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Developmental and Reproductive Toxicant Identification Committee
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Submitted by e-mail to: P65Public.Comments@oehha.ca.gov.

Re: Comments in Opposition to Listing Bisphenol A as a Female Reproductive Toxicant

Dr. Gold and Committee Members:

Please find attached written comments from the Polycarbonate/BPA Global Group of the American Chemistry Council (ACC)[1] on the Hazard Identification Materials for bisphenol A (BPA). The Polycarbonate/BPA Global Group consists of the leading global manufacturers of BPA and polycarbonate plastic, who for many years have sponsored scientific research to understand whether BPA has the potential to cause health or environmental effects and to support scientifically sound public policy.

As described in detail in our attached comments, the weight of evidence does not support a conclusion that BPA has been "clearly shown, through scientifically accepted testing conducted according to generally accepted principles to cause" female reproductive toxicity. Accordingly, BPA should not be listed as a female reproductive toxicant under Proposition 65. Key points that support this conclusion include:

- A 2014 assessment of BPA from the U.S. Food and Drug Administration, an authoritative body for purposes of Proposition 65, did not identify female reproductive toxicity as a hazard for BPA.[2]
- Human studies do not provide sufficient evidence to list BPA.

[1] ACC is a not-for-profit trade association representing the companies that make the products that make modern life possible, while working to protect the environment, public health, and security of our nation. ACC represents the leading companies engaged in the business of chemistry. The business of chemistry is a $674 billion a year enterprise and a key element of the nation's economy. ACC members are committed to improved environmental, health and safety performance through Responsible Care®, common sense advocacy designed to address major public policy issues, and health and environmental research and product testing.

[2] A complete copy of FDA's 2014 report was separately provided to all of the DART IC members. We respectfully request that you take the time to review the set of memoranda before the May 7 public hearing.

- Seven large-scale, comprehensive animal studies with conventional design consistently show that BPA is not a female reproductive toxicant.
- The weight of evidence from animal studies with unconventional design does not provide sufficient evidence to list BPA.
- Extensive metabolism and pharmacokinetic data suggest that female reproductive effects from BPA are not biologically plausible.

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 (202) 249-6624 or by e-mail at steve_hentges@americanchemistry.com.

Respectfully,

Steven G. Hentges, Ph.D.

Attachment

Comments
of the
Polycarbonate/BPA Global Group
American Chemistry Council

In Opposition To The Proposed Listing
of
Bisphenol A
as
"Clearly Shown" To Cause Female
Reproductive Toxicity Under Proposition 65

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Comments in Opposition to Listing Bisphenol A as a Female Reproductive Toxicant
Polycarbonate/BPA Global Group
April 20, 2015

1. Summary

The Developmental and Reproductive Toxicant Identification Committee ("DART IC" or "Committee") will meet on May 7 to consider whether bisphenol A ("BPA") has been "clearly shown through scientifically valid testing according to generally accepted principles to cause … [female] reproductive toxicity," which is the standard established by law under Proposition 65. As evidenced by the extensive Hazard Identification Materials ("HIM") provided by OEHHA, a large amount of relevant data is available to inform the Committee's decision.

Included in the HIM are numerous epidemiology and laboratory animal studies that cover a wide range of study designs and quality. Also included are reports from two authoritative bodies under Proposition 65, neither of which has formally identified BPA as a female reproductive toxicant. As described briefly in this summary, and in detail in the comments that follow, the available information does not provide sufficient evidence to support listing BPA as a female reproductive toxicant according to the statutory definition and the criteria established by the DART IC.

- **FDA, An Authoritative Body, Has Comprehensively Reviewed The Scientific Data On BPA And Does Not Identify Female Reproductive Toxicity As A Hazard**

One of the most significant documents provided in the HIM is a recent report on BPA from the U.S. Food and Drug Administration ("FDA"). This report is significant for the current DART IC proceeding for two reasons.

First, in the course of conducting its safety assessment, which is FDA's ultimate objective, FDA conducted a thorough hazard identification process, which is of direct relevance for Proposition 65 purposes. In this process, FDA applied well-defined hazard identification criteria to review substantially the same data that are now available for review by the DART IC. Detailed reviews of each study are included as part of FDA's extensive documentation. Based on the weight of evidence, FDA then identified hazards to evaluate in its subsequent safety assessment. Notably, FDA did not identify female reproductive toxicity as a hazard.

Second, FDA is designated as an "authoritative body" for purposes of Proposition 65. Had FDA "formally identified" BPA as a reproductive toxicant, OEHHA could have proposed to list BPA based on FDA's assessment, and there would be no reason for the Committee to consider the substance further. Although FDA's view does not bind the Committee, it is worthy of your consideration.

In addition to not formally identifying BPA as a reproductive toxicant, FDA reached a reassuring overall conclusion on safety. Based on its safety assessment, FDA unambiguously stated: "BPA is safe at the current levels occurring in foods."

This recent assessment from FDA is not the first time that an authoritative body has evaluated BPA. In 2008, the NTP-CERHR, which is also an authoritative body, issued its comprehensive report on BPA. As with the more recent FDA report, the NTP-CERHR report also did not identify BPA as a female reproductive toxicant.

The NTP-CERHR report was one of the primary documents reviewed by the DART IC at its July 2009 meeting when it evaluated whether BPA had been "clearly shown" to cause reproductive or developmental toxicity. At that meeting, the DART IC voted unanimously not to list BPA as a male or female reproductive toxicant, or as a developmental toxicant. The challenge for the DART IC in this second consideration of BPA is to evaluate the post-2009 data along with the data that formed the basis for the 2009 DART IC decision in a weight of evidence analysis.

- **Human Data Do Not Support A Finding That BPA Has Been "Clearly Shown" To Cause Female Reproductive Toxicity**

The HIM lists approximately 30 epidemiology studies related to BPA and various aspects of human female reproductive effects. Of the 7 studies available and reviewed by the DART IC in 2009, the Hazard Identification Document prepared by OEHHA for that proceeding reported that "[h]uman studies examining the effects of BPA on reproduction are of limited study design and correspondingly limited in their findings." The NTP-CERHR similarly stated that "[d]rawing firm conclusions about potential reproductive or developmental effects of bisphenol A in humans from these 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."

The epidemiology studies published since 2009 suffer from some of the same study design limitations. Similar to the OEHHA and NTP-CERHR conclusions in 2009, FDA stated in its recent assessment that "[n]o study demonstrated, nor was able to demonstrate based on its design, either a causal or a temporal relationship between exposure and measured outcome, since exposures to BPA were uncontrolled. … Therefore, no single study was able to definitively identify a hazard or exposure level that could be applied to the current BPA RA [risk assessment]." A similar conclusion was reached in a recent report on BPA from the European Food Safety Authority.

None of the human studies individually, nor all of them together, would support a finding that BPA is "clearly shown" to cause female reproductive toxicity. Since the available epidemiological evidence is not sufficient to support listing BPA, any decision to list BPA as a female reproductive toxicant would need to be supported by "sufficient evidence in experimental animals" alone.

- **Animal Data Do Not Support A Finding That BPA Has Been "Clearly Shown" To Cause Female Reproductive Toxicity**

There is an abundance of reproductive toxicity data on BPA from well-conducted studies of conventional design that are aimed at comprehensively evaluating reproductive effects. The

potential effects of BPA on fertility and reproductive performance have been investigated in seven high-quality reproductive toxicity studies in rats and mice, three of which are multi-generation studies that followed internationally validated guidelines. These studies used the oral route of exposure, which is the most relevant for expected human exposures and, thus, the most relevant for Proposition 65 purposes.

With the exception of two more recent studies, these studies were reviewed by the DART IC at its July 2009 meeting, and also in the 2008 NTP-CERHR report. Similar to the views of the DART IC, the NTP-CERHR report noted there was "[s]ome evidence of adverse effects," but only at very high doses that also caused significant maternal toxicity. The two female reproductive effects identified as "some evidence" in the NTP-CERHR report occurred only at dose levels that are more than two orders of magnitude higher than the NOAEL for systemic toxicity in multi-generational studies in rats and mice.

These well-conducted studies show conclusively that BPA is not a selective reproductive toxicant. None of these studies individually, nor all of them collectively, would support a finding that BPA is "clearly shown" to cause female reproductive toxicity.

A larger number of studies of unconventional design have also been conducted on BPA. These studies are often small-scale, screening studies that are conducted on small numbers of animals and may only test one dose level. Most of these studies were conducted at very low doses, often orders of magnitude lower than the NOAELs determined in conventional toxicity studies. Many of the studies use parenteral routes of exposure that are of little relevance for human exposure, and, thus, of little relevance for Proposition 65 purposes. The results from these studies have generally not been replicated or corroborated in independent laboratories.

Government body reviews (e.g., FDA, NTP-CERHR) have consistently regarded these "low-dose" studies as inadequate for a variety of reasons. While these experimental studies may be suitable for formulating hypotheses that can be tested in follow-up studies, they do not provide in their own right a reliable or adequate basis to conclude that BPA is "clearly shown" to cause female reproductive toxicity.

Other studies on BPA have examined molecular level events or other biological observations. Examples include studies on estrogenic activity of BPA, *in vitro* studies, and gene expression studies. The results of these studies are not reproductive effects *per se*, but rather provide potential mechanistic information that may be relevant for any demonstrated female reproductive effects. However, mechanistic studies alone are not a sufficient basis to support a conclusion that BPA is "clearly shown … to cause [female] reproductive toxicity."

- **Pharmacokinetic Data Suggest That Reproductive Effects In Humans Are Not Biologically Plausible And That Direct Extrapolation Of Rodent Data To Humans Is Not Appropriate**

The Committee's Guidance Criteria document notes that metabolic and pharmacokinetic data can "increase or decrease the confidence for classification of any agent as a reproductive

toxicant." For BPA, an abundance of high-quality pharmacokinetic and metabolism data are available from studies in rodents, non-human primates, and humans. Overall, pharmacokinetic differences between species and routes of exposure indicate that extrapolation of animal study results to humans is tenuous and that reproductive effects in humans are not biologically plausible.

The pharmacokinetic data illustrate that humans efficiently metabolize BPA after oral exposure (again, the most relevant route of exposure for humans) via "first-pass" conjugation into non-estrogenic metabolites that are rapidly and completely eliminated from the body through urinary excretion. As a result of this efficient process, BPA does not accumulate in the human body and has low bioavailability.

In contrast, 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, BPA has a longer half-life in rodents compared to humans. In addition, non-oral routes of exposure result in dramatically different pharmacokinetics compared to oral exposure. Because of these differences, the results of rodent toxicity studies using non-oral routes of exposure are of limited relevance to human health hazard identification.

- **BPA Has Not Been "Clearly Shown" To Be A Female Reproductive Toxicant And Should Not Be Listed Under Proposition 65**

Taking into account the weight of evidence, the statutory and regulatory criteria for listing, and the guidance articulated in the Committee's Guidance Criteria, BPA cannot reasonably be characterized as "clearly shown, through scientifically accepted testing conducted according to generally accepted principles to cause … [female] reproductive toxicity." The weight of evidence from human and animal studies, along with the lack of biological plausibility from pharmacokinetic studies, clearly indicates that BPA should not be listed.

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3. FDA, An Authoritative Body, Has Comprehensively Reviewed The Scientific Data On BPA And Does Not Identify Female Reproductive Toxicity As A Hazard

In November 2014, the U.S. Food and Drug Administration ("FDA") released a comprehensive safety assessment on the use of BPA in food contact applications. An integral part of the safety assessment is a detailed hazard assessment, which is of direct relevance for Proposition 65. Of particular significance, ***FDA did not identify female reproductive toxicity as a hazard for BPA***.

a. FDA Is An Authoritative Body For Purposes of Proposition 65

For purposes of Proposition 65, FDA is designated as an "authoritative body" for identifying reproductive toxicants under Proposition 65. Had FDA "formally identified" BPA as a reproductive toxicant, OEHHA could have proposed to list BPA based on FDA's assessment, and there would be no reason for this Committee to consider the substance further.

b. FDA's 2008 Assessment Confirmed That BPA Should Not Be Listed

In 2008, FDA released a comprehensive assessment of the safety of BPA for use in food-contact applications.[1] Additional updates were released by FDA in 2009. These reviews built on the 2008 NTP-CERHR Report discussed below, and included reviews of "low-dose" studies that had been published after the NTP-CERHR Report was published.

Based on the studies reviewed for the 2008 assessment, and similar to conclusions reached by other government bodies, "FDA concluded the appropriate no observed adverse effect level ("NOAEL") to be the NOAEL for ***systemic toxicity*** of 5 mg/kg bw/day … derived from two multigenerational rodent studies."[2] The 2008 assessment further noted that "the available data and information were insufficient to support other endpoints … as a point of departure for estimating margins of safety." Thus, the FDA assessment was in agreement, and supported the DART IC decision in 2009 that BPA should not be listed (see below).

c. FDA's 2014 Assessment Further Confirms That BPA Should Not Be Listed

Since January 2011, FDA has continued to systematically evaluate new studies on BPA through its BPA Joint Emerging Science Working Group ("Working Group"), which "performs systematic reviews of literature available on BPA using clearly defined criteria."[2] These evaluations have been well documented in a series of detailed memoranda. For its most recent memorandum, the working group consisted of 38 scientific experts drawn from throughout the FDA organization.

We have provided paper copies of FDA's 2014 report, through OEHHA, to all of the Committee members. The report includes the detailed memoranda that support FDA's overall

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[1] Since the primary source of human exposure to BPA is through the diet, FDA's primary focus has been on the use of BPA in food-contact applications.

[2] 2014 Updated safety assessment of Bisphenol A (BPA) for use in food contact applications. FDA Memorandum dated June 17, 2014 (emphasis added).

evaluation of BPA hazards and safety. Highly relevant for DART IC is FDA's systematic hazard assessment of BPA for all relevant toxicological endpoints. Along with an assessment of exposure to BPA, the hazard assessment supports FDA's November 2014 conclusion: "***FDA's current perspective, based on its most recent safety assessment, is that BPA is safe at the current levels occurring in foods***."[3]

d. The Recent FDA Assessment Did Not Identify Female Reproductive Toxicity As A Hazard

One of the key questions addressed by FDA's Working Group is directly relevant to Proposition 65: "what hazards should be added or removed from FDA's continuing review/research evaluation?" As part of its process to identify hazards, "[t]he Working Group reviewed studies employing various routes of administration for purpose of ***hazard assessment*** determination …"[2] and drew conclusions regarding hazards based on the weight of evidence.

The FDA Working Group "considered a study useful for hazard identification if it met the criteria detailed by the CERHR as an adequate study." This approach ensured continuity from the 2008 NTP-CERHR Report throughout the FDA assessment process.

Since its formation in early 2011, FDA's Working Group has issued a series of three detailed memoranda that "constitute a progressive series of evaluations with each subsequent memorandum building on the conclusions from the previous memoranda."[2] The most recent memorandum from the Working Group "can be considered as the current [2014] state of the science evaluation for use in Center specific product assessments"[2] and "the most current … hazard characterization of BPA."

In its ongoing evaluation of new studies, "[t]he Working Group did identify information relevant to hazard identification according to the Working Group review criteria; however, the Working Group qualified these hazard endpoints as 'maintained with low confidence due to study limitations, conflicting reports, and current understanding of potential for unintended exposure or contamination.'"[2,4]

Specifically, these low confidence hazard endpoints are "developmental neurotoxicity related to molecular or neuroanatomical endpoints with varying routes of administration, cardiovascular disease-related factors based on human epidemiology studies, and sperm/testicular/hormone related parameters based on very limited supporting animal data." Notably, the FDA Working Group ***did not identify female reproductive toxicity as a BPA hazard***, even with low confidence.

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[3] Bisphenol A (BPA): Use in Food Contact Application, http://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm064437.htm. (Emphasis added in quotations above)

[4] For the reasons mentioned briefly here, and in more detail in FDA's safety assessment documentation, it is highly unlikely that the studies supporting these low confidence hazards could satisfy the Proposition 65 requirements for "clearly shown, through scientifically accepted testing conducted according to generally accepted principles to cause" reproductive or developmental toxicity." It also is important to note that additional research is underway in FDA's laboratory that should help further our understanding of the potential for BPA to be a reproductive toxicant.

This finding from FDA is particularly noteworthy for purposes of Proposition 65 since FDA is designated as an authoritative body.

Overall, the most recent 2014 hazard assessment "reconfirms the previously identified NOAEL of 5 mg/kg bw/day for ***systemic toxicity*** from two multigenerational studies using rodents as the most appropriate NOAEL for a safety assessment of oral exposures. Available pharmacokinetic data and comparisons between ages and species further support use of this NOAEL as very conservative in extrapolating to humans."[2,5]

e. FDA's Findings On BPA Are Consistent With Those Of Other Government Bodies Worldwide

In January 2015, the European Food Safety Authority ("EFSA") released its comprehensive re-evaluation of BPA exposure and toxicity. The report thoroughly documents EFSA's expert panel review of many hundreds of studies and its overall weight of evidence conclusions on hazard identification. Overall, EFSA concluded that "BPA poses no health risk to consumers of any age group (including unborn children, infants and adolescents) at current exposure levels."[6]

The hazard conclusion from EFSA on the likelihood of reproductive effects of BPA in humans was "unlikely." Hazard conclusions were expressed on a standard scale with "unlikely" being the 6th level on a 7-level scale ranging from "very likely" to "very unlikely." Regarding reproductive and developmental toxicity in animals, the EFSA report stated: "[u]sing a WoE approach, the CEF Panel assigned a likelihood level of 'as likely as not' to reproductive and developmental effects of BPA at low doses," which were the primary focus of the evaluation.[7] The "as likely as not" conclusion is the 4th level on the 7-level scale.

The EFSA assessment, like FDA's recent assessment and previously the NTP-CERHR assessment, indicates that the underlying studies do not satisfy the Proposition 65 standard for "clearly shown, through scientifically accepted testing conducted according to generally accepted principles to cause" female reproductive toxicity.

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[5] Systemic effects observed at the LOAEL include reduced body weight gain and target organ effects in the liver and kidney.

[6] EFSA press release, January 21, 2015, http://www.efsa.europa.eu/en/press/news/150121.htm.

[7] In addition to the extensive evaluation and discussion of studies that examined low doses, EFSA referred to previous reports that discussed effects at very high doses observed in the presence of significant maternal toxicity. These are the same studies and high-dose effects previously reviewed by NTP-CERHR. Based on the same high-dose effects, BPA is currently classified for reproductive toxicity in Europe under category 2 (suspected) and may be reclassified under category 1B (presumed) according to EU guidance on new GHS classification criteria. BPA does not meet the criteria for category 1A (known).

4. The DART IC Recognized In 2009 That The Available Data Did Not Support Listing BPA As A Female Reproductive Toxicant

The DART IC considered BPA for listing at the DART IC meeting on July 15, 2009. At that meeting, the DART IC voted unanimously not to list BPA as a male or female reproductive toxicant, or as a developmental toxicant. As the data reviewed by the DART IC in 2009 must also be considered as part of the current weight-of-evidence review, it is important to understand why BPA was determined not to be a female reproductive toxicant in 2009.

a. The DART IC's Conclusions Were Consistent With The Conclusions Of The NTP-CERHR Report

One of primary documents that the DART IC considered in 2009 was the comprehensive NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A ("NTP-CERHR Report"). Like FDA, the NTP-CERHR is also designated as an "authoritative body" for identifying reproductive toxicants under Proposition 65. The formal identification of a chemical as a reproductive toxicant by an authoritative body can be the basis for a Proposition 65 listing. In its report, NTP-CERHR ***did not formally identify*** BPA as causing female reproductive toxicity.

To the contrary, the NTP-CERHR Report reached a number of reassuring conclusions with respect to BPA. Regarding human studies, the report 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."[8]

Regarding animal studies, the NTP-CERHR Report extensively discussed studies of traditional designs carried out to assess the toxicity of BPA. These studies used the oral route of administration, which is most relevant for expected human exposures and, thus, most relevant for Proposition 65 purposes. From these studies, which were considered to be of "high utility" by the NTP-CERHR expert panel, the NTP-CERHR Report noted there was "[s]ome evidence of adverse effects," but only at very high doses that also caused significant maternal toxicity. Specifically for female reproductive toxicity, the report noted "possible decreased fertility in mice (≥ 875 mg/kg bw/day)" and "altered estrous cycling in female rats (≥ 600 mg/kg bw/day)."[9,10] It is important to note that these dose levels are more than two orders of magnitude higher than the NOAEL for systemic toxicity in multi-generational studies in rats and mice.

Based on human exposure data provided in the report, the female reproductive effects in rats and mice were observed at dose levels that are more than 40,000 times higher than worst case estimates of potential daily intakes of BPA in infants and children less than 6 years of age. The differences in exposure are much greater, more than 1,900,000 times higher, when the dose

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[8] NTP-CERHR Report, NTP Brief at p. 9.
[9] NTP-CERHR Report, NTP Brief at p. 8.
[10] The NTP-CERHR Report characterized evidence of adverse effects on a 7-level scale with "some evidence" being the second level

levels are compared to estimated daily intakes for children ages 6-11 and adult women at the 95th percentile.[11]

The NTP-CERHR Report also discussed the diverse range of "low-dose" studies (defined by NTP-CERHR as < 5 mg/kg bw/day), many of which used parenteral routes of administration, which are of little or no relevance to expected human exposures. In general, the NTP-CERHR Report noted that many studies "were designed not as toxicology studies but rather to probe very specific experimental questions, and their results are not always easily interpreted with regard to how they contribute to the weight-of-evidence for human health risks."[8] Many of the studies had "technical or design shortcomings or their reports do not provide sufficient experimental details to permit an assessment of technical adequacy."[8]

More specifically, the NTP-CERHR expert panel stated that "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."[12] The expert panel concluded that many of these studies are "inadequate." Accordingly, it is highly unlikely that they would satisfy the Proposition 65 standard for "scientifically valid testing according to generally accepted principles."[13]

Based on the weight of evidence, the NTP-CERHR Report concluded for reproductive toxicity overall that there is "[n]egligible concern for adverse effects" in non-occupationally exposed adult men and women.[9,14]

b. The NTP-CERHR Conclusions Are Consistent With The Conclusions Of Numerous Government Bodies Worldwide

Along with NTP-CERHR, various other government bodies worldwide also conducted their own independent and comprehensive assessments of BPA at approximately the same time. Most prominently, these bodies include FDA, which published a report in 2008;[15] EFSA (reports published in 2006[16] and 2008[17]); the European Commission (report published in 2008[18]); Health

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[11] Relevant exposure data summarized in the NTP-CERHR Report, NTP Brief at p. 36.
[12] NTP-CERHR Report, Chapin *et al*., at p. 382.
[13] For a discussion of the standards for listing under Proposition 65, please see Section 6.
[14] The conclusions of the NTP-CERHR Report are expressed on a 5-level scale with "negligible concern for adverse effects" being the lowest level.
[15] U.S. Food and Drug Administration, Draft Assessment of Bisphenol A for Use in Food Contact Applications, August 2008.
[16] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A). The EFSA Journal (2006) 428.
[17] 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.
[18] UPDATED RISK ASSESSMENT OF 4,4'-ISOPROPYLIDENEDIPHENOL (Bisphenol-A). April 2008.

Canada (report published in 2008[19]); and the Japanese National Institute of Advanced Industrial Science and Technology (report published in 2005[20]).

All of these assessments included thorough reviews of the potential for BPA to cause reproductive or developmental toxicity. None of these government bodies reached a conclusion that would support listing BPA as "clearly shown, through scientifically valid testing according to generally accepted principles to cause … reproductive toxicity," which is the standard established by law under Proposition 65.[13]

In general, each of these government bodies had views similar to those of NTP-CERHR on the quality and utility of available human and low-dose animal studies. As a further point of similarity to NTP-CERHR, each of these bodies also found that the studies of highest utility were studies of traditional designs carried out to assess the toxicity of BPA. These studies were used as the basis for the overall conclusions that were reached in each assessment.

5. Evaluating The Post-2009 Data Along With The Data On Which The 2009 DART IC Decision Was Based

Since the 2009 DART IC decision, research on BPA has continued. As new studies have become available, government bodies worldwide also have continued to evaluate BPA. An overview of those reviews follows below, and demonstrates that the more recent studies often have the same limitations and deficiencies that were identified in 2009. Most importantly, none of the major government bodies that have reviewed BPA have reached any conclusion that would support listing BPA as a female reproductive toxicant under Proposition 65.

The challenge for the DART IC in this second consideration of BPA is to evaluate the post-2009 data along with the data that formed the basis of the 2009 DART IC decision. For reasons discussed below, the same stringent standard for listing and strict criteria for evaluating the data that were applicable in 2009 should be applicable in the current proceeding. In essence, the Committee must determine whether the overall weight of evidence, *considering all of the data*, demonstrate that BPA is "clearly shown, through scientifically valid testing according to generally accepted principles to cause … [female] reproductive toxicity."[13]

[19] Screening Assessment for the Challenge Phenol, 4,4' -(1-methylethylidene)bis- (Bisphenol A). October 2008.
[20] Bisphenol A Risk Assessment Document (AIST Risk Assessment Document Series No. 6). November 2005.

6. The Standards Under Proposition 65, Its Implementing Regulations, And The Committee's Guidance Criteria Authorize Listing Only If A Chemical Is "Clearly Shown" To Cause Reproductive Toxicity By Reliable Testing Data

The standard that this Committee must apply to determine 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 …"[21]

It is this Committee's task to determine whether this stringent statutory standard has been met in the case of BPA, specifically for female reproductive toxicity.

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."[22]

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."[23]
>
> "In determining whether a chemical is to be … listed as known … to cause reproductive toxicity, the ***biological plausibility*** of an 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 chemical. Conversely, confidence is decreased if the observed adverse effects are contradictory to the known characteristics of the particular chemical."[24]
>
> "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 …

[21] Health & Safety Code § 25239.8(b) (emphasis added).
[22] Cal. Code Regs., tit. 27 § 25305(b)(1) (emphasis added).
[23] Guidance Criteria at 1.D. (emphasis added).
[24] Guidance Criteria at 4.B. (emphasis added).

(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."[25]

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 human" 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."[26]

As discussed below, the available epidemiological evidence is not sufficient to support listing BPA. Therefore, ***any decision to list BPA as a female reproductive toxicant would need to be supported by "sufficient evidence in experimental animals" alone***. Whether animal studies are "sufficient evidence" to support extrapolation to humans, in most cases, is based on the adequacy of 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***, and in terms of timing, with regard to … sexual maturation, stage of pregnancy, or other critical periods for female reproductive toxicity …

(3) Number of dose levels, so that the presence of a ***dose-response relationship*** can be evaluated. It is desirable that the high dose level should elicit … systemic toxicity in female … reproductive studies, and that the low dose should elicit no observable adverse effect for adult and offspring.

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

(5) ***Number of tests or experimental animal species.***

(6) ***Other considerations***, including, but not limited to those listed below, which can increase or decrease the confidence for classification of an agent as a reproductive toxicant.

a. Severity or consistency of findings.

b. Metabolic and pharmacokinetic data.

c. Time course of events."[27]

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

[26] Guidance Criteria at 3.A.(1) (emphasis added).

[27] Guidance Criteria at 3.C(1)-(6) (emphasis added).

7. Pharmacokinetic Data Suggest That Reproductive Effects In Humans Are Not Biologically Plausible And That Direct Extrapolation Of Rodent Data To Humans Is Not Appropriate

As noted above in the Committee's Guidance Criteria (DART IC, 1993), metabolic and pharmacokinetic data can "increase or decrease the confidence for classification of an agent as a reproductive toxicant." In the case of BPA, an abundance of high-quality pharmacokinetic and metabolism data from studies in rodents, non-human primates, and humans demonstrate that humans metabolize and eliminate BPA more efficiently and rapidly than rodents, making direct extrapolation of rodent data to humans inappropriate.

Of particular importance are a series of studies conducted in FDA's own laboratory, the National Center for Toxicological Research (NCTR), over the last 5 years. This set of studies was specifically designed to generate a coherent set of data to support FDA's assessment of BPA. These studies have significantly reduced uncertainty regarding the potential for BPA to cause health effects.

A clear understanding of these data is critical to the determination of whether exposure to humans could result in female reproductive effects. Overall, pharmacokinetic differences between species and routes of exposure indicate that extrapolation of animal study results to humans is tenuous and that reproductive 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 rapidly and completely eliminated from the body through urinary excretion. Accordingly, serum concentrations of parent BPA in well-controlled human studies are far below levels that could result in estrogenic effects (Teeguarden *et al*., 2013).

In contrast, 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, BPA has a longer half-life in rodents compared to humans.

Finally, non-oral routes of exposure result in dramatically different pharmacokinetics compared to oral exposure. The consequences may 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 of limited relevance with respect to human health hazard identification.

a. BPA Metabolites Are Inactive And Non-Estrogenic

The primary metabolite of BPA is BPA-glucuronide, which has been shown to exhibit no estrogenic activity (Matthews *et al*., 2001). A secondary metabolite, BPA-sulfate, may be formed at lower levels but 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 systemic circulation following oral exposure have no known biological activity and, in particular, are not estrogenic. As noted above, the metabolites are rapidly eliminated from the body in urine with a terminal half-life of less than 6 hours.

b. Humans Efficiently Metabolize And Rapidly Eliminate BPA After Oral Exposure

Following oral exposure, BPA is efficiently conjugated, primarily to BPA-glucuronide (Teeguarden *et al*., 2015; Völkel *et al*., 2002). This biotransformation occurs both as BPA is absorbed through the intestinal wall and then in the liver before any significant levels of parent BPA (< 1% of the administered dose) can enter systemic circulation in the bloodstream (Fisher *et al*., 2011). 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 for BPA in the metabolic capacity of the intestine and the liver to prevent any toxicologically significant amount of parent BPA from entering systemic circulation.

The BPA-glucuronide metabolite, which is not estrogenic, is then rapidly eliminated from blood through urinary excretion with a terminal half-life of less than 6 hours. Elimination of the administered dose is complete within 24 hours post-ingestion (Teeguarden *et al*., 2015; Völkel *et al*., 2002).

The hypothesis that BPA may be sublingually absorbed during oral exposure, thereby bypassing efficient first-pass metabolism, was disproven in a high-quality human study (Teeguarden *et al*., 2015). Pharmacokinetic parameters, pharmacokinetic model simulations, and the significantly faster appearance half-life of BPA-glucuronide compared to parent BPA all provide evidence against meaningful absorption through any non-metabolizing tissue (< 1%) and no evidence for meaningful sublingual absorption.

The human pharmacokinetic data are very consistent with data from studies on non-human primates. In adult rhesus monkeys, concentration-time profiles following oral administration of BPA were remarkably similar to those previously reported in human volunteers given a similar dose (Doerge *et al*., 2010a).

Administration of BPA to pregnant rhesus monkeys resulted in serum pharmacokinetics that were quantitatively similar to those reported for non-pregnant female monkeys. In both pregnant and non-pregnant monkeys, oral administration resulted in low systemic bioavailability (< 1% of the administered dose). Internal exposures of the fetus to parent BPA were attenuated by maternal, placental, and fetal metabolism to less than half that in the dam. Levels of parent and conjugated BPA in whole placenta were consistent with a role in metabolic detoxification. These results indicate that the metabolism and pharmacokinetics of BPA are not altered during pregnancy and that internal exposures of the mother and fetus to parent BPA are very low (Patterson *et al*., 2013).

c. Non-Oral Routes Of Exposure Are Irrelevant To Humans

Experimental systems that use non-oral routes of exposure (i.e., parenteral routes) can result in blood BPA concentrations that are higher than those obtained when BPA is given via oral routes. Non-oral routes of exposure bypass the rapid and virtually complete first-pass presystemic conjugation that BPA undergoes in the intestine and liver following oral exposure. As a result, systemic concentrations of parent BPA are much higher and there is some evidence that the identity and distribution of metabolites are different (Zalko *et al*., 2003).

Because oral exposure is the relevant route of exposure for humans, toxicity studies involving non-oral routes of exposure are of limited relevance for assessing the hazards of BPA in humans. Government bodies worldwide that have evaluated BPA, including FDA and NTP-CERHR, have consistently applied this principle to hazard identification and risk assessment.

d. Human And Non-Human Primate Neonates Metabolize BPA Efficiently

A study in adult and neonatal rhesus monkeys showed only minimal pharmacokinetic differences between adult and neonatal monkeys for parent BPA. In both cases, circulating concentrations of parent BPA were < 1% after oral administration. In addition, no age-related changes in internal exposure metrics for parent BPA were observed. These results show that the enzymes necessary for metabolism of BPA (UDP glucuronosyl transferases) are well developed at birth and that infants and children are capable of efficiently metabolizing BPA (Doerge *et al*., 2010a).

A study on exposure of premature human infants to BPA demonstrated that BPA was eliminated through urine almost entirely in the form of conjugated BPA. These results show that premature human infants, similar to term infants, have the capability to metabolize and eliminate BPA (Calafat *et al*., 2003).

In contrast, studies on developing rats showed significant inverse age-related changes in measures of internal exposure to parent BPA and its elimination. The deficiencies observed in neonatal rats are due to immaturity of Phase II metabolism and renal excretion (Doerge *et al*., 2010b). These differences between developing rats and primates support the concept that any toxicological effects observed in rats from early postnatal exposures to BPA are likely to over-predict those possible in primates of the same age.

e. BPA Does Not Accumulate In The Human Body And Has Low Bioavailability

As discussed above, studies in humans and non-human primates consistently demonstrate that BPA has very low bioavailability, and very low bioactivity, due to efficient first-pass metabolism. In addition, the rapid elimination of conjugated BPA metabolites through urine indicates that BPA does not accumulate in the human body. Complete elimination of orally administered BPA within 24 hours post-ingestion provides evidence against the idea that there are any tissue depots for BPA (Teeguarden *et al*., 2015; Völkel *et al*., 2005, 2002).

A study in mice measured the pharmacokinetics of parent BPA in adipose tissue, which has been proposed as a depot for BPA. Parent BPA rapidly distributed out of circulation and the level of parent BPA in adipose tissue rapidly reached a maximal level in 0.25 hours that was below the serum maximum at the initial sampling time of 0.08 hours. The half-life for terminal elimination of parent BPA from adipose tissue (7.0 hours) was similar to that for conjugated BPA (6.6 hours) and < 0.01% of the administered dose remained in adipose tissue after 24 hours. The pharmacokinetic data are consistent with rapid equilibria and support the non-persistent nature of BPA (Doerge *et al*., 2012).

8. Human Data Do Not Support A Finding That BPA Has Been "Clearly Shown" To Cause Female Reproductive Toxicity

Studies of the potential reproductive effects of BPA in humans are extensive, and most of these studies have been reviewed by FDA and EFSA in their recent assessments. None of these studies individually, nor all of them together, support a finding that BPA is "clearly shown" to cause female reproductive toxicity.

a. Human Data Do Not Demonstrate That BPA Causes Reproductive Toxicity

Many of the human studies that attempt 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. Indeed, a standard epidemiology textbook acknowledges that cross-sectional studies have made significant contributions to public health for factors that are unchanging (e.g., race) or persistent (e.g., levels of lead in blood). The same textbook also cautions of a critical problem with cross-sectional data: "current exposure may have little relation to exposure during the time etiologically relevant to current disease" (Rothman and Greenland 1998). Because of the lack of a temporal connection between exposure and health effects, cross-sectional studies are incapable of establishing causality.

This limitation is prominent in studies on BPA, which has a very 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, or even at a different time of day.

Human exposure to BPA is most commonly measured by analysis of urine spot samples. Numerous studies in the last several years have evaluated the reproducibility of urinary BPA levels over time periods ranging from days to years. For example, Townsend *et al*. (2013) measured urinary BPA levels in two spot samples collected from each of 80 women over a 1-3 year time period. Temporal variability was assessed by calculating an intraclass correlation coefficient (ICC), which reflects the relationship between within- and between-person variance. The ICC can have a value between 0 and 1, with higher values indicating low within-person variance.

The study found high within-person variability of urinary BPA levels, with an ICC value of 0.14, meaning there is little correlation between BPA levels in spot urine samples collected 1-3 years apart. The study found slightly less, but still high, variability for samples taken < 25 months apart (ICC = 0.23) compared to samples taken > 25 months apart (ICC = 0.06), the latter samples showing almost no correlation at all. The implications of high variability for epidemiologic studies are quite significant. As stated by the authors, "investigation of associations between a single urinary bisphenol A measurement and disease risk may be

challenging in epidemiologic studies." Similar results have been reported in other studies that examined variability over time periods of days to years (for example, Lassen *et al*., 2013).

This same issue is relevant for studies with prospective design. Urine spot samples are reflective of BPA exposure at an instant in time, but provide very limited information on exposure at earlier or later times. Although less common in epidemiological studies, the same problem occurs with measurement of BPA in blood due to the short half-life of BPA in the human body.

i. Data And Evaluations Available To DART IC In 2009

The Hazard Identification Materials (HIM) that OEHHA provided to the DART IC for the current proceeding identify approximately 30 epidemiology studies related to BPA and various aspects of human female reproductive effects.[28] Of these studies, 7 were available and reviewed by the DART IC as part of the Hazard Identification Document (HID) that OEHHA prepared in 2009, as well as in the NTP-CERHR Report.[29]

Six of these studies used a cross-sectional study design and cannot provide sufficient evidence of causality.[30] Four of the six studies used the ELISA (i.e., enzyme-linked immunosorbent assay) method for measuring BPA in blood, which is unsuitable for this purpose because it does not positively identify BPA and it cross-reacts with other substances (e.g., phytoestrogens) likely to be present (Fukata *et al*., 2006). The single case-control study (Sugiura-Ogasawara *et al*., 2005) has several critical limitations including use of the ELISA method and the lack of an appropriate temporal relationship between the timing of BPA measurements and subsequent miscarriage or successful pregnancy.

The OEHHA 2009 Hazard Identification Document stated that "[h]uman studies examining the effects of BPA on reproduction are of limited study design and correspondingly limited in their findings."[31] Similarly, the NTP-CERHR Report stated that "[d]rawing firm conclusions about potential reproductive or developmental effects of bisphenol A in humans from these 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."[32] The NTP-CERHR Report further concluded that the "NTP concurs with findings of 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."[32]

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[28] The studies are listed in the OEHHA document titled "References on the Female Reproductive Toxicity of Bisphenol A provided to the Developmental and Reproductive Toxicant Identification Committee (DARTIC), February 2015."
[29] The HIM also identified two studies (Hanaoka *et al*., 2002; Li *et al*., 2010) that examined male workers. These studies are not relevant to the current proceedings on female reproductive toxicity and are not discussed here.
[30] These studies are Takeuchi and Tsutsumi, 2002; Takeuchi *et al*., 2004; Hiroi *et al*., 2004; Yang *et al*., 2006; Itoh *et al*., 2007; Wolff *et al*., 2008).
[31] OEHHA, 2009 HID at p. 66.
[32] NTP-CERHR Report, NTP Brief at p. 15.

ii. Data And Evaluations Available After The 2009 DART IC Meeting

Subsequent to the 2009 DART IC meeting, approximately 20 additional epidemiology studies related to BPA and various aspects of female reproductive effects have been published (see HIM provided by OEHHA).[29] As discussed in Section 3 above, FDA has continued to systematically evaluate new studies on BPA through its BPA Joint Emerging Science Working Group, which "performs systematic reviews of literature available on BPA using clearly defined criteria."

For evaluation of epidemiology studies, the criteria considered several key elements. These elements are briefly summarized below, and the full set of criteria is provided in Appendix 1 of these comments.

- Utility of study design … sufficiency of study size … use of multi-geographical approaches; adherence to proper statistical analyses.
- Measurement of BPA exposure and outcome metrics … possible environmental contamination … interference by other biological compounds.
- Appropriate treatment of the data with regard to non-detectables … adjustment of urinary concentrations … potential misclassification of LOD values … consideration of current state-of-the-science with regard to measurement of BPA in different biological matrices.
- Other factors: potential biologically plausible reverse causation; unconsidered confounders, risk factors and effect modifiers; and ascertainment of the correspondence between the measurement time and the relevant exposure window …

As described by FDA, "[e]ach study was reviewed for its ability to inform the HI [hazard identification] and RA [risk assessment] process for BPA. Links to animal data were used to understand generalization to or justification of the use of specific endpoints."

The evaluations of these epidemiology studies have been thoroughly documented in the series of detailed memoranda from FDA, discussed above. A synopsis of the summary comments on epidemiology studies from FDA's most recent memorandum is presented below and a complete copy of the summary comments is provided in Appendix 2. Complete copies of the summary comments from two previous memoranda are provided in Appendix 3 and Appendix 4. Detailed reviews of each individual study are provided in the three memoranda.

Of the studies reviewed by FDA, most were based on a cross-sectional study design. A variety of endpoints were examined and no two studies measured endpoints in an identical fashion. No associations were found in some studies while weak or possible associations/correlations were reported in other studies. The limitations in study design and analysis/interpretation that were found in these studies are significant. Rather than attempt to summarize FDA's analysis, we quote directly from the FDA memorandum below:

> "Critical review of the studies indicated significant limitations in study design that made the claims of association questionable or unsupportable. In some cases, an association was reported for transformed, but not the original, data, which casts further doubt on the

legitimacy of the conclusion. The most common limitations in study design included use of plastic sample containers of uncertain BPA leaching; small study sample size; use of a single exposure measure (*e.g.*, single spot urine); use of plasma concentration as measure of exposure; absence of control for possible confounders (*e.g.*, diet, BMI, age, race); lack of control for multiple statistical tests within study (favors false positives); inadequate description of timing of sample collection; lack of clinical significance of measured outcome (*e.g.*, decreased T4 without compensatory increase in TSH); use of highly variable outcome (gestational age); arbitrary/nonstandard definition of clinical outcome (*e.g.*, albuminuria); or inability to separate contribution of BPA from association with other measured compounds in a mixture."

"Major limitations in study analysis/interpretation included selective reporting of positive results; absence of dose (concentration)-response relationships; inconsistent within-study or between-study (*e.g.*, diabetes, serum T4/T3/TSH) results; equating a reported association/trend for transformed data (*e.g.*, square root of percentage breast density) or parameter estimates with an association for the original study data; lack of generalizability; possibility of reverse causation (*e.g.*, increased levels of lipophilic BPA may be the effect, not cause, of adiposity/obesity). NHANES data were explored in several reviewed studies, but as pointed out by LaKind *et al.* (2012), the use of NHANES data has given contradictory results in the past and may not be appropriate for drawing conclusions about BPA and chronic complex diseases due to the cross-sectional structure of that dataset. Reported associations often disappeared with addition of covariates/possible confounders."

"Our review indicated that ***no single study was able to make a definitive contribution to HI [hazard identification] or RA [risk assessment***]. No study demonstrated, nor was able to demonstrate based on its design, either a causal or a temporal relationship between exposure and measured outcome, since exposures to BPA were uncontrolled. And statistical techniques (*e.g.*, correlation analysis, linear regression), which attempt to quantify *mathematical* relationships between BPA exposure and outcome, are unable to test causal, *scientific* hypotheses. Therefore, no single study was able to definitively identify a hazard or exposure level that could be applied to the current BPA RA. Moreover, it would be inappropriate to selectively choose an isolated result, either positive or negative, out of its context." (emphasis added)

The summary comments from each of the three memoranda are similar. The available epidemiology studies relevant to female reproductive toxicity all have significant limitations that make them unsuitable, either individually or collectively, for hazard identification. These studies do not support a finding that BPA is "clearly shown" to cause female reproductive toxicity.

iii. The Recent EFSA Review Of BPA Concurs That Human Studies Do Not Show A Causal Link Between Exposure And Reproductive Effects

The European Food Safety Authority released its comprehensive review of BPA in January 2015.[33] Summary comments on its review of epidemiology studies, provided below, are very similar to the FDA comments above.

> "In their 2010 EFSA opinion, the CEF Panel concluded that the studies then available were not sufficient to draw any conclusion regarding BPA exposure and reproductive and developmental effects in humans. This conclusion was based on studies limited by mostly cross-sectional design, small sample size and other methodological weaknesses. Since then, a number of studies have been reported, but the limitations noted in the previous opinion are still prevalent. Of 22 new studies, only six had a prospective design. Some of the new studies were well powered (i.e. Galloway et al., 2010; Li et al., 2011; Miao et al., 2011a), but had large uncertainty in either exposure or outcome assessment. There are indications from several prospective studies that BPA exposure during pregnancy may have effects on fetal growth (two studies showed reduced fetal growth with increasing maternal BPA exposure, while one study reported increased fetal growth). There are also weak indications that BPA exposure during pregnancy may be associated with maternal and infant thyroid function. It cannot be ruled out, however, that these results are confounded by diet or concurrent exposure factors. ***The associations do not provide sufficient evidence to infer a causal link between BPA exposure and reproductive effects in humans***." (emphasis added)

In addition, EFSA noted that studies on women undergoing in vitro fertilization (IVF) treatment cannot be directly extrapolated to all infertile women or to the broader population of fertile women:

> "Of seven studies examining BPA exposure in relation to adult reproductive outcomes, five examined indicators such as embryo quality, fertilization and implantation failure in couples undergoing in vitro fertilization (IVF). The causes of infertility are very different in those who present for IVF compared with both fertile couples and couples seeking investigations and treatment for infertility. Notwithstanding the benefits of those undergoing IVF from a research practicability point of view, caution must therefore be exercised when extrapolating from an IVF study group to the general infertility population, let alone to the fertile population (Hull et al., 1985; Maheshwari et al., 2008; HFEA, 2011)."

The overall hazard conclusion from EFSA on the likelihood of reproductive effects of BPA in humans was "unlikely," which is the 6th level on a 7-level scale ranging from "very

[33] Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: PART II - Toxicological assessment and risk characterization. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). January 2015. p. 76.

likely" to "very unlikely." The EFSA's review of the available epidemiology studies does not support a finding that BPA is "clearly shown" to cause female reproductive toxicity.

9. Animal Data Do Not Support A Finding That BPA Has Been "Clearly Shown" To Cause Female Reproductive Toxicity

As in the case of human studies, none of the studies of BPA for reproductive effects in animals, nor all of them together, would support a finding that BPA is "clearly shown" to cause female reproductive toxicity. Most of the animal studies also have been reviewed by FDA and EFSA in their recent assessments.

All of the well-conducted conventional studies were designed to evaluate effects on reproduction are discussed separately in considerable detail below. The more numerous studies with diverse experimental designs are discussed in summary form rather than individually.

a. 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 studies of conventional design. The potential effects of BPA on fertility and reproductive performance have been investigated in seven 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 a two-generation study in the mouse); a second two-generation study in the mouse, a one-generation reproductive toxicity study in mice; the NTP continuous breeding study in mice; and a large-scale subchronic study conducted by FDA. None of these studies individually, nor all of them collectively, would support a finding that BPA is "clearly shown" to cause female reproductive toxicity.

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

This study evaluated BPA for reproductive toxicity from dietary exposure to three generations of CD Sprague-Dawley rats. The study was conducted according to U.S. EPA OPPTS test guidelines, with additional assessments beyond the guideline requirements, and it is regarded as one of the largest and most comprehensive multi-generation reproductive toxicity studies ever conducted for any substance. This study has been 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: Dietary (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 males and females per dosing level
- Endpoints examined: Multiple
- Genders evaluated for systemic toxicity: Maternal and paternal

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,[34] 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. A decrease in litter size and pup weight was observed in all three generations at the highest dose (500 mg/kg bw/day), which produced significant systemic toxicity.

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."[35] Importantly, if BPA was acting as an estrogen, 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.[36] The study concluded, however, that the "effects reported in F2 female AGD [anogenital distance] are considered of no biological significance and not due to BPA exposure."[37] 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."[37]

In summary, BPA did not cause selective reproductive toxicity in this study. The study showed 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 dose responses as claimed in some of the studies with unconventional design. The study concluded that "***BPA should not be considered a selective reproductive toxicant, based on the results of this study***."[38]

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[34] For completeness in describing the study results, male and female outcomes are presented here. Only the female reproductive outcomes are relevant for the current proceeding.
[35] Tyl *et al*., 2002a at p. 138.
[36] Anogenital distance was not measured in the F1 pups.
[37] Tyl *et al*., 2002a at p. 138.
[38] Tyl *et al*., 2002a at p. 121 (emphasis added).

ii. 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 OECD 416 guideline.

Strengths of this study include:

- Route of administration: Oral
- Number of dose levels: Four (0.2, 2, 20, 200 µg/kg bw/day)
- Number of animals: 25 males and females per dosing level
- Endpoints examined: Multiple (including a wide variety of hormonally sensitive behavioral measurements

Consistent with the three-generation rat study above, 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); neither were there non-monotonic dose responses, as claimed in some of the studies with unconventional design.

iii. Two-Generation Reproductive Toxicity Study In Mice (Tyl *et al*., 2008a)

A two-generation reproductive toxicity study of BPA was performed in CD-1 mice by Tyl *et al*. (2008a). The EU Risk Assessment Report of 2008 (EU RAR) refers to this study as the "gold standard."[39] 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*., 2008b) and a full two-generation (Tyl *et al*., 2008c) reproductive toxicity study on 17β-estradiol, which was used as a positive control in the BPA study.

Strengths of this study include:

- Route of administration: Dietary
- Number of dose levels: Six (verified dietary concentrations ranging from 0.018 to 3500 ppm)
- Number of animals: 28 males and females per dosing level
- Endpoints examined: Multiple, including thorough histological evaluation
- Positive control: 17β-estradiol
- Number of negative control groups: Two

[39] EU RAR, 2008 at p. 86 and 128 in Part II Human Health.

Mice were exposed to BPA in the diet at estimated dose 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). The limited evidence of reproductive toxicity at the highest dose (600 mg/kg bw/day) 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."[40] Gestational length also was increased by 0.3 days in the F1/F2 generations at the high dose. According to the study authors, "[t]he toxicological significance, if any, of this marginal difference is unknown."[40]

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*., 2008b, 2008c). 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 (Tyl *et al*. 2008a, 2008b, 2008c).

The systemic and reproductive NOAELs for BPA 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 (< 5 mg/kg bw/day); nor were there non-monotonic dose responses as claimed in some of the studies with unconventional design. The study concluded that "***BPA is not considered a selective reproductive or developmental toxicant in mice***."[40] (emphasis added)

[40] Tyl *et al*., 2008a at p. 362.

iv. Two-Generation Reproductive Toxicity Study in Mice (Kobayashi *et al.*, 2010)

A study of BPA in mice was conducted by the Japanese National Institute of Occupational Safety and Health to examine the effects of low-dose exposure to BPA on reproduction and development in two generations of mice. The study examined three dose levels of BPA by the oral route of administration (0.333, 3.3, and 33 ppm BPA, which correspond to approximately 0.05, 0.5, and 5 mg/kg bw/day). Pregnant female C57BL/6J mice (F0) were fed a diet containing BPA from gestational day 6 through postnatal day 22, and the weanlings (F1 and F2) from each F0 and F1 dam group, respectively, were also fed these same concentrations of bisphenol A ad libitum until sacrifice. There were no treatment-related changes in body weight, body weight gain, food consumption, gestation length, or the number of live births on postnatal day 1 in F0 dams between the control group and BPA groups. Sex ratio and viability were similar in all F1 pups. No treatment-related changes were observed in body weight, food consumption, developmental parameters, anogenital distance, or weight of any of the organs (liver, kidney, heart, spleen, thymus, testis, ovary, or uterus) in F1 and F2 adults in either sex. As reported by the authors, "[t]hese findings indicate that dietary exposure to bisphenol A between 0.33 and 33 ppm does not adversely affect reproduction or development as assessed in two generations of mice."

v. One-Generation Reproductive Toxicity Study In Mice (Tyl *et al.*, 2002b)

A one-generation reproductive toxicity study in mice was also performed by Tyl *et al.* (2002b). In the one-generation study, male and female mice were administered diets containing 0, 5,000 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 study described above. Parental systemic toxicity was clearly evident at both 5,000 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 post-implantation *in utero* loss, or on pup body weights per litter (sexes separately or combined)."[41]

vi. NTP Continuous Breeding Reproductive Toxicity Study In Mice (NTP, 1985 and Morrissey *et al.*, 1989)

In a study sponsored by the National Toxicology Program (Morrissey *et al.*, 1989), BPA was administered to mice in the diet at concentrations (estimated daily intakes in mg/kg bw/day) of 0.25% (438), 0.50% (875), or 1.00% (1,750). A continuous breeding protocol was used. Groups

[41] Tyl *et al.*, 2002b at p. 6.

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. Significant effects on the number of litters per pair and number of live pups per litter were reported at the two highest doses. Significant systemic toxicity was observed in the parental and offspring animals and, as noted by NTP: "some or all of the adverse effects on reproductive performance … may be secondary to the generalized toxicity of BPA."

vii. Subchronic Toxicity Study In Rats (Delclos *et al*., 2014; Churchwell *et al*., 2014; Camacho *et al*., 2015)[42]

A very large-scale subchronic toxicity study in Sprague-Dawley rats was conducted by FDA researchers at the National Center for Toxicological Research (NCTR). The primary goals of the study were to identify adverse effects attributable to BPA, in particular at low dose levels, to characterize the dose response for any observed effects, and to determine doses for a subsequent chronic study, which is now underway. To our knowledge, this is the largest published study of any type ever conducted on BPA (Delclos *et al*., 2014).

Strengths of this study include:

- Route of administration: Oral (daily dosing from GD 6 until start of labor, direct dosing of pups from PND 1 to termination
- Number of low dose levels: Seven equally spaced dose levels ranging from 2.5-2,700 µg/kg bw/day
- Number of high dose levels: Two (100,000 and 300,000 µg/kg bw/day
- Number of animals: 18-26/dose group
- Positive control: Ethinyl estradiol, two dose levels
- Negative control: Two (naïve and vehicle)
- Endpoints examined: Multiple

Rats were exposed to BPA at a wide range of dose levels including seven low dose levels, which were the primary focus of the study, and two high dose levels. As expected from previous studies, significant systemic toxicity was observed at the two high dose levels (e.g., depressed gestational and postnatal body weight gain). Effects on the ovary and estrous cycle were reported only at the highest dose and, as noted by the authors, "the interpretation of the high dose BPA effects [is] confounded by apparent systemic toxicity … at this dose." In the low dose range, "potential effects could not be clearly linked to treatment as they were observed sporadically across the dose groups and did not occur in a consistent grouping across organs …"[43]

[42] None of the 3 related studies were included in the Hazard Identification Materials provided by OEHHA.
[43] Delclos *et al*., 2014 at page 193.

Of particular relevance for female reproductive toxicity, the study found no effects from exposure to BPA in the absence of significant systemic toxicity on the time of vaginal opening, time to first estrus, anogenital distance, ovarian and uterine morphology, ovarian cyclicity or reproductive endocrinology. The study does not support a finding that BPA is a female reproductive toxicant.

The biological plausibility of the study results, in particular the negative findings with the low dose levels, was extensively examined in two additional studies conducted at NCTR (Churchwell *et al*., 2014; Camacho *et al*., 2015). As noted by Churchwell *et al*. (2014), "[a] limitation in the design of most BPA toxicity studies … is the lack of evaluation of internal dosimetry, particularly in the 'low dose region' … The comprehensive evaluation of tissue histopathology and reproductive and developmental endpoints in this subchronic BPA exposure study was matched by comprehensive evaluation of the levels of aglycone (i.e., unconjugated and receptor-active) and conjugated (i.e., inactive) BPA from daily oral administration throughout perinatal and adult life stages."

As stated by the FDA researchers (Churchwell *et al*., 2014): "A goal of these two studies was to evaluate whether lifestage-dependent internal dosimetry of estrogenic compounds could be used to evaluate critically the hypothesis that aberrant estrogenic signaling during critical developmental windows could manifest as phenotypical changes in the adult animal."

The comprehensive dosimetry analysis revealed that the internal doses of aglycone BPA in all of the low-dose treatment groups (2.5-2,700 µg/kg bw/day) provided estrogenic exposures well below levels at which estrogen receptor mediated signaling might be expected. This finding is fully consistent with the lack of effects, in particular estrogen-mediated effects, in the low-dose groups. Consistent with other studies discussed above, effects were only observed at the very high doses in the presence of significant systemic toxicity.

In the second additional study (Camacho *et al*., 2015), the NCTR researchers "assessed the global genomic DNA methylation and gene expression in the prostate and female mammary glands, tissues identified previously as potential targets of BPA, and uterus, a sensitive estrogen-responsive tissue." The purpose for this work was to "assess further the potential of the 'low BPA' doses to induce changes at the molecular level and characterize the dose response of such changes…"

Consistent with the toxicity outcomes reported by Delclos *et al*. (2014), both doses of the positive control (ethinyl estradiol) modulated the expression level of several genes, including known estrogen-responsive genes. These results confirmed the sensitivity of the NCTR Sprague-Dawley rat to the positive control.

In contrast for BPA, the NCTR researchers concluded "[t]he 'low BPA' doses modulated the expression of several genes; however, the absence of a dose response reduces the likelihood that these changes were causally linked to the treatment. These results are consistent with the toxicity outcomes."

Overall, the results from Camacho *et al*. (2015) and Churchwell *et al*. (2014) provide strong support for the findings reported by Delclos *et al*. (2014). In particular, the lack of effects at any of the low dose levels is fully consistent with the lack of changes at the molecular level and with the dosimetry results.

In summary, all seven conventional reproductive toxicity studies demonstrate that BPA is not a selective reproductive toxicant.

b. Studies Of Unconventional Design Are Of Questionable Toxicological Significance And Do Not Support Listing

The studies discussed in the section above follow "conventional" study designs, which are aimed at comprehensively evaluating reproductive performance. These studies generally follow validated test protocols and often are based on internationally accepted test guidelines for reproductive toxicity studies.

In contrast, the larger number of "unconventional" studies discussed in this section may be aimed at very specific questions related to reproduction and generally follow experimental protocols that have not been validated. These studies are often small-scale screening studies conducted on small numbers of animals and may only test one dosing level, which precludes assessment of a dose-response relationship. Complete experimental details may be lacking, which precludes complete analysis of the reported results. The results from these studies have generally not been replicated or corroborated in independent laboratories.

Most of the studies discussed in this section were conducted at very low doses, often orders of magnitude lower than the NOAELs determined in the conventional reproductive toxicity studies. In many of these studies, BPA was administered by parenteral routes of exposure (e.g., subcutaneous and intraperitoneal). As discussed in Section 7, non-oral routes of exposure are of no relevance to human exposure, which is generally accepted as occurring orally through the diet. In contrast to oral exposure, in which BPA undergoes efficient first-pass metabolism to inactive metabolites and rapid elimination, non-oral exposures result in much higher internal doses of biologically active parent BPA.

Government body reviewers (e.g., FDA, NTP-CERHR) have consistently regarded these "low-dose" studies as inadequate for a variety of reasons. Overall, the experimental studies discussed in this section may be suitable for formulating hypotheses that can be tested in follow-up studies. For purposes of Proposition 65, however, they do not provide a reliable or adequate basis to conclude that BPA is "clearly shown" to cause female reproductive toxicity.

i. A Weight-Of-Evidence Approach Is Required To Evaluate The Sufficiency of Evidence

As discussed in Section 6 above, the DART IC criteria document (DART IC, 1993) provides detailed guidance on the meaning of "sufficient evidence" and how it should be evaluated by the DART IC. An important over-arching principle is that "[i]n evaluating the sufficiency of data, a

'weight-of-evidence' approach ***shall*** be used to evaluate the body of information available for a given chemical" (emphasis added). To evaluate sufficiency of the evidence, the DART IC criteria document identifies a series of parameters to consider for individual human and animal studies.

It is obvious from the criterion above that the use of a weight-of-evidence approach to evaluate the "body of information available" means that a determination of "sufficient evidence" can be accomplished only if the body of information evaluated includes substantially all of the information available. This is particularly critical in light of the weight-of-evidence considerations outlined for animal (and human) studies. For example, the criteria document recites that data from a single study might be sufficient to justify listing a chemical "provided there are not equally well conducted studies which do not show an effect and which have sufficient power to call into question the repeatability of the observation in the positive study." In order to reach that conclusion, the review must include not just the "single study" reporting an effect but also the other data available to determine whether there "are not equally well-conducted studies which do not show an effect …"

The importance of weighing all relevant information has been well-illustrated in a series of publications that examined the weight of evidence for reproductive or developmental effects from low doses of BPA (Gray *et al*., 2004; Goodman *et al*., 2006; Goodman *et al*., 2009). As a starting point for the weight-of-evidence evaluations, all available data from rodent studies was collected and summarized in a series of tables for each endpoint examined. These summary tables have been updated with all data available for female reproductive effects as of January 1, 2015 and a complete set of updated tables is presented in Appendix 5.

To illustrate the utility of these tables, an extract from one of the tables showing all available data for two female reproductive endpoints relevant to Proposition 65 (onset of puberty and alterations in estrous cycle) is presented below. Although several data points indicate outcomes that are statistically significantly increased or decreased versus the controls, it is obvious at a glance that the vast majority of the data shows no statistically significant change in the outcomes.

It is readily apparent from this simple presentation of data that the "sufficiency of data" can be evaluated only if a complete body of information is included in the analysis. A conclusion derived from only those studies or data points that indicate statistically significant outcomes (or only studies that indicate no statistically significant outcomes) is not likely to be supported by the full weight of evidence.

Endpoint[(1)(2)]	Dose (mg/kg-d) Order of Magnitude					
	$\leq 10^{-5}$	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Age at first estrus			- 0 0	00 000	00 0	 0 0
Age at vaginal opening		0 0	0000 0 0 0000000	00000 0 0 000000- 00000+00-	0000 0 0+0 00000- 00000	 000 000
Estrous cycle (dams)		0	0 0	0 0	0 0	0 0
Estrous cycle (offspring)		0 0	0 0 0 000+	0 0 0 00000000-	0 0 -00 0000+	0 0-0 0+0

(1) Separate tables are prepared for studies with oral and non-oral routes of exposure. Data from all studies are sorted by endpoint and further sorted by the order of magnitude of the dose. Each data point is included in the appropriate table with a symbol: "0" indicates no statistically significant effect; "+" indicates an outcome statistically significantly higher than the control group; "-" indicates an outcome statistically significantly lower than the control group. See the Introduction section of the report in Appendix 5 for more details.

(2) The complete table from which the extract was taken is Table 3 (Outcome by Dose for Rat and Mouse – Oral Administration, Other Female Reproductive Effects) in the Appendix 5 report. The colors in the table indicate the timeframes when the data was collected, as described in the report.

It should also be noted that the table above and the complete tables in Appendix 5 are not weight-of-evidence evaluations in and of themselves. They are, however, a starting point that illustrates why a complete body of information must be included and why the "sufficiency of data" must be evaluated. As discussed above, the DART IC criteria document provides guidance on how to evaluate the "sufficiency of evidence." As discussed in the sections below, prominent government bodies have recently evaluated the weight-of-evidence for female reproductive toxicity.

ii. Data And Evaluations Available To The DART IC In 2009

The Hazard Identification Document provided to the DART IC for the 2009 meeting included numerous low-dose studies with unconventional designs. In the same timeframe as the 2009 DART IC meeting, various government bodies, including NTP-CERHR, FDA, and EFSA, had recently evaluated these studies and reached consistent conclusions. In general, all of the government bodies characterized the unconventional studies as inadequate or of limited utility for assessing risk.

As discussed in Section 4 above, the NTP-CERHR Report noted that many studies "were designed not as toxicology studies but rather to probe very specific experimental questions, and their results are not always easily interpreted with regard to how they contribute to the weight-of-evidence for human health risks."[44] Many of the studies had "technical or design shortcomings or their reports do not provide sufficient experimental details to permit an assessment of technical adequacy."[44]

More specifically, the NTP-CERHR expert panel stated that

> "… 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 exposure, 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."[45]

iii. Data And Evaluations Available After The 2009 DART IC Meeting

Subsequent to the 2009 DART IC meeting, numerous additional animal studies related to BPA and various aspects of female reproductive toxicity have been published (see HIM provided by OEHHA[28,46]). As discussed in Section 3 above, FDA has continued to systematically evaluate new studies on BPA through its BPA Joint Emerging Science Working Group, which "performs systematic reviews of literature available on BPA using clearly defined criteria."

To ensure continuity with the NTP-CERHR Report, the Working Group "considered a study useful for hazard identification if it met the criteria detailed by the CERHR as an adequate study." Additional criteria were applied to identify studies suitable for risk assessment. Further details on how FDA applied these criteria are summarized below:

> "As part of the weight-of-evidence assessment, findings from studies employing non-oral and oral exposures were to be considered for their relevance to hazard identification and

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[44] NTP-CERHR Report, NTP Brief at p. 8.
[45] NTP-CERHR Report, Chapin *et al*., at p. 382.
[46] The HIM also identified numerous studies that examined various effects in male laboratory animals. These studies, listed in the OEHHA document titled "References on the Female Reproductive Toxicity of Bisphenol A provided to the Developmental and Reproductive Toxicant Identification Committee (DARTIC), February 2015," are of questionable relevance to female reproductive toxicity, which is the sole focus of the current proceeding. Studies on male animals include: Akingbemi *et al*., 2004; Ashby and Lefevre, 2000; Atanassova *et al*., 2000; Cagen *et al*., 1999; Della Seta *et al*., 2006; Fritz and Lamartiniere, 1999; Gupta, 2000; Kato *et al*., 2006; Kawai *et al*., 2003; Manglesdorf *et al*., 2003; Negishi *et al*., 2004; Salian *et al*., 2009 (2 citations); Stoker *et al*., 1999; Takahashi *et al*., 2003; Tan *et al*., 2003; Thuillier *et al*., 2009; Timms *et al*., 2005; Tiwari and Vanage, 2013; Wistuba *et al*., 2003; Working, 1988.

> risk assessment based on the ability of pharmacokinetic data to inform dose translation. Data were also to be grouped among corroborating experiments to determine if they affected the strength or weakness of findings. Although studies were reviewed individually, collective findings were to be considered for their ability to indicate themes or identify potential hazards."
>
> "This updated literature review was carried out with the intent to identify any new information that could inform the hazard identification (HI) and/or risk assessment of BPA. A number of studies identified in the updated literature review reported biological changes/observations that are currently of unknown toxicological relevance. As part of the multistep review process, these studies were assessed for quality even though the impact of their findings is currently unknown. As these were generally considered to be mechanistic studies, where links to adverse effects or pathways leading to toxicity are unknown, they were not considered as meeting the criteria for identification as 'hazard'."

Several passages from FDA's overall conclusions are particularly informative for Proposition 65 hazard identification purposes. Rather than summarize them, we have quoted directly from the FDA memorandum below:

> "Uncertainty is inherent in any scientific study or evaluation. Hazard identification and risk assessments use criteria and methods to identify, reduce, or address uncertainty to provide confidence in weight-of-evidence conclusions. A major source of uncertainty in the field of "low-dose" BPA research has been the many discrepancies and conflicts in reported effects within and across species, even when studies are conducted over the same dose range. Following our extensive review of "low-dose" BPA literature, we are unable to construct a plausible or logical comprehensive toxicological profile or explanation for the many claimed effects of BPA, largely due to the inconsistencies that currently exist within this literature. As an example, while one study may report an effect on a specific endpoint, contradictory results are reported in other studies examining related endpoints that share pathways or mechanisms of action with the first reported effect. Similar issues arise when trying to compare various doses, routes of administration, and internal dosimetry."
>
> "To provide a comprehensive biological and toxicological evaluation of the effects of BPA in the low-dose range, very few studies (*e.g.*, Delclos *et al.* 2014) have evaluated an extensive range of endpoints (both discrete and related) across multiple systems. However, a few recent studies (reviewed herein) have employed new methods to elucidate the underlying causes of the observed variability in the BPA literature and thereby improve HI and RAs. The ability to correlate observed effects of BPA on various organ systems with levels of BPA in the affected tissues is crucial for demonstrating biological plausibility of adverse effects of low-dose BPA. Consequently, one of the most important findings was that the likelihood of sample contamination and inadvertent exposure by trace levels of environmental BPA is high in low-dose studies. For this

reason, the criterion *Environmental Contamination* (described in the Methods section of this memorandum), which is based on previous experiences with background contamination in testing for other compounds (*e.g.*, dioxin, acrylamide, genistein), was explicitly considered in this review. However, the extent to which biological samples can be contaminated with native aglycone BPA is only now being fully understood. Assessment of sample contamination and potential for inadvertent exposure must remain important considerations in performing laboratory and/or clinical studies, collecting and analyzing samples, and finally in the acceptance and interpretation of data."

"Thigpen *et al.* (2013) examined whether general estrogenic background levels and/or contamination could be a source of variability in the low-dose BPA literature. The study correlated higher and variable levels of estrogenic contamination sources with the inconsistencies in reported effects in the "low-dose" literature. The results of this study suggest that, if in a low-dose study examining endocrine effects of a weakly estrogenic chemical, background phytoestrogen and environmental estrogenic levels are not well controlled and/or measured, it becomes very difficult to exclude background contamination as a possible explanation for the reported effects. … Based on the findings from these two studies, we conclude that studies conducted at doses of 2.5 μg/kg bw/d and below must be interpreted with extreme caution due to the high probability of inadvertent exposure or contamination of samples by exogenous sources of BPA."

"The studies reviewed in this *Contamination* section support the need for strict criteria in hazard identification and risk assessment and in building weight-of-evidence evaluations. The high potential for inadvertent exposure or contamination by native BPA, confounding due to high or variable environmental estrogenic contamination or background, and methodological limitations in dose preparation significantly limits interpretation of studies that did not address these issues, leading to a high degree of uncertainty when trying to incorporate a given study's findings into an overall assessment."

Overall, FDA's analysis of the many unconventional studies that examine the potential for low doses of BPA to cause reproductive or developmental effects makes it clear that the available body of evidence is not sufficient to "clearly show" that BPA causes female reproductive toxicity. Accordingly, **the basis for FDA's current safety assessment is systemic toxicity** from two multigenerational studies. In particular, **female reproductive toxicity has not been identified as a BPA hazard** by FDA.

iv. The Recent EFSA Review Of BPA Confirms That Low-Dose Animal Studies Do Not Support Listing

In its January 2015 comprehensive review of BPA, EFSA reached a conclusion regarding low-dose animal studies consistent with that of FDA.[7] Regarding reproductive and developmental effects, EFSA concluded:

> "Overall, the better powered, better conducted studies in animals found few consistent effects of in-utero exposure to BPA on reproductive development at dose levels at or below 3.6 mg BPA/kg/day HED. On balance, the evidence remains contradictory and highly variable between studies. The CEF Panel noted that there is some evidence for effects of BPA exposure on several parameters indicative for changes in the reproductive system in adult male animals at dose levels below 3.6 mg/kg bw per day, although these effects were modest. It is not possible to conclude that these changes are reflective of changes in reproductive performance, since the studies rarely included a forced/continuous breeding phase in adulthood to establish reduced fertility. However, in several multigenerational studies no effects were observed at dose levels as low as 3 µg/kg bw per day up to at least 50 mg/kg bw per day."
>
> "Using a WoE approach, the CEF Panel assigned a likelihood level of "as likely as not" to reproductive and developmental effects of BPA at low doses (below the HED of 3.6 mg/kg bw per day)."

The "as likely as not" conclusion is the 4th level on the standard 7-level scale ranging from "very likely" to "very unlikely." Overall, EFSA's review is consistent with FDA's review; neither support a finding that BPA is "clearly shown" to cause female reproductive toxicity.

c. Estrogenic Activity And Mechanistic Studies Do Not Provide An Adequate Basis For Listing BPA In The Absence Of Sufficient Evidence Of Female Reproductive Effects

Some of the studies in the Hazard Identification Materials do not report effects on reproductive function or performance but rather examine molecular level events or other biological observations. Examples include studies on estrogenic activity of BPA, *in vitro* studies, and gene expression studies.

Regarding estrogenic activity, it has been known for decades that BPA is weakly estrogenic (for example, see Dodds and Lawson, 1936 and 1938, both cited in the HIM). To the extent that estrogenic activity is relevant, the estrogenic activity of BPA has been assessed in many *in vitro* and *in vivo* assays. Although the results vary between assays, the estrogenic activity of BPA is generally four to five orders of magnitude less potent than the endogenous estrogen 17β-estradiol. Based on this difference in estrogenic activity, BPA would be unlikely to cause female reproductive toxicity by an estrogenic mechanism. As noted previously, the potential for BPA to exhibit any estrogenic effect in humans is significantly further attenuated because orally ingested BPA is efficiently converted to non-estrogenic metabolites and rapidly excreted.

Many natural components of the human diet (i.e., phytoestrogens) have a similar or greater affinity than BPA to bind to estrogen receptors. Humans are exposed daily to a variety of such estrogenic substances. For example, genistein, a phytoestrogen found in soy, has a much higher affinity than BPA for both nuclear estrogen receptors (ERα and ERβ). Any trace amount 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, to bind with estrogen receptors.

More generally, as noted by FDA:

> "A number of studies identified in the updated literature review reported biological changes/observations that are currently of unknown toxicological relevance. As part of the multistep review process, these studies were assessed for quality even though the impact of their findings is currently unknown. As these were generally considered to be mechanistic studies, where links to adverse effects or pathways leading to toxicity are unknown, they were not considered as meeting the criteria for identification as 'hazard'"

Consistent with FDA's criteria for identification of reproductive hazards, the DART IC Guidance Criteria does not include molecular level events in the definition for female reproductive toxicity. Molecular level events, including the well-known weak estrogenic activity of BPA, is not a reproductive effect *per se*, but rather provide potential mechanistic information that may be relevant for any demonstrated female reproductive effects. Thus, mechanistic studies alone are not a sufficient basis to support a conclusion that BPA is "clearly shown … to cause [female] reproductive toxicity."

10. The Studies Summarized In Peretz *et al*. (2014) Do Not Provide Sufficient Evidence To Support A Finding That BPA Has Been "Clearly Shown" To Cause Female Reproductive Toxicity

A prominent component of the Hazard Identification Materials is a review paper on BPA and reproductive health (Peretz *et al*., 2014) that concludes (in the abstract) that BPA is a reproductive toxicant. The paper is a literature review that covers studies published in the 2008-2013 time period. As discussed below, the studies summarized in Peretz *et al*. (2014) do not provide "sufficient evidence" that BPA has been "clearly shown through scientifically valid testing according to generally accepted principles" to cause female reproductive toxicity.

a. Peretz *et al*. (2014) Does Not Apply A Weight-Of-Evidence Approach

As discussed in Section 9 above, the DART IC criteria document (DART IC, 1993) indicate that "[i]n evaluating the sufficiency of data, a 'weight-of-evidence' approach ***shall*** be used to evaluate the body of information available for a given chemical" (emphasis added). The document also provides detailed guidance on the meaning of "sufficient evidence" and how it should be evaluated. It is clear that the "body of information available" means that substantially all of the information available must be included in an evaluation of the sufficiency of the data.

It is similarly clear that the weight-of-evidence requirement cannot be met if only a subset of available studies is considered. By design, the Peretz *et al*. (2014) paper covered only the 2007-2013 time period; studies published before and after this time period are not included. In addition, as discussed in Section 9, the selection of studies is incomplete even for this time period. This approach may be suitable for conducting a survey of the literature over a defined period of time, but the resulting survey does not include all of the data that are necessary for a weight-of-evidence analysis.

b. Peretz *et al*. (2014) Does Not Evaluate Sufficiency Of Evidence

A decision to list a chemical as a reproductive toxicant requires that "sufficient evidence" is available to support the listing. To evaluate sufficiency of the evidence, the DART IC criteria document identifies a series of parameters to consider for individual human and animal studies (see Section 6 above and the DART IC Criteria Document).

The Peretz *et al*. (2014) review paper summarizes results reported in the studies that the authors surveyed, but the paper does not identify any criteria that were applied to evaluate studies for adequacy. In particular, there is no indication that criteria comparable to the DART IC criteria for adequacy were applied.

c. The Complete Body Of Evidence Must Be Evaluated To Determine If BPA Has Been "Clearly Shown" To Cause Female Reproductive Toxicity

Because it is incomplete and lacks a rigorous evaluation of adequacy, Peretz *et al*. (2014) is of very limited utility for the current proceeding, except as a brief summary of the included studies. The paper clearly cannot stand alone as the basis for a listing decision.

In contrast, as discussed above, both FDA and EFSA have evaluated the evidence thoroughly and both agencies identify significant limitations in many of the studies on BPA that are summarized in Peretz *et al*. (2014). The findings of these agencies do not support a conclusion that BPA has been "clearly shown" to cause female reproductive toxicity.

The limitations of the Peretz *et al*. (2014) review are highlighted below with several examples of how studies were analyzed by Peretz *et al*. (2014) and FDA (2014). In addition to the summaries below, complete text on these studies from Peretz *et al*. (2014) and FDA (2014) are provided in a side-by-side table in Appendix 6.

First, according to Peretz *et al*. (2014), the Hunt *et al*. (2012) study showed that low-dose BPA significantly disrupted synapsis and recombination between homologous chromosomes at the onset of meiosis, increased the number of oocytes present in the secondary and antral follicles at birth, and increased the incidence of unenclosed oocytes. However, Peretz *et al*. (2014) did not identify any uncertainties or limitations of this study. In contrast, FDA identified several critical limitations and uncertainties (see Appendix 6 for FDA's extensive analysis) and, based on these, concluded, "**This study has no utility for HI** [hazard identification] **and no utility for RA** [risk assessment]." (emphasis in original)

Second, Peretz *et al*. (2014) described the Veiga-Lopez *et al*. (2013) study in a single sentence: "In another study, Veiga-Lopez et al. (2013) reported that prenatal BPA exposure altered the fetal ovarian steroidogenic gene and microRNA expression that mediate gonadal differentiation and folliculogenesis in sheep." Again, Peretz *et al*. (2014) did not identify any uncertainties or limitations. In comparison, FDA (2014) identified numerous shortcomings of this study (see Appendix 6 for FDA's extensive analysis), and concluded, "**This study has no utility for HI and no utility for RA**." (Emphasis in original) FDA noted that only one dose level of BPA was evaluated, precluding any assessment of dose-response, and the group size was only 4-5 ewes. According to FDA, "the environmental conditions were not properly controlled. High phytoestrogen containing (alfalfa) hay was fed to the pregnant ewes through the entire experiment, and the content of phytoestrogen in the hay was not analyzed." In addition, the results were potentially confounded by chlortetracycline, which was given to the ewes to prevent abortions. BPA in the blood was measured at only a single point in time. FDA stated that "[a]lthough BPA seemed to down-regulate expression of a number of miRNAs, no changes in the gene expression of miRNA regulators, ovarian steroidogenic enzymes, receptors or growth factors were observed, except a transient up-regulation of *Cyp19* and *SRD5A1* on GD65, which returned to normal levels on GD90. At the present time, it is not clear whether those observed changes are due to BPA."

Finally, and once again, Peretz *et al*. (2014) summarized the Rivera *et* al. (2011) study but with no critical evaluation: "In neonatally exposed lambs, low-dose BPA was reported to increase the incidence of multioocyte follicles (Rivera et al., 2011). … BPA exposure also increased cell proliferation, indicative of follicular growth, in small antral follicles in neonatally exposed lambs and Wistar rats (Rivera et al., 2011; Rodriguez et al., 2010)." In contrast, FDA

noted that BPA was administered by subcutaneous injection, an inappropriate route of exposure for human hazard identification (see Appendix 6 for FDA's extensive analysis). The FDA also noted that only one dose level of BPA was evaluated, eliminating any possible assessment of dose-response, and the group size was small (i.e., 6 ewes in the BPA group and 10 control ewes). According to FDA, "This study is related to a previously reviewed study by Rodriguez *et al*, 2010 which was conducted in female rats. **Like the study by Rodriquez, this study is basically a mechanistic discovery study and is not useful for hazard identification or risk assessment. (**emphasis in original) The endpoints measured such as receptor protein expression, Ki67 and p27 biomarkers, and morphological counting of MOFs are at the cellular and molecular levels. These events are not clearly associated with adverse effects. The biological significance of these endpoints on follicular development and fertility in adult sheep needs to be determined. The study included only one dose of BPA and thus there was no dose-response measurement. It is difficult to translate dose by sc to the oral route."

Even if Peretz *et al*. (2014) were to be considered in its own right, the assertion recited in the abstract that BPA is a reproductive toxicant should not be considered as a conclusion *per se*, but rather a summary of the conclusions in the paper that follow. Here, the conclusion recited in the abstract does not reflect accurately and fully what is stated in the paper.[47] In the conclusion section of the paper, the authors explain that:

> "These conclusions, however, are not to be considered definitive without further investigation, especially with the gaps in clear results detailed throughout the review. In the experimental studies, strong, definitive conclusions often were difficult because study designs were so different. Experimental studies rarely used the same doses, timing, positive controls, and exposure routes; thus, the effects of BPA on exposure among animal strains and species could not effectively be compared."
>
> …
>
> "In the epidemiological studies, strong conclusions were difficult to determine because of study design and exposure parameters. For example, exposure assessments in most human studies relied on a single urine sample, which may introduce exposure misclassification and attenuate associations if they are present. Given the continuous and variable exposure to BPA, a single urine sample may not represent longer term exposure or exposure in the relevant etiological window. Finally, most of the human studies were cross-sectional, making it difficult to discern the temporal relationship of exposure with response. These limitations affect the interpretation of human studies."

[47] Peretz *et al*., 2014 at p. 783.

11. Conclusion

BPA should not be listed as a female reproductive toxicant under Proposition 65. Taking into account the weight of evidence, the statutory and regulatory criteria for listing, and the guidance articulated in the Committee's Guidance Criteria, BPA cannot reasonably be characterized as "clearly shown, through scientifically accepted testing conducted according to generally accepted principles to cause" female reproductive toxicity.

Applying those Proposition 65 standards to the testing data available on BPA and discussed in these comments, it is clear that the human data do not support listing. Therefore, any finding that BPA merits listing would have to rest on the animal data alone. The high-quality conventional animal data, summarized in Section 9, demonstrate consistently and convincingly that BPA is not a selective female reproductive toxicant in rodents. This conclusion is supported by recent comprehensive reviews of the same data by FDA, which is a Proposition 65 authoritative body, and EFSA. The weight of evidence from human and animal studies clearly indicates that BPA should not be listed.

12. References

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Appendix 1
FDA Criteria for Evaluation of Epidemiology Studies

The text below is quoted directly from: 2014 Updated Review of Literature and Data on Bisphenol A, Bisphenol A (BPA) Joint Emerging Science Working Group, U.S. Food and Drug Administration, June 6, 2014, p. 6.

"Epidemiology Criteria

For epidemiology studies, several key elements were considered. These included:

- Utility of study design [cross-sectional, case-control, cohort (prospective)], with more weight given to prospective studies; sufficiency of study size (consideration of uncertainty regarding size and representativeness of study sample and generalizability of results); use of multi-geographical approaches; adherence to proper statistical analyses.
- Measurement of BPA exposure and outcome metrics, with measurement uncertainty due to diurnal, seasonal, and individual variability, as well as possible environmental contamination, impact from lab plastics, interference by other biological compounds, and other factors being weighted in the analyses.
- Appropriate treatment of the data with regard to non-detectables [LOD values]; adjustment of urinary concentrations (creatinine or specific gravity, with the use of specific gravity preferred); consideration given to the potential misclassification of LOD values in the confidence of the finding; and consideration of current state-of-the-science with regard to measurement of BPA in different biological matrices.
- Other factors: potential biologically plausible reverse causation; unconsidered confounders, risk factors and effect modifiers; and ascertainment of the correspondence between the measurement time and the relevant exposure window were also to be considered in interpretation of the results.

Each study was reviewed for its ability to inform the HI and RA process for BPA. Links to animal data were used to understand generalization to or justification of the use of specific endpoints."

Appendix 2
FDA Summary Comments on Evaluation of Epidemiology Studies (2014)

The text below is quoted directly from: 2014 Updated Review of Literature and Data on Bisphenol A, Bisphenol A (BPA) Joint Emerging Science Working Group, U.S. Food and Drug Administration, June 6, 2014, p. 135-137.

"Epidemiology Studies

Summary

Forty eight (48) published epidemiologic studies utilizing largely cross-sectional, or to a limited degree case-control or cohort, designs, tested putative associations between BPA exposure (urine or plasma) and various health outcomes or molecular endpoints in samples of human subjects from around the world. Most studies did not account for extraneous contamination of BPA samples by the collecting device (Ye *et al.*, 2013). Several studies used blood or cord blood concentrations of BPA as the exposure variable; however, blood has been shown to be of limited reliability, since BPA has a short half-life in plasma and is therefore present at very low levels compared to urine (Calafat *et al.*, 2013). Furthermore, plasma BPA levels are unable to adequately discriminate between true signal and noise (background contamination). In almost all cases, measurements of exposure in the reviewed studies took place at a single point in time only, which provides a highly uncertain assessment of internal exposure.

Assessed outcomes were wide-ranging, and no two studies measured endpoints in an identical fashion. No associations (*i.e.*, negative results) were reported between BPA exposure and the following parameters: implantation failure in a prospective cohort of US women undergoing IVF; undescended testes (cryptorchidism) in a prospective cohort of French boys; endometriosis in a sample of US women; age of menarche in US adolescent girls; male idiopathic infertility or semen quality in a sample of adult Han-Chinese men; CYP19 gene expression levels in granulosa cells collected from a sample of US women undergoing IVF; congenital hypothyroidism in a sample of Korean infants; type 2 diabetes in a sample of Korean adults; cases of precocious puberty or metabolic changes in 84 measured hormones in a sample of Chinese girls; adverse coronary events in a sample of elderly Swedish adults; child or maternal health in a sample of Korean lactating mothers/newborns. In one study, although considerable amounts of BPA were shown to be eluted from various dialyzers, patients with chronic kidney disease who were undergoing hemodialysis with different BPA-eluting dialyzers did not show any notable change in the plasma BPA levels during a single course or over four weeks of treatment (Krieter 2013). Most studies reported some sort of weak or possible association/correlation between BPA exposure and measured endpoints, including increased heart rate, female fertility status, nuclear receptor gene expression in peripheral blood mononuclear cells, spontaneous abortion, fetal health/birth outcomes, thyroid function (both positive and negative associations reported), meningioma, oocyte number, BMI (both positive and negative associations depending on age at exposure), school-aged behavior, pre-diabetes,

peripheral arterial disease, hypertension, type 2 diabetes, metabolic syndrome, or obesity measure in various samples.

Critical review of the studies indicated significant limitations in study design that made the claims of association questionable or unsupportable. In some cases, an association was reported for transformed, but not the original, data, which casts further doubt on the legitimacy of the conclusion. The most common limitations in study design included use of plastic sample containers of uncertain BPA leaching; small study sample size; use of a single exposure measure (*e.g.*, single spot urine); use of plasma concentration as measure of exposure; absence of control for possible confounders (*e.g.*, diet, BMI, age, race); lack of control for multiple statistical tests within study (favors false positives); inadequate description of timing of sample collection; lack of clinical significance of measured outcome (*e.g.*, decreased T4 without compensatory increase in TSH); use of highly variable outcome (gestational age); arbitrary/nonstandard definition of clinical outcome (*e.g.*, albuminuria); or inability to separate contribution of BPA from association with other measured compounds in a mixture.

Major limitations in study analysis/interpretation included selective reporting of positive results; absence of dose (concentration)-response relationships; inconsistent within-study or between-study (*e.g.*, diabetes, serum T4/T3/TSH) results; equating a reported association/trend for transformed data (*e.g.*, square root of percentage breast density) or parameter estimates with an association for the original study data; lack of generalizability; possibility of reverse causation (*e.g.*, increased levels of lipophilic BPA may be the effect, not cause, of adiposity/obesity). NHANES data were explored in several reviewed studies, but as pointed out by LaKind *et al.* (2012), the use of NHANES data has given contradictory results in the past and may not be appropriate for drawing conclusions about BPA and chronic complex diseases due to the cross-sectional structure of that dataset. Reported associations often disappeared with addition of covariates/possible confounders.

Our review indicated that no single study was able to make a definitive contribution to HI or RA. No study demonstrated, nor was able to demonstrate based on its design, either a causal or a temporal relationship between exposure and measured outcome, since exposures to BPA were uncontrolled. And statistical techniques (*e.g.*, correlation analysis, linear regression), which attempt to quantify *mathematical* relationships between BPA exposure and outcome, are unable to test causal, *scientific* hypotheses. Therefore, no single study was able to definitively identify a hazard or exposure level that could be applied to the current BPA RA. Moreover, it would be inappropriate to selectively choose an isolated result, either positive or negative, out of its context. While we have concluded that the studies evaluated as part of this literature review cycle do not meet the necessary standard for regulatory HI, nor can they provide a quantitative point of departure for RA, they are, nevertheless, meaningful contributions to the growing body of hypothesis generating, epidemiological literature for BPA. The data "explorations" undertaken and reported in these studies might form the basis for more robust, well-designed prospective studies examining any range of chemicals in the future."

Appendix 3
FDA Summary Comments on Evaluation of Epidemiology Studies (2012)

The text below is quoted directly from: 2012 Updated Review of Literature and Data on Bisphenol A, Bisphenol A (BPA) Joint Emerging Science Working Group, U.S. Food and Drug Administration, August 22, 2013, p. 37-38.

"Epidemiology Studies

Summary

Twelve published epidemiologic studies utilizing a cross-sectional or cohort designs tested putative associations between BPA exposure (variably defined as serum, urinary, or past occupational) and individual endpoints in small samples of human subjects from around the world. Assessed outcomes spanned the gamut and included hormone levels, diabetes, obesity, vascular disease, developmental endpoints, or gene expression. No two studies measured endpoints in an identical fashion. No associations were found between BPA exposure and the following parameters: 1) embryo quality indicators in US women undergoing *in vitro* fertilization (IVF) procedures, 2) thyroid hormone levels in French boys born with or without cryptorchidism, 3) thyroid hormone levels in a sample of US adolescents; 4) glucose regulation/diabetes in Chinese adults; 5) estrogen/androgen signaling pathway-related gene expression in Italian adult males; 6) thyroid and various reproductive hormone levels in US male partners of subfertile couples; and 7) peak estradiol levels or oocyte counts in US women undergoing *in vitro* fertilization (IVF) procedures.

A few studies reported an association between BPA exposure and measured endpoints, including birth weight or anogenital distance in male offspring, estradiol level normalized to the number of mature-sized follicles in women undergoing IVF, diabetes mellitus, sperm damage, or obesity. However, critical review of these studies indicated significant limitations in study design that made the claims of association questionable or unsupportable. Limitations included absence of control for possible confounders, lack of reporting of statistical tests utilized, poor or absent reporting of BPA exposure levels, discrepancies in reporting time of measurement of BPA exposure or health outcome, measurement of BPA in biological media at a single point in time only, lack of clinical significance of measured outcome, inability to separate contribution of BPA from association with other measured compounds in a mixture, as well as many other limitations. Our review indicated that no single study was able to make a defining contribution to hazard identification or risk assessment at present, since no study demonstrated the causal relationship between exposure and outcome that is required by the hazard identification step within risk assessment[10].

While we have concluded that the studies evaluated as part of this literature review do not have current utility for hazard identification or risk assessment, information from these studies may have ancillary or supportive utility when viewed in the context of similar findings from previous

and future epidemiological studies, or in the context of the results from the animal toxicity studies. One study[11] was considered to have demonstrated a preliminary or suggestive positive association (trend) between paraben + BPA co-exposures and sperm damage in males. However, associations of BPA with serum hormone levels, semen quality parameters, and sperm DNA damage measures were not specifically assessed in the study. The possible association reported in this study was examined more closely in the context of adverse male reproductive effects, if any, reported in the mammalian toxicology database for BPA. The preliminary sperm findings from the NCTR 90-day subchronic rat toxicity study of BPA[12] show no significant effect on any sperm parameters at BPA doses of concern to human health. However, sperm DNA damage was not directly assessed. While a correlation between sperm DNA damage and infertility exists in humans and animals, the understanding of the causal relationships is generally poor and likely multifactoral. Overall from human studies, no consistent relation between sperm DNA damage and fertilization rates or embryo quality during IVF or IVF/ICSI procedures has been demonstrated. Evaluation of sperm DNA damage currently has only a modest predictive value."

Appendix 4
FDA Summary Comments on Evaluation of Epidemiology Studies (2011)

The text below is quoted directly from: Updated Review of the 'Low-Dose' Literature (Data) on Bisphenol A (CAS RN 80-05-7) and Response to Charge Questions Regarding the Risk Assessment on Bisphenol A. Bisphenol A (BPA) Joint Emerging Science Working Group, U.S. Food and Drug Administration, May 24, 2011, not paginated.

"Epidemiology Studies

Summary

Nineteen epidemiologic reports in the BPA literature were reviewed. Ten studies used a cross-sectional design, six studies used a case-control design (three used data from the same investigation conducted in China), and three studies used a cohort design. Approximately half of the studies assessed the relationship between BPA levels and specific diseases or adverse outcomes, such as cardiovascular disease, sexual dysfunction, lower semen quality, premature birth, and others. As described in the detailed reviews that follow, the three occupational studies by Li et al. (2010), which assessed sexual dysfunction and semen quality in workers with and without occupational BPA exposure are of interest, but of limited impact due to potential confounders in this population. The study by Melzer et al. (2010), which was a follow-up of a study by Lang et al. (2008) on cardiovascular disease and other endpoints and urinary BPA concentrations from NHANES, may subsequently have utility for HI. Some studies assessed the relationship between BPA and reproductive hormone levels or other physiologic measures and did not provide clear evidence of links to specific potential hazards. While such studies do not have current utility for HI or RA, information from these studies may be of future utility for HI in the context of similar findings from future studies.

Of particular concern with regard to the utility of the findings of many of the epidemiology studies is whether or not the methods used for BPA exposure measurement accurately characterized the true exposure of study subjects over time. For example, many studies used spot urine samples to assess BPA exposure. However, urine BPA concentrations are highly variable within individuals and over time, and the within-day variability of the spot test is greater than between-day/within-person variability; both are greater than the between-person variability (Ye, 2011). Because of the variability in urinary BPA excretion, exposure measurements based on a single urine sample may lead to bias in the exposure data and incorrect conclusions from analyses of these data. A related concern has to do with the timing of the exposure measurements. Most of the studies assessed exposure at the same time that health outcomes were assessed. Even if such measurements provided accurate estimates of current BPA exposure, such data may not be relevant with regard to the particular health outcome assessed."

Appendix 5

Tables for the Weight-of-Evidence Evaluation of Female Reproductive Effects of Low Doses of Bisphenol A

Report Prepared by Gradient

April 15, 2015

Tables for the Weight-of-Evidence Evaluation of Female Reproductive Effects of Low Doses of Bisphenol A

Prepared for
American Chemistry Council
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April 15, 2015

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Introduction

Many studies have been conducted that examine potential low-dose reproductive and developmental effects of bisphenol A (BPA). Gradient has participated in three weight-of-evidence reviews (Gray *et al*., 2004; Goodman *et al*., 2006, 2009) that have collectively found that the overall weight of the evidence does not support an association between low doses of BPA and adverse developmental or reproductive effects. The following tables represent an updated summary of the literature for female reproductive effects, using the same criteria for selecting and evaluating studies as in the prior three reports. These tables should be considered in conjunction with the earlier reviews for a complete assessment of the weight of evidence regarding the possible existence of low-dose female reproductive effects of BPA.

Relevant BPA toxicology studies were identified from the subset of literature from July 25, 2008 (the cutoff for the Goodman *et al*., 2009 review), to January 1, 2015. Studies were narrowed down to those that were peer-reviewed, evaluated *in vivo* findings in rodents, used doses of $\leq$ 5 mg/kg-d, and examined female reproductive endpoints (either as primary or secondary endpoints). Specific endpoints of interest include female reproductive organ weights, pubertal characteristics, and reproductive structure and function. We only considered studies that evaluated either morphologically evident effects pertinent to reproduction or reproductive endpoints themselves. We did not consider biochemical effects, such as changes in endocrine activity or gene expression, because these are not toxic endpoints in and of themselves (as discussed by Goodman *et al*., 2009). We chose all studies of all frequencies, durations, and routes of exposure with doses up to 5 mg/kg-d to ensure that no low-dose studies were excluded.

Tables 1-4 summarize the findings for each category of endpoints (female reproductive organ weights, organ morphology/cytology, and other female reproductive endpoints, including puberty) for both oral and non-oral rodent studies. Each row in Tables 1-4 summarizes results from all relevant studies of a single endpoint (*e.g.*, uterus weight), with a "0" indicating a dose group showing no statistically significant effects, a "-" indicating a dose group with an outcome statistically significantly lower than in control animals, and a "+" indicating a dose group with an outcome statistically significantly higher than in control animals. Dose groups are identified in "order of magnitude" categories across the columns (for example, each symbol in the 10^{-1} mg/kg-d column indicates an endpoint at a dose ranging from 0.1-0.99 mg/kg-d). If a study used two doses in one order of magnitude, this is represented by two symbols in the table. These tables include not only all studies included in the current analysis (shown in green), but also all studies reviewed by Gray *et al*. (2004) (shown in red), Goodman *et al*. (2006) (shown in black), and Goodman *et al*. (2009) (shown in blue). If, in a given study and for a particular dose group, what could be considered as a single endpoint was measured in several ways (*e.g.*, several measures of ovarian histology, organ weights measured at several time points or across generations), and the preponderance of outcomes are null while others are not, the outcome for that dose is indicated by a "0" in the table to account for the inconsistency. This is because, overall, this study does not provide consistent evidence for that specific endpoint at that particular dose. Tables 5 and 6 list the references for each endpoint category.

Table 1 Outcome by Dose for Rat and Mouse Studies – Oral Administration, Female Organ Weights and Histology/Cytology

Endpoint	Dose (mg/kg-d) Order of Magnitude					
	≤ 10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Organ Weight						
Cervix			0	000	00	
Ovaries		0 00	000 00 0 000	00000 000 0 0000000	0000 00+ 00 00000000	0 0 00 00000
Uterus		0 000	000 0000 0 00000	00000 0000+ 0 0000+0000	0000 000++ 0-0 00000000000	000 000 000 00+0000
Vagina			0	000 0	00 0	 0
Organ Morphology/Cytology						
Areola/nipple development			0	00	00	0
General genital tract					0	0
Mammary cell number		0	0+	+	0+	+
Mammary duct length				0		
Mammary glands		 -	0 00	0 -0000	0 00	0 0
Mammary gland hyperplasia				0		
Mammary gland proliferation				0	0	
Number of corpora lutea		 0		 0		0
Number of mammary alveolar buds				0	0	
Number of mammary lobules type 1				0	0	
Number of mammary terminal ducts				0	0	
Number of mammary terminal end buds		 0	 0+	0 0	0 00	 0
Number of oocytes per germ cell cyst				00+		
Number of oocytes per ovary section				000		
Number of oocytes per primordial follicle				00-		
Ovaries		 -	 0 00	 0 0 0000-	 0 0 000	0 00 0000
Uterine cell proliferation			0			
Uterine endometrial thickness				-	0	
Uterine epithelium apoptosis						-

Endpoint	Dose (mg/kg-d) Order of Magnitude					
	≤ 10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Uterine epithelium thickness				0	0+	0
Uterine glands (number)			0			
Uterine myometrial thickness				0	-	
Uterus						00
					+	
			0	0	0	00
			00	00	00	0+0
Vagina					-	0
					0-	0
			00	00	00	0
Urethral slit length/depth			0	0	0	
Urethro-vaginal distance			0	0	0	

Table 2 Outcome by Dose for Rat and Mouse Studies – Non-Oral Administration, Female Organ Weights and Histology/Cytology

Endpoint	Dose (mg/kg-d) Order of Magnitude					
	≤ 10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Organ Weight						
Ovaries	0	0		000 0	+00 0 00	00+00 0 --
Uterus	0 00	0 00 00 0	0 0	0 000 00 00	0+0 000 00 00	000 0000000 00 0
Vagina	00	-0				
Organ Morphology/Cytology						
Mammary Glands						
Absolute ductal area		+				
Alveolar buds	+	+		+	+	
Alveolar bud volume fraction		0	0	0		
Area subtended by the ductal tree		00				
Cribiform-like structures			0	0	+	+
Ductal extension		00				
Ductal hyperplasias			+	+0 +	0 0	0
Ductal migration rate				+	-	
Ducts/terminal ducts				+	+	
Duct volume fraction		0	0	0		
Incidence of beaded ducts		0	0	0		
Length of ductal tree	0	0				
Lobuloalveoli	+	+				
Mammary glands		+ 0	0	00	0 -	
Mammary gland proliferation				0	0	
Nipple/areola development			0	0	0	
Number and area of terminal end buds	0	+ 00				
Terminal duct volume fraction		0	0	0		
Terminal end buds	+	+		+	0	
Ovary						
Absence of corpus lutea (early time point only)					+	
Folliculogenesis				0		
Number of corpora lutea		0	0	0 000	0 00	0 00
Number of cystic ovaries				0	+	0

Endpoint	Dose (mg/kg-d) Order of Magnitude					
	≤ 10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Number of follicles				00		
Number of oocytes				00		+
Number of oocytes per ovary				0		
Number of paraovarian cysts				0	0	0
Oocyte diameter				++		
Ovarian bursa	-	-				
Ovarian cyst breakdown						0
Ovaries				00 0 0	00 0 0+0	00 0 0+-
Percent antral follicles	0	+				
Progressive proliferative lesions of the oviduct				0	0	0
Uterus						
Absolute volume of lamina propria	0	-				
Adenomyosis				0	0	0
Apoptosis in luminal epithelium	0	0				
Apoptosis glandular epithelium	0	0				
Atypical hyperplasia				0	0	0
Cystic endometrial hyperplasia				0	+	0
Endometriosis				00	++	0+
Leiomyoma				0	0	0
Stromal polyps (number)				0	0	0
Uterine hyperplasia				0	0	00
Uterine hypertrophy	0	0	0	0	0	000
Uterus					0+ 0	
				00 0	00 0+	00 0+
Volume of all other uterine tissues	0	0				
Wolffian remnants in the uterine wall				0	0	0
Vagina						
Number of vaginal epithelial cells	0	0				
Thickness of vaginal epithelium	0	0				
Vagina					0	
				0	0	0

Table 3 Outcome by Dose for Rat and Mouse Studies – Oral Administration, Other Female Reproductive Endpoints

Endpoint	Dose (mg/kg-d) Order of Magnitude					
	≤ 10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Age at first estrus			- 0 0	00 000	00 0	0 0
Age at vaginal opening		0 0	0000 0 0 0000000	00000 0 0 000000- 00000+00-	0000 0 0+0 00000- 00000	000 000
Estrous cycle (dams)		0	0 0	0 0	0 0	0 0
Estrous cycle (offspring)		0 0	0 0 0 000+	0 0 0 00000000-	0 0 -00 0000+	0 0-0 0+0
Fecundity			0	0	00	0
Fecundity index			0	0	0	0
Female fertility (based on % of pregnant females)	00	0 00	00 00 0 000	0000 000 0 0000000	000 00 0 000	0 0 0 0
Fertility index			0	0	0	0
Gestation period		 00	 0 000000	0 0 000000000 0000	 0 00000000	 0 00000
Litter size	 0	- 000000	00 00 0000000 000000	00 00 00000000000 000000000 0000000	0 000000 00000000 0000000	0000 0000 0000000 000
Mean number of zygotes				00	0	
Milk yield		0		0		
Number of live births		 00	0 00	00 000000000	0 0000	0 00
Number of resorption sites		0		+		
Number of stillbirths			0	0	0	0
Number of uterine implantations		 0	00 0 00000	00 0 000000	0 0 00000	 0
Percent of animals with resorptions			0 0	+ 0	+ 0	 0
Percent postimplantation loss			0 0	+ 0	+ 0	 0
Yield of oocytes in unfertilized females				00	0	

Table 4 Outcome by Dose for Rat and Mouse Studies – Non-Oral Administration, Other Female Reproductive Endpoints

Endpoint	Dose (mg/kg-d) Order of Magnitude					
	≤ 10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1†
Age at first estrus			0	-		
Age at mammary gland maturation	+					
Age at vaginal opening	0	00	0 0	-+ 0-0 -0-00	0 00 00-	00- 00 -0-
Estrous cycle (dosed directly)				0+0	00	000
Estrous cycle (offspring)	+	+ 0	+ 0	+ 0 0	+ 0	0
Female fertility (based on % of pregnant females)				0 0 0	0	
Gestation period			0 0	0 00 0	0 0	
Implantation rate				0	0	
Litter size	0	00 0	0 0	000 000 00	0 00 000	00 000 0
Litter size over time (during forced breeding)	0	0		-		
Number of full-term pregnancies					-	
Number of implantation sites				-0	00	
Number of litters per dam (during forced breeding)	0	0		-		
Number of offspring (live)	-	0	00	00 0-	00 00	0 -0
Number of offspring (total)				0 00	0	
Number of resorption site				0		
Number of uterine implantations				0 0	0 0	00
Ovulation					0	0
Percent of females giving birth				000	00	000
Percent of time in diestrus					+	
Percent of uterine implantations			0	0	0	0
Percent postimplantation loss			0	0	0	0
Time to first delivery	0	0		0		

Table 5 References for Studies with Oral Administration of BPA

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Al-Hiyasat, AS; Darmani, H; Elbetieha, AM. 2004. "Leached components from dental composites and their effects on fertility of female mice." *Eur. J. Oral Sci.* 112:267-272.	X		X
Anderson, OS; Peterson, KE; Sanchez, BN; Zhang, Z; Mancuso, P; Dolinoy, DC. 2013. "Perinatal bisphenol A exposure promotes hyperactivity, lean body composition, and hormonal responses across the murine life course." *FASEB J.* 27(4):1784-1792.			X
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.	X		X
Ashby, J; Odum, J. 2004. "Gene expression changes in the immature rat uterus: Effects of uterotrophic and sub-uterotrophic doses of bisphenol A." *Toxicol. Sci.* 82:458-467.	X		
Ayyanan, A; Laribi, O; Schuepbach-Mallepell, S; Schrick, C; Gutierrez, M; Tanos, T; Lefebvre, G; Rougemont, J; Yalcin-Ozuysal, O; Brisken, C. 2011. "Perinatal exposure to bisphenol a increases adult mammary gland progesterone response and cell number." *Mol. Endocrinol.* 25(11):1915-23.		X	X
Betancourt, AM; Mobley, JA; Russo, J; Lamartiniere, CA. 2010a. "Proteomic analysis in mammary glands of rat offspring exposed in utero to bisphenol A." *J. Proteomics* 73(6):1241-53.	X		X
Betancourt, AM; Eltoum, IA; Desmond, RA; Russo, J; Lamartiniere, CA. 2010b. "*In utero* exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat." *Environ. Health Perspect.* 118(11):1614-9.			X
Boudalia, S; Berges, R; Chabanet, C; Folia, M; Decocq, L; Pasquis, B; Abdennebi-Najar, L; Canivenc-Lavier, M. 2013. "A multi-generational study on low-dose BPA exposure in Wistar rats: Effects on maternal behavior, flavor intake and development." *Neurotoxicol. Teratol.* 41C:16-26.			X
Cagen, SZ; Waechter, JM; Dimond, SS; Breslin, WJ; Butala, JH; Jekat, FW; Joiner, RL; Shiotsuka, RN; Veenstra, GE; Harris, LR. 1999. "Normal reproductive organ development in CF-1 mice following prenatal exposure to bisphenol A." *Toxicol. Sci.* 50:36-44.			X
Christiansen, S; Axelstad, M; Boberg, J; Vinggaard, AM; Pedersen, GA; Hass, U. 2013. "Low dose effects of BPA on early sexual development of male and female rats." *Reproduction* doi: 10.1530/REP-13-0377.	X	X	X
deCatanzaro, D; Berger, RG; Guzzo, AC; Thorpe, JB; Khan, A. 2013. "Perturbation of male sexual behavior in mice (Mus musculus) within a discrete range of perinatal bisphenol-A doses in the context of a high- or low-phytoestrogen diet." *Food Chem. Toxicol.* 55:164-171.			X
Delclos, KB; Camacho, L; Lewis, SM; Vanlandingham, MM; Latendresse, JR; Olson, GR; Davis, KJ; Patton, RE; Gamboa de Costa, G; Woodling, KA; Bryant, MS; Chidambaram, M; Trbojevic, R; Juliar, BE; Felton, RP; Thorn, BT. 2014. "Toxicity evaluation of bisphenol A administered by gavage to Sprague-Dawley rats from gestation day 6 through postnatal day 90." Toxicol. Sci. 139(1):174-197.	X	X	X
Diel, P; Schmidt, S; Vollmer, G; Janning, P; Upmeier, A; Bolt, HM; Degen, GH. 2004. "Comparative responses of three rat strains (DA/Han, Sprague-Dawley and Wistar) to treatment with environmental estrogens." *Arch. Toxicol.* 78:183-193.	X	X	
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.	X		X
Ferguson, SA; Law, CD Jr; Abshire, JS. 2011. "Developmental treatment with bisphenol A or ethinyl estradiol causes few alterations on early preweaning measures." *Toxicol. Sci.* 124(1):149-60.			X
Ferguson, SA; Law, CD; Kissling, GE. 2014. "Developmental treatment with ethinyl estradiol, but not bisphenol A, causes alterations in sexually dimorphic behaviors in male and female Sprague-Dawley rats." *Toxicol. Sci.* 140(2):374-392.			X
Howdeshell, KL; Hotchkiss, AK; Thayer, KA; Vandenbergh, JG; vom Saal, FS. 1999. "Exposure to bisphenol A advances in puberty." *Nature* 401:763-764.			X

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Howdeshell, KL; vom Saal, FS. 2000. "Developmental exposure to bisphenol A: Interaction with endogenous estradiol during prgnancy in mice." *Am. Zool.* 40:429-437.			X
Howdeshell, KL; Furr, J; Lambright, CR; Wilson, VS; Ryan, BC; Gray, LE. 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(2):371-382.			X
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(3):165-171.			X
Jenkins, S; Raghuraman, N; Eltoum, I; Carpenter, M; Russo, J; Lamartiniere, CA. 2009. "Oral exposure to bisphenol a increases dimethylbenzanthracene-induced mammary cancer in rats." *Environ. Health Perspect.* 117(6):910-5.		X	X
Jenkins, S; Wang, J; Eltoum, I; Desmond, R; Lamartiniere, CA. 2011. "Chronic oral exposure to bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice." *Environ. Health Perspect.* 119(11):1604-9.	X		
Kabuto, H; Amakawa, M; Shishibori, T. 2004. "Exposure to bisphenol A during embryonic/fetal life and infancy increases to oxidative injury and causes underdevelopment of the brain and testis in mice." *Life Sci.* 74:2931-2940.			X
Kass, L; Altamirano, GA; Bosquiazzo, VL; Luque, EH; Muñoz-de-Toro, M. 2012. "Perinatal exposure to xenoestrogens impairs mammary gland differentiation and modifies milk composition in Wistar rats." *Reprod. Toxicol.* 33(3):390-400.		X	X
Kass, L; Durando, M; Altamirano, GA; Manfroni-Ghibaudo, GE; Luque, EH; Munoz-de-Toro, M. 2014. "Prenatal bisphenol A exposure delays the development of the male rat mammary gland." *Reprod. Toxicol.* doi: 10.1016/j.reprotox.2014.02.001.		X	X
Kendig, EL; Buesing, DR; Christie, SM; Cookman, CJ; Gear, RB; Hugo, ER; Kasper, SN; Kendziorski, JA; Ungi, KR; Williams, K; Belcher, SM. 2012. "Estrogen-like disruptive effects of dietary exposure to bisphenol A or 17α-ethinyl estradiol in CD1 mice." *Int. J. Toxicol.* 31(6):537-550.	X		X
Kim, P; Lee, A; Hwang, S. 2003. "The bisphenol A: Modulator of pregnancy in rats." *Korean J. Environ. Health. Soc.* 29(4):27-34.	X		X
Kobayashi, K; Ohtani, K; Kubota, H; Miyagawa, M. 2010. "Dietary exposure to low doses of bisphenol A: Effects on reproduction and development in two generations of C57BL/6J mice." *Congenit. Anom.* 50:159-170.	X		X
Kobayashi, K; Kubota, H; Ohtani, K; Hojo, R; Miyagawa, M. 2012. "Lack of effects for dietary exposure of bisphenol A during in utero and lactational periods on reproductive development in rat offspring." *J. Toxicol. Sci.* 37(3):565-73.	X		X
Krementsov, DN; Katchy, A; Case, LK; Carr, FE; Davis, B; Williams, C; Teuscher, C. 2013. "Studies in experimental autoimmune encephalomyelitis do not support developmental bisphenol A exposure as an environmental factor in increasing multiple sclerosis risk." *Toxicol. Sci.* 135(1):91-102.	X	X	
Kubo, K; Arai, O; Ogata, R; Omura, M; Hori, T; Aou, S. 2001. "Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat." *Neurosci. Lett.* 304:73-76.	X	X	X
Kubo, K; Arai, O; Omura, M; Watanabe, R; Ogata, R; Aou, S. 2003. "Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats." *Neurosci. Res.* 45:345-356.	X	X	X
Kumamoto, T; Oshio, S. 2013. "Effect of fetal exposure to bisphenol A on brain mediated by X-chromosome inactivation." *J. Toxicol. Sci.* 38(3):485-494.			X
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.	X	X	X

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
LaRocca, J; Boyajian, A; Brown, C; Smith, SD; Hixon, M. 2011. "Effects of in utero exposure to Bisphenol A or diethylstilbestrol on the adult male reproductive system." *Birth Defects Res. B Dev. Reprod. Toxicol.* 92(6):526-33.			X
Lee, HA; Shin, JA; Oh, BR; Park, S; Jeong, E; Lee, H; Gwak, HS; Kim, YJ; Park, H. 2013a. "Longitudinal changes in offspring body weight, fat mass and sex hormone levels according to maternal bisphenol A exposure during gestation and lactation." *Mol. Cell. Toxicol.* 9(3):285-293.			X
Lee, SG; Kim, JY; Chung, J-Y; Kim, Y-J; Park, J-E; Oh, S; Yoon, Y-D; Yoo, KS; Yoo, YH; Kim, J-M. 2013b. "Bisphenol A exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17β-estradiol synthesis via downregulation of aromatase in rat ovary." *Environ. Health Perspect.* 121(6):663-669.			X
Liu, X-L; Chen, X-Y; Wang, Z-C; Shen, T; Zhao, H. 2013a. "Effects of exposure to bisphenol A during pregnancy and lactation on the testicular morphology and caspase‐3 protein expression of ICR pups." *Biomed. Rep.* 1(3):420-424.			X
Ma, Y; Xia, W; Wang, DQ; Wan, YJ; Xu, B; Chen, X; Li, YY; Xu, SQ. 2013. "Hepatic DNA methylation modifications in early development of rats resulting from perinatal BPA exposure contribute to insulin resistance in adulthood. *Diabetologia* 56(9):2059-2067.			X
Mendoza-Rodríguez, CA; García-Guzmán, M; Baranda-Avila, N; Morimoto, S; Perrot-Applanat, M; Cerbón, M. 2011. "Administration of bisphenol A to dams during perinatal period modifies molecular and morphological reproductive parameters of the offspring." *Reprod. Toxicol.* 31(2):177-83.		X	X
Midoro-Horiuti, T; Tiwari, R; Watson, CS; Goldblum, RM. 2010. "Maternal bisphenol A exposure promotes the development of experimental asthma in mouse pups." *Environ. Health Perspect.* 118(2):273-277.			X
Miyawaki, J; Sakayama, K; Kato, H; Yamamoto, H; Masuno, H. 2007. "Perinatal and postnatal exposure to bisphenol A increases adipose tissue mass and serum cholesterol level in mice." *J. Atheroscler. Throm.* 14:214-221.			X
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.		X	
Nanjappa, MK; Simon, L; Akingbemi, BT. 2012. "The industrial chemical bisphenol A (BPA) interferes with proliferative activity and development of steroidogenic capacity in rat Leydig cells." *Biol. Reprod.* 86(5):135, 1-12.			X
Naule, L; Picot, M; Martini, M; Parmentier, C; Hardin-Pouzet, H; Keller, M; Franceschini, I; Mhaouty-Kodja, S. 2014. "Neuroendocrine and behanioral effects of maternal exposure to oral bisphenol A in female mice." *J. Endocrinol.* 220:375-388.	X	X	X
Negishi, T; Kawasaki, K; Takatori, A; Ishii, Y; Kyuwa, S; Kuroda, Y; Yoshikawa, Y. 2003. "Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats." *Environ. Toxicol. Pharmacol.* 14:99-108.			X
Negishi, T; Tominaga, T; Ishii, Y; Kyuwa, S; Hayasaka, I; Kuroda, Y; Yoshikawa, Y. 2004. "Comparative study on toxicokinetics of bisphenol A in F344 rats, monkeys (Macaca fascicularis), and Chimpanzees (Pan troglodytes)." *Exp. Anim.* 53:391-394.			X
Pacchierotti, F; Ranaldi, R; Eichenlaub-Ritter, U; Attia, S; Adler, ID. 2008. "Evaluation of aneugenic effects of bisphenol A in somatic and germ cells of the mouse." *Mutat. Res.* 651(1-2):64-70.			X
Palanza, P; Howdeshell, KL; Parmigiani, S; vom Saal, FS. 2002. "Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior of mice." *Environ. Health Perspect.* 110(Suppl 3):415-422.			X
Patel, BB; Raad, M; Sebag, IA; Chalifour, LE. 2013. "Lifelong exposure to bisphenol A alters cardiac structure/function, protein expression and DNA methylation in adult mice." *Toxicol. Sci.* 133(1):174-185.	X		X
Patel, BB; Di Iorio, M; Chalifour, LE. 2014. "Metabolic response to chronic bisphenol A exposure in C57bl/6n mice." *Toxicol. Reports.* 1:522-532.	X		

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Patisaul, HB; Sullivan, AW; Radford, ME; Walker, DM; Adewale, HB; Winnik, B; Coughlin, JL; Buckley, B; Gore, AC. 2012. "Anxiogenic effects of developmental bisphenol A exposure are associated with gene expression changes in the juvenile rat amygdala and mitigated by soy." *PLoS One* 7(9):e43890.			X
Patisaul, HB; Mabrey, N; Adewale, HB; Sullivan, AW. 2014. "Soy but not bisphenol A (BPA) induces hallmarks of polycystic ovary syndrome (PCOS) and related metabolic co-morbidities in rats." *Reprod. Toxicol.* 49:209-218.		X	X
Peknicova, J; Kyselova, V; Buckiova, D; Boubelik, M. 2002. "Effect of an endocrine disruptor on mammalian fertility. Application of monoclonal antibodies against sperm proteins as markers for testing sperm damage." *Am. J. Reprod. Immunol.* 47:311-318.			X
Poimenova, A; Markaki, E; Rahiotis, C; Kitraki, E. 2010. "Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A." *Neuroscience* 167(3):741-749.			X
Rosenfeld, CS; Sieli, PT; Warzak, DA; Ellersieck, MR; Pennington, KA; Roberts, RM. 2013. "Maternal exposure to bisphenol A and genistein has minimal effect on Avy/a offspring coat color but favors birth of agouti over nonagouti mice." *Proc. Natl. Acad. Sci.* 110(2):537-542.			X
Roy, A; Bauer, SM; Lawrence, BP. 2012. "Developmental exposure to bisphenol A modulates innate but not adaptive immune responses to influenza A virus infection." *PLoS One* 7(6): e38448.			X
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(7):675-680.	X	X	X
Ryan, BC; Hotchkiss, AK; Crofton, KM; Gray, LE Jr. 2010a. "*In utero* and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats." *Toxicol. Sci.*114(1):133-48.	X	X	X
Ryan, KK; Haller, AM; Sorrell, JE; Woods, SC; Jandacek, RJ; Seeley, RJ. 2010b. "Perinatal exposure to bisphenol-A and the development of metabolic syndrome in CD-1 mice." *Endocrinol.* 151(6):2603-2612.			X
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(7):675-680.			X
Sadowski, RN; Park, P; Neese, SL; Ferguson, DC; Schantz, SL; Juraska, JM. 2014. "Effects of perinatal bisphenol A exposure during early development on radial arm maze behavior in adult male and female rats." *Neurotoxicol. Teratol.* 42:17-24.			X
Schonfelder, G; Flick, B; Mayr, E; Talsness, C; Paul, M; Cahoud, I. 2002. "In utero exposure to low doses of bisphenol A lead to long-term deleterious effects in the vagina." *Neoplasia* 4(2):98-102.		X	
Schonfelder, G; Friedrich, K; Paul, M; Cahoud, I. 2004. "Developmental effects of prenatal exposure to bisphenol A on the uterus of rat offspring." *Neoplasia* 6(5):584-594.		X	
Seidlova-Wuttke, D; Jarry, H; Wuttke, W. 2004. "Pure estrogenic effect of benzophenone-2 (BP2) but not of bisphenol A (BPA) and dibutylphtalate (DBP) in uterus, vagina, and bone." *Toxicology* 205:103-112.	X	X	
Sobolewski, M; Conrad, K; Allen, JL; Weston, H ; Martin, K; Lawrence, BP; Cory-Schlecta, DA. 2014. "Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals." *Neurotoxicology*. 45:121-130.			X
Somm, E; Schwitzgebel, VM; Toulotte, A; Cederroth, CR; Combescure, C; Nef, S; Aubert, ML; Huppi, PS. 2009. "Perinatal exposure to bisphenol A alters early adipogenesis in the rat." *Environ. Health Perspect.* 117(10):1549-1555.			X

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Stump, DG; Beck, MJ; Radovsky, A; Garman, RH; Freshwater, LL; Sheets, LP; Marty, MS; Waechter, JM; Dimond, SS; Van Miller, JP; Shiotsuka, RN; Beyer, D; Chappelle, AH; Hentges, SG. 2010. "Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats." *Toxicol. Sci.* 115(1):167-182.			X
Talsness, C; Fialkowski, O; Gericke, C; Merker, HJ; Chahoud, I. 2000. "Effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring." *Congenit. Anom. (Kyoto)* 40:S94-S107.	X	X	X
Tinwell, H; Joiner, R; Pate, I; Soames, A; Foster, J; Ashby, J. 2000. "Uterotrophic activity of bisphenol A in the immature mouse." *Regul. Toxicol. Pharmacol.* 32:118-126.	X	X	
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.	X		X
Tyl, RW; Myers, CB; 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. 2002. "Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats." *Toxicol. Sci.* 68:121-146.	X		X
Tyl, RW; Myers, CB; Marr, MC; Sloan, CS; Castillo, NP; Veselica, MM; Seely, JC; Dimond, SS; Van Miller, JP; Shiotsuka, RS; Beyer, D; Hentges, SG; Waechter, JM Jr. 2008. "Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice." *Toxicol. Sci.* 104(2):362-384.	X	X	X
van Esterik, JC: Dolle, ME: Lamoree, MH; van Leeuwen, SP; Hamers, T; Legler, J; van der Ven, LT. 2014. "Programming of metabolic effects in C57BL/6JxFVB mice by exposure to bisphenol A during gestation and lactation." *Toxicology*. 321:40-52.			X
Wang, D; Gao, H; Bandyopadhyay, A; Wu, A; Yeh, IT; Chen, Y; Zou, Y ; Huang, C; Walter, CA; Dong, Q; Sun, LZ. 2014a. "Pubertal bisphenol A exposure alters murine mammary stem cell (MaSC) function leading to early neoplasia in regenerated glands." *Cancer Prev. Research.* 7:445-455.		X	
Wang, W; Hafner, KS; Flaws, JA. 2014b. "*In utero* bisphenol A exposure disrupts germ cell nest breakdown and reduces fertility with age in the mouse." *Toxicol. Appl. Pharmacol.* 276(2):157-164.			X
Wang, W; Jiang, C; Zhu, L; Liang, N; Liu, X; Jia, J; Zhang, C; Zhai, S; Zhang, B. 2014c. "Adsorption of bisphenol A to a carbon nanotube reduced its endocrine disrupting effect in mice male offspring." *Int. J. Molec. Sci.* 15(9):15981-15993.			X
Wang, J; Jenkins, S; Lamartiniere, CA. 2014d. "Cell proliferation and apoptosis in rat mammary glands following combinational exposure to bisphenol A and genistein." *BMC Cancer.* 14:379.	X	X	X
Weber Lozada, K; Keri, RA. 2011. "Bisphenol A increases mammary cancer risk in two distinct mouse models of breast cancer." *Biol. Reprod.* 85(3):490-497.		X	X
Wei, J; Lin, Y; Li, Y; Ying, C; Chen, J; Song, L; Zhou, Z; Lv, Z; Xia, W; Chen, X; Xu, S. 2011. "Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet." *Endocrinol.* 152(8):3049-3061.			X
Xu, X; Liu, Y; Sadamatsu, M; Tsutsumi, S; Akaike, M; Ushijima, H; Kato, N. 2007. "Perinatal bisphenol A affects the behavior and SRC-1 expression of male pups but does not influence on the thyroid hormone receptors and its responsive gene." *Neurosci. Res.* 58:149-155.			X
Xu, X-H; Wang, Y-M; Zhang, J; Luo, Q-Q; Ye, Y-P; Ruan, Q. 2009. "Perinatal exposure to bisphenol-A changes N-methyl-D-aspartate receptor expression in the hippocampus of male rat offspring." *Environ. Toxicol. Chem.* 29(1):176-181.			X
Xu, X; Tian, D; Hong, X; Chen, L; Xie, L. 2011. "Sex-specific influence of exposure to bisphenol-A between adolescence and young adulthood on mouse behaviors." *Neuropharmacol.* 61(4):565-573.	X		
Xu, X; Liu, X; Zhang, Q; Zhang, G; Lu, Y; Ruan, Q; Dong, F; Yang, Y. 2013. "Sex-specific effects of bisphenol-A on memory and synaptic structural modification in hippocampus of adult mice." *Horm. Behav.* 63(5):766-775.	X		

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Yoshida, M; Shimomoto, T; Katashima, S; Watanabe, G; Taya, K; Maekawa, A. 2004. "Maternal exposure to low doses of bisphenol A has no effects on the development of female reproductive tract and uterine carcinogenesis in Donryu rats." *J. Reprod. Dev.* 50(3):349-360.	X	X	X
Yoshino, S; Yamaki, K; Li, X; Sai, T; Yanagisawa, R; Takano, H; Taneda, S; Hayashi, H; Mori, Y. 2004. "Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice." *Immunology* 112:489-495.			X
Zhang, HQ; Zhang, XF; Zhang, LJ; Chao, HH; Pan, B; Feng, YM; Li, L; Sun, XF; Shen, W. 2012. "Fetal exposure to bisphenol A affects the primordial follicle formation by inhibiting the meiotic progression of oocytes." *Mol. Biol. Rep.* 39(5):5651-7.		X	X
Zoeller, RT; Bansal, R; Parris, C. 2005. "Bisphenol-A, an environmental contaminant that acts as a thyriod hormone receptor antagonist in vitro, increases serum thyroxine and alters RC3/neurogranin expression in the developing rat brain." *Endocrinology*. 146(2):607-612.			X

Table 6 References for Studies with Non-oral Administration of BPA

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Adewale, HB; Jefferson, WN; Newbold, RR; Patisaul, HB. 2009. "Neonatal bisphenol-a exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons." *Biol. Reprod.* 81(4):690-9.		X	X
Alonso-Magdalena, P; Vieira, E; Soriano, S; Menes, L; Burks, D; Quesada, I; Nadal, A. 2010. "Bisphenol-A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring." *Environ. Health Perspect.* 118(9):1243-1250.			X
Berger, RG; Hancock, T; deCatanzaro, D. 2007. "Influence of oral and subcutaneous bisphenol-A on intrauterine implantation of fertilized ova in inseminated female mice." *Reprod. Toxicol.* 23(2):138-144.			X
Berger, RG; Shaw, J; Decatanzaro, D. 2008. "Impact of acute bisphenol-A exposure upon intrauterine implantation of fertilized ova and urinary levels of progesterone and 17beta-estradiol." *Reprod. Toxicol.* 26(2):94-99.			X
Cabaton, NJ; Wadia, PR; Rubin, BS; Zalko, D; Schaeberle, CM; Askenase, MH; Gadbois, JL; Tharp, AP; Whitt, GS; Sonnenschein, C; Soto, AM. 2011. "Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice." *Environ. Health Perspect.* 119(4):547-52.			X
Chao, HH; Zhang, XF; Chen, B; Pan, B; Zhang, LJ; Li, L; Sun, XF; Shi, QH; Shen, W. 2012. "Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway." *Histochem. Cell Biol.* 137(2):249-59.		X	
Durando, M; Kass, L; Piva, J; Sonnenschein, C; Soto, AM; Luque, EH; Munoz de Toro, M. 2007. "Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats." *Environ. Health Perspect.* 115:80-86.		X	X
Durando, M; Kass, L; Perdomo, V; Bosquiazzo, VL; Luque, EH; Muñoz-de-Toro, M. 2011. "Prenatal exposure to bisphenol A promotes angiogenesis and alters steroid-mediated responses in the mammary glands of cycling rats." *J. Steroid. Biochem. Mol. Biol.* 127(1-2):35-43.		X	
Fernández, M; Bianchi, M; Lux-Lantos, V; Libertun, C. 2009. "Neonatal exposure to bisphenol a alters reproductive parameters and gonadotropin releasing hormone signaling in female rats." *Environ. Health Perspect.* 117(5):757-62.			X
Fernández, M; Bourguignon, N; Lux-Lantos, V; Libertun, C. 2010. "Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats." *Environ. Health Perspect.* 118(9):1217-22.	X	X	X
Honma, S; Suzuki, A; Buchanan, DL; Katsu, Y; Watanabe, H; Iguchi, T. 2002. "Low dose effect of in utero exposure bisphenol A and diethylstilbestrol on female mouse reproduction." *Reprod. Toxicol.* 16:117-122.			X
Iwasaki, T; Totsukawa, K. 2003. "Change in the sexual maturation and estrogen receptor in mouse fetuses exposed to bisphenol A." *Environ. Sci.* 10(4):239-246.	X		X
Karavan, JR; Pepling, ME. 2012. "Effects of estrogenic compounds on neonatal oocyte development." *Reprod. Toxicol.* 34(1):51-6.		X	
Kass, L; Durando, M; Altamirano, GA; Manfroni-Ghibaudo, GE; Luque, EH; Munoz-de-Toro, M. 2014. "Prenatal bisphenol A exposure delays the development of the male rat mammary gland." *Reprod. Toxicol.* doi: 10.1016/j.reprotox.2014.02.001.		X	X
Kim, K; Son, TG; Park, HR; Kim, SJ; Kim, HS; Kim, HS; Kim, TS, Jung, KK; Han, SY; Lee, J. 2009. "Potencies of bisphenol A on the neuronal differentiation and hippocampal neurogenesis." *J. Toxicol. Environ. Health, Part A.* 72:1343-1351.			X
Koda, T; Umezu, T; Kamata, R; Morohoshi, K; Ohta, T; Morita, M. 2005. "Uterotrophic effects of benzophenone derivatives and a p-hydroxybenzoate used in unltraviolet screens." *Environ. Res.* 98:40-45.	X		
Liu, J; Yu, P; Qian, W; Li, Y; Zhao, J; Huan, F; Wang, J; Xiao, H. 2013b. "Perinatal bisphenol A exposure and adult glucose homeostasis: Identifying critical windows of exposure." *PLoS One* 8(5):e64143.			X

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Losa-Ward, SM; Todd, KL; McCaffrey, KA; Tsutsui, K; Patisaul, HB. 2012. "Disrupted organization of RFamide pathways in the hypothalamus is associated with advanced puberty in female rats neonatally exposed to bisphenol A." *Biol. Reprod.* 87(2):28.			X
Markey, CM; Luque, EH; Munoz de Toro, M; Sonnenschein, C; Soto, AM. 2001. "In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland." *Biol. Reprod.* 65:1215-1223.		X	
Markey, CM; Coombs, MA; Sonnenschein, C; Soto, AM. 2003. "Mammalian development in a changing environment: Exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs." *Evol. Dev.* 5:67-75.	X	X	X
Markey, CM; Wadia, PR; Rubin, BS; Sonnenschein, C; Soto, AM. 2005. "Long-term effects of fetal exposure to low doses of the xenoestrogen bisphenol-A in the female mouse genital tract." *Biol. Reprod.* 72(6):1344-1351.	X	X	
Mehmood, Z; Smith, AG; Tucker, MJ; Chuzel, F; Carmichael, NG. 2000. "The development of methods for assessing the in vivo oestrogen-like effects of xenobiotics in CD-1 mice." *Food Chem. Toxicol.* 38:493-501.		X	
Monje, L; Varayoud, J; Muñoz-de-Toro, M; Luque, EH; Ramos, JG. 2009. "Neonatal exposure to bisphenol A alters estrogen-dependent mechanisms governing sexual behavior in the adult female rat." *Reprod. Toxicol.* 28(4):435-42.			X
Munoz-de-Toro, M; Markey, C; Wadia, PR; Luque, EH; Rubin, BS; Sonnenschein, C; Soto, AM. 2005. "Perinatal exposure to bisphenol A alters peripubertal mammary gland development in mice." *Endocrinology* 146(9):4138-4147.		X	
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(3):383-390.		X	X
Naciff, JM; Hess, KA; Overmann, GJ; Torontali, SM; Carr, GJ; Tiesman, JP; Foertsch, LM; Richardson, BD; Martinez, JE; Daston, GP. 2005. "Gene expression changes induced in the testis by transplacental exposure to high and low doses of 17-Ethynyl estradiol, genistein, or bisphenol A." *Toxicol. Sci.* 86(2):396-416.		X	X
Nah, WH; Park, MJ; Gye, MC. 2011. "Effects of early prepubertal exposure to bisphenol A on the onset of puberty, ovarian weights, and estrous cycle in female mice." *Clin. Exp. Reprod. Med.* 38(2):75-81.	X		X
Newbold, RR; Jefferson, WN; Banks, EP. 2007. "Long-term adverse effects of neonatal exposure to bisphenol A on the Murine female reproductive tract." *Reprod. Toxicol.* 24(2):253-258.		X	
Newbold, RR; Jefferson, WN; Padilla-Banks, E. 2009. "Prenatal exposure to bisphenol a at environmentally relevant doses adversely affects the murine female reproductive tract later in life." *Environ. Health Perspect.* 117(6):879-85.		X	X
Nikaido, Y; Yoshizawa, K; Danbara, N; Tsujita-Kyutoku, M; Yuri, T; Uehara, N; Tsubura, A. 2004. "Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring." *Reprod. Toxicol.* 18:803-811.		X	X
Noda, S; Muroi, T; Mitoma, H; Takakura, S; Sakamoto, S; Minobe, A; Yamasaki, K. 2005. "Reproductive toxicity study on bisphenol A, nonylphenol, and genistein in neonatally exposed rats." *J. Toxicol. Pathol.* 18:203-207.	X	X	X
Okada, A; Kai, O. 2008. "Effects of estradiol-17B and bisphenol A administered chronically to mice throughout pregnancy and lactation on the male pups' reproductive system." *Asian J. Androl.* 10:271-276.			X
Park, DH; Jang, HY; Park, CK; Cheong, HT; Kim, CI; Yank, BK. 2004. "Effect of bisphenol A administration on reproductive characteristic and blood metabolite in mice." *J. Anim. Sci. Technol.* 46(6):957-966.	X		
Park, DH; Jang, HY; Kim, CI; Choung, I; Cheong, HT; Park, CK; Yank BK. 2005a. "Effect of bisphenol A administration on reproductive toxicant of dam and sex ratio of pups in pregnant mice." *J. Toxicol. Public Health* 21(2):161-165.	X	X	X

Reference	Organ Weight	Organ Morphology/ Cytology	Reproductive Endpoints
Park, DH; Jang, HY; Kim, CI; Choung, I; Cheong, HT; Park, CK; Yang, BK. 2005b. "Studies on the reproductive toxicant and blood metabolite in pups born after bisphenol A administration in pregnant mice." *J. Toxicol. Public Health* 21(2):167-173.	X	X	
Rodríguez, HA; Santambrosio, N; Santamaría, CG; Muñoz-de-Toro, M; Luque, EH. 2010. "Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary." *Reprod. Toxicol.* 30(4):550-7.		X	
Saito, D; Minamida, G; Tani-Ishii, N; Izukuri, K; Ozono, S; Koshika, S; Teranaka, T. 2003. "Effect of prenatal exposure to dental composite resin monomers on testosterone production in the rat testis." *Environ. Sci.* 10:327-336.			X
Signorile, PG; Spugnini, EP; Mita, L; Mellone, P; D'Avino, A; Bianco, M; Diano, N; Caputo, L; Rea, F; Viceconte, R; Portaccio, M; Viggiano, E; Citro, G; Pierantoni, R; Sica, V; Vincenzi, B; Mita, DG; Baldi, F; Baldi, A. 2010. "Pre-natal exposure of mice to bisphenol A elicits an endometriosis-like phenotype in female offspring." *Gen. Comp. Endocrinol.* 168(3):318-25.		X	
Steinmetz, R; Mitchner, NA; Grant, A; Allen, DL; Bigsby, RM; Ben-Jonathan, N. 1998. "The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract." *Endocrinology* 139(6):2741-2747.	X	X	
Tinwell, H; Joiner, R; Pate, I; Soames, A; Foster, J; Ashby, J. 2000. "Uterotrophic activity of bisphenol A in the immature mouse." *Regul. Toxicol. Pharmacol.* 32:118-126.	X	X	
Toyama, Y. 2005. "Experimental effects of cleft lip and/or palate and thymic anomalies estimated bisphenol-A (BPA) in A/J mice." *Aichi Gakuin Daigaku Shigakkai Shi* 43:409-420.			X
Toyama, Y; Suzuki-Toyota, F; Maekawa, M; Ito, C; Toshimori, K. 2004. "Adverse effects of bisphenol A to spermiogenesis in mice and rats." *Arch. Histol. Cytol.* 67(4):373-381.			X
Vandenberg, LN; Maffini, MV; Wadia, PR; Sonnenschein, C; Rubin, S; Soto, AM. 2007. "Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland." *Endocrinology* 148(1):116-127.		X	
Vandenberg, LN; Maffini, MV; Schaeberle, CM; Ucci, AA; Sonnenschein, C; Rubin, BS; Soto, AM. 2008. "Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice." *Reprod. Toxicol.* 26:210-9.	X	X	X
Varayoud, J; Ramos, JG; Bosquiazzo, VL; Lower, M; Muñoz-de-Toro, M; Luque, EH. 2011. "Neonatal exposure to bisphenol A alters rat uterine implantation-associated gene expression and reduces the number of implantation sites." *Endocrinol.* 152(3):1101-11.		X	X
Wadia, PR; Vandenberg, LN; Schaeberle, CM; Rubin, BS; Sonnenschein, C; Soto, AM. 2007. "Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains." *Environ. Health Perspect.* 115:592-598.	X	X	
Xiao, S; Diao, H; Smith, MA; Song, X; Ye, X. 2011. "Preimplantation exposure to bisphenol A (BPA) affects embryo transport, preimplantation embryo development, and uterine receptivity in mice." *Reprod. Toxicol.* 32(4):434-441.			X
Xu, X; Gu, T; Shen, Q. 2014. "Different effects of bisphenol-A on memory behavior and synaptic modification in intact and estrogen-deprived female mice." *J. Neurochem.* doi: 10.1111/jnc.12998.	X		
Yamasaki, K; Sawaki, M; Noda, S; Wada, T; Hara, T; Takatsuki, M. 2002. "Immature uterotrophic assay of estrogenic compounds in rats given diets of phytoestrogen content and the ovarian changes with ICI 182,780 or antide." *Arch. Toxicol.* 76:613-620.	X		X
Yamasaki, K; Ishii, S. 2012. "Toxicity study of bisphenol A, nonylphenol, and genistein in rats neonatally exposed to low doses." *J. Clinic Toxicol.* 2:e109.			X

Appendix 6

Side-by-Side Comparison of Three Individual Study Analyses by Peretz *et* al. (2014) and FDA

Side-by-Side Comparison of Descriptions of Three Studies by Peretz *et al*. (2014) and FDA (2014)

Peretz *et al*. (2014)	FDA (2014)
Hunt *et al*. (2012) "In a macaque study designed to mimic serum levels of unconjugated BPA reported in human biomonitoring studies (Vandenberg et al., 2010), Hunt et al. (2012) observed that daily low-dose BPA exposure (measured < 1 ng/mL in maternal serum) significantly disrupted synapsis and recombination between homologous chromosomes at the onset of meiosis, which was consistent with previous findings (Susiarjo et al., 2007)." "Similarly, in gestationally exposed macaques, dietary low-dose BPA exposure increased the number of oocytes present in secondary and antral follicles at birth, and continuous BPA exposure (measured < 1 ng/mL in maternal serum) increased the incidence of unenclosed oocytes (Hunt et al., 2012)."	**Hunt *et al*. (2012)** "Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey PNAS (Hunt *et al.*, 2012) This study was intended to determine if BPA affects the onset of meiosis in the developing fetal ovary and formation of the ovarian follicles in the perinatal ovary. Adult rhesus monkeys, age 6-13 years were used. They were housed individually in stainless steel cages and fed Purina monkey chow *ad libitum*. Supplements consisted of seasonal produce, seeds, and cereal. Pachytene oocytes from ovaries in the "early treatment" group were scored for synaptic defects. MLH1 (*mutL* homolog 1) protein foci in pachytene cells were counted. Follicles from ovaries in the "late treatment" group were staged and the number of oocytes per follicle was recorded. *A. Single oral exposure per day: There were 2 cohorts: early treatment and late treatment:* The early treatment consisted of groups of 5 treated and 6 controls administered 400 µg deuterated bisphenol A (dBPA) in small pieces of fruit from GD 50-100. Fetuses were removed by C-section at the end of the period. The late treatment consisted of groups of 6 treated and 6 controls administered 400 µg dBPA each day in small pieces of fruit from GD 100-term.

	B. Silastic capsules intradermally implanted to release dBPA and produce serum levels from 2.2 to 3.3 ng/ml unconjugated dBPA: There were 2 cohorts: early treatment and late treatment: The early treatment consisted of groups of 6 treated and 2 controls from GD 50-100. Fetuses were removed by C-section at the end of the period. The early treatment consisted of groups of 6 treated and 2 controls from GD 100-term. *"Effects on meiotic chromosome behavior:* Due to technical difficulties, cells suitable for MLH1 staining could only be obtained on two exposed and one control animal from the group given a single daily exposure only. For continuously exposed females, the mean values for MLH1 were "significantly "different" for 33 cells from continuously exposed animals compared with 70 cells from placebo treated animals (50.4 +/- 7.0 *vs.* 42.2+/- 7.9.) However, there were a lot of overlapping values, and the range was wide. Synaptic defects (non-homologous chromosome associations) identified by SYCP3 staining, reportedly previously seen in mice, were not seen at significantly increased incidences in either single exposure or continuous exposure groups. The authors reported that the percentage of oocytes with centromere associations was not different between exposed and control animals in the group given the single daily dose, but that there was a significant increase in centromere associations in the continuous exposure group. It is noted that the control values for centromere associations were notably different for fetuses between the study with a single exposure per day and that with continuous exposure. *Effects on follicle formation:* The authors reported that they were

	unable to count single and multioocytes per primordial follicle, so they focused on counting secondary and antral follicles in the medullary region of the ovary. In females exposed continuously, the overall distribution of follicles containing 1 to >5 oocytes was not statistically different between exposed and control females, whereas for females exposed once per day there was a statistically significant result. For both groups, the number of follicles with >5 oocytes was significantly increased but the absolute number/incidence is a few percent. It is noted again that the control values for the number of oocytes per follicle were notably different between the single exposure per day fetuses and continuous exposure fetuses. The authors conclude that BPA disrupts key events of meiotic prophase and follicle formation. The effects were less pronounced than in the mouse. **This study has no utility for HI and no utility for RA.** Uncertainties and Limitations include the following: The intradermal implant route of exposure with silastic capsules are of questionable utility or relevance. The vehicle in the slow release implant capsule and other chemical components are not reported. The estrogenic content of the diet is unclear. For MHL1 counts; cells from fetuses were counted. Small numbers of animals (only two control dams) were used for some analyses, and only one dose was analyzed. The timing mimicked the developmental windows purportedly "showing effects for mice". However timing in primates would be different. Because of design deficits, it is not clear that a biologically meaningful difference has been identified to be a BPA hazard. Extrapolation of effects of BPA from rhesus to humans regarding any fetal effects in this study is confounded by differences between rhesus

	monkeys and humans: A. "The differences in metabolism of progesterone and pregnenolone in the placenta and fetus of the rhesus monkey and human" (Leung *et al.*, 1972). "In the ovary, only weak β-subunit (of inhibin) immunoreactivity was detected in granulosa cells of a few primary follicles from midgestational human fetal ovaries. In contrast, all three subunits were found in granulosa cells of numerous primary and secondary follicles in the late gestation rhesus monkey ovary" (Rabinovici *et al.*, 1991). B. Rhesus monkeys are seasonal breeders (only 11 days per year, usually fall-winter) thus hormonal control of ovulation differs markedly for rhesus monkeys and humans (Riesen *et al.*, 1971). That the single daily dosing and continuous dosing experiments were conducted in two different breeding seasons cannot be ruled out as a confounding factor in interpretation of the results since the control values for the number of oocytes per follicle were notably different between the single exposure per day fetuses and continuous exposure fetuses."
Veiga-Lopez *et al*. (2013) "In another study, Veiga-Lopez et al. (2013) reported that prenatal BPA exposure altered the fetal ovarian steroidogenic gene and microRNA expression that mediate gonadal differentiation and folliculogenesis in sheep."	**Veiga-Lopez *et al*. (2013)** "Developmental programming: gestational bisphenol-A treatment alters trajectory of fetal ovarian gene expression (Veiga-Lopez *et al.*, 2013) This study investigated the impact of gestational exposure to BPA on developmental changes in the ovarian transcriptome in ovine

	fetus. Pregnant Suffolk ewes (2-3 years old, n=4-5) were treated with 0 or 0.5 mg BPA (purity≥99%) /kg bw/day in corn oil by SC from gestational day (GD) 30 through GD90. The ewes were fed with shelled corn (0.5 kg/day) and alfalfa hay (1.0-1.5 kg/day) and given chlortetracycline 250 mg/day to prevent abortion. Fetal ovaries were collected from both groups on GD65 (Control, n=4 and BPA, n=5; dam was used as the experimental unit) and GD90 (Control & BPA, n=5). Gene expression of steroidogenic enzymes, hormone receptors, growth factors and their receptors, and signal transduction factors, microRNA regulators, and micro RNAs were measured by quantitative RT-PCR. On GD90, the level of BPA in umbilical arterial blood was measured by HPLC coupled with mass spectrometer. All RT-PCR data were analyzed by 2-way ANOVA followed by a Bonferroni post hoc test, and Student's *t*-test was used for internal BPA concentration. No difference was reported in number of fetuses per dam between control and BPA groups at either GD. Concentration of free BPA in the umbilical artery was measured on GD90 only, which was significantly higher in BPA treated group than control (2.672 *vs.* 0.43 ng/ml). Age-dependent changes in ovarian mRNA expression were observed in steroidogenic enzymes, hormone receptors, growth factors and insulin-related biomarkers in both control (most up-regulated, except *17βHSD*) and BPA (most up-regulated, except *17βHSD* and *Cyp19*) groups. No differences in expression of these genes were observed between control and BPA groups at either GD, except *Cyp 19* (upregulated by BPA at GD 65 only) and *SRD5A1* (up-regulated by BPA at GD65 only). Age-dependent (both treated and untreated) and BPA treatment-related (both GDs) changes were also noticed in fetal ovine

	ovarian miRNAs. BPA down-regulated all measured miRNAs (from 2 to 275 fold) on both GDs, but had no effect on gene expression of miRNA regulators (endoribonuclease enzymes, *Drosha* and *Dicer*) at either GD. In this study, the environmental conditions were not properly controlled. High phytoestrogen containing (alfalfa) hay was fed to the pregnant ewes through the entire experiment, and the content of phytoestrogen in the hay was not analyzed. Other activities of chlortetracycline are not known in addition to its antibiotic effect. Other limitations include a single dose treatment, and one time point blood sample collection and measurement. Although BPA seemed to down-regulate expression of a number of miRNAs, no changes in the gene expression of miRNA regulators, ovarian steroidogenic enzymes, receptors or growth factors were observed, except a transient up-regulation of *Cyp19* and *SRD5A1* on GD65, which returned to normal levels on GD90. At the present time, it is not clear whether those observed changes are due to BPA. Moreover, the biological significance of these transient changes in expression of aromatase, 5α-reductase, and various miRNAs to fetal ovarian development and function remains to be determined. **This study has no utility for HI and no utility for RA.**
Rivera *et al*. (2011) "Studies have also suggested that BPA interferes with germ cell nest breakdown in animal models. In neonatally exposed lambs, low-dose BPA was reported to increase the incidence of multioocyte follicles (Rivera et al., 2011)."	**Rivera *et al*. (2011)** "Neonatal exposure to bisphenol A or diethylstilbesterol alters the ovarian follicular dynamics in the lamb (Rivera *et al*., 2011) This study evaluated the effect of neonatal exposure to BPA on follicle development in neonatal female lambs. Corriedale ewes

"BPA exposure also increased cell proliferation, indicative of follicular growth, in small antral follicles in neonatally exposed lambs and Wistar rats (Rivera et al., 2011; Rodriguez et al., 2010). Taken together, the data suggest that BPA enhances the recruitment and growth of primordial and primary follicles across species."	(2-4 years old) were mated to Hampshire Down rams. Female lambs born during August and September were randomly assigned to one of three treatment groups. Female lambs were dosed by sc injection on Postnatal Days 1 to 14 with DES at 5 µg/kg/day (n=6) or BPA at 50 µg/kg/day (n=6). The controls were dosed with the vehicle, corn oil (n=10). The experiments were conducted in an experimental farm belonging to a university in Argentina. The ewes grazed pasture with low rate of clover but the phytoestrogen content in the pasture was not analyzed. All control and treated lambs were sacrificed on PND 30. Female lambs were weighed and ovaries were removed, weighed, and further processed. Lambs were observed for clinical signs of acute or chronic toxicity. Ovaries were serially sectioned (5 µm thick) and one slide out of every 40 sections was stained with picosirius-hematoxylin. Follicular dynamics were evaluated by morphometry for the percentage of primordial and/or recruited follicles and the incidence of multioocyte follicles (MOFs). Ovarian sections were used to evaluate protein expression of ERα, ERβ, AR, Ki67, and p27 by immunohistochemistry. Hormone assays were done by RIA for serum levels of E2 or T. The results were analyzed statistically by the Kruskall-Wallis and Dunn post hoc test. Reported findings in the BPA and DES groups included significantly decreased ovarian weights, decreased primordial follicle reserve, and increased growing follicles (transitional and primary follicles). The increased incidence of MOFs which were mostly in the primordial stage was statistically significant only in the BPA group and non-statistically significant in the DES group. There was a higher proliferation rate in the large preantral cells

	(granulosa cells) and small antral follicles (granulosa and theca cells) as determined by immunoreactivity for Ki67. Both BPA and DES groups showed a significantly higher expression of p27 in granulosa cells of small antral follicles. Both groups showed an increased number of small antral atretic follicles. There were no effects in the BPA or DES groups on the expression of ERα, ERβ, and AR or the serum levels of E2 or T. This study is related to a previously reviewed study by Rodriguez *et al*, 2010 which was conducted in female rats. **Like the study by Rodriquez, this study is basically a mechanistic discovery study and is not useful for hazard identification or risk assessment.** The endpoints measured such as receptor protein expression, Ki67 and p27 biomarkers, and morphological counting of MOFs are at the cellular and molecular levels. These events are not clearly associated with adverse effects. The biological significance of these endpoints on follicular development and fertility in adult sheep needs to be determined. The study included only one dose of BPA and thus there was no dose-response measurement. It is difficult to translate dose by sc to the oral route."