American Chemistry Council

June 30, 2009

Cynthia Oshita
Office of Environmental Health Hazard Assessment
Proposition 65 Implementation
P.O. Box 4010
1001 I Street, 19th floor
Sacramento, California 95812-4010

Re: Comments on Hazard Identification Materials for Bisphenol A

Dear Ms. Oshita:

Please find attached written comments from the Polycarbonate/BPA Global Group on the Hazard Identification Materials for bisphenol A. The Polycarbonate/BPA Global group consists of the leading global manufacturers of bisphenol A and polycarbonate plastic, who for many years have supported and conducted scientific research to understand whether bisphenol A has the potential to cause health or environmental effects and to support scientifically sound public policy.

I also confirm that we will be present at the DART IC meeting on July 15 to present and discuss our comments.

Please do not hesitate to contact me if I can be of further assistance to clarify any of the information provided or if additional information is needed. I can be reached at (703) 741-5588 or by e-mail at steve_hentges@americanchemistry.com.

Regards,

Steven G. Hentges, Ph.D.
Executive Director
Polycarbonate/BPA Global Group

COMMENTS

OF THE

AMERICAN CHEMISTRY COUNCIL

POLYCARBONATE / BPA GLOBAL GROUP

IN OPPOSITION TO THE PROPOSED LISTING

OF

BISPHENOL-A

AS

"CLEARLY SHOWN TO CAUSE REPRODUCTIVE TOXICITY" UNDER PROPOSITION 65

F. Jay Murray, Ph.D., D.A.B.T.
MURRAY & ASSOCIATES

Arthur L. Lawyer, Ph.D.
TECHNOLOGY SCIENCES GROUP INC.

Christian Volz
Stanley W. Landfair
MCKENNA LONG & ALDRIDGE LLP

EXECUTIVE SUMMARY

When the Developmental and Reproductive Toxicant Identification Committee ("DART IC" or "Committee") convenes to consider Bisphenol-A ("BPA") for listing under Proposition 65,[1] it will not do so in a vacuum. It will not be possible to ignore the controversy that has developed among advocacy groups and the popular media concerning this chemical, or the many legislative initiatives to ban the use of products that contain BPA. Equally conspicuous, however, is the fact that at least six respected agencies of at least four different governments and international governing authorities independently and exhaustively have reviewed BPA for reproductive and developmental toxicity and have issued detailed assessments.

Agencies that have recently reviewed BPA for potential to cause reproductive and developmental effects include the:

- ***National Toxicology Program*** Center for the Evaluation of Risks to Human Reproduction ("NTP" or "CERHR") (an "authoritative body" for purposes of Proposition 65);
- ***U. S. Food and Drug Administration*** ("FDA") (an authoritative body);
- ***European Food Safety Authority*** ("EFSA");
- ***European Union Institute for Health and Consumer Protection*** *("EU")*;
- ***Japanese National Institute of Advanced Industrial Science and Technology*** ("NIAIST"); and
- ***Health Canada.***

None of these expert agencies has reached any regulatory conclusion that would support a decision by this Committee to list BPA as "clearly shown, through scientifically valid testing according to generally accepted principles to cause . . . reproductive toxicity," the standard established by law under Proposition 65.[2]

The conclusions of these agencies are not dispositive by themselves, because the function of each agency is to assess risks according to regulatory standards, rather than to identify hazards without concern for risk as Proposition 65 requires. Nevertheless, these unbiased agency analyses of the data are extremely relevant for two reasons. First, these agencies reviewed the ***same data*** that the DART IC is charged to review now. Second, risk assessment requires an assessment of hazard, and therefore the agency findings necessarily include detailed analyses of the human and animal data. Thus, the determinations of the NTP, for example, that exposure of pregnant women to BPA presents a ***"negligible concern"*** of fetal or neonatal mortality or birth defects, or that exposure to non-occupationally exposed adults presents a ***"negligible concern"*** of reproductive effects, or that exposure to the fetus presents in the worst case only ***"some concern"*** of developmental effects, would be extraordinarily inconsistent with a finding by this Committee that BPA is "clearly shown" to cause any of these effects, based on the same data.

---

[1] Proposition 65 is the popular name for California's Safe Drinking Water & Toxic Enforcement Act of 1986, Cal. Health & Safety Code § 25249.5 *et seq.*

[2] Cal. Health & Safety Code § 25249.8(b) (emphasis added).

To assist the Committee in its review of the data, we have summarized in Section I of our detailed comments the pertinent portions of five agency reviews. These assessments, singly or collectively, do not support a finding that BPA is "clearly shown to cause reproductive toxicity" for purposes of Proposition 65. Thus, as the Committee evaluates the data, we urge you to consider these agency findings, from which the following observations are clear:

1. ***There is an abundance of consistent, high quality data from multiple well-conducted conventional studies that support the conclusion that BPA is not a selective developmental or reproductive toxicant.*** BPA has been studied more extensively than most chemicals. In Sections IV and V of our detailed comments, we have summarized five comprehensive reproductive toxicity studies encompassing both low and high doses and three comprehensive developmental toxicity studies that the reviewing agencies all accepted, indeed praised. These include a three-generation reproductive study in rats (Tyl *et al.,* 2002b); a two-generation reproductive study in rats (Ema *et al.*, 2001); a two-generation reproductive study in mice (Tyl *et al.*, 2008b); a one-generation reproductive study in mice (Tyl *et al.,* 2002a); a continuous breeding reproductive study in mice by the NTP (Morrissey *et al.,* 1989); developmental studies in mice and rats by the NTP (Morissey *et al.*, 1987); and a developmental study in rats (Kim *et al.*, 2001).

Collectively, these studies consistently demonstrate that:

- BPA is not a selective developmental or reproductive toxicant in rats or mice;
- BPA does not cause treatment-related effects at low doses;
- Treatment-related effects were seen only at high doses that cause significant systemic toxicity in the parental animals, and these effects were attributed to systemic toxicity; and
- BPA does not produce the reproductive effects that were observed with the estrogen 17β-estradiol in multigeneration reproductive toxicity studies by the same investigators.

Regarding the quality of these studies, FDA audited the Tyl *et al.* multigeneration studies thoroughly in 2009, found them scientifically valid, and described them, along with Ema *et al.*, 2001, as "most useful in a safety assessment because of their size, comprehensive endpoint evaluation, rigorous attention to the certification of doses, and control of experimental conditions."[3] The EU Institute for Health and Consumer Protection referred to Tyl *et al.*, 2008b as the "gold standard" for measuring reproductive effects of BPA.

CONCLUSION: In the words of Proposition 65 and its implementing regulations, the data from these ***"scientifically valid"*** tests conducted according to ***"generally accepted principles"*** do not support listing BPA as a developmental or reproductive toxicant.[4]

---

[3] FDA, 2008 at p. 29.

[4] Cal. Health & Safety Code § 25249.8(b); Cal. Code Regs., *tit.* 22, § 12305(b)(1).

2. ***The unconventional, "low-dose" studies that suggest BPA may cause adverse reproductive or developmental effects have been characterized as inadequate or of limited utility for assessing risk by every regulatory agency that has reviewed them.*** NTP, FDA, EFSA, EU, NIAIST and Health Canada reviewed both the high quality studies addressed above and many unconventional, "low-dose" studies of BPA and judged the latter studies to be inadequate or of limited utility. No regulatory agency has relied on an NOAEL from any of these unconventional studies.

FDA and NTP, as well as other reviewers, regarded these "low-dose" studies as inadequate for assessing risk, for a variety of reasons, including:

- insufficient replication by independent investigators;
- unsuitability of various experimental approaches;
- undetermined relevance of the animal model in evaluating human risks; and
- incomplete understanding or agreement on the adverse nature of reported effects.

Many investigators did not attempt to replicate their studies; in some cases, independent investigators concluded that ***findings were not reproducible***. Many used ***unvalidated experimental protocols***. In many of these studies, BPA was administered by injection and other ***routes of exposure not relevant to human*** **exposure**. Additional experimental shortcomings include ***small group size***, administration of ***only one dose***, ***inappropriate control groups, inadequate reporting of experimental details***, and ***failure to consider the appropriate statistical unit*** (the litter rather than the pup).

The majority of the unconventional studies were "low-dose" studies that investigated the hypothesis that very low doses of BPA are capable of disrupting the development or functioning of the reproductive system. The weight of the evidence does not support the "low-dose" hypothesis, particularly in light of the robust "conventional" studies summarized above. As CERHR stated:

> ". . . . the ***failure of BPA to produce reproducible adverse effects*** via a ***relevant route of exposure,*** coupled with ***the lack of robustness*** of the many of the low dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the ***inability to reproduce many of these effects*** of *(sic)* any adverse effect strains the credibility of some of these study results. They need to be replicated using appropriate routes of exposures, adequate experimental designs and statistical analyses and linked to higher dose adverse effects if they are to elevate our concerns about the effects of BPA on human health."[5]

CONCLUSION: Returning to the words of Proposition 65 and its implementing regulations, the unconventional, "low-dose" studies are not ***"scientifically valid tests*** conducted according to ***generally accepted principles"*** for purposes of the statute. Even if they did satisfy that standard, they do not provide sufficient evidence to support a finding that BPA is "***clearly shown*** to cause developmental or reproductive toxicity" for purposes of Proposition 65.[6]

---

[5] CERHR, 2008, Chapin *et al.,* at p. 382 (emphasis added).

[6] Cal. Health & Safety Code § 25249.8(b); Cal. Code Regs., *tit.* 22, § 12305(b)(1) (emphasis added).

3. ***The metabolism and pharmacokinetic properties of BPA indicate that extrapolation of animal study results to humans is tenuous, and that reproductive or developmental effects from BPA are not biologically plausible in humans.*** The metabolism and pharmacokinetic properties of BPA have been well characterized in numerous studies in humans, non-human primates and rodents. Several key aspects of these properties have been applied consistently by regulatory agencies for assessing hazard and risk, and are relevant for the Committee for assessing hazard under Proposition 65.

In humans, "first pass" metabolism of BPA to non-estrogenic metabolites (primarily BPA-glucuronide) is essentially complete after oral exposure (the route of exposure relevant to humans), and the metabolites are rapidly excreted into urine with a half-life of 4-6 hours. Efficient metabolism has been demonstrated in adults and premature infants, indicating that infants and children also have the ability to deactivate and eliminate BPA after oral exposure.

In rodents, biliary excretion is the predominant elimination pathway, resulting in extensive enterohepatic recirculation, higher bioavailability and a significantly longer half-life in rodents than in humans. In addition, significant route-dependent differences in metabolism and pharmacokinetics have been demonstrated in rodent studies. Most notably, the efficient "first pass" metabolic pathway that is operative following oral exposure, which is most relevant to humans, is by-passed when exposure occurs via non-oral routes.

For human hazard assessment, these properties lead to several significant conclusions:

- The weak estrogenicity of BPA that is observed *in vitro* is not relevant *in vivo*, because BPA is converted to non-estrogenic metabolites after oral exposure, which limits the biological plausibility of reproductive or developmental effects in humans by an estrogenic mechanism.
- Due to significant inter-species differences between rodents and humans, which result in higher bioavailability and longer half-life in rodents, the extrapolation of any effects observed in rodents to humans is extremely conservative (*i.e.*, effects in rodents would over-predict potential responses in humans, because the doses that elicited those responses in rodents would not elicit the same responses in humans).
- Significant route-dependent differences indicate that rodent studies with non-oral routes of exposure are of limited relevance to humans.
- Due to the very short half-life of BPA in the human body, cross-sectional epidemiology studies that rely on measurement of BPA or its metabolites in single urine or blood spot samples cannot determine exposure levels in the past (or future) during the onset and development of a disease.

CONCLUSION: In the words of this Committee's Guidance Criteria, animal data alone will support listing only if there is "[s]ufficient evidence in experimental animals (mammals), such that extrapolation to humans is appropriate."[7] Extrapolation from effects observed in rodent studies on BPA to humans is tenuous at best because the pharmacokinetic data show significant differences between species and routes of exposure. These differences indicate that reproductive or developmental effects in humans from exposure to BPA are not biologically plausible.

---

[7] DART IC, 1993, Section 4.B, at p. 6.

4. ***The weak estrogenic activity of BPA is not an adequate basis for listing BPA in the absence of sufficient evidence of developmental and reproductive toxicity.*** Scientists have known for decades that BPA binds weakly to the estrogen receptor. A weak potential to bind to estrogen receptors is not enough to list a chemical under Proposition 65. Rather, the chemical must be clearly shown to cause developmental or reproductive toxicity.

The estrogenic activity of BPA has been assessed in many *in vitro* and *in vivo* assays. Although the results vary, the estrogenic activity of BPA appears to be four to five orders of magnitude less potent than the endogenous estrogen 17β-estradiol. Based on this difference in estrogenic activity alone, BPA would not be expected to cause developmental or reproductive toxicity by an estrogenic mechanism. And, as noted previously, there is no significant opportunity for BPA to exhibit an estrogenic effect in humans because orally ingested BPA is converted efficiently to non-estrogenic metabolites and excreted rapidly.

Many components of the human diet, such as phytoestrogens and lignans, have a similar or greater affinity than BPA to bind to estrogen receptors. Humans are exposed daily to significant levels of many estrogenic chemicals from foods such as nuts, cereals, and bread. For example, genistein, a phytoestrogen found in soy, has a much higher affinity than BPA for both estrogen receptors (ERα and ERβ). Any trace amounts of BPA that might be available *in vivo* must compete with endogenous estrogen and much higher levels of these dietary phytoestrogens, both of which have a higher affinity than BPA, for the ability to bind with estrogen receptors.

CONCLUSION: Turning again to the Committee's Guidance Criteria, the term "reproductive toxicity," even in its sub-categories of "developmental toxicity," "female reproductive toxicity" and "male reproductive toxicity," do not include estrogenic activity.[8] Thus, estrogenic activity is not a reproductive effect ***per se***, but rather a potential mechanism that might induce such an effect under certain circumstances. The many well-conducted conventional reproductive and developmental toxicity studies, and the pharmacokinetic data show that those circumstances are not present in the case of BPA. Therefore, the weak estrogenic activity of BPA is not a sufficient basis to support a conclusion that BPA is "clearly shown . . . to cause reproductive toxicity."

5. ***The weight of the evidence does not support a finding of developmental or reproductive toxicity for any of the endpoints of concern identified in the Hazard Identification Document.*** The OEHHA Hazard Identification Document (OEHHA, 2009 ("HID")) identifies effects in a variety of reproductive endpoints that reportedly were caused by exposure to low doses of BPA in one or more studies. Sections IV and V of our detailed comments summarize these data briefly by endpoint, and demonstrate why the data do not provide an adequate basis for listing BPA.

As a starting point, the weight of the scientific evidence in the most reliable studies consistently indicates that BPA is not a selective developmental or reproductive toxicant. Proceeding endpoint by endpoint, the "low-dose" studies suggest thought-provoking and intriguing hypotheses regarding potential developmental or reproductive effects, but are not suitable for proving hypotheses because of significant study design limitations and flaws. In

---

[8] *Id.* Section 2.C (defining "developmental toxicity"), Section 2.D (defining "female reproductive toxicity") and Section 2.E. (defining "male reproductive toxicity").

addition, their results conflict frequently with the consistent results from the robust high quality studies. Finally, the results of many of the low-dose studies are inconsistent among themselves, and most of these endpoints are not affected in a consistent or reliable manner.

CONCLUSION: In general, these studies do not represent "scientifically valid testing according to generally accepted principles," as required by Proposition 65. Regulatory agencies have consistently asserted that these studies need to be replicated using appropriate routes of exposure, adequate experimental designs and statistical analyses and linked to higher-dose adverse effects if they are to be considered more than suggestive evidence.

CONCLUSION

Taking into account the weight of the evidence, the statutory and regulatory criteria for listing, and the guidance articulated in the Committee's Guidance Criteria, BPA cannot be characterized as "clearly shown, through scientifically accepted testing conducted according to generally accepted principles, to cause" developmental or reproductive toxicity.

Applying those Proposition 65 standards to the data on BPA, it is undisputed that the human data do not support listing. Therefore, any finding that the chemical merits listing would have to rest on the animal data alone. The high-quality conventional animal studies, summarized in Sections IV and V of our detailed comments, demonstrate consistently and convincingly that BPA is not a selective developmental or reproductive toxicant in rodents.

The unconventional, "low-dose" studies, characterized as inadequate or of limited utility for risk assessment purposes by every regulatory agency that has reviewed them, do not provide a basis for listing because they provide no consistent or compelling evidence, for all of the reasons discussed in Sections IV.B.3 and V.B.3 of our detailed comments. Indeed, many of the unconventional studies do not represent "scientifically valid testing according to generally accepted principles," and cannot be relied upon here for that reason alone. In addition, strong pharmacokinetic data show that the effects observed in rodents are not directly applicable to humans because humans metabolize and eliminate BPA more rapidly and efficiently than rodents. As a result, developmental and reproductive effects are biologically implausible in humans.

COMMENTS IN OPPOSITION TO PROPOSED LISTING OF BPA

TABLE OF CONTENTS

Page

I. THE FINDINGS BY AGENCIES THAT HAVE EXAMINED BPA WOULD NOT SUPPORT A FINDING THAT BPA SHOULD BE LISTED 1

A. *NTP-CERHR Evaluation (2008)* 1

B. *European Food Safety Authority Evaluations (EFSA, 2006; 2008)* 2

C. *FDA Draft Assessment (2008)* 4

D. *European Union Risk Assessment Report (EU RAR 2008)* 6

E. *Health Canada (2008)* 8

II. PROPOSITION 65 AUTHORIZES LISTING ONLY IF BPA IS "CLEARLY SHOWN" TO CAUSE REPRODUCTIVE TOXICITY 9

III. PHARMACOKINETIC DATA SHOW THAT REPRODUCTIVE OR DEVELOPMENTAL EFFECTS IN HUMANS ARE NOT BIOLOGICALLY PLAUSIBLE AND THAT DIRECT EXTRAPOLATION OF RODENT DATA TO HUMANS IS NOT APPROPRIATE 11

A. *Humans Metabolize and Eliminate BPA Efficiently* 11

B. *BPA Metabolites Are Inactive and Non-Estrogenic* 11

C. *BPA Does Not Accumulate in the Human Body and Has Low Bioavailability* 12

D. *Metabolism and Elimination of BPA Are More Efficient in Humans Than in Rodents* 12

E. *Neonates Metabolize BPA Efficiently* 12

F. *Non-Oral Routes of Exposure Are Irrelevant to Humans* 12

IV. THE DATA DO NOT SUPPORT A FINDING THAT BPA HAS BEEN "CLEARLY SHOWN" TO CAUSE DEVELOPMENTAL TOXICITY 12

A. *The Human Data Do Not Show an Association Between BPA and Developmental Toxicity* 13

B. *The Animal Data Do Not Demonstrate That BPA Causes Teratogenicity or Selective Developmental Toxicity* 14

V. THE DATA DO NOT SUPPORT A FINDING THAT BPA HAS BEEN "CLEARLY SHOWN" TO CAUSE MALE OR FEMALE REPRODUCTIVE TOXICITY 19

A. *The Human Data Do Not Demonstrate That BPA Causes Reproductive Toxicity* 19

B. *The Animal Data Do Not Demonstrate That BPA Is a Selective Reproductive Toxicant* 21

VI. CONCLUSION 32

I. THE FINDINGS BY AGENCIES THAT HAVE EXAMINED BPA WOULD NOT SUPPORT A FINDING THAT BPA SHOULD BE LISTED

A. *NTP-CERHR Evaluation (2008)*

The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction ("CERHR") recently completed an exhaustive review of the human and animal studies on BPA that are relevant to reproduction and development. In September 2008, CERHR issued a monograph summarizing its findings ("CERHR Monograph"), which consists of: (1) the NTP Brief and (2) the NTP-CERHR Expert Panel Report, which was also published in the scientific literature (Chapin *et al.,* 2008). While the CERHR Monograph evaluates both hazard and exposure data, the majority of this document is focused on hazard identification.

CERHR Conclusions Relevant to Proposition 65

CERHR has not formally identified BPA as causing developmental or reproductive toxicity.[9] To the contrary, CERHR reached a number of reassuring conclusions. Regarding human studies, NTP concluded that "evidence from the limited number of studies in humans exposed to bisphenol A is not sufficient to reach conclusions regarding possible developmental or reproductive hazard."[10] Regarding animal studies, CERHR extensively discussed both the relatively standard reproductive toxicity and developmental toxicity studies using oral administration, and the widely variant and numerous "low-dose" (defined by CERHR as < 5 mg/kg bw/day) studies that commonly used parenteral routes of administration. CERHR observed that: "[reproductive effects in rats and mice] are observed at dose levels that are more than 3,500-times higher than 'worst case' daily intakes of bisphenol A in infants and children less than 6 years of age . . . . The differences in exposure are much greater, more than 160,000-times different, when the high oral dose level is compared to estimated daily intakes for children ages 6-11 and adult women . . . ."[11]

"Low-Dose" Studies

With respect to the low-dose studies, CERHR stated: "the failure of BPA to produce reproducible adverse effects via a relevant route of exposure, coupled with the lack of robustness of the many of the low-dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the inability to reproduce many of these effects of *(sic)* any adverse effect strains the credibility of some of these study results."[12] CERHR concluded that many of these studies are "inadequate," which means it is highly unlikely that they would satisfy

---

[9] The NTP Brief within CERHR, 2008 designated "clear evidence of adverse effects" for "'high' dose developmental toxicity" based on reduced survival in fetuses or newborns, reduced fetal or birth weight or growth of offspring early in life, and delayed puberty in female rats. CERHR, 2008, NTP Brief at pp. 6-8. However, this does not mean that BPA meets the Proposition 65 listing standard. First, and most importantly, all of these effects were seen only at high doses associated with systemic toxicity. Second, many of these effects were observed in studies with both prenatal and postnatal exposure; under Proposition 65, developmental toxicity is limited to effects caused by prenatal exposure only.

[10] CERHR, 2008, NTP Brief at p. 9.

[11] *Id.* at p. 36.

[12] CERHR, 2008, Chapin *et al.*, at p. 382

Proposition 65's requirements for "scientifically valid testing according to generally accepted principles."

CERHR Levels of Concern

CERHR normally classifies its level of concern into five categories: serious concern, concern, some concern, minimal concern and negligible concern. The NTP summarized its conclusions regarding the "possible effects of exposure to bisphenol A" as follows:

- The NTP has ***negligible concern*** that exposure of pregnant women to bisphenol A will result in fetal or neonatal mortality, birth defects, or reduced birth weight and growth in their offspring.
- The NTP has ***negligible concern*** that exposure to bisphenol A will cause reproductive effects in non-occupationally exposed adults and ***minimal concern*** for workers exposed to higher levels in occupational settings.[13]
- The NTP has ***minimal concern*** for effects on the mammary gland and an earlier age for puberty for females in fetuses, infants, and children at current human exposures to bisphenol A.
- The NTP has ***some concern*** for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to bisphenol A.

The highest level of concern for BPA for any endpoint was "some concern," and this does not equate to a finding that BPA causes reproductive toxicity under the "clearly shown" standard that applies under Proposition 65. For example, while CERHR expressed "some concern" for effects on the brain and behavior, CERHR said that "additional research is needed" and "the current literature cannot yet be fully interpreted for biological or experimental consistency or for relevance to human health."[14]

B. *European Food Safety Authority Evaluations (EFSA, 2006; 2008)*

The European Food Safety Authority Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food ("EFSA") evaluated the developmental and reproductive toxicity of BPA in 2006 and updated its evaluation in 2008. In general, the findings and conclusions in the EFSA evaluations are consistent with those of the CERHR assessment.

High-Quality Data

Like CERHR, EFSA chose to use the results of two multigeneration reproductive toxicity studies, both of which examined doses ranging from very low to very high, to derive a Tolerable Daily Intake ("TDI"). After reviewing the available data, EFSA concluded that the TDI should be based on the NOAELs for systemic toxicity (not reproductive toxicity) derived from the three-generation rat reproductive study (Tyl *et al.,* 2002b) and the two-generation mouse reproductive study (Tyl *et al.,* 2008b). In both studies, the NOAEL for systemic toxicity was 10-fold below the NOAEL for other toxic effects.

---

[13] *Id.* at p. (vii).

[14] *Id.* at p. 20.

"Low-Dose" Studies

EFSA "considered that low-dose effects of BPA in rodents have not been demonstrated in a robust and reproducible way, such that they could be used as pivotal studies for risk assessment." Due to "species differences in toxicokinetics, whereby BPA . . . is less bioavailable in humans than in rodents," and "the high sensitivity of the mouse to oestrogens," ***the Panel found "considerable doubts about the relevance of any low-dose observations in rodents for humans."***[15]

Pharmacokinetics

The Panel observed that the "toxicokinetics of BPA in adult humans and in animals are well characterized. (EFSA, 2006; Willhite, *et al.*, 2008). In humans, orally administered BPA is well absorbed and undergoes complete first-pass metabolism in the liver to BPA-glucuronide as major metabolite, which is rapidly excreted in the urine, with a half-life of less than 6 hours (Völkel *et al.*, 2002, 2008). . . . Because this first-pass metabolism is so effective, there is extremely low systemic availability of free BPA in humans after oral exposure. BPA-glucuronide and the minor urinary metabolite BPA-sulphate do not interfere with hormonal regulation of reproduction (Snyder *et al.*, 2000; Shimizu *et al.*, 2002; Willhite *et al.*, 2008). Therefore, these conjugation reactions represent detoxication pathways. In rats, BPA is also predominantly glucuronidated, with sulphation representing a minor pathway (Pottenger *et al.*, 2000), but the BPA-glucuronide formed is excreted from the liver via bile into the gastrointestinal tract, cleaved back to BPA and reabsorbed into the blood. Thus it undergoes enterohepatic recirculation resulting in slower elimination of BPA including its conjugate in rodents compared with humans (EFSA, 2006) . . . . The enterohepatic cycling and decreased first pass metabolism of BPA in rats results in higher plasma levels of unconjugated BPA in rats compared to humans given the same dose." These differences reflect the known species difference in molecular mass threshold for biliary elimination in rats and humans. (Hirom *et al.*, 1976; Walton *et al.*, 2001; Ghibellini *et al.*, 2006).[16]

In 2008, EFSA updated its evaluation of BPA in response to a request by the European Commission, based on new information. According to EFSA, sulphation of BPA is a major metabolic pathway in fetuses and infants, and "sulphation of BPA in fetuses and neonates is also expected to efficiently detoxify BPA."[17] EFSA concluded that "there is sufficient capacity in the neonate to conjugate BPA at doses below 1 mg/kg/day . . . . In addition, the Panel notes that because of the metabolic differences described, exposure to free BPA in adult, fetal and neonatal rats will be greater than in humans and that rats would therefore be more susceptible to BPA-induced toxic effects than humans on a equivalent dose basis."[18]

---

15 EFSA, 2006 at p. 5 (emphasis added).

16 *Id,* at pp. 4-10.

17 *Id.* at pp. 5 – 10, 6 – 10.

18 *Id.* at pp. 6-10.

C. *FDA Draft Assessment (2008)*

In early 2008, the FDA formed a Task Force to evaluate the safety of all FDA-regulated products that contain BPA, focusing on the endpoints of carcinogenesis and reproductive and developmental toxicity. The Agency issued a Draft Assessment of Bisphenol A for use in Food Contact Applications on August 14, 2008.[19] The FDA Task Force concentrated on endpoints of concern identified in the NTP-CERHR evaluation and determined that the data on endpoints such as the prostate gland and developmental neural and behavioral toxicity "were insufficient to provide a basis to alter the NOAEL used to calculate the margins of safety." Similar to EFSA, FDA used the systemic toxicity NOAEL from two multigeneration reproductive toxicity studies in rats and mice (Tyl *et al.*, 2002b; Tyl *et al.,* 2008b) as the basis to conclude "that an adequate margin of safety exists for BPA at current levels of exposure from food contact uses."[20] Subsequent to the release of the draft assessment, FDA staff conducted an extensive on-site audit of the two multigeneration studies to confirm their scientific validity. Portions of FDA's draft assessment that are pertinent to the Committee's review are summarized below.

Pharmacokinetics

The FDA Task Force noted that the absorption, disposition, metabolism and elimination of BPA have been evaluated thoroughly in a number of studies, and that the primary metabolite of BPA, BPA-glucuronide ("BPAG"), has no significant estrogenic activity in either *in vitro* or *in vivo* test systems. Thus, to evaluate the safety of products that contain BPA, the Task Force reviewed the cross-species pharmacokinetic properties of BPA, considering *in vivo* data generated in 12 studies in rats, 3 studies in mice, 4 studies in monkeys and 3 studies in humans, and observed that "all indicate rapid intestinal absorption, and very rapid conjugation of BPA with UDP-glucuronic acid, forming BPAG. BPAG formation is followed by a slower process of its elimination. Human (and monkey) BPAG elimination is relatively rapid (t1/2 = 3-4 hr) and primarily via urine. Elimination of BPAG in rodents is complicated by the fact that it is routed primarily into bile rather than urine (as in primates), and consequently enters the intestines, where bacterial glucuronidases hydrolyze BPAG to re-form free BPA, allowing BPA to be reabsorbed and re-circulated. The extent of re-absorption of BPA from rat intestines may be less than 50%, but is sufficiently large to make kinetic measurements in the rat confusing and emphasizes the fact that additional free, estrogenically-active BPA may be available in this animal model as compared to humans."[21]

Acceleration of Puberty in Female Rodents

The Task Force reviewed the two multi-generational studies by Tyl *et al.* (2002b, 2008b discussed above) and Ema *et al.* (2001).

> "All three studies were conducted under GLP conditions and examined only the day of vaginal opening as the endpoint for determination of the onset of puberty

---

[19] FDA, 2008. It is important to recognize that this is a draft, and "it does not represent and should not be construed to represent any agency determination or policy."

[20] *Id.* at p. 2.

[21] *Id.* at p. 17.

in the female. Tyl *et al.* (2008b) used mice; the other studies used rats. The study by Ema *et al.* (2001) reported no effects on the day of vaginal opening at oral doses up to 200 μg/kg bw/day. Although the authors did not identify a NOAEL, it appears that 200 μg/kg bw/day would be a NOAEL for the timing of female puberty; this was the maximum dose used in this study. Tyl *et al.* (2002b) reported a delay in vaginal opening at 7500 ppm; this appeared to be due to a decrease in body weight. Tyl *et al.* (2008b) used CD-1 mice and reported no effect on the day of vaginal opening at any dose, including the maximum dose of 3500 ppm; however, as indicated in Table 9 of the published study absolute day of acquisition was not statistically significant at 3500 ppm. Day of acquisition was statistically significantly accelerated when adjusted by body weight on PND 21 for F1 (only animals measured). Again, no findings were reported at the lower doses. Due to the very thorough nature of these studies, FDA has a high level of confidence in their results."[22], [23]

Altered Prostate and Urinary Tract Development in Males

The Task Force concluded that the two Tyl studies (Tyl *et al.*, 2002b; Tyl *et al.*, 2008b) "show no evidence of selective reproductive toxicity or effects on male development or prostate at doses at or below 750 ppm (approximate intake of 50 mg/kg bw/day) in the rat or 300 ppm (approximate intake of 50 mg/kg bw/day) in the mouse" and that "the NOAEL for reproductive and offspring toxicity was 50 mg/kg bw/day." Moreover, the study by Ema (Ema *et al.*, 2001) "likewise found no effect on prostate weight or histology at doses up to 200 μg/kg bw/day." FDA observed that "these studies clearly contain datasets that are most useful in a safety assessment because of their size, comprehensive endpoint evaluation, rigorous attention to the certification of doses, and control of experimental conditions. The study of Tyl *et al.* (2008b) is particularly important because it utilizes a strain of mouse that has been reported by others to be sensitive to BPA under different treatment conditions. These studies indicate that perinatal BPA exposure does not adversely affect prostate weight or histology at doses of 0.2 - 50 mg/kg bw/day."[24]

The Task Force again acknowledged "conflicting results" in other studies, including "smaller studies . . . us[ing] non traditional dose routes and endpoints." The Task Force discounted these results because they were "not repeatable" because the use of a low dose of "DES as a positive control . . . is questionable," and concluded that "the relevance of these findings to safety assessment is unresolved."[25]

---

22 *Id.* at p. 26.

23 The Task Force also reviewed other studies determining puberty by the first estrus, but concluded them to be "of questionable significance." *Id.* at p. 27.

24 *Id.* at p. 28.

25 *Id.* at p. 29.

Developmental Neurotoxicity

Finally, the Task Force addressed "recently released assessments," noting "concerns for developmental exposure and neural and behavioral effects of BPA." FDA thus performed its own "updated review for neurotoxicity as a whole."[26]

In the Agency's view, the most appropriate route of exposure is oral. Several studies reviewed by the Task Force dosed the test animals by a variety of unconventional routes (including subcutaneous and intraperitoneal injection). Unconventional exposure regimens were used in which test animals were given BPA "for various intervals during gestation, lactation and/or after weaning." According to the Task Force, the "varied treatment-related findings in a majority of the reviewed studies collectively appear to suggest that developmental BPA exposure in rodents may have the potential to alter brain development and behavior. However, in view of the limitations in study design and study conditions that confound the interpretability of the study findings, without appropriate confirmation of these findings using well-designed experimental protocols and/or clarification of their biological significance, the utility and relevance of the study findings to an assessment of the safety of BPA from food contact uses is unknown. Until these disparate experimental findings are examined in well-designed safety assessment studies using appropriate biomarkers of effect, FDA has concluded that the reviewed studies are inadequate for use in supporting a safety assessment determination or regulatory decision for BPA."[27]

D. *European Union Risk Assessment Report (EU RAR 2008)*

The European Union's Institute for Health and Consumer Protection updated its Risk Assessment Report (RAR) in 2008 to consider the new two-generation mouse study (Tyl *et al.,* 2008b), and other new data that had been developed since 2003 in the fields of human exposure and pharmacokinetics, among others (EU RAR, 2008). This review resulted in the EU's "Updated Risk Assessment of BPA," which was completed in April 2008 and is awaiting publication, and is referred to herein as the "EU RAR 2008."[28]

Reproductive Toxicity

Regarding reproductive toxicity, the EU RAR 2008 states: "The effects of BPA on fertility have been investigated in three good quality studies: two generation and multigeneration studies in the rat, and a continuous breeding study in the mouse. . . . [N]o effect on fertility was seen in the rat two-generation study [although at low doses]. In the multigeneration study, an effect on fertility . . . was seen in all three generations at the top dose of 500 mg/kg. Although this effect was seen only at a dose level causing parental toxicity . . . it is not clear whether or not the finding could be a secondary consequence of parental toxicity, or a direct effect of BPA. . . . ***No effects on fertility were seen at 50 mg/kg***."[29]

---

26 *Id.*

27 *Id.* at p. 30.

28 EU RAR, 2008 at p. 1.

29 *Id.* at p. 84 (emphasis added).

Developmental Toxicity

Regarding developmental toxicity, the report concludes that "in standard development studies in rodents, ***there is no convincing evidence that BPA is a developmental toxicant.***" The authors acknowledged some disagreement regarding "apparently conflicting data from studies conducted using low doses . . . based on differing views about the uncertainties surrounding the reproducibility of the findings and their biological significance, if any, to human health."[30]

"Low-Dose" Studies

In addition to Tyl *et al.* (2008b), the report also considered "approximately 40-50" studies "investigat[ing] the same standard reproductive and developmental endpoints as those examined by Tyl *et al.* (2008b[31]) . . . . performed on a range of animal species and strains, at different life stages, over a wide array of doses, using a variety of exposure routes, for varying exposure durations, and . . . investigat[ing] a large assortment of endpoints." The authors reported that the "majority of these studies have reported only small changes (unrelated to dose) in organ weight, tissue architecture, receptor expression or hormone levels of unknown pathophysiological consequences. Some have found no effect, but, overall, no consistent, reproductive, adverse effects have been observed. Furthermore, the results from these studies have been in contrast to the results of investigations conducted according to internationally recognized guidelines and in compliance with GLP, including the recent 2-generation study in the mouse by Tyl *et al* (2008b)," which the report then referred to as the "gold standard, definitive study of the reproductive toxicity of BPA . . . ." Therefore, the EU RAR (2008) does not evaluate those studies in detail.[32]

No Observable Adverse Effects Level

Upon referral of this issue to the Competent Authorities after the completion of the original risk assessment in 2003, the EU Member States requested the two-generation mouse study (Tyl *et al.,* 2008b) to address uncertainties regarding potential reproductive and developmental toxicity. Based on the data available at the time, "it ***was agreed that a provisional NOAEL of 50 mg/kg/day for developmental effects***, derived from the rat-multigenerational study, ***should be used in the risk characterization in the interim, whilst awaiting the outcome of further testing***, with the aim of identifying those scenarios which are clearly of concern irrespective of the outcome of the further testing."[33]

Thus, the NOAEL was set for provisional purposes for risk assessment, and was not intended as a statement that BPA actually does cause reproductively toxic effects by any pre-determined standard. The requested two-generation mouse study conducted by Tyl (Tyl *et al.*, 2008b) effectively disposed of any concerns for reproductive effects at the so-called "low doses."

---

30 *Id.* at pp. 85-86 (emphasis added).

31 Note that EU RAR (2008) refers to the two-generation reproductive toxicity study on mice, published and referred to in the HID and our Comments as "Tyl *et al.,* 2008b" as "Tyl *et al.,* 2007", which refers to the unpublished report that preceeded the published version. They are equivalent references.

32 *Id.* at p. 86.

33 *Id.* at p. 86 (emphasis added).

Thus having ruled out any low-dose concerns, the EU left in place the NOAEL of 50 mg/kg/day, to be used for risk assessment purposes.

E. *Health Canada (2008)*

In October 2008, Health Canada issued a "final screening assessment report" on the potential for BPA to cause harm to human health. (Health Canada, 2008). The report surveyed the same animal data reviewed in the NTP, FDA and EFSA reports discussed above, and in fact extensively referred to those reports. Health Canada concluded that the collective weight of non-traditional, low-dose animal studies suggested a potential for neurobehavioral effects on humans. The agency's summary of its "Characterization of Risk to Human Health," however, makes it quite clear that it did not find that the animal data "clearly show" developmental effects.

Weight of Evidence

Similar to other agencies that have reviewed the evidence, Health Canada chose the multigeneration studies in rats and mice as the basis for their overall conclusions: "The NOAELs from the multigeneration reproductive toxicity studies in Sprague- Dawley rats and CD-1 mice of 5 mg/kg-bw per day for systemic effects (reduced body weight gain in rats and minimal to mild hepatocyte hypertrophy in adult male and female mice) and 50 mg/kg-bw per day for reproductive and developmental toxicity (Tyl *et al.*, 2002b; 2008b) are considered an appropriate departure point for characterizing risk to human health from exposure to bisphenol A."[34]

Health Canada's views on low-dose studies, in particular neurodevelopmental and behavior studies, also are similar to the views of other agencies.

> "While collectively these studies provide evidence that exposure to bisphenol-A during gestation and early postnatal life ***may be*** affecting neural development and some aspects of behaviour in rodents, ***the overall weight of evidence was*** considered limited from the perspective of rigour (*e.g.*, study design limitations such as conduct of behavioral assessments as a single time point); power (*e.g.*, limited number of animals per test group), corroboration/consistency (limited consistency of studies) and biological plausibility (*e.g.*, certain studies involve use of a single dose, lack of dose response relationship). These limitations make it difficult to determine actual ***significance of findings to human health risk assessment."***
>
> "The neurodevelopmental and behavioral dataset in rodents, ***though highly uncertain***, is ***suggestive*** of ***potential effects*** at doses at the same order of magnitude to 1-2 orders of magnitude higher than exposures. Given that toxicokinetics and metabolism data indicate potential sensitivity to the pregnant woman/fetus and infant, and that animal studies ***suggest a trend*** towards heightened susceptibility during stages of development in rodents, it is ***considered appropriate to apply a precautionary approach*** when characterizing risk. As such, it is concluded that bisphenol A be considered as a substance that may be

[34] Health Canada, 2008 at p. 72.

entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health."[35]

In essence, then, Health Canada's screening assessment reached conclusions very consistent with those in the assessments conducted by the other regulatory agencies discussed above. The subsequent policy actions of the Canadian government were based upon the application of the precautionary principle, which is required under Canadian law.

II. PROPOSITION 65 AUTHORIZES LISTING ONLY IF BPA IS "CLEARLY SHOWN" TO CAUSE REPRODUCTIVE TOXICITY

The standard that this Committee must apply in determining whether to list a chemical as a reproductive toxicant under Proposition 65 is stringent. The statute provides that:

> "A chemical is known to the state to cause cancer or reproductive toxicity . . . if in the opinion of the state's qualified experts it has been ***clearly shown*** through ***scientifically valid testing*** according to ***generally accepted principles*** to cause cancer or reproductive toxicity . . . ."

Health & Safety Code § 25249.8(b) (emphasis added). The question before this Committee is restricted to reproductive toxicity, of course. Nevertheless, it is this Committee's task to determine whether this stringent statutory standard has been met in the case of BPA.

Specifically, it is the Committee's duty to:

> "Render an opinion . . . as to whether specific chemicals have been ***clearly shown, through scientifically valid testing according to generally accepted principles,*** to cause reproductive toxicity."

Cal. Code Regs., tit. 27, § 25305(b)(1) (emphasis added).

Although this statutory standard is clearly stringent, it is not very specific, so this Committee has developed Guidance Criteria (DART IC, 1993) that provide both general principles and specific factors for the Committee to weigh in assessing whether the scientific data on a particular chemical satisfy the "clearly shown" standard. The general principles include the following:

> "In evaluating the sufficiency of data, a ***weight of evidence approach*** shall be used to evaluate the body of information available for a given chemical."

Guidance Criteria at 1.D. (emphasis added).

> "In determining whether a chemical is to be . . . listed as known . . . to cause reproductive toxicity, the ***biological plausibility*** of the association between the adverse reproductive effects observed and the chemical in question should be considered. Confidence is increased when . . . a sound scientific basis exists for the observed adverse effects and the known characteristics of the particular

---

[35] *Id.* at p. 73 (emphasis added).

chemical. Conversely, confidence is decreased if the observed adverse effects are contradictory to the known characteristics of the particular chemical."

Guidance Criteria at 4.B. (emphasis added).

"Developmental, and female and male reproductive effects ***shall meet at least one of the following criteria*** for recommendation as known to the State to cause reproductive toxicity.

(a) ***Sufficient evidence*** in humans. . . .

(b) Limited evidence or suggestive evidence in humans, supported by sufficient experimental animal (mammalian) data . . . .

(c) ***Sufficient evidence*** in experimental animals (mammals), such that extrapolation to humans is appropriate."

Guidance Criteria at 3.A.-C. (emphasis added).

The Guidance Criteria identify specific factors for the Committee to consider in evaluating whether the available data on a particular compound qualify as "sufficient evidence in humans" or "sufficient evidence in experimental animals."

"Sufficient evidence in humans," in the case of epidemiology studies, means studies that provide:

"***convincing*** evidence to support ***a causal relationship*** between exposure to the chemical and the . . . effect in question. This requires ***accurate exposure and toxicity endpoint classification*** and ***proper control of confounding factors***, bias, and effect modifiers."

Guidance Criteria at 3.A.(1) (emphasis added).

As discussed below, there is no epidemiological evidence that would support listing BPA. Therefore, ***any decision to list BPA as a reproductive toxicant would need to be supported on the basis of "sufficient evidence in experimental animals" alone***. Whether animal studies are "sufficient evidence" to support extrapolation to humans, in most cases, is based on the following:

"(1) The ***experimental design,*** including overall protocol and numbers of animals and ***presence of appropriate controls***.

(2) The exposure, in terms of ***route of administration***, is ***relevant to expected human exposures*** . . . .

(3) Number of dose levels, so that the presence of a ***dose-response relationship*** can be evaluated . . . .

(4) ***Consideration of maternal and systemic toxicity***."

Guidance Criteria at 3.C.(1)-(4) (emphasis added).

Finally, with respect to developmental toxicity in particular, Proposition 65 regulates developmental effects caused only by pre-natal exposures, and not by post-natal exposures. (OEHHA General Counsel William Soo Hoo, 1996.)

III. Pharmacokinetic Data Show That Reproductive Or Developmental Effects In Humans Are Not Biologically Plausible And That Direct Extrapolation Of Rodent Data To Humans Is Not Appropriate

A clear understanding of the pharmacokinetics of BPA is critical to the determination of whether exposure to humans could result in reproductive or developmental effects. The pharmacokinetics and metabolism of BPA have been very well characterized in rodents, humans and non-human primates. Differences between species and routes of exposure in the pharmacokinetics and metabolism of BPA indicate that extrapolation of animal study results to humans is tenuous and that reproductive or developmental effects in humans are not biologically plausible.

The pharmacokinetic data illustrate that humans efficiently metabolize BPA after oral exposure, which is most relevant to humans, via "first pass" conjugation into non-estrogenic metabolites that are eliminated from the body rapidly and completely through urinary excretion. Accordingly, serum concentrations of parent BPA in controlled human studies are non-detectable. Comparative data show that hepatic conjugation is more efficient in humans than in rodents. In addition, biliary excretion is the primary route of elimination in rodents, leading to enterohepatic recirculation after hydrolysis of conjugated BPA metabolites in the intestines and re-absorption of parent BPA. As a result, parent BPA can be detected transiently in the blood of rodents following oral dosing. Finally, non-oral routes of exposure result in dramatically different pharmacokinetics compared to oral exposure, the consequences of which include a significantly longer half-life, higher circulating concentrations of systemically available parent BPA, and a different spectrum of metabolites. Because of these differences, the results of rodent toxicity studies using non-oral routes of exposure are irrelevant to human health hazard identification.

A. *Humans Metabolize and Eliminate BPA Efficiently*

Following oral exposure in humans, BPA is biotransformed by the intestine walls and the liver to BPA-glucuronide (Völkel *et al.*, 2002). This extensive "first-pass" metabolism virtually precludes parent BPA from entering the systemic circulation. Metabolism at both sites takes place before BPA in any form can enter into circulation in the body. This is a classic example of presystemic elimination or the "first pass effect" that limits systemic exposure and minimizes toxic potential (Lehman-McKeeman, 2008). In essence, the human body has two layers of protection in the metabolic capacity of the intestine and the liver to prevent any toxicologically significant amount of parent BPA from entering the systemic circulation.

B. *BPA Metabolites Are Inactive and Non-Estrogenic*

The primary metabolite of BPA, BPA-glucuronide ("BPAG"), has been shown to exhibit no estrogenic activity (Mathews *et al.*, 2001). A secondary metabolite, BPA-sulfate, may be formed at lower levels and also does not have estrogenic activity (Shimizu *et al.*, 2002). These

studies indicate that BPA is not likely to cause estrogenic effects in humans, because the metabolites that actually enter the body following oral exposure have no known biological activity and, in particular, are not estrogenic.

C. *BPA Does Not Accumulate in the Human Body and Has Low Bioavailability*

BPA has very low bioavailability in humans since very little, if any, parent BPA actually enters circulation in the body (due to efficient first-pass metabolism). In addition, the rapid elimination of BPA metabolites (the half-life for elimination of BPA is approximately 4-6 hours) indicates that BPA does not bioaccumulate in the human body (Völkel *et al.*, 2005, 2002).

D. *Metabolism and Elimination of BPA Are More Efficient in Humans Than in Rodents*

By excreting the conjugated BPA metabolites into urine, humans eliminate BPA more rapidly than rodents, which predominately rely on biliary excretion (Kurebayashi *et al.*, 2003; Sakamoto *et al.*, 2002). In rodents, BPA undergoes enterohepatic recirculation where the BPA metabolites are hydrolyzed in the intestines back to parent BPA, which can then be absorbed, metabolized and excreted multiple times before eventual elimination in feces. Enterohepatic recirculation results in a longer half-life and higher bioavailability in rodents compared to humans. In addition, humans have been shown to have significantly greater hepatic capacity than rodents to metabolize BPA (Pritchett *et al.*, 2002).

E. *Neonates Metabolize BPA Efficiently*

Metabolism of BPA to non-estrogenic metabolites occurs in rodent and human neonates as well as adults (Calafat *et al.*, 2009; Domoradzki *et al.*, 2003). The data indicate that infants and children also are capable of efficiently metabolizing and eliminating BPA.

F. *Non-Oral Routes of Exposure Are Irrelevant to Humans*

Experimental systems that utilize non-oral routes of exposure (*i.e.,* parenteral routes) have a significant difference in the bioavailability of BPA than systems that utilize oral routes. Non-oral routes of exposure by-pass the rapid and complete first-pass presystemic conjugation that BPA undergoes following oral exposures. As a result, systemic concentrations of parent BPA are much higher and the identity and distribution of metabolites are different (Zalko *et al.*, 2003). Because oral exposure is the relevant route of exposure for humans, studies of toxicity or estrogenic potency involving non-oral routes of exposure in laboratory animals are of limited relevance for assessing the hazards of BPA in humans.

IV. THE DATA DO NOT SUPPORT A FINDING THAT BPA HAS BEEN "CLEARLY SHOWN" TO CAUSE DEVELOPMENTAL TOXICITY

The many studies that have evaluated the potential of BPA to cause developmentally toxic effects are discussed below. For the most part, these are the same studies reviewed so thoroughly by the NTP, the FDA, EFSA, the EU Institute and Health Canada. These data do not support a finding that BPA is "clearly shown" to cause developmental toxicity for purposes of Proposition 65.

The HID identified three developmentally toxic endpoints of concern:

- Offspring viability
- Sex-differentiation of exploratory and affective behavior
- Immune hyper-responsiveness

The first endpoint, offspring viability, has been evaluated in multiple robust, well-conducted studies of both developmental and reproductive toxicity. The HID states: "In general these studies suggest that fetal viability is sensitive to BPA at doses >500 mg/kg bw/day by the oral route in these experimental paradigms. Based on limited data, malformations and variations do not seem to be affected by BPA."[36] Importantly, reduced viability was observed only at the high dose and only in the presence of significant maternal toxicity, including maternal death. By any measure, even putting the stringent Proposition 65 standard aside, these studies clearly demonstrate that BPA is ***not*** a selective developmental toxicant.

Effects on the other two endpoints, sex-differentiation of exploratory and affective behavior and immune hyper-responsiveness, have been elicited only in unconventional studies which, as the CERHR, EFSA assessments, FDA draft assessment, EU RAR and the Health Canada assessment noted, have major limitations and inadequacies. None of these studies would support a finding that BPA has been clearly shown to cause developmental toxicity.

A. *The Human Data Do Not Show an Association Between BPA and Developmental Toxicity*

Epidemiological studies of exposure to BPA and developmental outcomes consist of two recent studies (Padmanabhan *et al.*, 2008; Wolff *et al.*, 2008b). As noted in the HID, "neither study reported an association between BPA and birth outcomes . . . ." Thus, there are no human data to suggest an association between BPA and developmental toxicity.

Most of the human studies attempting to correlate exposure to BPA with adverse health outcomes are of cross-sectional design. This design is practical and inexpensive for investigators because all exposure and health information are collected simultaneously. As noted in a standard epidemiology textbook, cross-sectional studies have demonstrated contributions to public health for factors that are unchanging (*e.g.,* race) or persistent (*e.g.,* blood lead levels). However, the critical problem with cross-sectional data is that "current exposure may have little relation to exposure during the time etiologically relevant to current disease" (Rothman KJ and Greenland S. 1998).

This flaw is prominent in the studies on BPA, with its short half-life in the human body. The primary source of BPA is the diet, which varies day-to-day and over longer periods of time. Without a known source of consistent but heterogeneous exposure, investigators cannot know, for example, if persons at the upper quartile of exposure would remain in the same quartile when sampled even a few days later. Studies of other short-lived chemicals with repeated measures on individuals have shown that variability within individuals is high (MacIntosh *et al.*, 1999) and persons with the highest levels on day one may be near the 50th percentile on day two (Hoppin *et*

---

[36] OEHHA, 2009 HID at p. 31.

al., 2002). Therefore, cross-sectional epidemiology studies are of limited value, because the investigator cannot adequately characterize exposure prior to or during the onset and development of disease.

The two studies that attempted to correlate developmental toxicity in humans with exposure to BPA found no association between maternal BPA levels and infant health indicators (*i.e.,* birth weight, birth length). The study by Padmanabhan *et al.* (2008) had low statistical power, with only 40 subjects, and was cross-sectional, measuring maternal blood at the time of delivery. The larger study by Wolff *et al.* (2008b) collected maternal urine prospectively in the third trimester.

B. *The Animal Data Do Not Demonstrate That BPA Causes Teratogenicity or Selective Developmental Toxicity*

Three conventional developmental toxicity studies were reported in the scientific literature and are discussed below. Each was based on oral administration of BPA during pregnancy. None of these studies demonstrated teratogenicity or selective developmental toxicity. In addition to studies conducted for developmental toxicity *per se*, data from reproductive toxicity studies also may be useful in evaluating the potential for developmental toxicity. Thus, the findings of four well-conducted conventional studies for reproductive toxicity also are discussed below. The unconventional studies that purport to "suggest" developmental effects are discussed separately, and are identified by the effect that they purport to identify, rather than by study title.

1. *Well-Conducted Conventional Developmental Toxicity Studies Show No Adverse Effects in the Absence of Maternal Toxicity*

The National Toxicology Program conducted developmental toxicity studies in rats and mice (Morrissey *et al*, 1987). BPA was administered by gavage on gestation days ("GD") 6-15. Fetotoxicity was observed in mice, but not in rats, at a maternally toxic dose during organogenesis. Fetotoxicity (increased resorptions and decreased fetal body weight) was observed only in mice, and only at the high dose (1250 mg/kg bw/day), which was associated with severe maternal toxicity, including maternal death (18%). Administration of lower doses to mice produced maternal toxicity, but not fetotoxicity. Signs of maternal toxicity in the rat were reported at all doses up to 640 mg/kg bw/day (the high dose), but no evidence of developmental toxicity was observed at any dose.

Another rat developmental toxicity study showed no evidence of teratogenicity in the offspring of dams administered BPA by gavage on GD 1-20. The study reported fetotoxicity (increased resorptions and decreased fetal body weight) and "severe maternal toxicity" at the high dose (1000 mg/kg bw/day). Fetotoxicity was not observed in the absence of significant maternal toxicity (Kim *et al.*, 2001).

IN SUMMARY, the evidence of developmental toxicity in these studies in animals is limited to fetotoxicity (increased resorptions and decreased fetal body weight) at dose levels associated with severe maternal toxicity, including maternal death.

2. *Well-Conducted Conventional Reproductive Toxicity Studies Also Demonstrate That BPA Is Not a Developmental Toxicant*

As noted above, there are four well conducted, reproductive toxicity studies. Three of these studies were conducted by Dr. Rochelle W. Tyl and colleagues at Research Triangle Institute and the fourth was performed at the Safety Research Institute for Chemical Compounds (Sapporo, Japan) and was supported by grants from the Japanese Ministry of Health and Welfare (Ema *et al.*, 2001). Three of the four studies exceed regulatory guidelines. (For example, Tyl *et al.*, 2002b and 2008b included additional low-dose levels that the guidelines did not require. Similarly, Ema *et al.* measured behavioral endpoints beyond those required under the guidelines.) None of these studies indicate that BPA is a selective developmental toxicant. All four demonstrate that BPA does not produce developmental toxicity on PND 0, or that any symptoms of developmental toxicity occur only in association with significant adult systemic toxicity.[37]

(a) Three-Generation Reproductive Toxicity Study in Rats (Tyl et al., 2002b)

Tyl *et al.* (2002b) investigated the potential for reproductive toxicity in a three-generation study in rats. This is one of the most comprehensive studies conducted for BPA or any chemical. Rats were exposed to BPA in the diet at concentrations of 0, 0.015, 0.3, 4.5, 75, 750 and 750 ppm, the approximate equivalent to doses of 0, 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg bw/day. A decrease in litter size at birth was observed in all three generations, but only at the high dose (500 mg/kg bw/day), which exceeded the maximum tolerated dose in the parental rats. Doses of 50 mg/kg bw/day or greater were associated with significant systemic toxicity, including decreased body weight, weight gain and organ weight changes. The LOAELs for developmental and systemic toxicity were 500 and 50 mg/kg bw/day, respectively. The study concluded: "Based on the absence of reproductive and developmental effects in offspring in this study, at doses where there was no significant maternal systemic toxicity, BPA should not be considered a selective reproductive or developmental toxicant."[38]

(b) Two-Generation Reproductive Toxicity Study in Rats (Ema et al., 2001)

Ema *et al.* (2001) investigated the potential for reproductive toxicity in a two-generation study in rats. The animals were exposed to BPA at doses of 0.2, 2, 20, 200 μg/kg bw/day. There was no evidence of developmental or adult systemic toxicity attributable to the test material in this study (Ema *et al.*, 2001).

---

[37] Proposition 65 defines developmental effects as effects that occur as a result of prenatal (not postnatal) exposure. Accordingly, effects observed at PND 0 in a reproductive toxicity study may be relevant to the determination of developmental toxicity under Proposition 65. However, effects occurring after PND 0 in reproductive toxicity studies are of questionable relevance to a determination of developmental toxicity under Proposition 65 since they may be due to postnatal exposure.

[38] Tyl *et al.*, 2002b at p. 144.

(c) Two-Generation Reproductive Toxicity Study in Mice (Tyl et al., 2008b)

Tyl *et al.* (2008b) investigated the potential for reproductive toxicity in a two-generation study in mice. The animals were exposed to BPA in the diet at concentrations of 0, 0.018, 0.18, 1.8, 30, 300 and 3500 ppm, approximately equivalent to doses of 0, 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg bw/day. Adult systemic toxicity, including decreased body weight, increased organ weights (liver, kidney), centrilobular hepatocyte hypertrophy, and renal nephropathy in males was observed at the high dose (600 mg/kg bw/day). Centrilobular hepatocyte hypertrophy was observed at 50 mg/kg bw/day.

There was no evidence of developmental toxicity at birth at any dose level in either generation. Pup survival on PND 0 was not significantly affected.[39],[40] The study reported no effects in the F1/F2 generations on the number of implantation sites per litter; total number of live litters on PND 0; live birth index; and the number of total, live, and dead pups and sex ratio (% males) per litter on PND 0. Postimplantation loss per litter and still birth index were statistically equivalent across all groups. Pup weight at birth was not significantly affected. Other changes were reported among pups, but none of these were observed at or near the time of birth; these findings are discussed later in the sections on reproductive toxicity. The study concluded: "BPA is not considered a selective reproductive or developmental toxicant in mice."[41]

(d) One-Generation Reproductive Toxicity Study in Mice (Tyl et al., 2002a)

Prior to conducting the two-generation study in mice discussed above, Tyl performed an "abbreviated" one-generation reproductive toxicity study in mice (Tyl *et al.,* 2002a). Male and female mice were administered diets containing BPA at concentrations of 0, 5000 or 10,000 ppm for two weeks prior to and during mating, and the females were exposed throughout gestation. Dams and litters were necropsied on PND 0.

Maternal toxicity was observed at both 5000 and 10,000 ppm. Fetotoxicity was observed "only at 10,000 ppm, expressed as slightly (statistically significant) reduced total and live pups/litter, with no significant effects on pre- or postimplantation *in utero* loss or on pup body weights per litter (sexes separately or combined)."[42] At 10,000 ppm, BPA produced significant maternal toxicity, including decreased body weight, decreased body weight gain, decreased food consumption, increased relative liver and kidney weights, and altered histopathology of the liver and kidneys. The results of the one-generation reproductive toxicity study are consistent with those of the two-generation study, Tyl *et al.* (2008b). Both studies showed that BPA is not a selective developmental toxicant in mice.

---

39 *See* footnote 37.

40 Although the HID indicates that this study "found significant treatment ANOVAs for a number of variables reflecting pup survival," the ANOVAs included the positive control group (estradiol). As noted in the HID, none of the BPA-treated groups was statistically significantly different from the negative control group.

41 Tyl *et al.,* 2008b at p. 362.

42 Tyl *et al.,* 2002a at p. 6.

IN SUMMARY, all four reproductive toxicity studies demonstrate that BPA is not a selective developmental toxicant.

3. *The Unconventional Studies Are of Questionable Toxicological Significance and Do Not Support Listing*

As discussed in Section II above, BPA has been evaluated for a variety of developmental toxicity endpoints in many studies that are aptly described as "unconventional." Without enumerating the flaws of those studies here, it must be emphasized that many of the studies employed unvalidated methodologies. One of the most common flaws was the use of an inappropriate route of exposure. The primary route of exposure to BPA in humans is oral, and BPA is not metabolized and eliminated as rapidly with non-oral routes of exposure compared to the oral route. Many of the unconventional studies administered BPA by parenteral routes (*i.e.*, intraperitoneal and subcutaneous) and are thus irrelevant to human hazard identification. In addition, as the HID notes, most of these unconventional studies have not been replicated. Indeed, in a number of cases, investigators in independent laboratories were unable to replicate the original results. More generally, results from these studies have not been found or corroborated consistently in similar studies conducted in independent laboratories.

Most of these studies have been conducted at very low doses, which are orders of magnitude lower than the NOAELs in the conventional developmental and reproductive toxicity studies. In many cases, only a single low dose was used, precluding any evaluation of dose-response. FDA, NTP and other reviewers have regarded these "low-dose" studies as inadequate for a variety of reasons, including: insufficient replication by independent investigators (many investigators did not attempt to replicate their studies; in some cases, independent investigators concluded that findings were not reproducible); unsuitability of various experimental approaches; undetermined relevance of the specific animal model in evaluating potential human risks; and incomplete understanding or agreement on the potential adverse nature of reported effects.[43] Consequently, these unconventional studies do not provide a reliable or adequate basis to conclude that BPA is clearly shown to cause developmental toxicity.

a. *Sex Differentiation of Exploratory and Affective Behavior*

CERHR reviewed these "low-dose" studies and concluded: "Rodent studies ***suggest*** that bisphenol A causes neural and behavioral alterations related to disruptions in normal sex differences in rats and mice."[44] The NTP-CERHR Brief states: "NTP also concurs with the CERHR Expert Panel on Bisphenol A that ***additional research is needed*** to more fully assess the functional, long-term impacts of exposures to bisphenol A on the developing brain and behavior. Overall, ***the current literature cannot yet be fully interpreted for biological or experimental consistency or for relevance to human health***. Part of the difficulty for evaluating consistency

---

43 CERHR, 2008, NTP Brief at p. 9.

44 CERHR, 2008, Chapin *et al.*, at p. 381 (emphasis added).

lies in reconciling findings of different studies that use different experimental designs and different specific behavioral tests to measure the same dimension of behavior."[45]

Results of studies evaluating these endpoints are inconsistent. Only three of the 19 studies of potential effects on sex-differentiation in Tables B7-B9 of the HID used exclusively prenatal exposure. All three of these studies were reviewed in detail by CERHR, which determined that ***each of these studies is "inadequate for the evaluation process"*** due to problems with statistical methodology, lack of experimental detail, and/or insufficient sample size.[46]

In the two studies identified in Table B-11 of the HID, pregnant mice were administered a low dose (10 μg/kg bw/day) by gavage on GD 14-18 (Palanza *et al.*, 2002) or GD 11-18 (Laviola *et al.*, 2005). These studies provide evidence that is only ***"suggestive"*** of behavioral effects. The studies examined completely different endpoints, and there were inconsistencies in results within each study. In addition, the administration of only one dose level precludes evaluation of a dose-response relationship.

b. *Immune Hyper-Responsiveness*

The HID identified only two studies that examined immune response after prenatal exposure (and in the absence of post-natal exposure).[47] Neither supports a conclusion that BPA is clearly shown to cause developmental toxicity.

The first study (Yoshino *et al.,* 2004) reported hypersensitivity in weanlings after immunization with protein antigen following prenatal exposure of mice to BPA "for 17 days, beginning 1 day before mating." CERHR concluded that ***this study is "inadequate for the evaluation process"*** due to "small sample size (n=5) and lack of clarity regarding statistical analysis of factors such as litter and sex effects."[48]

The second study (Yan *et al.,* 2008) reported that mice exposed to BPA prenatally or in adulthood showed a dose-dependent increase in footpad swelling after being infected with Leishmania major, among other immune system effects. CERHR did not review this study, because it was not published until after the CERHR evaluation was completed. The study suffers from many of the major limitations and inadequacies that CERHR attributed to other studies, however. For example, the number of animals per group (n=3-4) was inadequate. No information on the statistical unit was provided in the prenatal experiment. Furthermore, the time of exposure was unusual for a study evaluating prenatal exposure because the exposure period included only the early days of gestation (prior to organogenesis). According to the study authors, female mice were exposed to BPA in drinking water for two weeks, then mated, and then given BPA for another week. The offspring used in this experiment were born within 16 to 19 days after the BPA treatment was finished. Since the gestation period of the mouse is

---

45 CERHR, 2008, NTP Brief at p. 20 (emphasis added).

46 CERHR, 2008, Chapin *et al.*, at pp. 243 – 44, 259 – 60 (emphasis added).

47 OEHHA, 2009 HID at p. 41. The HID identified four studies that examined immune response, two of which included both prenatal and post-natal exposure.

48 *Id.* at p. 288 (emphasis added).

typically not more than 19 days, this indicates that prenatal exposure to BPA was limited to only the first few days of gestation.

IN SUMMARY, the data taken as a whole, including both the well-conducted conventional studies and the unconventional studies, do not demonstrate that BPA is clearly shown to cause developmental toxicity.

V. THE DATA DO NOT SUPPORT A FINDING THAT BPA HAS BEEN "CLEARLY SHOWN" TO CAUSE MALE OR FEMALE REPRODUCTIVE TOXICITY

The many studies of BPA for reproductive effects are discussed below. None of these studies, nor all of them together, would support a finding that BPA is "clearly shown to cause reproductive toxicity." As in the case of developmental toxicity discussed above, most of these studies have been reviewed and were the basis of conclusions reached by CERHR, FDA, EFSA, the EU Institute, and Health Canada. All of the well-conducted conventional studies are discussed separately below, in considerable detail. As above, the unconventional studies are discussed in summary. Finally, we provide below a summary of all of the data relevant to the endpoints identified in the HID, and demonstrate why the weight of the evidence does not support listing based on adverse effects in any of those endpoints.

A. *The Human Data Do Not Demonstrate That BPA Causes Reproductive Toxicity*

Studies in humans do not demonstrate that BPA causes male or female reproductive toxicity. The HID states that "[h]uman studies examining the effects of BPA on reproduction are of limited study design and correspondingly limited in their findings."[49] CERHR similarly states that "[d]rawing firm conclusions about potential reproductive or developmental effects of bisphenol A in humans from these [epidemiological] studies is difficult because of factors such as small sample size, cross-sectional design, lack of large variations in exposure, or lack of adjustment for potential confounders."[50] CERHR thus concluded (as noted earlier), that the "NTP concurs with findings of the recent evaluations that while these studies may suggest directions for future research, there is currently insufficient evidence to determine if bisphenol A causes or does not cause reproductive toxicity in exposed adults."[51]

As discussed in Section IV.A., the cross-sectional design of these studies imposes a significant limitation on their usefulness. Due to the short half-life of BPA in humans, cross-sectional studies that rely on measurements of BPA or its metabolites in single urine or blood spot samples are of limited value, because the study cannot adequately characterize exposure prior to or during the onset and development of disease.

---

49 OEHHA, 2009 HID at p. 66.

50 CERHR, 2008, NTP Brief at p. 15.

51 *Id.* at p. 15.

1. *Female Reproductive Toxicity*

There are seven epidemiology studies that examined associations between BPA and female reproduction. Six of these studies are cross-sectional and therefore do not provide sufficient evidence of causality. (Takeuchi and Tsutsumi, 2002; Takeuchi *et al.*, 2004; Hiroi *et al.*, 2004; Yang *et al.*, 2006; Itoh *et al.*, 2007; Wolff *et al.*, 2008a) Four studies used the ELISA analytical method for measuring BPA in blood. This method was subsequently found to be unsuitable for measuring BPA in human blood samples, because it does not positively identify BPA and cross-reacts with other substances (e.g., phytoestrogens) likely to be present (Fukata *et al.*, 2006).

The single case-control study by Sugiura-Ogasawara *et al.*, 2005 does not show a clear relationship between exposure and effect and has some critical limitations. First, it is one of the studies that used the ELISA method; therefore the validity of the measured concentrations of BPA is suspect. Second, the authors state that serum BPA levels were collected serially in patients with a history of recurrent miscarriages (n=45) and controls who had never been pregnant (n=32). No data are provided, however, on the number or timing of serial samples or the time of sample collection in relation to subsequent miscarriages or successful pregnancies. The absence of these data prevents any understanding of a connection between BPA levels and pregnancy or miscarriage. BPA has a very short half-life in the body (4-6 hours), so the timing of the measurements is critical. It seems unlikely that serum samples were collected in a time-frame that would be relevant to the serial miscarriages since the patients were selected after their history of miscarriage had been established. Notably, the median BPA levels for the cases and controls are virtually identical.

The authors also report that 35 of the 45 patients subsequently became pregnant and 17 (48.6%) miscarried again. The authors highlight the fact that the mean BPA levels in the women who miscarried again tended to be higher, albeit without significance, than the levels in those who had successful pregnancies. However, the ***median*** BPA levels were actually higher in the women with successful pregnancies, which means that at least half the women with successful pregnancies had higher BPA levels than half of those who miscarried again, which is inconsistent with the hypothesis that BPA levels are associated with increased risk of miscarriages.

2. *Male Reproductive Toxicity*

There are two epidemiology studies related to male reproduction. Both are occupational studies of epoxy resin sprayers and demonstrated BPA levels approximately twice the levels of the unexposed comparison group. Both are cross-sectional. As summarized in Table D1 of the HID, the findings of the two studies are directly opposed. Hanaoka *et al.* (2002) identified *lower* FSH in the exposed workers, and Cha *et al.* (2008) reported *higher* levels. Together, these studies do not show an association between exposure to BPA and male reproductive adverse effects.

B. *The Animal Data Do Not Demonstrate That BPA Is a Selective Reproductive Toxicant*

CERHR described the weight of the evidence that BPA causes adverse reproductive effects in laboratory animals as only "some evidence" (not "clear evidence"). In a recent review of the scientific literature on the reproductive and developmental toxicity of BPA, Willhite *et al.* (2008) stated: "There is little evidence for reduced fertility, impaired reproductive performance, or adverse pregnancy outcome in the rodent oral developmental toxicity, reproductive toxicity, and multigeneration studies — even at low (Ema *et al.*, 2001) or high oral bolus (Kwon *et al.,* 2000; Ichihara *et al.,* 2003), drinking-water (Elswick *et al.*, 2000b), or dietary exposures (Tyl, *et al.,* 2002b, 2008b)."[52]

1. *The Well-Conducted Conventional Studies Show That BPA Is Not a Selective Reproductive Toxicant*

There is an abundance of reproductive toxicity data on BPA from well-conducted, conventional studies. The potential effects of BPA on fertility and reproductive performance have been investigated in five high-quality conventional reproductive toxicity studies in rats and mice, three of which used internationally validated guidelines (the three-generation and two-generation studies in the rat and the two-generation study in the mouse); a one-generation reproductive toxicity study in mice; and the NTP continuous breeding study in mice. None of these studies, nor all of them collectively, would support a finding that BPA is "clearly shown to cause reproductive toxicity."

a. *Three-Generation Reproductive Toxicity Study in Rats (Tyl et al., 2002b)*

This study evaluated BPA for reproductive toxicity from dietary exposure to three generations of CD Sprague-Dawley rats. The study was conducted according to the US EPA OPPTS test guidelines, with additional assessments beyond the guideline requirements., and is is regarded as one of the largest and most comprehensive multi-generation reproductive toxicity studies ever conducted for any substance. This study is relied upon by regulatory agencies around the world in risk assessments and as the basis for establishing NOAELs for BPA.

Strengths of this study include:

- Route of Administration: Oral (most relevant for human exposure)
- Number of Dose Levels: Six (0.001, 0.02, 0.3, 5, 50, and 500 mg/kg bw/day)
- Number of Animals: 30 per dosing level
- Endpoints Examined: Multiple
- Genders Evaluated for Systemic Toxicity: Maternal and paternal

[52] Willhite *et al.*, 2008 at p. 117.

Doses of 50 and 500 mg/kg bw/day were associated with significant systemic toxicity in all generations of parental rats, including decreased body weight, weight gain and organ weight changes. With respect to reproductive endpoints, exposure to BPA had no effect on: mating, fertility, gestational indices; ovarian primordial follicle counts; estrous cyclicity; precoital interval; gestational length; offspring sex ratios; postnatal survival; nipple/areolae retention in preweanling males; epididymal sperm number, motility, or morphology; daily sperm production ("DSP"), or efficiency of DSP. Reproductive organ histopathology and function were unaffected. As noted in the discussion of developmental toxicity above, a decrease in litter size and pup weight was observed in all three generations at the high dose (500 mg/kg bw/day). This dose produced significant systemic toxicity and exceeded the maximum tolerated dose in the parental rats.

At the highest dose (500 mg/kg bw/day), vaginal patency and preputial separation were delayed in all three generations of offspring. The study concluded, however, that reduced body weights are "most likely the cause of the significant delay in acquisition of puberty in both sexes."[53] Importantly, if BPA was acting as an estrogen, an acceleration, not a delay, in vaginal patency would be expected.

Anogenital distance was statistically significantly increased among some dose groups (not dose-related) among the F2 female pups, but not among the F3 female pups or F2 or F3 male pups.[54] The study concluded, however, that "the effects reported on F2 female AGD [anogenital distance] are considered of no biological significance and not due to BPA exposure."[55] The authors arrived at this conclusion because (1) the effect was not observed in the F3 female pups, (2) "the magnitude of the difference was minimal," (3) "these changes along with the similarly minor delays in acquisition of [preputial separation] and [vaginal patency] are not associated with any alterations in reproductive organ structures or function in the animals exhibiting them," (4) anogenital distance is under androgenic control and is not affected by estrogens, and (5) "BPA was shown to be neither an androgen nor anti-androgen in vivo."[56]

IN SUMMARY, BPA did not cause selective reproductive toxicity in this study. The study showed that the NOAELs for systemic and reproductive toxicity were 5 and 50 mg/kg bw/day, respectively. No treatment-related effects were found at doses ranging from 0.001 to 5 mg/kg bw/day, and this study did not corroborate effects claimed to occur in the low dose range (<5 mg/kg bw/day); nor were there non-monotonic does responses as claimed in unconventional studies. The study concluded that ***"BPA should not be considered a selective reproductive toxicant, based on the results of this study."***[57]

---

53 Tyl *et al.*, 2002b *at* p. 137.

54 Anogenital distance was not measured in the F1 pups.

55 Tyl *et al.*, 2002b at p. 138.

56 *Id.* at p. 138.

57 *Id.* at p. 121 (emphasis added).

b. *Two-Generation Reproductive Toxicity Study in Rats (Ema et al., 2001)*

A two-generation reproductive toxicity study of BPA in rats was conducted by the Safety Research Institute for Chemical Compounds (Sapporo, Japan) and supported by grants from the Japanese Ministry of Health and Welfare (Ema *et al.*, 2001). This study evaluated a range of low doses and followed the internationally accepted OCED 416 guidelines.

Strengths of this study included:

- Route of Administration: Oral
- Number of Dose Levels: Four (0.2, 2, 20, 200 μg/kg bw/day)
- Number of animals: 25 per dosing level
- Endpoints examined: Multiple (including a wide variety of hormonally sensitive behavioral measurements)

Consistent with the three-generation rat study, no treatment-related effects were found in the low dose range used in this study. Some statistically significant changes were observed. Those changes were sporadic, however, and were inconsistent or not dose-dependent. Accordingly, findings were considered non-treatment-related. This study did not corroborate findings of effects that were claimed to occur in the low dose range (<5 mg/kg bw/day); nor were there non-monotonic does responses as claimed in unconventional studies.

c. *Two-Generation Reproductive Toxicity Study in Mice (Tyl et al., 2008b)*

A two-generation reproductive toxicity study of BPA was performed in CD-1 mice by Tyl *et al.* (2008b). The EU RAR 2008 refers to this study as the "gold standard."[58] The study followed the internationally accepted OECD 416 guidelines, with additional assessments beyond the guidelines. The study was preceded by a one-generation (Tyl *et al.*, 2008a) and a full two-generation reproductive toxicity study on 17β-estradiol (Tyl *et al.,* 2008b), which also was also used as a positive control in the BPA study.

Strengths of this study include:

- Route of Administration: Oral
- Number of Dose Levels: Six (verified dietary concentrations ranging from 0.018 to 3500 ppm)
- Number of animals: 28 per dosing level
- Endpoints examined: Multiple, including a thorough histological evaluation
- Positive control: 17β-estradiol

---

58 EU RAR, 2008 at p. 86 and 128 in Part II Human Health.

- Number of Negative Control Groups: Two

Mice were exposed to BPA in the diet at levels of 0, 0.003, 0.03, 0.3, 5, 50 and 600 mg/kg bw/day. Systemic toxicity was observed at the two highest doses. At the highest dose (600 mg/kg bw/day), adult systemic toxicity included: decreased body weight, increased organ weights (liver, kidney), centrilobular hepatocyte hypertrophy, and renal nephropathy in males. At 50 mg/kg bw/day, the evidence of adult systemic toxicity was limited to a liver effect (*i.e.,* centrilobular hepatocyte hypertrophy).

There were no BPA-related effects on adult mating, fertility or gestational indices, ovarian primordial follicle counts, estrous cyclicity, precoital interval, offspring sex ratios or postnatal survival, sperm parameters or reproductive organ weights or histopathology (including the testes and prostate). There was limited evidence of reproductive toxicity at the highest dose (600 mg/kg bw/day), which was associated with significant systemic toxicity. At lower doses (50 mg/kg bw/day or less), there were no treatment-related reproductive effects.

The study reported, at the highest dose, reduced weanling body weight, reduced weanling spleen and testes weights (with seminiferous tubule hypoplasia) in the F1/F2 generations, and slightly delayed preputial separation in the F1 generation. In addition, there appeared to be a transient increase at the high dose in the incidence of treatment-related, undescended testes in weanlings, which did not result in adverse effects on adult reproductive structures or functions. This finding is considered a developmental delay in the normal process of testes descent. According to the study authors, "[i]t is likely that these transient effects were secondary to (and caused by) systemic toxicity."[59] Gestational length also was increased by 0.3 days in F1/F2 generations at the high dose. According to the study authors, "[t]he toxicological significance, if any, of this marginal difference is unknown."[60]

The same investigators conducted a one- and two-generation reproductive toxicity study of 17β-estradiol in mice to demonstrate that their test model is sensitive to the effects of estrogenic substances (Tyl *et al*, 2008a, 2008c).[61] Many reproductive effects were produced by 17β-estradiol in these studies, and the NOAEL for the developmental toxicity of 17β-estradiol was approximately 0.001 mg/kg bw/day, or approximately 50,000 times lower than the NOAEL for developmental toxicity for BPA in the same test system. Thus, unlike BPA, 17β-estradiol clearly caused reproductive toxicity in conventional reproductive toxicity studies by Tyl *et al.* (2008a,b,c).

The systemic and reproductive NOAELs in this study were 5 and 50 mg/kg bw/day, respectively. This study did not corroborate findings of effects that were claimed to occur in the low dose range (commonly defined as <5 mg/kg bw/day); nor were there non-monotonic dose responses as claimed in unconventional studies. The study concluded that "BPA is not considered a selective reproductive or developmental toxicant in mice."[62]

---

59 Tyl *et al.,* 2008b at p. 362.

60 *Id.*

61 Tyl *et al.,* 2008b at pp. 144–160

62 *Id.*

d. *One-Generation Reproductive Toxicity Study in Mice (Tyl et al., 2002a)*

As noted above, Tyl also performed an "abbreviated" one-generation reproductive toxicity study in mice (Tyl *et al.,* 2002a) prior to conducting her two-generation study in mice. In the one-generation study, male and female mice were administered diets containing 0, 5000 or 10,000 ppm of BPA for 2 weeks prior to and during mating, and the females were also exposed throughout gestation.

The results of the one-generation reproductive toxicity study are consistent with those of the two-generation mouse study. Parental systemic toxicity was clearly evident at both 5000 and 10,000 ppm. At 10,000 ppm, BPA produced significant maternal toxicity, including decreased body weight, decreased body weight gain, decreased food consumption, increased relative liver and kidney weights, and altered histopathology of the liver and kidneys. The evidence of reproductive toxicity was limited to a decrease in litter size observed "only at 10,000 ppm, expressed as slightly (statistically significant) reduced total and live pups/litter, with no significant effects on pre- or postimplantation *in utero* loss or on pup body weights per litter (sexes separately or combined)."[63]

e. *NTP Continuous Breeding Reproductive Toxicity Study in Mice (Morrissey et al., 1989)*

In a National Toxicology Program study, Morrissey *et al.* (1989) administered BPA to mice in the diet at doses of 0, 470, 900, or 1880 mg/kg bw/day and used a continuous breeding protocol. Groups of 20 males and 20 females at each dose were fed the diet for one week, after which the mating trial began and cohabitation continued for 14 weeks. The offspring of up to five litters were evaluated and the last litter delivered was used for the second generation. The data were compared to a concurrent control group of 40 mice of each gender. The only significant changes observed were increased fertility indices and elevated combined male and female pup body weights at the two highest doses.[64]

IN SUMMARY, all five conventional reproductive toxicity studies demonstrate that BPA is not a selective reproductive toxicant.

2. *The Unconventional Studies Are of Questionable Significance and Do Not Support Listing*

As discussed in Section IV.B.3. above, many studies have been conducted to evaluate the potential of BPA to cause reproductive toxicity, which are appropriately categorized as "unconventional." To summarize, many are small-scale, screening studies and/or used experimental protocols that have not been validated. Some were conducted on small numbers of animals; some used only one dosing level (so a dose-response relationship could not be determined), and some lacked experimental details that would allow complete analysis of the reported results. BPA was often administered by injection and other routes of exposure not

---

63 Tyl *et al.,* 2002a at p. 6.

64 Willhite *et al.*, 2008 at p. 100.

relevant to human hazard evaluation. Additional experimental shortcomings included selective use of only male or female offspring, inadequate control procedures, lack of positive controls, absence of correlative morphological and functional endpoints, and failure to consider litter as the appropriate statistical unit.[65]

The majority of the unconventional studies were "low-dose" studies that investigated the hypothesis that very low oral doses of BPA are capable of disrupting the development or functioning of the reproductive system. However, the "conventional" studies described above robustly examined validated endpoints at low doses and found no effects. The weight of the scientific evidence does not currently support the "low-dose" hypothesis. For example, CERHR stated:

> "Hence, the failure of BPA to produce reproducible adverse effects via a relevant route of exposure, coupled with the lack of robustness of the many of the low dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the inability to reproduce many of these effects of any adverse effect strains the credibility of some of these study results. They need to be replicated using appropriate routes of exposures, adequate experimental designs and statistical analyses and linked to higher dose adverse effects if they are to elevate our concerns about the effects of BPA on human health. ***The lack of reproducibility of the low-dose effects, the absence of toxicity in those low-dose-affected tissues at high-doses, and the uncertain adversity of the reported effects led the panel to express 'minimal' concern for reproductive effects.***"[66]

After reviewing the weight of evidence for "low-dose" reproductive and developmental studies, Goodman *et al.* (2009) reached a similar conclusion: "We found some statistically significant positive findings, but these were all countered by null findings in more numerous studies, have not been replicated, do not exhibit coherence and plausibility, and/or do not show consistency across species, doses and time points. In addition, many of the effects noted were not corroborated by the large, robust, multigenerational studies."[67]

IN SUMMARY, for all the reasons above, the unconventional reproductive toxicity studies of BPA do not provide a basis to conclude that BPA has been clearly shown to cause reproductive or developmental toxicity.

3. *The Data Do Not Support Listing for Any of the Reproductive Endpoints Identified in the HID*

The HID identified a variety of reproductive endpoints that were reportedly affected by exposure to BPA in one or more studies. The discussion below explains briefly, endpoint by endpoint, why the data do not support a conclusion that BPA should be listed.

---

65 FDA, 2008 at p. 25.

66 CERHR, 2008, Chapin *et al.*, at p. 382 (emphasis added).

67 Goodman *et al.,* 2009 at p. 68.

(a) Uterine and Estrogenic Effects

It is well documented that BPA binds weakly to estrogen receptors. However, the estrogen binding affinity of BPA is orders of magnitude lower than that of endogenous estrogen.

In vitro studies evaluating the estrogen receptor binding capacity of BPA indicate that BPA is 4-5 orders of magnitude less potent than endogenous estrogen (17β-estradiol). A standard screening test for estrogenic activity is the measurement of changes in uterine weight in ovariectomized rodents. *In vivo* studies of the effects of oral BPA on uterine weight confirm that BPA is typically 4-5 orders of magnitude less potent than endogenous estrogen. Based on this difference in estrogenic activity alone (in non-human assays), BPA would not be expected to cause developmental or reproductive toxicity via an estrogenic mechanism. Furthermore, as noted previously, there is no significant opportunity for BPA to exhibit an estrogenic effect in humans because orally ingested BPA is rapidly metabolized to BPA-glucuronide in the intestines and during its first pass through the liver and because BPA-glucuronide does not exhibit estrogenic activity.

In addition, and very importantly, the potency of BPA would be expected to be lower in humans than rodents, because BPA undergoes enterohepatic recirculation in rodents. Uterotrophic evaluations are typically performed twenty-four hours after the last dose of the test agent is administered. Laws *et al.* (2000) showed no significant effect from exposure to BPA at doses up to 400 mg/kg bw/day, administered orally on rat uterine wet weight assessed 24 hours after administering the last dose. Many components of the human diet, such as phytoestrogens and lignans, have a similar or greater affinity than BPA to bind to estrogen receptors. For example, genistein, a phytoestrogen found in soy, has a much higher affinity than BPA for both estrogen receptors (ERα and ERβ): Any BPA available *in vivo* competes with endogenous estrogen and with much higher levels of these dietary phytoestrogens for the ability to bind with estrogen receptors.

Estrogenic activity in itself is not an adverse reproductive toxic or developmental effect ***per se***. Rather it is a mechanism that might induce such an effect under certain circumstances, which are not present in the case of BPA. Therefore, the weak estrogenic activity of BPA is not a sufficient basis to support a conclusion that BPA is "clearly shown to cause reproductive toxicity" for purposes of Proposition 65. Moreover, even if estrogenic activity were a basis for listing a chemical, there is no significant opportunity for BPA to exhibit an estrogenic effect in humans, because orally ingested BPA is efficiently metabolized to BPA-glucuronide in the intestines and during its first pass through the liver, and BPA–glucuronide does not exhibit estrogenic activity.

(b) Estrous Cycle Effects

Reports of estrous cycle alterations in "low-dose" studies (Talsness *et al.,* 2000; Rubin *et al.*, 2001) are inconsistent and unreliable. The findings in Talsness *et al.* (2000) varied depending on whether the estrous phase length and reproductive cycles phase length were evaluated per female rat in each group or as a percent of total cycles in each group. In addition, it has been reported that the Talsness *et al.* (2000) controls were not run concurrently with the

animals exposed to BPA. NTP, 2001. Regarding the findings in Rubin *et al.* (2001), changes in the estrous cycle were qualitatively inconsistent across individual animals.[68] Estrous cycle alterations were not reported in most studies of BPA, including the multigeneration reproductive toxicity studies in rats and mice (Tyl *et al*, 2002b, 2008b; Ema *et al.*, 2001), at low or high doses.[69] Overall, there is no consistent evidence in rodents to support an association between BPA and alterations in estrous cycle.

(c) Onset of Puberty

CERHR concluded: "There are sufficient data to conclude that bisphenol A does not change the age of puberty in male or female rats [NOAELs of 0.2 mg/kg bw/day (Ema *et al.*, 2001) and 1823 mg/kg bw/day (Tyl *et al.*, 2002b)]. While limited data available suggest an effect on the onset of female puberty in mice [LOAEL 0.2 mg/kg bw/day (Ryan and Vandenbergh, 2006) and 0.002 mg/kg bw/day (Howdeshell *et al.*, 1999)], the data are insufficient to conclude that bisphenol A accelerates puberty in female mice. The limited data that are available suggest, but are insufficient to conclude, that bisphenol A slightly delays the age of puberty in male mice at a LOAEL of ca. 550-800 mg/kg bw/day (Tyl *et al.*, 2008b)."[70]

FDA reached a similar conclusion after reviewing the available scientific evidence: "Taken together, these results suggest that within the context of laboratory animal studies, limited evidence exists regarding an acceleration of puberty . . . ."[71]

(d) Neural and Behavioral Alterations

A number of studies screened for neural and behavioral alterations in rats and mice following BPA exposure. As noted in the HID, "comprehensive screening of BPA for developmental neurotoxicity has not yet been conducted. Routine guidelines are available for developmental neurotoxicity screening but have not yet been used for BPA.[72] (Note, however, that Ema *et al.* (2001) conducted a limited behavioral screen on offspring in their two-generation study and found no effects.) Nonetheless, most of the neurotoxicity screening studies of BPA conducted to date are unconventional, low-dose studies that included both prenatal and postnatal exposure. At most, they provide suggestive evidence, and suggestive evidence does not meet the "clearly shown to cause" standard.

Thus, CERHR concluded: "Rodent studies ***suggest*** that bisphenol A: causes neural and behavioral alterations related to disruptions in normal sex differences in rats and mice."[73] The NTP-CERHR Brief states: "NTP also concurs with the CERHR Expert Panel on Bisphenol A that ***additional research is needed*** to more fully assess the functional, long-term impacts of exposures to bisphenol A on the developing brain and behavior. ***Overall, the current literature cannot yet be fully interpreted for biological or experimental consistency or for relevance to***

---

68 Goodman *et al.,* 2009 at p. 54.

69 *Id.* at pp. 37-40.

70 CERHR, 2008, Chapin *et al.*, at p. 329.

71 FDA, 2008 at p. 27.

72 OEHHA, 2009 HID at p. 63.

73 CERHR, 2008, Chapin *et al.*, at p. 381 (emphasis added).

human health. Part of the difficulty for evaluating consistency lies in reconciling findings of different studies that use different experimental designs and different specific behavioral tests to measure the same dimension of behavior."[74]

FDA also reviewed studies in the scientific literature on BPA and neural and behavioral alteration:

> "The varied treatment-related findings in a majority of the reviewed studies collectively ***appear to suggest*** that developmental BPA exposure in rodents may have the potential to alter brain development and behavior. However, ***in view of the limitations in study design and study conditions that confound the interpretability of the study findings, without appropriate confirmation of these findings using well-designed experimental protocols and/or clarification of their biological significance, the utility and relevance of the study findings to an assessment of the safety of BPA from food contact uses is unknown.*** Until these disparate experimental findings are examined in well-designed safety assessment studies using appropriate biomarkers of effect, FDA has concluded that ***the reviewed studies are inadequate for use in supporting a safety assessment determination or regulatory decision for BPA***."[75]

(e) Anogenital Distance

CERHR stated: "Although some sporadic effects were reported for anogenital distance in male and female rats, study authors concluded that the endpoint was not affected by prenatal, lactational, and/or post-weaning exposure to bisphenol A (Ema *et al.,* 2001; Tinwell *et al.,* 2002; Tyl *et al.*, 2002b)."[76] In evaluating the effects estradiol on reproduction, Tyl *et al.* (2008b) also found: "It is clear that AGD on PND 0 or 21 is not an indicator of estrogenicity in rodents, consistent with the evidence that AGD is driven by androgen (5α-dihydrotestosterone; DHT) secretion rather than by estrogens (Imperato-McGinley *et al*, 1992)."

Honma *et al.* (2002) reported a *longer* anogenital distance (masculinized) in offspring after prenatal BPA exposure. In contrast, Talsness *et al.* (2000) reported *shorter* anogenital distance in male rats after prenatal exposure to BPA (although, as noted above, the Talsness *et al.* controls were not run concurrently with the BPA treated animals (NTP, 2001)). And another study using similar doses and time of exposure but with a smaller sample size (Tinwell *et al.*, 2002), found no effect. In high dose studies, no consistent differences in anogenital distance were reported.

The magnitude of the differences in anogenital distance in these studies was very small. As noted in the HID, anogenital distance can be difficult to measure accurately depending on how the animal is restrained for the measurement, the measuring tool, etc.[77] Newborn anogenital distance is approximately 5 mm in rats and 1 mm in mice, making accuracy an important issue.

---

74 CERHR, 2008, NTP Brief at p. 20 (emphasis added).

75 FDA, 2008 at p. 30 (emphasis added).

76 CERHR, 2008, Chapin *et al.*, at p. 328.

77 OEHHA, 2009 HID at p. 43.

(f) Testis

The weight of the evidence indicates that testis weight was not affected by exposure to BPA. The HID states: "The majority of studies that measured this endpoint did not find any significant reduction, regardless of the strain of animals, exposure period, doses, or dosing methods."[78] Similarly, for rats, the HID states: "As was the case with studies in mice, most studies in rats either did not report testis weight or found no effect on it."[79]

The HID further states that *"[i]n vitro* studies consistently show effects of BPA on cultured cells or tissues from the testis (Sertoli or Leydig cells) or the prostate."[80] The results of these *in vitro* studies are not useful for human hazard or risk assessment, however, since *in vitro* studies provide no opportunity for the rapid metabolism of BPA known to occur *in vivo* in the intestines and liver (first pass effect). More importantly, the weight of evidence from *in vivo* studies does not demonstrate adverse effects consistently on the testis or prostate.

(g) Prostate

The HID correctly notes that BPA did not cause effects on the prostate when evaluated by traditional methods such as organ weight or routine histopathological evaluation.[81] However, the HID states that prostate effects "can be detected with advanced molecular and/or cellular approaches."[82] In fact, these effects were seen in only three out of 14 studies that evaluated the histopathology of the prostate, and in all three of these studies, BPA was administered via a subcutaneous implant. Two of the three studies were found to be "inadequate" by CERHR. The third study, which was published after the review by the CERHR Expert Committee, suffers from the same flaws.[83] These studies are not useful for human hazard identification, because administration of BPA in the form of a subcutaneous implant is designed to provide a constant blood level of BPA, which is very different from the rapidly declining or undetectable blood levels of BPA in humans (due to the efficient metabolism and rapid elimination of ingested BPA). Furthermore, subcutaneous administration to rats has been shown to produce unique metabolites not present following oral administration (Pottenger *et al.*, 2000) that may contribute to the production of effects not relevant for oral exposure.

Regarding prostate weight, CERHR concluded: "There are sufficient data to conclude that bisphenol A exposure during development does not permanently affect prostate weight in

---

78 *Id.* at p. 136.

79 *Id.*

80 OEHHA, 2009 HID at p. 136.

81 *Id* at p. 144.

82 *Id.*

83 The HID identified 33 studies that evaluated the prostate in rats or mice exposed to BPA in Tables D6 and D7. Of these 33, 14 specifically evaluated the histopathology of the prostate. The three studies that appeared to show effects on the prostate were Ramos *et al.* (2001, 2003) and Ogura *et al.* (2007). CERHR evaluated the two studies by Ramos *et al.* and considered them to be "inadequate" for multiple reasons. The Ogura *et al.* (2007) study was published after the CERHR Expert Panel completed its review, and it suffers from many of the same serious limitations as the Ramos *et al.* studies, including the use of a subcutaneous pellet as the route of administration.

adult rats or mice."[84] Decreased prostate weight or changes in prostate ducts were reported in a small number of studies. However, no effects on the prostate were observed in more robust studies with multiple dose levels and larger group sizes.

(h) Fertility

CERHR concluded that BPA "does not alter male or female fertility after gestational exposure up to doses of 450 mg/kg bw/day in the rat and 600 mg/kg bw/day in the mouse (highest dose levels evaluated)."[85] As noted earlier, effects on litter size were observed in studies at higher dose levels that exceeded the maximum tolerated dose and caused significant systemic toxicity.

(i) Sperm

The NTP-CERHR Brief concluded that effects on sperm "are not reported consistently enough to be considered reproducible."[86] According to CERHR, "Effects on rat sperm parameters were inconsistent."[87] A number of "low-dose" studies identified changes in rates of genital maturation or sperm counts and motility in rats and mice given low doses of as little as 2-20 μg/kg bw/day (Sakaue *et al.*, 2001; Chitra *et al.*, 2003a, 2003b; Al Hiyasat *et al.*, 2002). Others, however, were unable to reproduce these findings in rodents (Ashby *et al.*, 2003). In fact, most studies of BPA that evaluated sperm did not find effects on sperm parameters following exposure during development at either low or high dose levels ranging from 0.0002 to 600 mg/kg bw/day (Cagen *et al.*, 1999a, 1999b; Ashby *et al.*, 1999; Kwon *et al.*, 2000; Takashima *et al.*, 2001; Tyl *et al.,* 2008b; Ema *et al.*, 2001; Kato *et al.*, 2006; Howdeshell *et al.*, 2008; Nagao *et al.*, 2002; Kim *et al.*, 2002).

In some of the studies reporting changes at very low doses, the control daily sperm production data are more variable than those generally reported; variability between control populations and the care and source of the animals may account for some of the discrepancies in the rodent data (Ashby *et al.*, 2003).

(j) Mammary Gland

Various changes in the mammary gland were reported in several unconventional "low-dose" studies. The NTP-CERHR Brief, which reviewed these studies (including Moral *et al.,* 2008; Durando *et al.*, 2007; Murray *et al.,* 2007), observed that: "[t]he current literature is not sufficient to establish the reproducibility of the ductal lesion findings by multiple independent investigators."[88] Thus, the NTP-CERHR Brief concluded that additional data in better-designed studies is needed:

---

84 CERHR, 2008, Chapin *et al.*, at p. 384.

85 *Id.* at p. 381.

86 CERHR, 2008, NTP Brief at p. 30.

87 CERHR, 2008, Chapin *et al.,* at p. 328.

88 CERHR, 2008, NTP Brief at p. 23.

> "The NTP concurs with recent reviews that additional data are needed to more completely understand the possible long-term consequences of disrupting mammary gland development in animals by bisphenol A exposure and its significance for human health. Namely, long-term follow-up studies with sufficient statistical power should be conducted to evaluate if the ductal hyperplasia and carcinoma *in situ* progress to mammary gland tumors, preferably without the use of a secondary chemical challenge in adulthood.[89]

Only one of the unconventional studies (Moral *et al.*, 2008) administered BPA orally. This study reported changes in gene expression and an increased number of mammary gland terminal ducts in the female offspring of rats treated during gestation at 0.025 or 0.25 mg/kg bw/day. However, there was no consistent dose-response relationship, and the results of this study have not been replicated by others.

In all of the other studies of mammary gland effects, BPA was administered by subcutaneous injection or by surgically implanting a subcutaneous osmotic mini-pump. The CERHR Expert Panel considered these studies to be inadequate. For example, Durando *et al.* (2007) "is inadequate for inclusion [in its evaluation] due to the use of 99.9% DMSO as a vehicle to administer bisphenol A via s.c. osmotic pump."[90] Murray *et al.* (2007) "was inadequate due to small sample size, route of administration, and lack of clarity on statistical analysis."[91] Further, the NTP Brief noted that "a number of pathologists questioned the classification of the [mammary gland] lesions" observed in both of these studies. The EU RAR (2008) was also critical of both Durando *et al.* (2007) and Murray *et al.* (2007): "due to the small sample size, lack of clarity on statistical analysis and use of a single dose level, it is difficult to establish whether the effects reported [in the Durando *et al.* (2007) study] were due to chance or were real, treatment-related effects. Furthermore, because of the subcutaneous route of administration, it is questionable whether the reported findings are relevant to normal routes of exposures."[92]

VI. CONCLUSION

BPA should not be listed. Taking into account the weight of the evidence, the statutory and regulatory criteria for listing, and the guidance articulated in the Committee's Guidance Criteria, the chemical simply cannot be characterized as "clearly shown, through scientifically accepted testing conducted according to generally accepted principles, to cause" developmental or reproductive toxicity.

Applying those Proposition 65 standards to the testing data available here, it is undisputed that the human data do not support listing. Therefore, any finding that the chemical merits listing would have to rest on the animal data alone. The high-quality conventional animal data, summarized in Sections IV and V, demonstrate consistently and convincingly that BPA is not a selective developmental or reproductive toxicant in rodents.

---

89 *Id.* at pp. 23 – 24.

90 CERHR, 2008, Chapin *et al.*, at p. 247.

91 *Id.* at p. 246.

92 EU RAR, 2008 at p. 82.

The unconventional, "low-dose" studies, characterized as inadequate for risk assessment purposes by every regulatory agency that has reviewed them, do not provide a basis for listing because they provide no consistent or compelling evidence, for all of the reasons discussed in Sections IV.B.3 and V.B.3. Indeed, many of the unconventional studies do not represent "scientifically valid testing according to generally accepted principles," and cannot be relied upon here for that reason alone. In addition, strong pharmacokinetic data show that the effects observed in rodents are not directly applicable to humans, because humans metabolize and eliminate BPA more rapidly and efficiently than rodents. As a result, the developmental and reproductive effects observed in rodents are biologically implausible in humans. Therefore, the weight of the evidence does not support a conclusion that BPA is "clearly shown to cause reproductive toxicity."

We urge the Committee to apply these standards in reviewing the data for BPA. Indeed, they are the only standards that the Committee may apply under Proposition 65. In applying these standards, the Committee can take comfort that other highly respected agencies have examined the same data and have reached well-supported conclusions that BPA may be used safely, including in food contact applications. The data support those findings, and similarly support a finding that BPA should not be listed under Proposition 65.

Respectfully submitted,

AMERICAN CHEMISTRY COUNCIL

BY:

STEVEN G. HENTGES, PH.D.
EXECUTIVE DIRECTOR
POLYCARBONATE/BPA GLOBAL GROUP

MURRAY & ASSOCIATES

By: _____
F. JAY MURRAY, PH.D.

MCKENNA LONG & ALDRIDGE LLP

By: _____
CHRISTIAN VOLZ

_____
STANLEY W. LANDFAIR

TECHNOLOGY SCIENCES GROUP INC.

By: _____
ARTHUR L. LAWYER, PH.D.

REFERENCES

Al-Hiyasat AS, Darmani H, Elbetieha AM. 2002. Effects of bisphenol A on adult male mouse fertility. Eur J Oral Sci. 110:163–167. [Erratum: Eur J Oral Sci 2003;2111:2547].

Ashby J, Tinwell H, Haseman J. 1999. Lack of effects for low dose levels of bisphenol A and diethylstilbestrol on the prostate gland of CF1 mice exposed in utero. Regul Toxicol Pharmacol. 30:156–166.

Ashby J, Tinwell H, Lefevre PA, Joiner R, Haseman J. 2003. The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisphenol A between postnatal days 91–97. Toxicol Sci. 74:129–138.

Cagen SZ, Waechter Jr JM, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR. 1999a. Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A. Toxicol Sci. 50:36–44.

Cagen SZ, Waechter Jr JM, Dimond SS, Breslin WJ, Butala JH, Jekat FW, Joiner RL, Shiotsuka RN, Veenstra GE, Harris LR. 1999b. Normal reproductive organ development in Wistar rats exposed to bisphenol A in the drinking water. Regul Toxicol Pharmacol. 30: 130–139.

Calafat AM, Weuve J, Ye X, Jia LT, Hu H, Ringer S, Huttner K, Hauser R. 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environmental Health Perspectives. 117(4):639-644.

CERHR, 2008. Center for the Evaluation of Risks to Human Reproduction. NTP-CERHR Monograph on the potential human reproductive and developmental effects of bisphenol A. NIH Publication No. 08-5994. September 2008.

Cha BS, Koh SB, Park JH, Eom A, Lee KM, Choi HS. 2008. Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters. Mol Cell Toxicol. 4:230-234.

Chapin RE, Adams J, Boekelheide K, Gray LE Jr, Hayward SW, Lees PSJ, McIntryr BS, Portier KM, Schnorr TM, Selevan SG, Vandenbergh GE, Woskie SR. 2008. Expert Panel Report: NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. Birth Defects Research (Part B). 83:157-395.

Chitra KC, Latchoumycandane C, Mathur PP. 2003a. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology. 185:119–127.

Chitra KC, Rao KR, Mathur PP. 2003b. Effect of bisphenol A and co-administration of bisphenol A and vitamin C on epididymis of adult rats: a histological and biochemical study. Asian J Androl. 5:203–208.

DART-IC, 1993. Developmental and Reproductive Toxicity Identification Committee. Criteria for recommending chemicals for listing as known to the State to cause reproductive toxicity. November 1993.

Domoradzki JY, Pottenger LH, Thornton CM, Hansen SC, Card TL, Markham DA, Dryzga MD, Shiotsuka RN, Waechter Jr JM. 2003. Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages. Toxicol Sci. 76:21–34.

Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, Mun˜oz-de-Toro M. 2007. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environ Health Perspect. 115:80–86.

EFSA, 2006. Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to bisphenol A. The EFSA Journal (2006) 428, 1-75.

EFSA, 2008. Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission on the toxicokinetics of bisphenol A. The EFSA Journal (2008) 759, 1-10.

Elswick BA, Janszen DB, Gould JC, Stedman DB, Welsch F. 2000b. Effects of perinatal exposure to low doses of bisphenol A in male offspring of Sprague-Dawley rats. Toxicol. Sci. 54:256.

Ema M, Fujii S, Furukawa M, Kiguchi M, Ikka T, Harazono A. 2001. Rat two-generation reproductive toxicity study of bisphenol A. Reprod Toxicol. 15:505–523.

EU-RAR, 2008. European Union Risk Assessment Report. European Union updated risk assessment of 4,4'-isopropyldineddiphenol (bisphenol-A), final approved version awaiting for publication. April, 2008.

FDA, 2008. United States Food and Drug Administration. Draft Assessment of Bisphenol A for Use in Food Contact Applications. August 14, 2008.

Fukata H, Miyagawa H, Yamazaki N, Mori C. 2006. Comparison of ELISA and LC-MS based methodologies for the exposure assessment of bisphenol A. Toxicol Mech Methods. 16:427–430.

Ghibellini,G, Leslie EM, Brouwer KL. 2006. Methods to evaluate biliary excretion of drugs in humans: an updated review. Mol.Pharm.. 3, 198-211.

Goodman JE, Witorsch RJ, McConnell EM, Sipes IG, Slayton TM, Yu CJ, Franz AM, Rhomberg LR. 2009. Weight-of-evidence evaluation of reproductive and development effects of low doses of bisphenol A. Critical Reviews in Toxicology. 39:1-75.

Hanaoka T, Kawamura N, Hara K, Tsugane S. 2002. Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. Occup Environ Med. 59:625–628.

Health Canada, 2008. Screening Assessment for the Challenge,
Phenol, 4,4' - (1-methylethylidene)bis-(Bisphenol A), Chemical Abstracts Service Registry Number 80-05-7, Environment Canada, Health Canada, October 18, 2008.

Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, Yokota H, Taketani Y. 2004. Differences in serum bisphenol A concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocr.J. 51:595–600.

Hirom PC, Millburn P, Smith RL. 1976. Bile and urine as complementary pathways for the excretion of foreign organic compounds. Xenobiotica. 6:55-64

Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T. 2002. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod Toxicol. 16:117–122.

Hoppin JA, Brock JW, Davis BJ, Baird DD. 2002. Reproducibility of urinary phthalate metabolites in first morning urine samples. Environ Health Perspect. 110:515-518.

Howdeshell KL, Furr J, Lambright CR, Wilson VS, Ryan BC, Gray LE, Jr. 2008. Gestational and lactational exposure to ethinyl estradiol, but not bisphenol A, decreases androgen-dependent reproductive organ weights and epididymal sperm abundance in the male Long Evans Hooded rat. Toxicol Sci. 102:371 – 382.

Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, Inaguma S, Suzuki S, Shirai T. 2003. Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. J Toxicol Sci. 28:165–171.

Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S. 2007. Urinary bisphenol A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study. Environ Health Prev Med. 12:258 – 264.

Kato H, Furuhashi T, Tanaka M, Katsu Y, Watanabe H, Ohta Y, Iguchi T. 2006. Effects of bisphenol A given neonatally on reproductive functions of male rats. Reprod Toxicol. 22:20–19.

Kim JC, Shin HC, Cha SW, Koh WS, Chung MK, Han SS. 2001. Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy. Life Sci. 69:2611–2625.

Kim HS, Han SY, Kim TS, Kwack SJ, Lee RD, Kim IY, Seok JH, Lee BM, Yoo SD, Park KL. 2002. No androgenic/anti-androgenic effects of bisphenol-A in Hershberger assay using immature castrated rats. Toxicol Lett. 135:111–123.

Kurebayashi H, Harada R, Stewart RK, Numata H, Ohno Y. 2002. Disposition of a low dose of bisphenol a in male and female cynomolgus monkeys. Toxicol Sci. 68:32-42.

Kurebayashi H, Betsui H, Ohno Y. 2003. Disposition of a low dose of 14C bisphenol A in male rats and its main biliary excretion as BPA glucuronide. Toxicol Sci. 73:17–25.

Kwon S, Stedman DB, Elswick BA, Cattley RC, Welsch F. 2000. Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. Toxicol Sci. 55:399–406.

Laws SC, Carey SA, Ferrell JM, Bodman GJ, Cooper RL. 2000. Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Toxicol Sci. 54:154–167.

Lehman-McKeeman. 2008. Chapter 5, Absorption, Distribution, and Excretion of Toxicants, in Casarett and Doull's Toxicology The Basic Science of Poisons, Seventh Edition. Ed., C.D. Klaassen. McGraw-Hill Medical, New York.

MacIntosh DL, Needham LL, Hammerstrom KA, Ryan PB. 1999. A longitudinal investigation of selected pesticide metabolites in urine. J Expo Anal Environ Epidemiol. 9:494-501.

Matthews JB, Twomey K, Zacharewski TR. 2001. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chem Res Toxicol. 14:149–157.

Moral R, Wang R, Russo IH, Lamartiniere CA, Pereira J, Russo J. 2008. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. J Endocrinol. 196:101 – 112.

Morrissey RE, George JD, Price CJ, Tyl RW, Marr MC, Kimmel CA. 1987. The developmental toxicity of bisphenol a in rats and mice. Fundam Appl Toxicol. 8:571–582.

Morrissey RE, Lamb JC, Morris RW, Chapin RE, Gulati DK, Heindel JJ. 1989. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. Fundam Appl Toxicol. 13:747–777.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. 2007. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol. 23: 383–390.

Nagao T, Saito Y, Usumi K, Yoshimura S, Ono H. 2002. Low-dose bisphenol A does not affect reproductive organs in estrogen-sensitive C57BL/6N mice exposed at the sexually mature, juvenile, or embryonic stage. Reprod Toxicol. 16:123–130.

NTP, 2001. The National Toxicology Program Endocrine Disruptors Low-Dose Peer Review Final Report, August, 2001," available on the Internet at http://ntp.niehs.nih.gov/ntp/htdocs/liason/LowDosePeerFinalRpt.pdf, specifically p. A-57.

OEHHA, 2009. HID Office of Environmental Health Hazard Assessment. Evidence on the developmental and reproductive toxicity of bisphenol A. Draft, May 2009.

Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, Tao L, Kannan K. 2008. Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol. 28:258 – 263.

Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter Jr JM. 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent on the route of administration. Toxicol Sci. 54:3–18.

Pritchett JJ, Kuester RK, Sipes IG. 2002. Metabolism of bisphenol a in primary cultured hepatocytes from mice, rats, and humans. Drug Metab Dispos. 30:1180–1185.

Rothman KJ, Greenland S. 1998. Modern Epidemiology (2nd Ed) Lippincott-Raven, Philadelphia, PA.

Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect. 109:675–680.

Ryan BC, Vandenbergh JG. 2006. Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice. Horm Behav. 50:85–93.

Sakamoto H, Yokota H, Kibe R, Sayama Y, Yuasa A. 2002. Excretion of bisphenol A-glucuronide into the small intestine and deconjugation in the cecum of the rat. Biochim Biophys Acta. 1573:171–176.

Sakaue M, Ohsako S, Ishimura R, Kurosawa S, Kurohmaru M, Hayashi Y, Aoki Y, Yonemoto J, Tohyama C. 2001. Bisphenol-A affects spermatogenesis in the adult rat even at a low dose. J Occup Health. 43:185–190.

Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y, Ozawa S. 2002. Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. Toxicol In Vitro. 16:549–556.

Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SC, Fennell TR. 2000. Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol. 168:225–234.

Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. 2005. Exposure to bisphenol A is associated with recurrent miscarriage. Hum Reprod. 20:2325–2329.

Takashima Y, Tsutsumi M, Sasaki Y, Tsujiuchi T, Kusuoka O, Konishi Y. 2001. Lack of effects of bisphenol A in maternal rats or treatment on response of their offspring to N-nitrosobis(2-hydroxypropyl)amine. J Toxicol Pathol 14:87–98.

Takeuchi T, Tsutsumi O. 2002. Serum bisphenol a concentrations showed gender differences, possibly linked to androgen levels. Biochem Biophys Res Commun. 291:76–78.

Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. 2004a. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr. J 51:165–169.

Talsness CE, Fialkowski O, Gericke C, Merker HJ, Chahoud I. 2000. The effects of low and high-doses of bisphenol A on the reproductive system of female and male rat offspring. Congenit Anom. 40: 94–107.

Tinwell H, Haseman J, Lefevre PA, Wallis N, Ashby J. 2002. Normal sexual development of two strains of rat exposed in utero to low doses of bisphenol A. Toxicol Sci. 68:339–348.

Tyl R, Myers CB, Marr MC. 2002a. Abbreviated one-generation study of dietary bisphenol A (BPA) in CD-1^{s} (Swiss) mice. Research Triangle Park, NC: RTI (sponsored by the Society of the Plastics Industry, Inc.). Report nr 65C.07036.312.

Tyl RW, Myers CB, Marr MC, Thomas BF, Keimowitz AR, Brine DR, Veselica MM, Fail PA, Chang TY, Seely JC, Joiner RL, Butala JH, Dimond SS, Cagen SZ, Shiotsuka RN, Stropp GD, Waechter JM. 2002b. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci. 68:121–146.

Tyl RW, Myers CB, Marr MC, Castillo NP, Veselica MM, Joiner RL, Dimond SS, Van Miller JP, Stropp GD, Waechter Jr JM, Hentges SG. 2008a. One-generation reproductive toxicity study of dietary 17beta-estradiol (E2; CAS No. 50-28-2) in CD-1 (Swiss) mice. Reprod Toxicol. 25: 144-60.

Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter Jr JM. 2008b. Two-generation reproductive toxicity study of dietary bisphenol A (Bisphenol A) in CD-1(R) (Swiss) mice. Toxicol Sci. 104:362 – 384.

Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RS, Stropp GD, Waechter JM, Jr., Hentges SG. 2008c. Two-generation reproductive toxicity evaluation of dietary 17beta-estradiol. (E2; CAS No. 50 – 28 – 2) in CD-1 (Swiss) mice. Toxicol Sci. 102:392 – 412.

Völkel W, Colnot T, Csanady GA, Filser JG, Dekant W. 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem Res Toxicol. 15:1281–1287.

Völkel W, Bittner N, Dekant W. 2005. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. Drug Metab Dispos. 33:1748–1757.

Walton K, Dorne JL, Renwick AG. 2001. Uncertainty factors for chemical risk assessment: interspecies differences in glucuronidation. Food Chem.Toxicol. 39, 1175-1190.

Willhite CC, Ball GL, McLellan CJ. 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. J Toxicol Environ Health B Crit Rev. 11:69 – 146.

Wolff MS, Britton JA, Boguski L, Hochman S, Maloney N, Serra N, Liu ZS, Berkowitz G, Larson S, Forma J. 2008a. Environmental exposures and puberty in inner-city girls. Environ Res. 107:393-400.

Wolff M, Engel S, Berkowitz G, Ye X SM, Zhu C, Wetmur J, Calafat A. 2008b. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect. 116:1092 – 1097.

Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP. 2003. Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environ Health Perspect. 111:309–319.

SF:27371501.1