel noted that some studies conducted on developing animals have suggested other BPA-related effects of possible toxicological relevance, in particular biochemical changes in brain, immune-modulatory effects and enhanced susceptibility to breast tumours. These studies had many shortcomings. At present the relevance of these findings for human health cannot be assessed, though should any new relevant data become available in the future, the Panel will reconsider the current opinion.

A minority opinion expressed by a Panel member is presented in an Annex to the opinion.