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Rochelle W. Tyl, Ph.D., DABT
Distinguished Fellow
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Center for Pharmacology & Toxicology
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Dear Dr. Tyl:

Thank you for your letter of May 12, 2010, responding to the Request for Relevant Information on the possible listing of bisphenol A (BPA) under Proposition 65[1]. BPA is a candidate for listing as known to cause reproductive toxicity. The potential listing would be by the authoritative bodies provision[2] of Proposition 65 and based on findings by the National Toxicology Program (NTP). NTP made its findings in a report[3] by the NTP Center for the Evaluation of Risks to Human Reproduction that BPA causes developmental toxicity at "high" doses.

OEHHA has carefully reviewed the comments you submitted. A document providing our responses to your comments is enclosed.

After review of all the submissions received in response to the Request for Relevant Information, OEHHA has determined that BPA meets the criteria for listing under the authoritative bodies provision of Proposition 65. Accordingly, a Notice of Intent to List BPA will be published in the near future. Following its publication, there will be a 30-day period for submission of public comments regarding the possible listing.

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[1] The California Safe Drinking Water and Toxic Enforcement Act of 1986, California Health and Safety Code section 25249.5 et seq.
[2] Health and Safety Code section 25249.8(b) Title 27, Cal. Code of Regulations, section 25306.
[3] National Toxicology Program – Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR, 2008). *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A.* NIH Publication No. 08 – 5994.

Comments should focus on whether or not the criteria for listing have been met.[4] In the event that OEHHA finds the criteria have not been met after review of the comments, the chemical will be referred to the Developmental and Reproductive Toxicant Identification Committee (DARTIC) for its consideration as required by regulation.[5]

Thank you for your interest in Proposition 65. If you have any questions or concerns, please contact me at (916) 322-6325 or by email at Lauren.Zeise@oehha.ca.gov.

Sincerely,

Lauren Zeise, Ph.D.
Deputy Director for Scientific Affairs

Enclosure:

Response to Comments from Rochelle W. Tyl on the Request for Relevant Information on Bisphenol A as a Chemical under Consideration for listing under Proposition 65.

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[4] Title 27, Cal. Code of Regulations, section 25306.
[5] Title 27, Cal. Code of Regulations, section 25306(i)

Response to Comments from Rochelle W. Tyl on the Request for Relevant Information on Bisphenol A as a Chemical under Consideration for listing under Proposition 65

Office of Environmental Health Hazard Assessment

January 2013

On February 12, 2010, the Office of Environmental Health Hazard Assessment (OEHHA) published in the California Regulatory Notice Register (CRNR) a Request for Relevant Information for Bisphenol A (BPA) for possible listing as a chemical known to cause reproductive toxicity under Proposition 65.[1] The listing was proposed under the authoritative bodies provision of the regulations[2] based on findings by the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) that BPA causes developmental toxicity at "high" doses (NTP-CERHR, 2008).

On May 12, 2010, OEHHA received comments concerning the listing of BPA under Proposition 65 from Rochelle W. Tyl of Research Triangle Institute (RTI) International, supported by the American Chemistry Council. This document provides a response to those comments.

For authoritative bodies listings, a chemical must be listed under Proposition 65 when the following criteria are met:

1) **Formal Identification**: An authoritative body formally identifies the chemical as causing reproductive toxicity (Section 25306(d)[3]).
2) **Sufficiency of Evidence**: The evidence considered by the authoritative body meets the sufficiency criteria contained in the regulations (Section 25306(g)). However, the chemical is not listed if scientifically valid data which were not considered by the authoritative body clearly establish that the sufficiency of evidence criteria were not met (Section 25306(h)).

Dr. Tyl's comments primarily address OEHHA's role in examining **sufficiency of evidence,** specifically considerations of maternal toxicity as stated in the regulations, section 25306(g)(2):

> "Studies in experimental animals indicate that there are sufficient data, taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (codified at Health and Safety Code section 25249.5 *et seq*.).
[2] Title 27, Cal. Code of Regulations, section 25306.
[3] All referenced sections are from Title 27 of the Cal. Code of Regulations.

adverse reproductive effects in humans and the toxic agent in question is biologically plausible."

Detailed responses are provided below using headers from Dr. Tyl's comments.

Comment:

I. Definition of Maternal Toxicity:

The comments provide a definition of maternal toxicity, as follows:

> "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)."

Response: The comments appear to be Dr. Tyl's opinion and interpretation of the U.S. Environmental Protection Agency (U.S. EPA, 1991) Guidelines for Developmental Toxicity Risk assessment, as the exact wording of the comments does not appear in the guidelines document. The Guidelines provide a list of endpoints of maternal toxicity, along with guidance as to their interpretation. The list of endpoints is as follows (U.S. EPA 1991, pp 8-9):

> "Mortality
> Mating index [(no. with seminal plugs or sperm/no. mated) × 100]
> Fertility index [(no. with implants/no. of matings) × 100]
> Gestation length (useful when animals are allowed to deliver pups)
> Body weight
> - Day 0
> - During gestation
> - Day of necropsy
>
> Body weight change
> - Throughout gestation
> - During treatment (including increments of time within treatment period)
> - Post-treatment to sacrifice

Corrected maternal (body weight change throughout gestation minus gravid
uterine weight or litter weight at sacrifice)

Organ weights (in cases of suspected target organ toxicity and especially when supported by adverse histopathology findings)
Absolute
Relative to body weight
Relative to brain weight

Food and water consumption (where relevant)

Clinical evaluations
Types, incidence, degree, and duration of clinical signs
Enzyme markers
Clinical chemistries

Gross necropsy and histopathology"

There are a number of important differences between the definition provided in the comments and that provided by U.S. EPA. In several bullets the comments include the wording "dose-related", which is not found in the U.S. EPA Guidelines. The incidence of maternal mortality is not addressed in the U.S. EPA list of endpoints; rather, the parenthetical phrase "no greater than 10%" is used in the Guidelines in connection with selection of doses in experimental studies that do not exceed a "minimal" level of maternal toxicity. The parenthetical "especially if associated with reduced body weight" is not found in the Guidelines. The phrase "known to be associated with adverse outcomes" is not used in the Guidelines in reference to clinical observations. The phrase "especially if there is confirmatory evidence of histopathology" is not found in the Guidelines. Thus, OEHHA considers the definition of maternal toxicity in the comments to be Dr. Tyl's opinion and interpretation of the Guidelines, rather than a direct quote from the guidance document.

Comment:

II. Background:

A series of comments concerning maternal toxicity are presented under this header. The general theme of these comments is that maternal toxicity caused by a chemical could influence the fetus. The comments state that "[t]he current consensus is that maternal toxicity in toxicology studies is the major cause of embryo fetal effects observed," and provides a series of quotations addressing the possible relationship between extreme maternal toxicity and adverse developmental outcome. For example,

> "…[e]xtreme maternal toxicity may result in embryo-fetal loss *in utero…"*…
>
> "[s]ometimes, 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".

A second theme of the comments is that, if this occurs, the chemical cannot be identified as causing developmental toxicity.

Response: Although the comments state "[t]he current consensus is that maternal toxicity in toxicology studies is the major cause of embryo fetal effects observed," no documentation supporting this statement is included in the comments. All of the quotations provided in the comments address only the possibility that extreme maternal toxicity might influence developmental outcome.

As regards the relationship between maternal and developmental toxicity, OEHHA relies on generally accepted scientific principles as expressed by regulatory agencies. Two examples are given below:

> "Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are especially of concern because the developing organism is affected but toxicity is not apparent in the adult. However, the more common situation is when adverse developmental effects are produced only at doses that cause minimal maternal toxicity; in these cases, the developmental effects are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity" U.S. EPA (1991) Guidelines for Developmental Toxicity Risk Assessment

> "Developmental effects, which occur even in the presence of maternal toxicity, are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case by case basis that the developmental effects are secondary to maternal toxicity." United Nations Globally Harmonized System of Classification and Labeling of Chemicals (Section 3.6.2.4.2, 2009)

This same principle is also expressed by individual reviews of the issue, such as the review by Carney (1997) cited in the comments:

> "[T]here currently remains a considerable burden of proof lying with the investigator if developmental effects are suspected to be secondary to altered maternal physiology. This burden is justifiable in that maternal toxicity is not always associated with developmental toxicity. Thus a cause and effect relationship between the two is not automatic."

Comment:

III. Bisphenol A

This section of the comments reviews the maternal and developmental toxicity data from six of the eight studies cited by NTP-CERHR to support their finding of “clear” evidence of the developmental toxicity of BPA at “high” doses. The comments reiterate various findings related to maternal and developmental toxicity in the studies discussed in the NTP-CERHR document. In each case the comments discuss the co-occurrence of maternal and developmental effects, and conclude that BPA is not a selective developmental toxicant. The comments also discuss a recent developmental neurotoxicity study by Stump et al. (Stump et al, 2010) that was not considered by the authoritative body.

Response: As described above, OEHHA relies on the generally accepted scientific principle that developmental toxicity occurring at the same doses as maternal toxicity is not to be dismissed as secondary to maternal toxicity. The comments provide the opinion of the commenter about the relationship between maternal and developmental effects; however, no evidence is provided in the comments that the developmental toxicity of BPA was secondary to maternal toxicity. The comments also do not provide any information beyond that which was considered by the authoritative body.

The study by Stump et al. includes oral doses of BPA greater than 50 mg/kg/d (identified as “high dose” by NTP-CERHR) and administered during gestation and lactation in rats. No effects were reported on survival of newborns, or birth weight. However, this study was conducted under a protocol that differed from those of the studies where effects were reported. For example, the dosing period is not as long as in studies with dietary administration cited by NTP-CERHR.

Section 25306(h) specifies that “[t]he lead agency shall find that a chemical does not satisfy the definition of ‘as causing reproductive toxicity’ if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria [specified in regulations]”. However, this single study conducted under a different experimental protocol does not meet this criterion.

Comment:

IV. Additional Comments and Concerns

This section contains comments concerning oral vs. parenteral administration, noting 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”.

The section also comments on differences in metabolism between humans and rodents. In humans, the comments note, oral administration of BPA results in essentially 100% metabolism of the parent to BPA glucuronide. 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. The comments state that there is a longer half-life and higher bioavailability in rodents compared to humans and conclude that, 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. Finally, this section discusses the variable response of mouse and rat strains to estrogen.

Response: As regards oral vs. parenteral administration, all of the studies cited by NTP-CERHR in connection with its "clear evidence" conclusion used oral administration. Thus, consideration of differences in metabolism resulting from differences in route of administration is not relevant to the listing stage of the Proposition 65 process since the route of exposure in the relevant animal studies is the same as the expected route of exposure in humans.

As regards species differences in metabolism, the relevance of these differences in metabolism to Proposition 65 listing is not clearly spelled out by the commenter. OEHHA reviewed the discussion of metabolism in the NTP-CERHR document and did not find any information that conflicted with the finding of adverse developmental effects at high doses. The comments do cite one study not reviewed by NTP-CERHR (Calafat et al. 2009). However, this report concerns BPA exposure of premature infants from medical devices, which is not relevant to Proposition 65 since post-natal exposures are not considered when assessing the toxicity of a chemical under Proposition 65's criteria.

Regarding differences in response to estrogen, the NTP-CERHR document notes that:

> "Bisphenol A is most commonly described as being "weakly" estrogenic; however, an emerging body of molecular and cellular studies indicate the potential for a number of additional biological activities. These range from interactions with cellular receptors that have unknown biological function to demonstrated effects on receptor signaling systems known to be involved in development." (NTP-CERHR, p. 1)
>
> "The NTP does not necessarily consider it appropriate to consider the reported biological effects of bisphenol A exclusively within the context of estrogen receptor α or β binding. An increasing number of molecular or cell-based ("*in vitro*") studies suggest that attributing the effects of bisphenol A solely to a classic estrogenic mechanism of action, or even as a selective estrogen receptor modulator (SERM), is overly simplistic." (NTP-CERHR, p. 10)

Thus, it is apparent that NTP considered the estrogenic activity of BPA in reaching its weight of evidence conclusion based on studies of several mouse and rat strains.

Comment:

V. Summary and conclusions:

The comments summarize four reasons why the commenter "strongly believes, based on scientific data, that embryo-fetal offspring toxicity from exposure to high doses of BPA is caused by maternal toxicity". The comments also note that

> "[t]he 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."

1. "…BPA is not a selective developmental toxicant."

Response: The term "selective developmental toxicant" is not specifically defined in the comments, nor is the term contained in Proposition 65 regulations, in the NTP-CERHR conclusions concerning BPA, or in the regulatory guidelines for performing and evaluating developmental toxicity studies. No "selective developmental toxicants" are named to provide examples. From the context of the comments it may be inferred that the intended meaning of the term "selective developmental toxicant" is a chemical that causes developmental toxicity in the absence of any discernible toxicity in the dam. As discussed above, developmental toxicity that co-occurs with maternal toxicity is generally regarded as developmental toxicity unless the maternal toxicity is excessive.

Comment:

2. "BPA is not a developmental neurotoxicant…"

Response: Developmental neurotoxicology studies were not among the studies cited by NTP-CERHR in connection with their conclusion concerning "clear evidence" for BPA developmental toxicity at "high "doses. Neither the Proposition 65 "Request for relevant information" nor the NTP-CERHR "clear evidence" conclusions mention developmental neurotoxicity. Thus OEHHA is unable to take this statement into account in evaluating the adequacy of the data which supports the identification of bisphenol A as a developmental toxicant by the authoritative body.

Comment:

3. "…BPA is not a selective reproductive toxicant."

Response: This point is not relevant to the Request for Information which is limited to the developmental toxicity of BPA.

Comment:

4. "Since intentional feed restriction 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 BPA studies."

Response: This comment suggests that reduced food intake was a prominent component of the maternal toxicity produced by BPA in the studies cited by NTP-CERHR in reaching their weight of evidence conclusion concerning developmental toxicity. OEHHA's review of the maternal toxicity data found minimal reports of effects on maternal food intake during gestation. Three of the studies (Morrissey et al. 1987; NTP 1985; Tinwell et al. 2002) cited by NTP-CERHR did not present, analyze or describe gestational food intake. One developmental toxicity study (Kim et al. 2001) reported lower food intake on gestation day (gd) 4 in terms of g/day, but no effects on food intake on gd 1, 11, 15 or 18. The three-generation rat study (Tyl et al. 2002b) reported no treatment related effects on food intake. The one-generation mouse studies (Tyl et al. 2002a) reported reduced food intake from gd 14 to 17 in terms of g/day but not g/kg/day(corrected for body weight), with no effects reported for gd 7-10, gd 10-24 or overall gestation (gd 0-17). A similar finding was reported in the second generation of the two-generation mouse study (Tyl et al. 2008b) with no food intake effects in the first generation. In no case was food intake reduced by 20% relative to controls as in the study cited in the comments (Waalkens-Berendsen et al. 1990).

The comments cite recent literature concerning the relationship between maternal food intake and developmental toxicity (Beyer et al. 2011; Chernoff et al. 1987; Chernoff et al. 2008). This literature is very clear in recommending that a pair-fed group is necessary to determine the possible role of reduced maternal food intake in causing developmental toxicity for any individual chemical. No such pair-fed control groups were included in the studies cited by NTP-CERHR to support their conclusion concerning developmental toxicity. Thus, there is no empirical basis for concluding that reduced maternal food intake had any influence on the developmental outcomes reported in these studies.

With regard to national and international regulatory test guidelines for developmental toxicity specifically requiring demonstrable maternal toxicity at the top dose, OEHHA agrees that this is the case. OEHHA also notes that it is generally recognized that "[i]n order to interpret the data fully, an integrated evaluation must be performed considering all maternal and developmental endpoints" (U.S. EPA, 1991). Although the commenter has offered her opinion of the relationship between maternal and developmental effects in the studies relied upon by NTP, she has provided no factual information to demonstrate either that NTP failed to consider maternal toxicity in concluding that there

is clear evidence that BPA causes developmental toxicity, or that NTP made factual errors in doing so.

References

Beyer, B. K., Chernoff, N., Danielsson, B. R., Davis-Bruno, K., Harrouk, W., Hood, R. D., Janer, G., Liminga, U. W., Kim, J. H., Rocca, M., Rogers, J. and Scialli, A. R. (2011). ILSI/HESI maternal toxicity workshop summary: maternal toxicity and its impact on study design and data interpretation. Birth Defects Res B Dev Reprod Toxicol 92(1): 36-51.

Calafat, A. M., Weuve, J., Ye, X., Jia, L. T., Hu, H., Ringer, S., Huttner, K. and Hauser, R. (2009). Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environ Health Perspect 117(4): 639-44.

Carney, E. (1997). Maternal physiological disruption. Drug Toxicity and Embryonic Development. K. RJ and D. GP. New York, Springer. **1:** 573-594.

Chernoff, N., Kavlock, R. J., Beyer, P. E. and Miller, D. (1987). The potential relationship of maternal toxicity, general stress, and fetal outcome. Teratog Carcinog Mutagen 7(3): 241-53.

Chernoff, N., Rogers, E. H., Gage, M. I. and Francis, B. M. (2008). The relationship of maternal and fetal toxicity in developmental toxicology bioassays with notes on the biological significance of the "no observed adverse effect level". Reprod Toxicol 25(2): 192-202.

Kim, J. C., Shin, H. C., Cha, S. W., Koh, W. S., Chung, M. K. and Han, S. S. (2001). Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy. Life Sciences 69(22): 2611-25.

Morrissey, R. E., George, J. D., Price, C. J., Tyl, R. W., Marr, M. C. and Kimmel, C. A. (1987). The developmental toxicity of bisphenol A in rats and mice. Fundam Appl Toxicol. **8:** 571-82.

National Toxicology Program – Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR, 2008). "NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A." NIH Publication No. 08 – 5994.

National Toxicology Program (NTP, 1985). Bisphenol A: reproduction and fertility assessment in CD-1 mice when administered in the feed. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC.

Stump, D. G., Beck, M. J., Radovsky, A., Garman, R. H., Freshwater, L. L., Sheets, L. P., Marty, M. S., Waechter, J. M., Jr., Dimond, S. S., Van Miller, J. P., Shiotsuka, R. N.,

Beyer, D., Chappelle, A. H. and Hentges, S.G (2010). Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. Toxicol Sci 115(1): 167-82.

Tinwell, H., Haseman, J., Lefevre, P. A., Wallis, N. and Ashby, J. (2002). Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicological Sciences 68(2): 339-48.

Tyl, R., Myers, C. and Marr, M. C. (2002a). Abbreviated One-Generation Study of Dietary Bisphenol A (BPA) in CD-2 (Swiss) Mice. Research Triangle Park, NC, Reproductive and Developmental Toxicology Laboratory, Center for Life Sciences and Toxicology, RTI International**:** 193.

Tyl, R. W., Myers, C. B., Marr, M. C., Sloan, C. S., Castillo, N. P., Veselica, M. M., Seely, J. C., Dimond, S. S., Van Miller, J. P., Shiotsuka, R. N., Beyer, D., Hentges, S. G. and Waechter, J. M., Jr. (2008b). Two-Generation Reproductive Toxicity Study of Dietary Bisphenol A (BPA) in CD-1 (Swiss) Mice. Toxicol Sci 2008b 104(2): 362-84.

Tyl, R. W., Myers, C. B., Marr, M. C., Thomas, B. F., Keimowitz, A. R., Brine, D. R., Veselica, M. M., Fail, P. A., Chang, T. Y., Seely, J. C., Joiner, R. L., Butala, J. H., Dimond, S. S., Cagen, S. Z., Shiotsuka, R. N., Stropp, G. D. and Waechter, J. M. (2002b). Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicological Sciences 68(1): 121-46.

U.S. Environmental Protection Agency (U.S. EPA, 1991). Guidelines for Developmental Toxicity Risk Assessment, U.S. Environmental Protection Agency, Risk Assessment Forum, Washington DC.

Waalkens-Berendsen, D. H., Koeter, H. B. and van Marwijk, M. W. (1990). Embryotoxicity/teratogenicity of isomalt in rats and rabbits. Food Chem Toxicol 28(1): 1-9.