Toxicokinetics of Bisphenol A[1]

Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC)

(Question No EFSA-Q-2008-382)

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PANEL MEMBERS

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SUMMARY

The Panel has been asked to reconsider the possible age-dependent toxicokinetics of BPA in animals and humans and their implication for hazard and risk assessment of BPA in food. The Panel concluded that the exposure of a human fetus to free BPA would be negligible due to the maternal capacity for conjugation whereas the fetal rat would be exposed to free BPA from the maternal circulation. Taking account of data in human neonates on compounds structurally related to BPA which undergo glucuronidation/sulphation, the Panel considers that there is sufficient capacity in the neonate to conjugate BPA at doses below 1 mg/kg bw (the Panel noted that exposures at the TDI of 0.05 mg/kg bw are 20 fold lower than this).

Therefore, the Panel concluded that there is sufficient capacity for biotransformation of BPA to hormonally inactive conjugates in neonatal humans at exposures to BPA that were considered in the EFSA opinion of 2006 and the European Union Risk Assessment Report (EC, 2003, 2008).

In addition, the Panel notes that because of the metabolic differences described, exposure to free BPA in adult, fetal and neonatal rats will be greater than in humans and that rats would therefore be more susceptible to BPA-induced toxic effects than humans on a equivalent dose basis.

The Panel therefore considers that its previous risk assessment based on the overall NOAEL for effects in rats and using a default uncertainty factor of 100 can be considered as conservative for humans. The Panel concluded that the differences in age-dependent toxicokinetics of BPA in animals and humans would have no implication for the EFSA 2006 risk assessment of BPA.

Key words: Bisphenol A, BPA, 2,2-bis(4-hydroxyphenyl)propane, 4,4'-isopropylidendiphenol, CAS no. 80-05-7, toxicokinetics, neonate, glucuronidation

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BACKGROUND

In its opinion of November 2006, EFSA re-evaluated Bisphenol A (BPA) for use in food contact materials. The Panel's conclusions were based on the then available, extensive database on repeated-dose toxicity, reproductive and developmental toxicity of BPA in rodents and on the comparison of toxicokinetics in primates, including humans, and rodents. The Panel concluded that the new studies provided a basis for revising the uncertainty factors that were used by the SCF to derive the temporary TDI of 0.01 mg/kg bw in 2002. In particular, the Panel considered that the database concerning reproduction and development had been considerably strengthened and that the additional uncertainty factor of 5, introduced by the SCF in 2002 for the uncertainties in the database on reproduction and development, was no longer required. The Panel also concluded, in view of the well described species differences in toxicokinetics, showing a low level of free BPA in humans compared with rats, that a default uncertainty factor of 100 applied to the overall NOAEL from the rodent studies could be considered as conservative.

Since then, there have been ongoing discussions on the reported low-dose effects of BPA, particularly neurodevelopmental and behavioural effects in laboratory animals, and on the immaturity of metabolic pathways in the fetus and neonate, which are important issues for risk assessment. A recent Draft Screening Assessment on BPA by the Canadian Government highlighted the possible sensitivity of the fetus and the infant due to insufficiently developed enzymes for clearing BPA from the body. The Canadian risk assessment takes a precautionary approach for these sensitive life stages, taking into account the findings in the low-dose studies, although commenting that these are limited in rigor, consistency and biological plausibility.

TERMS OF REFERENCE

The Commission asks EFSA to assess possible age-dependent toxicokinetics of BPA in animals and humans and their implication for hazard and risk assessment of BPA in food as soon as possible taking into account the most recent information and data available.

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ASSESSMENT

Toxicokinetics and metabolism of BPA in adult animals and humans

The toxicokinetics of BPA in adult humans and in animals are well characterized (EFSA, 2006; Willlhite *et al.*, 2008). In humans, orally administered BPA is well absorbed and undergoes complete first-pass metabolism in the liver to BPA-glucuronide as major metabolite, which is rapidly excreted in the urine, with a half-life of less than 6 hours (Völkel *et al.*, 2002, 2008). Bisphenol A-sulphate has been reported as a minor urinary metabolite of BPA in humans (Ye *et al.*, 2005, 2006). Because this first-pass metabolism is so effective, there is extremely low systemic availability of free BPA in humans after oral exposure. BPA-glucuronide and the minor urinary metabolite BPA-sulphate do not interfere with hormonal regulation of reproduction (Snyder *et al.*, 2000; Shimizu *et al.*, 2002, Willlhite *et al.*, 2008). Therefore, these conjugation reactions represent detoxication pathways.

In rats, BPA is also predominantly glucuronidated, with sulphation representing a minor pathway (Pottenger *et al.*, 2000), but the BPA-glucuronide formed is excreted from the liver via bile into the gastrointestinal tract, cleaved back to BPA and reabsorbed into the blood. Thus it undergoes enterohepatic recirculation resulting in slower elimination of BPA including its conjugate in rodents compared with humans (EFSA, 2006), with terminal elimination half-lives between 20 and 80 h. The enterohepatic cycling and decreased first pass metabolism of BPA in rats results in higher plasma levels of unconjugated BPA in rats compared to humans given the same dose.

These differences reflect the known species difference in molecular mass threshold for biliary elimination in rats and humans. The molecular mass of the BPA-glucuronide (484 D) is well above the threshold for rats (300 – 400 D) but below that of humans (500 - 600 D) (Hirom *et al.*, 1976; Walton *et al.*, 2001; Ghibellini *et al.*, 2006).

Neonates and toxicokinetics of xenobiotics

In human neonates, some metabolic pathways, e.g. glucuronidation (2-5 fold lower in premature neonates), and some excretory functions, e.g. glomerular filtration (1.7 fold lower), have a lower efficiency compared to adults; these functions reach their full capacities only within one and seven months after birth, respectively (Renwick *et al.*, 2000; Dorne *et al.*, 2005; Benedetti *et al.*, 2007; Dorne, 2007).

In the rat, activity of uridine diphosphate glucuronosyltransferases (UDPGT) is also low at birth (Matsumoto et al.., 2002), and remains reduced until post-weaning (Renwick *et al.*, 2000; Heaton and Renwick, 1991). Considering the more rapid attainment of critical developmental points in rats compared with humans, it can be concluded that reduced activity of UDPGT persists for a relatively longer period, relative to the equivalent development period in humans.

In contrast to UDPGT, sulphotransferases (SULT), which often share xenobiotic substrates with UDPGT, are present with high activity already in the developing fetus and are fully functional at birth (Richard *et al.*. 2001; Strolin and Baltes 2003; Duanmu *et al.*, 2006; Pacifici, 2007; Blake *et al.*, 2005). BPA is a substrate for several human SULT which catalyse BPA-sulphate formation with high efficiency (Nishiyama *et al.*, 2002).

Levels of human UDPGT activity gradually increase during fetal and neonatal development, including the weeks and months following birth. In rodents, several studies indicate that maternal liver glucuronidation activity is lower during pregnancy. However, in humans, as evidenced by increased oral clearance of lorazepam, paracetamol and lamotrigine, glucuronidation activities during pregnancy are induced (Chen *et al.*, 2005; Papini *et al.*, 2006 Miners *et al.*, 1986; Pennell *et al.*, 2008), This increase in glucuronidation capacity results in a reduction in the plasma concentrations of drugs depending on glucuronidation capacity as the major metabolic pathway. By analogy with related compounds in humans the maternal conjugation capacity is likely to result in negligible fetal exposure to free BPA. However, in rats metabolite profiles in non-pregnant and pregnant animals were similar, and therefore there would be systemic availability of free BPA (Domoradzki *et al.*, 2003) which could result in significant potential fetal exposure to free BPA.

Biotransformation of BPA in neonatal animals and humans

In neonatal rats, orally administered BPA (1 or 10 mg BPA/kg bw at postnatal days 4, 7, or 21) is metabolised to BPA-glucuronide at all three ages. Generally, BPA-glucuronide and BPA concentrations in the plasma were higher in neonates than in adults, but elimination of BPA-glucuronide in plasma was more rapid in neonatal animals than in adults. A dose-dependency of BPA biotransformation to the glucuronide in neonatal rats was observed, but BPA-glucuronide accounted for 94 to 100 % of radioactivity in blood at the 1 mg/kg bw dose indicating sufficient capacity in neonatal rats to efficiently metabolise low doses of BPA to the glucuronide. (Domoradzki *et al.*, 2004).

A second study (Taylor *et al.*, 2008) investigated the blood concentrations of ^{14}C-BPA in neonatal mice after oral administration and subcutaneous (sc) injection of doses of 35 and 395 microg/kg bw. Peak levels of BPA in blood and blood areas under the curve (AUC) were reported as identical after both routes of application. The available information in the publication is limited. BPA was determined only in ether extracts of blood samples, and no information on total radioactivity in blood or concentrations of BPA-conjugates in the blood samples is presented. Moreover, no mass balance and only a very small portion, less then 5 % of the applied dose, is recovered. Therefore, no conclusions can be drawn from this study on the systemic availability of unconjugated BPA and the efficiency of first pass metabolism.

No information on the biotransformation of BPA in human neonates is available. However, BPA is a simple phenolic structure without steric hindrance of the OH groups and therefore data on other phenols would be equally applicable. Hence, qualitative conclusions on the biotransformation of BPA in human neonates may be drawn from toxicokinetic data for xenobiotics undergoing sulphation and/or glucuronidation such as the analgesic acetaminophen (paracetamol) (Arana *et al.*, 2001; van der Marel, 2003).

In adults, paracetamol metabolism (expressed as percentages of an oral dose) by glucuronidation accounts for 50-60% of biotransformation and sulphation for 25-40%, whereas oxidation and renal excretion account for approximately 15 %. In human neonates, biotransformation of paracetamol by sulphation and glucuronidation is dose-dependent. Sulphation of paracetamol is more pronounced (65-68% expressed as a percentage of dose) after a comparatively high oral dose of 10-12 mg/kg bw, but glucuronidation remains a relevant pathway (18-22%) (Levy *et al.*, 1975; Miller *et al.*, 1976). After exposure via breast milk to doses of approximately 0.3 mg/kg b.w. glucuronidation remained the most important (54 %) pathway of paracetamol biotransformation in neonates, and sulphation accounted for only 11 %, (Notarianni *et al.*, 1987). Depending on post-conceptional and postnatal age (Allegaert *et*

al., 2005), the ratio between the amount of sulphate and glucuronide in paracetamol metabolism was between 2 and 3 in neonates as compared to 0.6 in adults. In summary, these data show that in human neonates, despite lower activity of UDPGT, there is considerable capacity for biotransformation of xenobiotics by this pathway. Moreover, sulphation of BPA in humans has been shown in primary hepatocytes; several SULT-enzymes efficiently catalyze BPA-sulphate, and BPA-sulphate is a human urinary metabolite of BPA (Pritchett *et al.*, 2002). Therefore, sulphation of BPA in fetuses and neonates is also expected to efficiently detoxify BPA.

CONCLUSIONS

The Panel has been asked to reconsider the possible age-dependent toxicokinetics of BPA in animals and humans and their implication for hazard and risk assessment of BPA in food. The Panel concluded that the exposure of a human fetus to free BPA would be negligible due to the maternal capacity for conjugation whereas the fetal rat would be exposed to free BPA from the maternal circulation. Taking account of data in human neonates on compounds structurally related to BPA which undergo glucuronidation/sulphation, the Panel considers that there is sufficient capacity in the neonate to conjugate BPA at doses below 1 mg/kg bw (the Panel noted that exposures at the TDI of 0.05 mg/kg bw are 20 fold lower than this). Therefore, the Panel concluded that there is sufficient capacity for biotransformation of BPA to hormonally inactive conjugates in neonatal humans at exposures to BPA that were considered in the EFSA opinion of 2006 and the European Union Risk Assessment Report (EC, 2003, 2008).

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GLOSSARY / ABBREVIATIONS

BPA	bisphenol A
NOAEL	no observed adverse effect level
SULT	sulphotransferase
TDI	tolerable daily intake
UDPGT	uridine diphosphate glucuronosyltransferase