COMMENTS ON THE PROPOSED LISTING OF BISPHENOL A
AS A REPRODUCTIVE OR DEVELOPMENTAL TOXICANT
UNDER PROPOSITION 65

Submitted by

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May 12, 2010

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Qualifications
I am an obstetrician-gynecologist and reproductive toxicologist with specific expertise in the evaluation of the medical and scientific literature concerning reproductive and developmental toxicology and concerning teratology. I am Adjunct Professor of Obstetrics and Gynecology and of Biochemistry and Molecular Biology at Georgetown University, where I teach and Clinical Professor of Obstetrics and Gynecology at George Washington University, where I teach and provide clinical services. I am employed by Tetra Tech Sciences, a health and environmental consulting firm. I am also Director of the Reproductive Toxicology Center, A Non-Profit Foundation. The Reproductive Toxicology Center operates a data base called REPROTOX®, which is a reference source for information on the reproductive and developmental effects of chemical and other agents on all aspects of reproduction. REPROTOX® is used by physicians, genetic counselors, industry scientists, and government agencies in the US and in other countries.

I am the founding editor of the peer-reviewed journal Reproductive Toxicology, which is published by Elsevier Science. I was Editor-in-Chief of Reproductive Toxicology for 17 years. I continue to serve as an editor for articles submitted to Reproductive Toxicology as well as for articles submitted to other scientific journals including Birth Defects Research, Obstetrics and Gynecology, the New England Journal of Medicine, and the American Journal of Obstetrics and Gynecology, among others.

From 1983 to 2004, I was a full-time faculty member at Georgetown University School of Medicine, achieving the ranks of Professor of Obstetrics and Gynecology and Professor of Biochemistry and Molecular Biology. I was also Director of the Residency Program in Obstetrics and Gynecology at Georgetown University Hospital.

I have served as an advisor or consultant on the subject of developmental toxicology and reproductive medicine for organizations such as the US Food and Drug Administration (FDA), the World Health Organization, the Environmental Protection Agency (EPA), and the Occupational Safety and Health Administration, among others. My activities at the FDA include teaching a course for medical officers on the interpretation and use of developmental toxicity data and service as a consultant to the Pregnancy Labeling Task Force, the purpose of which was to revise the pregnancy portion of the medication labeling to make it more useful and understandable for clinicians.

I have been active in national and international professional societies concerned with reproductive medicine and toxicology. These societies include the Teratology Society, of which I am a past president, the Organization of Teratology Information Specialists, the Society of Toxicology, of which I am a member of the Reproductive and Developmental Toxicology Specialty Section, the American College of Obstetricians and Gynecologists, and the American Society for Reproductive Medicine.

I have counseled men and women about the reproductive effects of exposures to chemicals and other agents and have authored, coauthored, or edited original scientific studies, review articles, book chapters, and books in reproductive medicine, including reproductive and developmental toxicology. I have been a regular contributor to the scientific literature on the effects of chemicals and other agents on reproduction. I am
very familiar with methods of risk assessment in reproductive and developmental toxicology and with the interpretation of findings that are confounded by parental or adult toxicity.

During 2004–2007, I was Principal Investigator on the National Toxicology Program (NTP) Center for Evaluation of Risks to Human Reproduction (CERHR) contract held by my employer. In this capacity, I organized and supported the writing of Expert Panel reports. I am very familiar with the goals of CERHR and the methodology used in the development of Expert Panel reports.

Purpose

It is my understanding that a petition has been submitted seeking the listing of bisphenol A as a known reproductive and developmental toxicant under Proposition 65. The basis of this petition is the purported determination by NTP through CERHR that bisphenol A is a reproductive and developmental toxicant. This determination was described by the petitioner as being based on the results of studies conducted at parentally/adult toxic bisphenol A exposure levels.

It is my purpose in this commentary to correct the misunderstanding that these studies demonstrate the reproductive or developmental toxicity of bisphenol A. Moreover, it is not the case that CERHR determined that bisphenol A is a reproductive or developmental toxicant, nor would CERHR make such a determination. I will explain how CERHR did its business and what determinations were made by CERHR.

The Effect of Systemic Toxicity on Reproduction

The reproductive system in mammals is sensitive to stress induced by general toxicity. The function of the ovaries and testes depends on appropriate stimulation by the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The pituitary gonadotropins are released in response to pulsatile release of gonadotropin-releasing hormone (GnRH) by the arcuate nucleus in the hypothalamus. There are a number of neural inputs to the arcuate nucleus that can modify or eliminate GnRH production. One such system, for example, is the opioidergic system, which turns off the arcuate nucleus in response to stress (Yen et al., 1985). In addition, a recently identified group of gonadotropin-inhibiting substances have been identified. These hormones are produced by the adrenal gland in response to stress and inhibit the gonadotropic system through direct action on the pituitary and hypothalamus (Tsutsui 2009).

Embryo development has been known for decades to be sensitive to the effects of maternal toxicity. Khera (1984) reviewed teratology studies performed in mice, rats, hamsters, and rabbits and determined that resorptions and embryo death were common end points associated with maternal toxicity. Chernoff et al. (1990) showed that toxicant-induced maternal toxicity can be associated with resorptions and decreased fetal weight. Inadequate maternal nutrition, which can be due to inappetence or inefficient processing of ingested feed, can produce deficits in fetal body weight and viability. Feeding restriction studies in rats have shown effects on pup weight and function that persist well
after birth (Chernoff et al., 2009). Human experience from famines has confirmed that inadequate maternal dietary intake can produce impairments in fetal growth and postnatal function (Stein et al., 2004; Kyle and Prichard, 2006). In clinical obstetrics, it is axiomatic that the best environment for growing a healthy baby is a healthy mother.

Because maternal/adult toxicity can confound the results of reproductive and developmental toxicology studies, recommendations for study design include the selection of a dose range that does not cause excessive parental/adult toxicity. For example, the Guidelines for Developmental Toxicity Risk Assessment of the US EPA (1991) state with respect to dose selection:

“The high dose is selected to produce some minimal maternal or adult toxicity (i.e., a level that at the least produces marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality). At doses that cause excessive maternal toxicity (that is, significantly greater than the minimal toxic level), information on developmental effects may be difficult to interpret and of limited value.”

More recently, the Organisation for Economic Cooperation and Development (2007) published similar guidelines for developmental neurotoxicity studies, specifying:

“Unless limited by the physico-chemical nature or biological properties of the substance, the highest dose level should be chosen with the aim to induce some maternal toxicity (e.g., clinical signs, decreased body weight gain (not more than 10%) and/or evidence of dose-limiting toxicity in a target organ).”

Therefore, decreases weight of body weight gain of more than 10% are considered to represent dose levels higher than the maximum tolerated dose (MTD).

**The CERHR Approach**

CERHR adopted an evaluative process that differs fundamentally from the Proposition 65 process. While Proposition 65 entails consideration of whether a chemical should be listed as a reproductive or developmental toxicant based on effects that occur as a result of prenatal exposure, CERHR characterizes the conditions under which reproductive or developmental toxicity occur and determines a level of concern for human exposure based on a comparison of anticipated human exposure conditions and those represented in experimental studies. The guidelines for the CERHR approach have been published (Shelby, 2005). The statement template set forth in these guidelines is:

“There is (sufficient, insufficient) evidence in (animals and/or humans) that (chemical X) (does or does not) cause (developmental, reproductive) toxicity when exposure is (route, dose, timing, duration). The data are (relevant, assumed relevant, irrelevant) to human risk.”

As indicated in the Shelby paper, this template was taken from a National Research Council Report published in 2001. I was a coauthor of that report. There was a distinct intention to avoid a prescription for list-making by the members of the committee that

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generated the National Research Council Report and, consequently, determined the basis of the CERHR process. The CERHR process by its design could not have listed bisphenol A as a reproductive or developmental toxicant because it does not create lists.

As is discussed amply in the CERHR report on the relevant bisphenol A studies, the context of the reproductive and developmental effects associated with bisphenol A experiments included high dose level exposures with consequent maternal toxicity. I will review each of these studies.

**The Studies**

In each of the six studies that have been identified as showing reproductive or developmental toxicity after treatment with bisphenol A, parental or adult toxicity explains the reproductive or developmental effects that were described. The studies are presented in chronological order except for a 2002 study that is placed after the 1985 study that it attempted to reproduce.

**NTP (1985)**

This report describes a mouse reproduction study performed by continuous breeding and using dietary dosing with bisphenol A. This type of study design was developed by the National Toxicology Program and consists of four tasks. Task 1 was intended to identify an appropriate high dose at which there would be some impairment of body weight gain but not excessive toxicity. Task 2 was the fertility study in which male and female mice were placed on test diets for a 7-day pre-cohabitation period followed by a 98-day cohabitation period and a 21-day separation period (to permit birth of the last litter). Diets contained 0, 0.25, 0.5, or 1.0% bisphenol A resulting in estimated ingestion of 0, 437.5, 875, or 1750 mg/kg bw/day. Litters were removed from dams after birth to permit continued breeding. Task 3 was a cross-over trial in which animals in the high dose group were mated with animals in the control group. Task 4 was a mating trial of F1 offspring that had been exposed via their dams during pregnancy and lactation and directly in the diet between weaning and sexual maturity.

Adult toxicity was not systematically assessed in all dose groups due to the nature of the study design. Adult body weights were measured at specified intervals without regard to pregnancy status, making it difficult to identify possible treatment-related effects on body weight. Dam weight after pregnancy, which was reduced in the high-dose dams, was not adjusted for litter size, which was also reduced by treatment in the middle and high dose groups. It would be expected that the bearing of a smaller litter would be associated with an increase in postpartum maternal weight compared to control, and the lack of such an increase may represent adult toxicity. Necropsy weight at the end of the study was reduced 4% in the high-dose animals but was not reported for the other dose groups. It should be noted that in other rodent feeding studies (Tyl et al., 2002b, 2008), substantial weight decrements were seen in the first two weeks after onset of dosing; therefore, the 4% weight decrement at necropsy in this underestimates the degree of adult toxicity occurring with treatment. Organ weights and histopathology evaluations were obtained
only in high-dose animals and demonstrated significant toxicity, especially involving the liver and kidney.

Developmental toxicity in the high dose group included a reduction in number of litters per pair and number of live pups per litter. Based on the crossover matings, these effects appeared to be due to the female. The substantial adult toxicity in the high dose group was sufficient to cause the small decrease in litters per mating pair and live pups observed in these animals.

The magnitude of adult toxicity in the middle dose group, at which there was a small decrease in litters/pair and live pups/litter, cannot be assessed directly from Task II, but can be assessed from Task IV (mating of F1 animals on the same diet as their parents). The middle dose group demonstrated liver and kidney toxicity in the experiment, characterized by alterations in organ weights and histologic abnormalities. In spite of this adult toxicity, there was no treatment effect on reproductive success in this single-mating study. The study authors concluded, “It is possible, therefore, that some or all of the adverse effects on reproductive performance observed in the present study may be secondary to the generalized toxicity of BPA.” These effects were further investigated by Tyl et al. (2002a), discussed below.

**Tyl et al., 2002a**

This study was designed to further investigate the reproductive effects of bisphenol A as described in the 1985 NTP continuous breeding study. Adult male and female mice were given diets containing 0, 0.5%, or 1% bisphenol A, with estimated bisphenol A intake levels of 0, 1055, and 1988 mg/kg bw/day in females during the prebreeding period, and 0, 870, and 1716 mg/kg bw/day in females during pregnancy. These dietary concentrations are the same as in the NTP (1985) continuous breeding study. Exposure to the diet occurred during a 2-week prebreeding period and a 1-week breeding period. Body weights and food consumption were recorded periodically and dams in both dose groups underwent necropsy at the end of the study.

Weight gain was decreased during gestation by 16% in the low dose group and 19% in the high dose group. In both dose groups, hepatic and renal abnormalities were noted on histological examination and relative organ weights were increased. There was a 15% decrease in number of total pups and live pups per litter in the high dose group but no alteration in these end points in the low dose group. This study confirmed the developmental events reported by the NTP study with the 1% dietary bisphenol A level but did not reproduce the developmental effects reported at the 0.5% level. The authors noted that the degree of maternal toxicity in both dose groups was considerable, and the developmental effects at the high exposure level were consistent with this maternal toxicity.

**Morrissey et al. (1987)**

This paper describes a standard developmental toxicity study in which pregnant rats and mice were treated with bisphenol A by gavage from gestation day 6 through 15. Dose
levels for rats were 0, 160, 320, 640, and 1280 mg/kg bw/day. Dose levels for mice were 0, 500, 750, 1000 and 1250 mg/kg bw/day. Pregnant animals were weighed at specified intervals during pregnancy and at termination on gestation day 20 (rats) or 17 (mice). Fetuses were counted, weighed, and examined using standard techniques for external, visceral, and skeletal abnormalities.

Maternal toxicity was prominent at all bisphenol A dose levels in both rats and mice. Maternal toxicity in rats was manifested as a decrease in body weight gain and a decrease in maternal carcass weight; that is, body weight excluding the pregnant uterus. The highest dose level produced a 14% decrease in maternal body weight gain over the course of the pregnancy. In spite of this substantial toxicity, there was no developmental toxicity at any dose in the rat.

Maternal toxicity in mice was more severe, resulting in deaths in all dose groups. In the three lower dose groups, there were one or two deaths among 29 to 34 dams. At the highest dose level, maternal mortality was 18% (6 deaths among 33 dams), which is considered excessive toxicity. Among survivors in the high dose group, maternal body weight was decreased 43%, which is also excessive. Fetal body weight per litter and resorptions per litter were increased in the high dose group, findings that are fully consistent with the degree of maternal toxicity produced at this excessive dose level. The authors concluded, “…post implantation exposure to BPA (gavage) did not cause external, visceral, or skeletal malformations at doses that caused significant maternal toxicity (rats) or mortality (mice).”

Kim et al., 2001

In this study, pregnant rats were treated from the day after evidence of mating through gestation day 20 with gavage doses of bisphenol A at 0, 100, 300, and 1000 mg/kg bw/day. Maternal body weight was assessed at standard intervals and food consumption was measured. Pregnant animals were killed on gestation day 21 and fetuses removed for external examination and for visceral or skeletal evaluation. Body weight gains in the middle and high dose group were decreased by 35 and 52%, respectively, well above the 10% decrease in body weight gain that is considered an appropriate degree of maternal toxicity. Some animals were reported to be emaciated. Fetal death and resorption were increased and fetal body weights were decreased 36% in the high dose group. Fetal body weight was decreased about 14% in male fetuses in the middle dose group. Skeletal ossification sites were decreased in the high dose group. The decrease in fetal weight and viability is typical for the degree of maternal toxicity produced in the high dose group. The decrease in skeletal ossification sites represents a delay in ossification rather than a developmental anomaly and is commonly due to maternal toxicity. Delayed ossification has been shown to be transient, resolving as delayed pups catch up during the lactation period (Carney and Kimmel, 2007).

Tyl et al., 2002b

This paper describes a three-generation dietary study of bisphenol A in rats. Dose groups were 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm resulting in ingestion of approximately
0.001, 0.02, 0.3, 5, 50, and 500 mg/kg bw/day bisphenol A. Parental animals (F₀) were exposed to the test diets for 10 weeks before mating, during mating, and, for females, during pregnancy and lactation. After weaning, the offspring (F₁) were exposed to the test diet for ten weeks prior to mating. The test diet was continued during mating and, for females, during pregnancy and lactation. Offspring of these matings (F₂) were treated in the same manner as their parents and mated after a ten week exposure period. Again, dietary treatment of females continued during pregnancy and lactation. Offspring of these matings (F₃) were examined at weaning or, after continued dietary exposure, at about 17 weeks of age. Standard methods were used including culling of litters on postnatal day 4, interval weighing, evaluation of pubertal landmarks, and necropsy of F₀, F₁, and F₂ adults.

Adult toxicity was seen at the highest two dose groups and consisted of decreases in body weights. Weaning weight in all generations was consistently decreased in the high dose group. There was a decrease in the number of pups per litter in the highest dose group, which could not be explained by post-implantation loss. It is possible that there was an increase in preimplantation loss or a decrease in the number of follicles that ovulated, both of which are expected consequences of adult toxicity, mediated by disruption of gonadotropins, which are necessary for ovulation, or suppression of prolactin, which is important in pregnancy maintenance in the rat. A decrease in ovarian weight in the highest dose group was noted, but ovarian histology was not compromised and the number of primary follicles was not altered by treatment. The decrease in ovarian weight is of uncertain significance, particularly given the known effects of stress on decreasing gonadotropin-releasing hormone and pituitary gonadotropins, thereby decreasing ovarian activity.

This study is remarkable for the lack of reproductive or developmental toxicity over three generations in the face of prominent adult toxicity in the high dose group. The authors concluded that bisphenol A should not be considered a selective reproductive or developmental toxicant.

_Tyl et al., 2008_

This paper describes a two-generation reproductive study in mice dosed with bisphenol A through the diet. Dietary levels were 0, 0.018, 0.18, 1.8, 30, 300, or 3500 ppm, and estimated bisphenol A intakes were 0, 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day. Parental (F₀) animals were given treated feed for at least eight weeks prior to mating, during the mating period and, for females, throughout pregnancy and lactation. Offspring were continued on treated feed after weaning for at least eight weeks prior to mating and during mating, and females were continued during gestation and weaning. Their offspring (F₂) were evaluated and terminated at weaning after potential exposure through the dams during pregnancy and the lactation period and potential direct exposure to treated feed during the end of the lactation period. Standard methods were used to standardize litters on postnatal day 4.

There was no evidence of developmental toxicity in this study except for transient delay in testis descent in weanlings, attributed to maternal toxicity. Parental toxicity was prominent in the highest dose group, manifested as abnormal kidney and liver organ

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weights and histopathology. The authors concluded that bisphenol A is not a selective reproductive or developmental toxicant.

Conclusions

Reproductive or developmental effects due to parental or adult toxicity do not warrant consideration of a chemical as a reproductive or developmental toxicant. Indeed, there is a well-established tradition in the field of avoiding excessive parental or adult toxicity in study design in order to avoid obtaining findings that cannot be interpreted. In the case of the six studies identified as showing reproductive or developmental effects, the effects occurred with exposure levels that produced clear parental/adult toxicity of a degree sufficient to explain the reproductive or developmental effects; moreover, the developmental effects were those expected to occur from adult toxicity. The CERHR Bisphenol A Expert Panel called attention to the parental/adult toxicity at high doses of bisphenol A. By design, the CERHR process does not result in a listing of chemicals as reproductive or developmental toxicants, and neither the CERHR Expert Panel nor the NTP listed bisphenol A as a reproductive or developmental toxicant. In my opinion, the data do not support the listing of bisphenol A as a reproductive or developmental toxicant under Proposition 65.

Disclosure

For the time spent in writing this commentary, my employer is being compensated by the American Chemistry Council.
References


