

May 12, 2010

Ms. Cynthia Oshita
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-19B
Sacramento, CA 95812-4010

Re: Response to Request for Relevant Information on Bisphenol A

Dear Ms. Oshita,

Attached are my comments in response to the OEHHA Request for Information of February 12, 2010 (Request for Relevant Information on a Chemical Being Considered for Listing by the Authoritative Bodies Mechanism: Bisphenol-A). From this notice I understand that a petition has been submitted to OEHHA proposing to list bisphenol A (BPA) as a reproductive toxicant under Proposition 65. The basis for the petition is the NTP-CERHR report on BPA, which includes summaries of certain studies that report developmental toxicity at high levels of exposure.

I have reviewed the six relevant BPA studies, with a particular focus on the role of maternal toxicity on embryo-fetal/offspring toxicity. Notably, I am the first author and study director of three of these studies (one-, two- and three-generation reproductive toxicity studies on BPA and estradiol) and am a co-author on another of the studies (Morrissey et al., 1987).

Based on my intimate knowledge of these studies and my review of other relevant studies, I conclude that BPA is not a selective reproductive or developmental toxicant. As detailed in the attached comments, reproductive or developmental effects occur only at very high BPA doses in the presence of profound maternal toxicity. At lower doses with less, but still significant, maternal toxicity, there are no reproductive or developmental effects. Based on other relevant studies, it is apparent that maternal toxicity is most likely the critical determinant of embryo-fetal/offspring toxicity observed at high doses of BPA. Consequently, BPA does not satisfy the criteria for listing under Proposition 65.

My review relies on my broad experience and recognized expertise, encompassing more than 40 years of experience in reproductive and developmental biology and toxicology. I am currently a Distinguished Research Fellow in Developmental and Reproductive Toxicology (DART) and

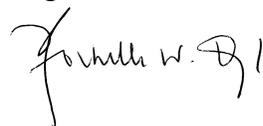
Principal Investigator of DART studies in the Center for Pharmacology and Toxicology at RTI International. I am also currently an Adjunct Professor in the Curriculum for Toxicology at the University of North Carolina at Chapel Hill. I have served as Study Director for more than 50 multigeneration studies in rodents and more than 150 developmental toxicity studies in rodents and rabbits, as well as numerous other related reproductive and developmental toxicity studies. I have authored or co-authored more than 100 peer-reviewed articles, 18 book chapters, more than 100 abstracts, and hundreds of study reports.

I am currently an Editorial Board member for *Reproductive Toxicology*, have previously served as an Editorial Board member for *Fundamental and Applied Toxicology*, and am a manuscript reviewer for *Toxicological Sciences*, *Reproductive Toxicology*, *Birth Defects Research*, and other journals. I have also served in relevant professional society leadership positions including as President of the Reproductive and Developmental Toxicology Specialty Section of the Society of Toxicology (2007-2008), President of the Teratology Society (2003-2004), and as an elected member of the American College of Toxicology's Long-Range Planning Committee (2007-2009).

I have advised numerous governmental and private sector organizations on matters within my area of professional expertise. For the time spent in preparing my comments, my employer is being compensated by the American Chemistry Council.

Please feel free to contact me if I can be of further assistance in this matter.

Regards,

A handwritten signature in black ink, appearing to read "Rochelle W. Tyl". The signature is fluid and cursive, with a large initial "R" and "T".

Rochelle W. Tyl, Ph.D., DABT
Distinguished Fellow

**Comments on the Proposed Listing of Bisphenol A as a
Reproductive Toxicant Under Proposition 65**

Rochelle W. Tyl, Ph.D., DABT

May 12, 2010

I. Definition of Maternal Toxicity

Maternal systemic toxicity has been classically defined (U.S. EPA guidelines, 1991) as one or more of the following effects:

- Dose-related maternal mortality (no greater than 10%)
- Dose-related reduced body weight(s)
- Dose-related reduced body weight gain(s)
- Dose-related reduced feed and/or water consumption (especially if associated with reduced body weights)
- Adverse clinical observations or clinical observations known to be associated with adverse outcomes
- Necropsy observations such as changes in organ weights (increased or decreased), especially if there is confirmatory evidence of histopathology (e.g., liver, kidneys, adrenal glands, etc.), both absolute and relative to terminal body weight or brain weight, to correct for any confounding from changes in body weight (U.S. EPA Guidelines, 1991; pp. 7-9).

NOTE: Increased liver weight, due to increased cell sizes (hypertrophy) and/or increased cell numbers (hyperplasia), may be due (at least in part) to induction of metabolizing enzymes (Conney, 1967), and therefore an adaptive response rather than an adverse response.

II. Background

The attention to and concern for the role of maternal toxicity on embryo-fetal/offspring toxicity have a long history. Khera (1984 [mice], 1985 [rats and rabbits]) presented data and naively proposed that maternal toxicity was a “possible etiologic factor” in fetal malformations (in mice, rats, and rabbits) and in intra-uterine deaths and congenital malformations in humans (Khera, 1987a). Khera (1987b) also presented an overview of the role of maternal toxicity on fetal development in humans and animals. Many other noted developmental toxicologists weighed in on the role of maternal toxicity on embryo-fetal effects (e.g., Kavlock et al., 1985; Kimmel and Francis, 1987; Chernoff et al., 1989; Black and Marks, 1992; Chahoud et al., 1999; Chernoff et al., 2008; Beyer et al., 2010, etc.).

The current consensus is that maternal toxicity in toxicology studies is the major cause of embryo-fetal effects observed, including those effects which are adverse or those which are not. Some effects observed in offspring that are linked to maternal toxicity are: reduced fetal/offspring body weights, increased fetal variations, and delays in acquiring landmarks of pubertal development. Extreme maternal toxicity may result in embryo-fetal loss *in utero* (Carney et al., 2004). A variation on this theme is that maternal stress associated with maternally toxic doses of a test material “can be expected to result in associated, often transient, fetal abnormalities that may not be the result of deviant organogenesis...” “Sometimes, the toxicity toward the pregnant animal, including her embryos/fetuses... is severe enough to result in the resorption of the embryo or absorption of the fetus. Therefore, it is possible that embryo lethality and other indications of developmental toxicity, produced by some drugs and chemicals, may be the result of mechanism(s) other than selective toxicity toward the embryo” (Black and Marks, 1992). Also, alteration/disruption of maternal homeostasis can result in disruption of embryonic support and therefore fetal effects (e.g., toxicity, increased variations, etc.), but are not, in fact, due to the test chemical per se (Black and Marks, 1992). A review of the role of maternal gestational stress in enhancing developmental toxicity of chemicals (Hougaard and Hansen, 2007) confirmed the importance of maternal stress on developmental toxicity from chemical insult.

As Rogers (1987) and others (e.g., Schwetz and Moorman, 1987) have commented, the indicators of maternal toxicity in developmental toxicity studies are generally limited to “significant maternal mortality, dose-related weight loss, or obvious external observations” (see Section I above). It is possible that additional indicators of maternal toxicity are present but either not evaluated or missed. Better and more uniform assessment of maternal toxicity may aid in the interpretation of developmental toxicity and the role of maternal toxicity.

The argument that maternal toxicity is the major cause of developmental toxicity in many developmental reproductive toxicity studies is bolstered by two additional papers. A paper by Waalkens-Berendsen et al. (1990) evaluated a test chemical (Isomalt) in developmental toxicity studies of Wistar rats and NZW rabbits. They included an *ad libitum* negative control for both species and, in rats, a second control group was feed restricted to 80% of the *ad libitum* feed presented to the negative control group. What is germane is that the dams in the feed-restricted control group exhibited significantly reduced feed consumption (down to ~80% of the *ad libitum* negative control group), significantly reduced gestational weight gains, and reduced gravid uterine weights at term. This group also exhibited significantly increased postimplantation loss, increased numbers of small fetuses,

decreased fetal and placental weights, and significantly reduced skeletal ossification in cervical thoracic vertebral bodies. Therefore, clearly nonchemical-induced maternal toxicity caused the embryo-fetal toxicity.

A paper by Ryan et al. (2001) exposed SD rats to phenol at 0, 200, 1000, or 5000 ppm in the drinking water for a two-generation reproductive toxicity study and immunotoxicity screen. Phenol has been shown not be estrogenic in the E-SCREEN assay (Soto et al., 1995), so the effects observed in this study at high doses are not expected to be endocrine mediated. These effects with a known nonestrogen provide a useful comparison to assess whether the results from BPA, which is weakly estrogenic, in the same rat strain and study design are endocrine mediated or the result of maternal toxicity. In the phenol study, there were significant reductions in feed and water consumption and in body weights and weight gains at 5000 ppm (the top dose) in both sexes and both parental generations (F0 and F1). Acquisition of vaginal patency and preputial separation were both delayed in F1 animals at 5000 ppm, considered “secondary to the reduced F1 body weights.” Litter survival of both F1 and F2 generations was also significantly reduced at 5000 ppm. Absolute uterine and prostate weights were significantly reduced in all dose groups for the F1 generation, with no underlying pathology and no functional deficit in any parameter of reproductive performance. Comparable parental and offspring findings were observed in the top dose of the SD rat, three-generation study of BPA (see Section III below), indicating they are also likely not endocrine mediated and more likely secondary to maternal toxicity.

III. Bisphenol A (BPA)

BPA is a monomer used in the manufacture of epoxy resins and polycarbonate plastics. Epoxy resins are corrosion resistant and are used in interior coatings of cans and drums, reinforced pipes, flooring, water main filters, dental sealants, and some food packaging materials. Polycarbonate plastic uses include disposable dishware, water bottles, compact discs, eyeglass lens, and electronics. In 2009, OEHHA in California issued a draft Hazard Identification Document (HID) from the Office of Environmental Health Hazard Assessment, entitled “Evidence on the Developmental and Reproductive Toxicity of Bisphenol A,” with expressed concern that the offspring effects of BPA were not due to maternal toxicity. However, interest in BPA arose long before 2009.

Due to widespread occupational and consumer exposure, in the 1980s, the National Toxicology Program (NTP) undertook general toxicology and carcinogenicity studies of BPA in rats and mice (both types of studies were negative in both species). The reproductive and developmental toxicity studies

are most relevant to this discussion of maternal versus embryo-fetal/offspring toxicity. A reproductive assessment by continuous breeding (RACB) study of BPA in mice was performed (Reel et al., 1985) at dietary concentrations of 0, 0.25, 0.5, and 1.0%. Decreased numbers of litters per breeding pair and reduced numbers of live pups/litter were observed at 0.5 and 1.0%. A crossover mating trial (1.0% males x 0% females and 0% males x 1.0% females) indicated that reproductive processes of both male and female mice were adversely affected at this very high dose.

Morrissey et al. (1987) subsequently performed a standard developmental toxicity evaluation of BPA administered orally by gastric intubation on GD 6 through 15 to Sprague Dawley rats at 0, 160, 320, 640, and 1280 mg/kg/day and at 0, 500, 750, 1000, or 1250 mg/kg/day to CD-1 mice. The timed-pregnant females were sacrificed one day prior to expected parturition, and all term fetuses were evaluated for external, visceral, and skeletal effects. Maternal toxicity was also characterized. In **rats**, maternal weight gain during gestation, gestational weight gain corrected for gravid uterine weight (GUW), and weight gain during the treatment period were significantly reduced at all BPA doses. GUW and mean fetal body weight/litter were unaffected. There were maternal clinical signs of toxicity in all the BPA groups versus the control groups. There were no increased percentages of resorptions/litter or of malformed fetuses/litter.

In **mice**, maternal mortality occurred at all BPA doses reaching 18% at the top dose. Clinical signs of toxicity were also present at all BPA doses. There was a trend towards reduced maternal weight gain during gestation and the treatment period, with mice at the top dose gaining significantly less weight. Corrected weight gain (weight gain during gestation minus GUW) was unaffected across groups. There was a trend towards reduced GUW with increasing BPA dose; the high-dose GUW was significantly lower than the control group GUW. Maternal absolute liver weight was significantly increased at 500, 750, and 1000 mg/kg/day, and maternal relative liver weight (liver weight/body weight) was dose dependently and significantly increased at all doses. There were no differences among groups in the number of uterine implantation sites/litter, but the number of ovarian corpora lutea/dam (reflecting the number of eggs ovulated) was decreased in a dose-related pattern. The percent resorptions/litter increased at 1000 and 1250 mg/kg/day (the latter statistically significant). Seven litters in the high-dose group were totally resorbed. BPA had no effect on the number of live fetuses/litter (for litters with live fetuses) or on sex ratio.

Also in mice, there were dose-dependent decreases in average fetal body weight/litter with increasing BPA dose, with the value at 1250 mg/kg/day significantly less than the control group values.

There was no effect of BPA on the percentage of fetuses malformed/litter or on the percentage of litters with malformed fetuses. There were no significant treatment effects on any measure of teratogenicity. BPA exposure on GD 6 through 15 had no effect on the incidence of external, visceral, or skeletal malformations. BPA exposure was also not associated with any particular malformation or group of malformations in any dose group. In both rats and mice, the increased embryo-fetal response occurred only at maternally toxic doses at the higher end of the dose-response curve.

In a more recent paper, Kim et al. (2001) administered BPA by gavage at 0, 100, 300, and 1000 mg/kg/day to maternal female Sprague-Dawley rats once daily on GD 1 through 20 of gestation. The day of sperm in the vagina was designated GD 0, the dams were terminated on GD 21, and the fetuses were examined externally, viscerally, and skeletally. At 1000 mg/kg/day, there was significant maternal toxicity, including abnormal clinical signs, decreased maternal body weights and body weight gain, and reduced feed consumption. Also at this dose, there was an increase in pregnancy failure (in sperm-positive females), increased embryo deaths, increased postimplantation loss, reduced litter sizes, and reduced fetal body weights. In addition, at this dose there were decreased numbers of fetal ossification centers in several skeletal districts. There were no effects of BPA at any dose on the ovarian corpora lutea (number of eggs ovulated), on uterine implantation sites (i.e., preimplantation loss), or on fetal morphological findings. At 300 mg/kg/day, maternal body weights, body weight gains, and feed consumption were also reduced. Reduced body weights of the male fetuses were also observed. At 100 mg/kg/day, there was no maternal or developmental toxicity observed. Therefore, in the presence of severe maternal toxicity from BPA exposure through pregnancy, there were increased pregnancy failures, increased pre- and postimplantation loss, and fetal developmental delay, but no embryo-fetal dysmorphogenesis at an oral dose of 1000 mg/kg/day.

The same (or better) evaluations of maternal, paternal, and offspring toxicity are made from GLP-compliant, regulatory guideline-compliant, one-, two- and three-generation reproductive toxicity studies of BPA in CD (SD) rats and CD-1 mice.

1. Three-generation reproductive toxicity study by Tyl et al. (2002b) of BPA in the diet of CD (SD) rats at 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm (equivalent to 0, ~0.001, 0.02, 0.3, 5.0, 50.0, or 500.0 mg/kg/day), with 30 adult animals/sex/group. In the parental animals (F0, F1, F2) and F3 retained adults, there was demonstrable toxicity as follows:

Adult Toxicity

Body weights:

- There were reduced body weights and weight gains in both sexes of F0, F1, and F2 parents and retained F3 adults.
- There were reduced body weights during gestation and lactation for F0, F1, and F2 females at 7500 ppm and at 750 ppm for F0 and F2 females.
- There were reduced body weights during lactation for F1 females at 750 ppm.
- There were reduced body weights at terminal sacrifice for both males and females in all generations at 7500 ppm, for F1 females at 750 ppm, and for F2 and F2 males at 750 ppm.

Feed consumption: Variable

Clinical observations: None treatment related

Organ weights at necropsy: Absolute weights reduced at 7500 ppm: liver, kidneys, ovaries, adrenal glands, spleen, pituitary, and brain

Relative organ weights: Increased at 7500 ppm or unaffected

Histopathology:

- At 7500 ppm, slight to mild renal tubular degeneration and chronic hepatic inflammation in F0, F1, and F2 females
- Reproductive organ histopathology or function not affected

Reproduction and Offspring

- There were no effects on mating, fertility, gestational indices, ovarian primordial follicle counts, estrous cyclicity, precoital interval, gestational length, offspring sex ratio, postnatal survival, nipple/areolae retention in preweanling males, epididymal sperm number, motility or morphology, daily sperm production (DSP), or efficiency of DSP.

Offspring Toxicity

- Vaginal patency (VP; female) and preputial separation (PPS; male) were both delayed in F1, F2, and F3 offspring, associated with reduced body weights at 7500 ppm.
- Anogenital distance (AGD) on postnatal day (PND) 0 was unaffected for F2 and F3 males and F3 females. F2 female AGD increased at some doses but not at 7500 ppm (considered not biologically relevant).

Conclusions

- Adult systemic NOAEL: 75 ppm (5 mg/kg/day)
- Reproductive and postnatal NOAEL: 750 ppm (50 mg/kg/day)
- There were no treatment-related effects in the low-dose region (0.001 – 5 mg/kg/day) for any parameters and no evidence of non-monotonic dose-response curves across generations for either sex. “BPA should not be considered a selective reproductive toxicant based on the results of this study.”

A very recent developmental neurotoxicity study of BPA in the diet of SD rats at 0, 0.15, 75, 750, and 2250 ppm, from GD 0 to PND 21 (Stump et al., 2010), reported systemic toxicity to dams and offspring, expressed as reduced body weights at 750 and 2250 ppm (NOAEL, 75 ppm), with no treatment-related effects on gestational lengths, parturition, litter sizes, acquisition of developmental landmarks, or on acquisition of puberty in either sex at any dose. There was also no evidence of any treatment-related neurobehavioral effects, of altered neuropathology, or of altered brain morphometry in offspring through PND 72. The NOAEL for developmental neurotoxicity was 2250 ppm, the highest dose tested. The authors concluded that there was no evidence that BPA is a developmental neurotoxicant in rats.

2. We also performed an abbreviated one-generation study (Tyl et al., 2002a, unpublished), designed to duplicate the species/strain (CD-1 mice), route (diet), and the top two dietary BPA concentrations (5,000 and 10,000 ppm) employed in the BPA RACB study (Reel et al., 1985), and to provide an enhanced evaluation of parental systemic and reproductive toxicity at the top two doses previously employed, to provide the context for the F1 offspring effects at the doses previously reported. CD-1 mice (F0 generation, 20/sex/group) were exposed to dietary BPA for two weeks prebreed, one-week mating (with the F0 males terminated after mating), and through gestation to parturition (PND 0 of the F1 offspring, with F0 females exposed to BPA for 5-6 weeks). F0 females and their F1 pups were necropsied on PND 0. At necropsy, F0 female body, liver, paired kidneys, paired ovaries, and uterine weights were reduced, and maternal livers and kidneys were fixed and evaluated histopathologically. Maternal blood was taken and serum evaluated for a full panel of BUN, creatinine, total protein, albumin, inorganic phosphate, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sodium, potassium, and chloride.

F0 parental systemic toxicity was present at both dietary BPA doses: increased absolute liver and kidney weights in both sexes (except for F0 male kidney weight at 10,000 ppm not statistically significantly different), increased relative liver and kidney weights at 10,000 ppm for both sexes, increased relative liver weights at 5000 ppm for both sexes, and increased relative kidney weights for F0 females at 5000 ppm. Maternal toxicity also included reduced body weights, reduced weight gains, reduced feed consumption and food efficiency during pregnancy, and significantly prolonged gestation at both doses (prolonged for ~10 hours at both doses; significance, if any, unknown). No effects were observed for precoital interval or ovarian or uterine absolute or relative weights. The F0 dams also exhibited histopathologic lesions in the liver (dose-related hepatocellular hypertrophy), kidneys (dose-related renal tubular epithelial degeneration, necrosis and regeneration), elevated BUN at 10,000 ppm, and reduced serum, sodium, potassium, and chloride at 5000 ppm, consistent with renal toxicity.

F1 offspring toxicity was observed only at 10,000 ppm, expressed as slightly (statistically significantly) reduced total and live pups/liver, with no significant effects on pre- or postimplantation *in utero* loss or on pup weights/litter (separate or combined) on PND 0 (day of birth).

These data confirmed the F1 litter size effects at 10,000 ppm observed in the RACB study for the first F1 litters. This study also demonstrated parental systemic toxicity (specifically to the liver and kidneys) at both doses; worse at 10,000 ppm. These data also support the interpretation of F1 litter size effects at 10,000 ppm in the RACB study and in this study as caused by the compromised status of the F1 dams after only 5-6 weeks of exposure in the present study, while the lesser maternal systemic toxicity observed at 5000 ppm did not cause litter size effects after the 5- to 6-week exposure regimen in this study. Note that F1 litter size effects were observed at 5000 ppm in the RACB study for only the fourth and fifth litters after a much longer parental exposure duration (>98 days, >14 weeks).

3. Two-generation reproductive toxicity study of dietary BPA in CD-1 (Swiss) mice by Tyl et al. (2008b) of BPA in the diet at 0, 0.018, 0.18, 30, 300, or 3500 ppm (equivalent to 0, 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg/day), with 28 adults/sex/group, plus concurrent positive control group of dietary 17 β -estradiol at 0.5 ppm (~ 0.080 mg/kg/day)

Adult Toxicity

- At 300 ppm: minimal centrilobular hepatic hypertrophy
- At 3500 ppm: reduced body weights, increased liver and kidney weights, minimal/mild centrilobular hepatocyte hypertrophy, and renal nephropathy in males
- There were no effects at any dose on adult mating, fertility or gestational indices, ovarian primordial follicle counts, estrous cyclicity, precoital intervals, sperm parameters (number,

motility, morphology), or reproductive organ weights or histopathology, including the testes and prostate. F1 absolute paired epididymal weights were reduced at 3500 ppm.

Offspring Toxicity

- At 3500 ppm, BPA reduced F1 and F2 weanling body weights, reduced weanling spleen and testes weights (with seminiferous tubule hypoplasia), slightly delayed PPS, and apparently increased the incidence of treatment-related undescended testes only in weanlings. This is considered a developmental delay in the normal process of testes descent, since all adult offspring had descended testes and normal reproductive structures and functions. It is likely that these transient effects were secondary to and caused by systemic toxicity. Gestational length was increased by 0.3 days in F1 and F2 generations. The toxicological significance, if any, is unknown. At low doses (0.018 to 30 ppm, 0.03 to 30 mg/kg/day), there were no effects and no evidence of non-monotonic dose response curves for any parameter.

Conclusions

- Adult systemic NOAEL: 30 ppm (~5 mg/kg/day)
- Reproductive and postnatal NOAEL: 300 ppm (~50 mg/kg/day)
- “Therefore, BPA is not considered a selective reproductive or developmental toxicant in mice.”

Other researchers have also published robust studies where low BPA doses, in the absence of maternal toxicity, do not cause effects on the prostate in mice (Ashby et al., 1999), in reproductive organ development in mice (Cagen et al., 1999), on sexual development in rats (Tinwell et al., 2002), or on pubertal development in rats (Kwon et al., 2000). The strongly supported view that low oral BPA doses do not represent a risk has also been reflected in various national evaluatory and regulatory decisions.

IV. Additional Concerns and Aspects

There are two additional scientific concerns for studies used to evaluate the maternal and developmental toxicity of BPA. One is the profound difference in metabolism of BPA when administered orally (by gavage or dosed feed) versus parenterally. Non-oral routes of administration (e.g., subcutaneous or intravenous injection, intracisternal [brain] injections, subcutaneous implants, etc.) bypass the rapid and essentially complete first pass presystemic metabolic conjugation of BPA observed with oral exposures. Therefore, systemic concentrations of parent BPA are much higher, with higher and longer bioavailability, and the identities of the metabolites are different by these non-oral routes (Zalko et al., 2003). Confirmation of the lack of toxicity of orally administered BPA is reported in two

recent studies performed in a U.S. EPA NHEERL laboratory. In one of them, oral administration of BPA during gestation and lactation did not alter sexually dimorphic behavior, puberty, fertility, or anatomy in female rats, while ethinyl estradiol caused all of these effects (Ryan et al., 2010). In the other study, gestational and lactational oral exposure to BPA also did not affect androgen-dependent organ weights or epididymal sperm counts in male rats, while ethinyl estradiol caused all of these effects (Howdeshell et al., 2008). Since oral exposure is the relevant human route, use of non-oral routes in laboratory animals may inform hazard (the intrinsic capacity of the chemical to do harm) but not risk (effects by relevant routes, at relevant doses, during sensitive life stages).

A second scientific concern is the difference in metabolism from oral administration in humans versus rodents. It has been shown that in humans, oral administration of BPA results in essentially 100% metabolism of the parent to BPA glucuronide. In adults, BPA from oral exposure is not detected in the blood. BPA is glucuronidated in the intestinal walls and liver (prior to systemic exposure) into BPA glucuronide which is not estrogenic and rapidly excreted via the urine, with no bioaccumulation and a short elimination half-life of approximately 4-6 hours (Mathews et al., 2001; Volkel et al., 2005). This “first pass” effect (presystemic elimination) occurs from oral exposure but not from other routes of exposure (Pottenger et al., 2000). A secondary BPA metabolite, BPA sulfate, is formed at much lower concentrations and is also not estrogenic (Shimizu et al., 2002). Metabolism of BPA into non-estrogenic BPA glucuronide also occurs in rodent neonates (Domoradzki et al., 2003), in human neonates in hospital intensive care units, and in infants and children (Calafat et al., 2009). In rats, oral administration of BPA results in glucuronidation of BPA in the intestine and liver (less efficiently than in humans), but there is enterohepatic recirculation after hydrolysis of the conjugated BPA metabolites in the intestines and reabsorption of parent BPA. Therefore, parent BPA from oral dosing is transiently detected in rodent blood, and there is a longer half-life and higher bioavailability in rodents compared to humans. Consequently, since the weight of evidence indicates that BPA is not a selective reproductive or developmental toxicant in rodents, it is highly unlikely that BPA could be a reproductive or developmental toxicant in humans.

One important aspect of animal testing and human exposure to BPA is the presence of molecules with much higher affinity (versus BPA) for the estrogen receptor. Many components of the human and rodent diet, such as phytoestrogens (e.g., genistein found in soy), have similar or higher affinity than BPA for the estrogen receptors. Therefore, any bioavailable BPA with weak affinity for the estrogen receptor (4000- to 5000-fold less potent than endogenous estrogen) would compete with

endogenous estrogen (17 β -estradiol) and/or dietary phytoestrogens (e.g., the soy phytohormones genistein, daidzein, glycitein), all with much greater affinity for the estrogen receptor than BPA. As a result, it is not biologically plausible that BPA could cause reproductive or developmental effects in humans, in particular endocrine-mediated effects.

A second important aspect is the inaccurate and unfounded assertions that the rat and mouse strains employed in the large, robust studies on BPA are not sensitive to estrogens. We have performed and published a three-generation reproductive toxicity study of dietary nonylphenol (Tyl et al., 2006) in CD (SD) rats with an E2 positive control group, and one-generation (Tyl et al., 2008a) and two-generation (Tyl et al., 2008c) reproductive toxicity studies of E2 in the diet of CD-1 mice. In these studies, E2 exposure caused the estrogenic effects anticipated at low dietary doses in both rats and mice. This is in stark contrast to the absence of BPA effects at low dietary doses. In fact, governmental laboratories have also reported no effects at low doses of BPA, but they did report effects from low-dose endogenous (E2) or synthetic (e.g., 17 α -ethinyl estradiol; EE2) estrogens in rats (Howdeshell et al., 2008; Ryan et al., 2010).

V. Summary and Conclusions

Summary

1. The scientific evidence clearly indicates that BPA is not a selective developmental toxicant. Developmental toxicity occurs only at very high oral BPA doses in the presence of profound maternal toxicity. At lower doses with less, but still significant, maternal toxicity, there is no developmental toxicity.
2. BPA is not a developmental neurotoxicant, even in the presence of maternal toxicity.
3. The scientific evidence clearly indicates that BPA is not a selective reproductive toxicant. Reproductive and/or offspring effects from BPA, if they occur, occur only in the presence of substantial maternal toxicity. In the presence of less, but still significant, maternal toxicity, there is no reproductive or offspring toxicity.
4. Since intentional feed restriction, per se, results in maternal toxicity and subsequent embryo-fetal toxicity in rats and rabbits, it is likely that maternal toxicity is the critical determinant of embryo-fetal toxicity in the BPA studies as well.
5. Therefore, BPA does not satisfy the criteria for listing under Proposition 65.

Conclusions

This reviewer strongly believes, based on scientific data, that embryo-fetal offspring toxicity from exposure to high doses of BPA is caused by maternal toxicity.

The importance and central role of maternal toxicity in the causation and evaluation of embryo-fetal and postnatal offspring toxicity are explicitly acknowledged in all the national and international regulatory test guidelines for developmental toxicity. All of them specifically require demonstrable maternal toxicity at the top dose (see Section I for the list of findings encompassing maternal toxicity). The requirement for parental toxicity at the top dose is also present in all the national and international test guidelines for reproductive toxicity assessment. Reproductive or developmental effects that occur at high doses must be evaluated through the lens of maternal toxicity. These test guidelines include:

- FDA Segment II study (Goldenthal, 1966; FDA Redbook, 2000)
- FDA Segment III study (Goldenthal, 1966)
- FDA two-generation reproduction plus teratogenicity study
- EPA OPPTS developmental toxicity study (870.3700; 1998)
- EPA OPPTS reproduction and fertility effects (870.3800; 1998)
- OECD teratogenicity (TG 414; 1981, 2001a)
- OECD one-generation reproduction toxicity study (TG 415; 2001b)
- OECD two-generation reproduction toxicity study (TG 416; 2001c)
- ICH test guidelines (FDA, 1994, 1996)
- ICH test guideline, embryo-fetal development (like FDA Segment II) (ICH 4.1.3)
- ICH test guideline, two-study design in rodents (like FDA Segment III) (ICH 4.3; ICH 4.1.1 plus 4.1.2)

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