June 11, 2009

Cynthia Oshita
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Proposition 65 Implementation
P.O. Box 4010
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Re: Comments on the Bisphenol A (BPA) Proposition 65 Docket

Dear Ms Oshita:

I have reviewed the draft report by California OEHHA titled Evidence on the Developmental and Reproductive Toxicity of Bisphenol A, May 2009. I have also reviewed other aspects of the docket with respect to the potential listing of BPA on the California Proposition 65 list for developmental and reproductive toxicants. As a toxicologist and specialist in metabolism and pharmacokinetics, I have reviewed the OEHHA draft report with a particular focus on the pharmacokinetics of BPA. This letter is to transmit ways in which the draft document could be strengthened. These comments are also meant to be helpful to the Developmental and Reproductive Toxicant Identification Committee (DARTIC) during its July deliberations on BPA.

The OEHHA draft report covers the pharmacokinetics of BPA beginning on Page 169 in a rather matter-of-fact review sidestepping several important pharmacokinetic issues that have been raised by the European Food Safety Authority (EFSA, 2008). These issues have implications for interpretation of BPA health effects as follows:

1) Does BPA's rapid conjugative metabolism ensure inconsequential exposure in humans to the endocrine-active parent compound? Certain studies (e.g., Volkel et al. 2002, 2006) have failed to detect parent compound in the blood of human volunteers receiving labeled (deuterated) BPA. This is compatible with rapid metabolism and is seen as evidence that there is not enough internal dose of parent compound to be of concern in humans (Volkel et al. 2006; Dekant and Volkel 2008; EFSA 2008).

2) Is the BPA dose response information developed in rats relevant to humans given that the glucuronide metabolite undergoes enterohepatic circulation in rats but not in humans, leading to substantially longer half life in rats (EFSA 2008)?

3) Is sulfation capacity sufficiently developed in neonates to offset the immaturity in glucuronidation at this age? EFSA (2008) makes the plausible argument that sulfation in early life will rapidly remove BPA. While the OEHHA draft document does bring up neonatal immaturity in glucuronidation, it fails to acknowledge that sulfation may be a compensatory BPA removal process. Further, the physiologically-based pharmacokinetic (PBPK) modeling study relied upon by OEHHA to describe BPA internal dose in neonates (Edginton and Ritter, 2009) is not reliable for several reasons as discussed below.

The fact that the OEHHA draft report does not explicitly address these pharmacokinetic issues is a deficiency. These issues should be a special focus of the pharmacokinetics section and should also appear in the Executive Summary, which right now has no statements pertaining to pharmacokinetics. Wherever possible, the report should stipulate whether free BPA or BPA-glucuronide is what was detected as this is an important toxicological distinction.

My review of Gary Ginsberg leads to the conclusion that these issues do not substantially decrease health concerns or the relevance of rodent studies for human health risk assessment. This is in spite of rapid glucuronidation and even considering the potential for sulfation in early childhood. The logic for this conclusion is attached based upon the 3 issues identified above. Please let me know if you need additional information regarding this submission.

Sincerely

Gary Ginsberg, Ph.D.
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CT Dept Public Health

Issues Related to BPA Pharmacokinetics

1) **Does BPA's rapid conjugative metabolism ensure inconsequential exposure in humans of the endocrine-active parent compound?**

There is still ample opportunity for human exposure to free BPA in spite of rapid first pass glucuronide conjugation from oral exposure. That's because β-glucuronidases exist not only in the intestines, but throughout the body, including the placenta and fetal liver. This creates the potential for local activation of the conjugated form back to free BPA in numerous tissues. This is analogous to the situation for endogenous estrogens, which are transported as sulfate conjugates and can be cleaved to active hormone by tissue sulfatases (Iwamori et al. 2005). Conjugation-deconjugation cycling that does not involve enterohepatic recirculation has been documented for the drug diflunisal (Brunelle and Verbeeck 1997). Further, beta-glucuronidase-mediated deconjugation of a variety of other xenobiotic metabolites was documented in human liver preparations, including acetaminophen (Bohnenstengel et al. 1999) and the aromatic amines benzidine and 4-aminobiphenyl (Zenser et al. 1999). β-Glucuronidase protein levels and enzyme activity were readily detectable and varied widely in human liver and kidney samples taken from 30 and 18 individuals, respectively (Sperker et al. 1997).

There are also data which question how rapidly BPA is eliminated from humans. A reanalysis of the NHANES BPA urinary biomonitoring results from 1469 adult participants suggested a longer than expected half-life of BPA (Stahlhut et al. 2009). While the terminal half-life is reported to be only 5.3 hours in humans after deuterated-BPA administration (Volkel et al. 2002), that time course shows no additional d-BPA removal at the later time points (additional data from Volkel et al. 2002 presented in Teeguarden et al. 2005). This is consistent with delayed excretion due to long-term tissue storage and/or conjugation/deconjugation cycling. The terminal half-life in cynomolgus monkeys dosed orally with ^{14}C-BPA was 9.7 hours (Kurebayashi et al. 2002). Thus, the rat to human difference in terminal half-life of BPA-glucuronide may not be as large as stated (EFSA 2008).

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Developmental studies suggest rapid ontogeny of β-glucuronidase, since it is detected prenatally in liver, kidney, and lung in a variety of laboratory species, with activity particularly high in placenta (Lucier et al. 1977). Human placenta also has considerable β-glucuronidase activity

and this enzyme is critical for proper in utero development (Collier et al. 2009; Paigen 1989; Sperker et al. 1997). An inherited deficiency leads to hydrops fetalis, a birth defect related to improper fetal breakdown of mucopolysaccharides and water accumulation. Because glucuronidation capacity is immature in early life, the net balance tends to be towards deconjugation in the animal models and tissues studied (Lucier et al. 1977). This makes BPA deconjugation a potentially important pharmacokinetic factor during the perinatal period.

Thus it is apparent that one has to consider the potential for β-glucuronidase-mediated deconjugation of BPA glucuronide in placental and fetal tissues. Even though glucuronidation may be rapid, the reported elimination half-life of circulating BPA glucuronide, 5.3 hours, affords ample opportunity for transport to placenta and deconjugation back to BPA. This and the fact that the fetus itself contains β-glucuronidase increases the chances for substantiative fetal exposure to free BPA. However, the potential for beta-glucuronidase-mediated generation of free BPA has yet to be addressed in BPA pharmacokinetic models (Teeguarden et al. 2005; Edginton and Ritter 2009) or regulatory determinations.

In terms of actual evidence for exposure to free BPA, this has been shown in humans and rodents in adult, placental and fetal tissues (Table 1). While Volkel et al. (2002, 2008) failed to detect free BPA in blood after administration of labeled compound to human volunteers, numerous other studies have detected free BPA in tissues either coming from general background exposure or in experiments where rodents were dosed with BPA (Table 1). This is described to some extent in the OEHHA draft document but could that aspect could stand expansion. Dekant and Volkel (2008) argue that the detection of free BPA in such studies may result from background contamination from labware and indoor dust. Further some free BPA may be formed by cleavage from the glucuronide present in the sample when readying the sample for analysis (Dekant and Volkel 2008).

However, review of the studies that found free BPA suggests that this result is not artifactual. Instead, these data suggest patterns of occurrence that have important implications for BPA risk assessment. Specifically, studies in rodents, monkeys and humans show that free BPA is greatest in placenta with fetal and maternal concentrations comparable (Schonfelder et al. 2002; Domoradski et al. 2003; Takahashi and Ohni, 2000; Uchida et al. 2002). The glucuronide to free BPA ratio showed a continuous decline when going from maternal to placental to fetal compartment (Domoradzki et al. 2003). These trends have a plausible biological basis in that

placenta has extensive β-glucuronidase activity (see above) and so may be an important site of metabolite deconjugation and resultant fetal exposure. Thus, in spite of rapid BPA conjugation in the maternal system, free BPA is detectable in both placenta and fetus.

2) Is the BPA dose response information developed in rats relevant to humans given that the glucuronide metabolite undergoes enterohepatic circulation in rats but not in humans, leading to substantially longer half life in rats (EFSA 2008)?

Enterohepatic recirculation is only one factor that could modulate BPA internal dose. As discussed above, beta-glucuronidase-mediated deconjugation may produce free BPA in targeted locations (e.g., placenta, fetus). How this ultimately affects the overall pharmacokinetics of BPA and local tissue dose is something that PBPK models can attempt to address. However, the currently available models (Teeguarden et al. 2005; Edginton and Ritter, 2009) do not account for deconjugation reactions and do not simulate fetal and neonatal exposures to free BPA. Ideally, the longer half-life of BPA-glucuronide in rodents would be accounted for via PBPK models that can adjust the internal dose across species. Such pharmacokinetic adjustment would not invalidate the effects seen in rodent low dose studies but would merely modify the dose response by some factor.

3) Is sulfation capacity sufficiently developed in neonates to offset the immaturity in glucuronidation at this age?

EFSA also considers dietary BPA exposures in neonates to be insignificant due to rapid metabolism, in this case not because of glucuronidation, but because of sulfation. Based upon analogy with acetaminophen, BPA may be conjugated with sulfate rather than glucuronide in neonates due to the earlier development of sulfotransferases. However, sulfation does not end the biological activity of endogenous hormones and so there is no reason to believe it will do so for sulfated BPA. Sulfated estrogens, mainly in the form of estrone sulfate, have a long half-life in blood where their concentration is much higher than the active hormone (Nakamura et al. 2005). These conjugates act as a circulating reservoir of inactive hormone that can be deconjugated in local tissues by arylsulfatase C, a widely expressed microsomal enzyme that is especially prevalent in estrogen-responsive tissues (Reed et al. 2005; Tobacman et al. 2002). Given that sulfotransferases also exist in these tissues, the balance between conjugation and deconjugation at a particular life stage and in a specific tissue is a key determinant of local

estrogen dose. This has not been studied for BPA, but it is reasonable to assume that its sulfate conjugate would also be subject to deconjugation in a manner similar to endogenous sulfated estrogens. This is pertinent to the postnatal period as arylsulfatase C activity develops in utero and is readily detectable in human neonatal liver (Richard et al. 2001). Thus, sulfation of BPA in neonates does not guarantee negligible internal dose as recently assumed (EFSA 2008).

The OEHHA draft report addresses neonatal BPA dosimetry primarily by relying upon a recent pharmacokinetic modeling assessment (Edginton and Ritter, 2009). However, that assessment doesn't consider the compensatory sulfation pathway and it assumes that the human enzyme responsible for BPA glucuronidation is UGT2B7. This assumption is central to the entire analysis as the ontogeny of UGT2B7 is governs BPA metabolic clearance in the neonatal model. The assumption of 2B7 involvement comes from the fact that UGT2B1 is the key BPA enzyme in rats and there are certain similarities between 2B1 in rats and 2B7 in humans. However, a recent paper by Hanoika et al. (2008) shows that in humans, UGT2B15 is likely to be of greatest importance. While I have not explored the ontogeny of 2B15, this is something that CalOEHHA should do before relying upon the analysis of Edginton and Ritter (2009). Even if the ontogeny of UGT2B15 and 2B7 are similar, the lack of consideration of neonatal sulfation should decrease reliance on Edginton and Ritter (2009). Instead a major focus should be on the potential for deconjugation to lead to local tissue doses of free BPA in the fetus and neonate, with emphasis on studies which have evaluated free BPA in human and rodent tissues (e.g., Table 1).

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Table 1. Studies Evaluating Free BPA in Biological Fluids

Study	Exposure	Analytical Method	Results	Comments
Domoradzki et al. 2003	Pregnant rats gavaged ^{14}-C BPA10mg/kg days 11, 13 or 16 gestation	Radiochemical HPLC LOD ~ 35 ug/L Tests run to ensure that extraction procedures didn't cleavage BPA-glucuronide to BPA	Free BPA not detectable in many samples, especially early in gestation; Day 16 free BPA detectable in all tissues: placenta > maternal plasma > fetus; ratio of free/conjugated BPA was: fetus (1.4) > placenta (0.27) > maternal plasma (0.04).	Although fetus had 3.6 fold less free BPA than maternal plasma, free/conjugated BPA ratio was fetus > > maternal plasma ; may reflect altered conjugation/deconjugation balance in fetal compartment These measurements were from early time points before enterohepatic circulation created a secondary Cmax.
Ikezuki et al. 2002	Humans (mother/fetus) background exposure (N=32-38)	ELIZA, details not given, but they checked accuracy against traditional HPLC method	Free BPA detected in maternal serum, ovarian follicular fluid, cord blood and amniotic fluid at similar levels except for amniotic fluid which was 5 times higher in early but not late pregnancy	Possible explanation for higher free BPA in amniotic fluid early in pregnancy related to changing composition: from maternal plasma early, changing to fetal urine late; free BPA may be in maternal plasma but unlikely to be in urine
Schonfelder et al. 2002	Humans (mother/fetus) background exposure (N= 37)	GC-MS LOD = 0.01 ug/L LOQ = 0.1 ug/L	Free BPA detected in placenta > maternal plasma > fetal plasma but fetal > maternal in 14 of 37 samples; free BPA in male fetus > female fetus	Methods avoided BPA leaching from labware into sample. Differences across tissues and gender suggest free BPA biologically based rather than from background contamination
Takahashi and Oishi 2000	Pregnant rats dosed day 18 gestation with 1 g/kg gavage	HPLC LOD = 5 ug/L	Free BPA detected in maternal tissues > fetal tissue ≥ maternal blood; fetal t½ 3x >maternal t½	High dose rat study has limited relevance to human BPA exposure. However, it demonstrates distribution to and concentration in fetal compartment. Enterohepatic recirculation likely affects t½ in both fetus and mother but doesn't explain longer t½ in fetus
Takeuchi et al. 2004	Rats background exposure N=10 per sex	HPLC Details not clear but appears to involve standard solvent extraction	Free BPA detected in males > females	Gender differential corresponds to lower BPA conjugating capacity in male liver microsomes and lower expression of UGT2B1
Takeuchi et al. 2002	Humans background exposure N=11 men, 14 women	ELIZA with accuracy checked against HPLC method	Free BPA detected in males > females	Free BPA correlated with serum testosterone in both men and women suggesting androgen effect on BPA fate in humans
Tan and Mohd 2003	Humans background exposure N=180 women	GC-MS LOD = 0.05 ug/L	Free BPA detected in cord blood in 88% of samples	Demonstrated potential utility of biomonitoring free BPA and other alkylphenols in cord blood

Volkel et al. 2002	Humans dosed orally with 5 mg deuterated BPA (N=4)	LC-MS LOD_{blood} = 2.3 ug/L LOD_{urine} = 1.4 ug/L	No detection of free BPA even though deut-BPA-gluc high (160 ug/L blood) Deut-BPA gluc removal half life from blood and urine = 5.3 hr.	Lack of free BPA attributed to "practically complete" first pass metabolism and lack of enterohepatic circulation. Detection of free BPA in background human samples attributed to plastics contamination of the sample.
Volkel et al. 2008	Humans background exposure (N=287)	LC-MS LOD_{urine} = 0.3 ug/L LOQ_{urine} = 1.25-5 ug/L	10% of urine samples with low detection (>LOD, <LOQ)	Free BPA in urine attributed to contamination from house dust or plastics since BPA also found in the blanks and deuterated BPA experiment did not find free BPA in urine.
Volkel et al. 2008	Human dosed orally with 5 mg deuterated BPA (N=1)	HPLC-MS LOD_{urine} for deuterated BPA was not stated	Free deuterated BPA not detected in any urine samples from this one subject.	Results from this one subject used to assert that if free BPA is detected in urine, it is from contaminated sample and not free BPA in vivo.
Yamada et al. 2002	Humans background exposure (N=200 women)	ELIZA LOD = 0.2 ug/L, method previously validated against HPLC method	2nd trimester serum samples in Japanese women showed continuous decline in free BPA from 1989 to 1998	No clear explanation of decreasing BPA in serum but results significant ($p<0.001$). Amniotic fluid free BPA much lower than maternal serum