April 20, 2015

Via E-Mail

Ms. Monet Vela
Office of Environmental Health Hazard Assessment
P. O. Box 4010
1001 I Street
Sacramento, California 95812-4010

Re: Bisphenol A -- Hazard Identification Material for DARTIC Review

Dear Ms. Vela:

The North American Metal Packaging Alliance, Inc. (NAMPA)\(^1\) submits this response to the Office of Environmental Health Hazard Assessment’s (OEHHA) announcement of the availability for public review of hazard identification materials on bisphenol A (BPA) and female reproductive toxicity. NAMPA provides below citations for additional information to be considered by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) and highlights specific studies that were found to be insufficient for hazard review. NAMPA believes this information supports the conclusion that existing scientifically valid BPA studies conducted to generally accepted principles do not meet the requisite standard for listing as a reproductive toxicant under Proposition 65 because they do not “clearly demonstrate that BPA causes female reproductive toxicity,” and as such, BPA should not be listed as a reproductive toxicant under Proposition 65.

**Additional Review Materials for DARTIC Consideration**

In addition to the materials identified in the OEHHA document, “Hazard Identification Materials for Consideration of the Female Reproductive Toxicity of Bisphenol A, February 2015,” NAMPA notes the following comprehensive regulatory reviews on BPA should also be provided to DARTIC for consideration:

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\(^1\) NAMPA is a not-for-profit corporation committed to protecting health through the safety of metal packaging and metal packaged foods. NAMPA’s membership includes companies and associations representing various sectors along the supply chain for the food and beverage packaging industry.
U.S. Food and Drug Administration (FDA) 2014 Updated Review of Literature and Data on Bisphenol A (2014)
The review of reproductive and developmental toxicity studies can be found on pages 84 through 112.

FDA 2012 Updated Review of Literature and Data on Bisphenol A (2013)
The review of reproductive and developmental toxicity studies can be found on pages 22 through 32.

Updated Review of the ‘Low-Dose’ Literature (Data) on Bisphenol A and Response to Charge Questions Regarding the Risk Assessment on Bisphenol A (2011)
The review of reproductive and developmental toxicity studies can be found on pages 28 through 36.

The review of reproductive and development toxicity studies can be found on pages 71 through 91.

Additional Studies for DARTIC Consideration

Studies on the metabolism of BPA are critical in determining whether exposures present a toxicological hazard. Toward that end, DARTIC members should include recent pharmacokinetic and metabolism studies in their review of the BPA literature, including the following studies posted on the FDA BPA website:


**DARTIC Should Make Note of Study Limitations**

In its multiple assessments of BPA, FDA scientists provided indications as to whether studies assessed were useful for hazard identification (HI) or risk assessment (RA). The following studies were referenced in “Bisphenol A and Reproductive Health: Update of Experimental and Human Evidence, 2007-2013” that is listed as a primary resource for DARTIC’s consideration. As highlighted below, when FDA scientists reviewed these same studies, the vast majority were found to have little or no utility for HI for the reasons listed. NAMPA urges DARTIC members to consider the scientifically valid concerns raised by FDA in their review.

Low dose bisphenol A impairs spermatogenesis by suppressing reproductive hormone production and promoting germ cell apoptosis in adult rats (Jin et al., 2013).

*FDA conclusion in 2014 updated review of literature and data:* This study is primarily a mechanistic study. Potential sources of environmental contamination in the study were not addressed, i.e., diet, water, cage, etc. The study only used one dose level and thus could not identify a dose-response for the parameters measured. Based on the mechanistic nature of the study, other limitations cited, and use of one dose level, this study has no utility for HI and no utility for RA.²

Lack of effects for dietary exposure of bisphenol A during in utero and lactational periods on reproductive development in rat offspring (Kobayashi et al., 2012).

*FDA conclusion in 2014 updated review of literature and data:* The study design has strengths and limitations. The strengths of the study include the use of three BPA treatment groups, two time points for the parameters as well

as n values of at least 10, and appropriate statistical methods. One weakness of the study methods is that there was no discussion of control for environmental BPA exposure in the cages or phytoestrogen level in the diet. Another weakness of the study is that the methods did not clearly state how the offspring were selected from the litters for the parameters measured. **This study has limited utility for HI and none for RA.**

- 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 (Lee et al., 2013).

  FDA conclusion in 2014 updated review of literature and data: **This study has no utility for HI and no utility for RA.** A dose-response could not be determined because the study methods used a limited dose range. Sample size was small; 3-5 animals per analysis. Certain study details that could impact the results of the estrous cycle analysis were not specified. For example, the study methods did not state the number of shipments of animals, how the animals were assigned to treatment groups, and did not evaluate cycling status of each animal before treatment to ensure that regularly cycling animals were assigned to control and treated groups. BPA purity and potential environmental sources of contamination were not addressed.

- Exposure to bisphenol A disrupts meiotic progression during spermatogenesis in adult rats through estrogen-like activity (Liu et al., 2013).

  FDA conclusion in 2014 updated review of literature and data: **This study has no utility for HI and no utility for RA.** Earlier FDA reviews have discussed inconclusive data concerning effects of BPA on spermatogenesis. Uncertainties and Limitations include: The statistical analysis described consists of multiple t-tests, without correction for multiple comparisons. The animal numbers are limited, and the mechanistic data are limited to a single dose group. The authors note differences in the actions of E2 and BPA (e.g., serum T) and discuss the differences in receptor affinities of the compounds and possible differences in receptor type and location distributions at various seminiferous epithelium stages, but they do not discuss the likely differences in active compound that reach the target organ given the differing routes of administration.

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3 Id. at 91.

4 Id. at 92.
used for E2 (SC) and BPA (gavage). An E2 plus ICI group would have been useful to support the mechanistic similarities of BPA and E2.5

- The industrial chemical bisphenol A (BPA) interferes with proliferative activity and development of steroidogenic capacity in rat Leydig cells (Nanjappa et al., 2012).

**FDA conclusion in 2014 updated review of literature and data:**

**This study has no utility for HI or for RA** because of limitations in the experimental design and methods. Although the study protocol used soy protein free diets and polypropylene cages, the vehicle was olive oil, which has been reported to have 5-alpha reductase inhibition activity. Dose response could not be assessed because three or more dose levels were not used. Although tissues were pooled from multiple F1 offspring, in the description of the study methods it is not clear if n values of 10 or more were used. The study protocol did not describe what method was used when male pups were assigned to litters within treatment groups, selected for measurements, or pooled for the molecular studies.6

- Decreased androgen receptor expression may contribute to spermatogenesis failure in rats exposed to low concentrations of bisphenol A (Qiu et al., 2013).

**FDA conclusion in 2014 updated review of literature and data:**

**This study has limited utility for HI but no utility for RA.** As previous FDA reviews have discussed, inconsistent or even conflicting effects of BPA on spermatogenesis have been reported. Potential sources of contamination in caging, water, and food were not addressed. The widely spaced doses of BPA provide limited dose-response information.7

- Bisphenol A differentially activates protein kinase C isoforms in murine placental tissue (Tan et al., 2013).

**FDA conclusion in 2014 updated review of literature and data:**

**This study has no utility for HI and no utility for RA.** Sample size was small, 3-5 animals per analysis. Potential sources of contamination in caging, water, and food were not evaluated. In the statistical analysis, the standard deviations are

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5 Id. at 93.
6 Id. at 95.
7 Id. at 96.
small and uniform in each analysis, which is unexpected for the endpoints measured.8

- Mutagenic effect of Bisphenol A on adult rat male germ cells and their fertility (Tiwari and Vanage, 2013).
  
  **FDA conclusion in 2014 updated review of literature and data:**
  
  **This study has no utility for HI and no utility for RA.** The number of males treated in each group is marginal (n=7), and sperm parameters and the comet assay were done in fewer animals (n=4-5). The dose spacing and number of doses in all assays is inadequate. The results in the comet assay may be secondary to toxicity to the sperm (due to apoptosis/necrosis and not a direct effect of BPA on DNA). Also, the comet assay did not include untreated controls or a positive control, and arbitrary units are given rather than absolute percent tail DNA. No justification was presented for the length of the treatment period. The authors took some care to avoid environmental exposure to BPA with regard to diet and water; although soy-free chow was used, it was prepared in-house, and quality control measures were not described. Additionally, caging and the possible presence of arsenic, which has adverse effects on sperm, in the paddy (rice) husk bedding is possible, were not described.9

- Bisphenol A exposure modifies methylation of imprinted genes in mouse oocytes via the estrogen receptor signaling pathway (Chao et al., 2012).
  
  **FDA conclusion in 2014 updated review of literature and data:**
  
  **This study has no utility for HI and no utility for RA** due to a lack of clarity in the description of the experimental design and methods. The exact number of mice per group was not presented, but 110 mice were utilized in Experiment 1 and 73 mice in Experiment 2. The study protocol did not describe how female pups were grouped with the dams nor did it describe how many female pups were assigned to treatment groups, selected for measurements, or pooled for the molecular studies. Thus, the study methods stated that oocytes from 10-12 ovaries were used in the analysis, but the number of F1 female pups or number of litters from F0 parental females was not stated. From the description in the study methods, it is not clear if N values of 10 or more were used in the statistical analysis and how pooled tissues were used for statistical analysis. Because only two treated groups were used, a dose response could not be assessed. The route of administration was not described completely, and no information was presented.

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8 Id. at 97.

9 Id. at 98.
on housing conditions, the type of 100 chow administered, the source and purity of BPA and the source of the animals.  

- Effects of transplacental 17-α-ethinyl estradiol or bisphenol A on the developmental profile of steroidogenic acute regulatory protein in the rat testis (Horstman et al., 2012). 
  
  **FDA conclusion in 2014 updated review of literature and data:**
  
  **This study has no utility for HI and no utility for RA.** Potential sources of contamination in water and food were not evaluated. The distribution of the doses was skewed, with two doses being fairly close to each other (0.02 and 0.5 mg/kg/day) and one dose being substantially higher (400 mg/kg bw/day). Samples within the litters were pooled and the litter was used as the statistical unit. The actual group sizes were unspecified (at least 6 litters used per group), however. Because an effect was seen in the QRT-PCR with the highest dose of BPA at GD20, the authors only examined that dose at the other time points.

- Effects of early prepubertal exposure to bisphenol A on the onset of puberty, ovarian weights, and estrous cycle in female mice (Nah et al., 2011). 
  
  **FDA conclusion in 2014 updated review of literature and data:**
  
  **This study has no utility for HI and no utility for RA.** The use of SC route, rather than oral route, in these PND8 animals would be expected to lead to higher internal doses of free BPA and should be a consideration in evaluating the reported effects (Doerge et al. 2011). As noted in previous FDA reviews, inconsistent findings on vaginal opening and ovarian weight changes have been reported in mice treated with BPA. The estrous cycle measurement in this study is inadequate since mice, not like rats, have not established their normal cycle yet at PND20-29 (Nelson et al., 1982). The stage of estrous cycle in the animals at sacrifice was not described, which can confound the ovarian morphology and weights profoundly. Other limitations include the lack of environmental control (diet, water, cage, etc.) and few animals (n=5) used for statistics.

- Developmental programming: gestational bisphenol-A treatment alters trajectory of fetal ovarian gene expression (Veiga-Lopez et al., 2013). 
  
  **FDA conclusion in 2014 updated review of literature and data:**

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10 *Id.* at 99 and 100.

11 *Id.* at 102.

12 *Id.* at 105.
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 \textit{Cyp19} and \textit{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. 

\textbf{This study has no utility for HI and no utility for RA.}\footnote{Id. at 107.}

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- Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey \textit{PNAS} (Hunt \textit{et al.}, 2012).

\textbf{FDA conclusion in 2014 updated review of literature and data:}

The authors conclude that BPA disrupts key events of meiotic prophase and follicle formation. The effects were less pronounced than in the mouse. This \textbf{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.” Timing in primates would be different, however. 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 \textit{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.\textsuperscript{14}

- Effects of in utero exposure to Bisphenol A or diethylstilbestrol on the adult male reproductive system (Larocca et al., 2011).

\textit{FDA conclusion in 2012 updated review of literature and data:}
The authors have demonstrated that in utero exposure of C57/Bl6 mice to BPA from GD 10 to 16 at 50 or 1000 μg/kg did not induce statistically significant changes in the parameters examined in this study. DES at 2 μg/kg did affect some endpoints. Limitations of this study include small sample sizes for most measured endpoints (n = 4-10), lack of control for potential environmental (PC cages) and dietary estrogen exposure (soy-based chow), one time point measurements (hormone, mRNA, etc), uncertainty of the biological and functional interpretation of the measurements/changes at the molecular levels, \textit{i.e.}, relationship of change in mRNA levels to expected functional changes in the reproductive system. The data in this study are in themselves not useful for either HI or RA.\textsuperscript{15}

- Oral exposure to low-dose bisphenol A aggravates testosterone-induced benign hyperplasia prostate in rats (Wu et al., 2011)

\textit{FDA conclusion in 2012 updated review of literature and data:}
This study has limited utility for hazard identification and no utility for risk assessment. Castration/supplementation methods are useful for mechanism of action information, but this study does not have any notable new findings. The castrated/supplemented rodent is not an appropriate model for evaluating human BPH. Systemic levels of BPA and potential sources of contamination in caging,

\textsuperscript{14} \textit{Id.} at 110.

water, and food were not evaluated. The amounts of phytoestrogens in the food were not reported.\textsuperscript{16}

- **Bisphenol-A exposure alters endometrial progesterone receptor expression in the nonhuman primate.** (Aldad \textit{et al.}, 2011).

  \textit{FDA conclusion in 2012 updated review of literature and data:}

  The authors demonstrated that BPA could inhibit estrogen-induced PR gene expression in the endometrium of the African green monkey. This is the first report of suppression of the PR gene’s expression in an adult primate, albeit surgically altered. This would be consistent with the known estrogen-dependent regulation of PR and suppression by many functional anti-estrogens. Other than this basic observation, little can be concluded since the methods section was inadequate for an evaluation of the system’s validity. Oophorectomy/supplementation methods are useful for mechanism of action information, but only a single dose of BPA was used, EB treatment used silastic implants and BPA was administered through an Alzet minipump. The dose of EB, BPA source, and animal husbandry information were not provided. Systemic levels of BPA and potential sources of contamination in caging, water, and food were not evaluated. \textit{This study has limited utility for hazard identification and no utility for risk assessment.}\textsuperscript{17}

- **Neonatal exposure to bisphenol A or diethylstilbesterol alters the ovarian follicular dynamics in the lamb** (Rivera \textit{et al.}, 2011).

  \textit{FDA conclusion in 2012 updated review of literature and data:}

  This study is related to a previously reviewed study by Rodriguez \textit{et al}, 2010, which was conducted in female rats. \textit{Like the study by Rodriguez, this study is basically a mechanistic discovery study and is not useful for HI or RA}. 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.\textsuperscript{18}

\textsuperscript{16} \textit{Id.} at 27.

\textsuperscript{17} \textit{Id.} at 27 and 28.

\textsuperscript{18} \textit{Id.} at 30.
Neonatal exposure to Bisphenol A alters rat uterine implantation-associated gene expression and reduces the number of implantation sites (Varayoud et al., 2011). FDA conclusion in 2012 updated review of literature and data: This study has some utility for hazard ID (a long-lasting reduction of the number of implantation sites but only at a neonatal dose of 20 mg/kg BPA). A possible mode of action is suggested (through Hox signalling). The uterine environment of the rats exposed neonatally to 20 mg/kg BPA is not normal.  

Preimplantation exposure to bisphenol A (BPA) affects embryo transport preimplantation embryo development, and uterine receptivity in mice. (Xiao et al., 2011). FDA conclusion in 2012 updated review of literature and data: The authors concluded that high doses of BPA (40 or 100 mg/kg bw/day) by the sc route have adverse effects on processes critical for embryo implantation. This study is a mechanistic study which is not useful for hazard ID and for RA. Study limitations for risk assessment: no doses less than 40 mg/kg or 100 mg/kg were used (except study design 1), low and uneven n values in the study groups, rodent chow, which contains phytoestrogens, non-oral route (sc), study designs with only one or two treatment groups, no information on type of water bottle, possible effects of sesame oil vehicle, lack of adequate controls, and publication did not report analytical work on drinking water for contaminants or dosing solutions for added BPA level.

Endocrine disrupter bisphenol A increases in situ estrogen production in mouse urogenital sinus (Arase et al., 2011). FDA conclusion in 2011 updated review of “low dose” literature: The data in this report are in themselves not useful for HI or RA based on the small sample size and lack of clarity on the pooling of the pups for analysis.

Dietary exposure to low levels of bisphenol A: effects on reproduction and development in two generations of C57BL/6J mice (Kobayashi et al., 2010).

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19 Id. at 30.

20 Id. at 31 and 32.

21 FDA Bisphenol A (BPA) Joint Emerging Science Working Group Memorandum, “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” (May 24, 2011) at 29.
FDA conclusion in 2011 updated review of “low dose” literature:
This study meets the criteria for providing data of utility for HI, but not RA
due to concerns in the reporting of the study, including lack of confirmation of the
dietary dose levels and a lack of description of possible environmental
confounders.22

- Administration of bisphenol A to dams during perinatal period modifies
molecular and morphological reproductive parameters of the offspring (Mendoza-
Rodriguez et al., 2011).

FDA conclusion in 2011 updated review of “low dose” literature:
Despite the findings in the report, this study in itself did not meet the criteria for
HI or RA due to the lack of description of environmental contamination control
and offspring treatment (culling/cross-fostering), use of an inappropriate test unit
(pups instead of litter), small sample size, and the use of only a single dose
level.23

- Gene Expression in the Fetal Mouse Ovary is Altered by Exposure to Low Doses
of Bisphenol A (Lawson et al., 2011).

FDA conclusion in 2011 updated review of “low dose” literature:
While potentially providing mechanistic data, the study was not considered to
be useful for HI or RA given the statistical issue of pooling ovaries across litters
and the lack of a clear understanding of the biological relevance of the reported
gene changes.24

- Generation of reactive oxygen species in sperm of rats as an earlier marker for
evaluating the toxicity of endocrine-disrupting chemicals (Minamiyama et al.,
2010).

FDA conclusion in 2011 updated review of “low dose” literature:
While these results suggest possible mechanisms of action, they were not
considered appropriate for either HI or RA. The lack of a dose-response is also
problematic for interpretation of the results.25

22 Id. at 30.
23 Id. at 30.
24 Id. at 31.
25 Id. at 31.
Perinatal Exposure of Rats to Bisphenol A Affects the Fertility of Male Offspring (Salian et al., 2009).

FDA conclusion in 2011 updated review of “low dose” literature:
This study was not considered to be of utility for HI or RA given the lack of understanding that the F0 dam was the experimental unit. The use of an in-house bred strain of rat together with an in-house prepared diet also limits its usefulness because replication outside of this laboratory may be problematic.26

Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin-releasing hormone neurons (Adewale et al., 2009).

FDA conclusion in 2011 updated review of “low dose” literature:
With respect to the reproductive effects, this study is of low utility for HI and not useful for RA. While the high dose of BPA had clear effects on the ovary, low dose data are difficult to interpret due to insufficient control of potential litter effects and the limited evaluation of the data (no statistical evaluation of estrous cycle data, qualitative histological evaluation of low dose ovaries).27

Perinatal exposure to environmentally relevant levels of bisphenol-A decreases fertility and fecundity in CD-1 mice (Cabaton et al., 2011).

FDA conclusion in 2011 updated review of “low dose” literature:
This study is not useful for HI given the inappropriate solvent used for dose delivery and the unusual dose-response. Forced breeding, which alters maternal hormones, negates the use of the data for risk assessment since it may increase sensitivity to BPA through mechanisms that cannot readily be extrapolated to humans.28

Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary (Rodriguez et al., 2010).

FDA conclusion in 2011 updated review of “low dose” literature:
Considering the lack of information on BPA purity, potential phytoestrogen exposure from the diet, and dosing regime (two dose levels and 48 hrs dosing interval), the data in this study are not useful for HI or RA.29

26 Id. at 32.
27 Id. at 32.
28 Id. at 33.
29 Id. at 33 and 34.
Prenatal exposure of mice to bisphenol A elicits an endometreosis-like phenotype in female offspring (Signorile et al., 2010).  

*FDA conclusion in 2011 updated review of “low dose” literature:*  
**The study did not meet the criteria for HI or RA** based on the statistical analysis, which did not adequately address litter effects. Use of an isolated mouse colony and unusual diet are also possible concerns.30

Neonatal exposure to bisphenol A and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats (Fernandez et al., 2010).  

*FDA conclusion in 2011 updated review of “low dose” literature:*  
While experimental design issues, including the use of an in-house SD rat, housing pups in different dose groups in the same cage, and approximate dosing (i.e., the animals were not weighed before dosing) are of concern, the data do show a dose-response. **The data were thus considered to be of utility for HI based on the dose-response.**31

Neonatal Exposure of Male Rats to Bisphenol A Impairs Fertility and expression of Sertoli Cell Junctional Proteins in the testis (Salian et al., 2009a).  

*FDA conclusion in 2011 updated review of “low dose” literature:*  
Because of these statistical issues, this study was considered **not useful for HI or RA.** The reported effects of BPA on sperm numbers and motility and fertility may warrant further investigation, however.32

The following studies were included in the document, “Studies Relevant to the Female Reproductive Toxicity of Bisphenol A Published Subsequent to the Review by Peretz et al., 2014,” that was also provided as a reference to DARTIC members. As with the studies referenced in Peretz 2014 above, FDA scientists found concerns with several of these studies during its latest review, as noted below:

*The influence of endocrine disruptors in a selected population of infertile women (Caserta et al., 2013a).  

*FDA conclusion in 2014 updated review of literature and data:*  
Limitations of this study include the observational design, small sample size, and insufficient statistical power, thereby limiting statistical analyses to the Chi

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30 *Id.* at 34.
31 *Id.* at 35.
32 *Id.* at 35.
Square test, which cannot adjust for potential confounders such as age. Increasing age is associated with female infertility and also potentially with increased exposure to substances such as BPA. The mean age of the control group was notably lower (32.6 +/- 5.2 yrs) than the cases (36.0 +/- 4.7 yrs). In addition, the recruitment process for the controls was not well described, and there is potential for selection bias. Finally, there may be potential for residual confounding and reverse causation in this relationship, if unmeasured factors related to fertility status are also related to BPA metabolism. This study has no utility for HI or RA.  

Parental phenols exposure and spontaneous abortion in Chinese population residing in the middle and lower reaches of the Yangtze River (Chen et al., 2013).

**FDA conclusion in 2014 updated review of literature and data:**

This study has no utility for HI and no utility for RA. This is a small sample size study, and the case-control study design limits the ability to make a causal conclusion based on its results. Also, the study looked at only one urinary BPA sample, defining low and high exposure based on LOD, and it is unclear when the urinary samples were obtained in relation to the time of spontaneous abortion. The study was conducted in a population from a limited area of China, and study subjects were obtained from hospitalized patients because urine samples were required. Thus, it is unlikely that the reported findings are generalizable.

Maternal Urinary Bisphenol A during Pregnancy and Maternal and Neonatal Thyroid Function in the CHAMACOS Study (Harley et al., 2013). [NOTE: Harley is listed as the primary author in the FDA review. In the OEHHA documentation, Chevrier is listed as the primary author.]

**FDA conclusion in 2014 updated review of literature and data:**

Study strengths include a large sample size, observational cohort design, and two BPA measurements. Limitations include that while a majority of statistical tests did not find a statistical significance, authors generalize from the minimal positive results to say that there is therefore a relationship between maternal BPA and thyroid hormones without explaining the negative results. This study has no utility for HI and no utility for RA.


34 Id. at 145.

35 Id. at 147.
Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure (Kundakovic et al., 2013).

*FDA conclusion in 2014 updated review of literature and data:*
The study suggests prenatal BPA exposure as a possible hazard that may affect neuronal epigenetic pathways and behavioral outcomes. The results would need to be confirmed by other laboratories. **Thus, the data have limited utility for HI and no utility for RA.**

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 (Lee et al., 2013).

*FDA conclusion in 2014 updated review of literature and data:*
**This study has no utility for HI and no utility for RA.** A dose-response could not be determined because the study methods used a limited dose range. Sample size was small; 3-5 animals per analysis. Certain study details that could impact the results of the estrous cycle analysis were not specified. For example, the study methods did not state the number of shipments of animals, how the animals were assigned to treatment groups, and did not evaluate cycling status of each animal before treatment to ensure that regularly cycling animals were assigned to control and treated groups. BPA purity and potential environmental sources of contamination were not addressed.

Sex specific impact of perinatal bisphenol A (BPA) exposure over a range of orally administered doses on rat hypothalamic sexual differentiation (McCaffrey et al., 2013).

*FDA conclusion in 2014 updated review of literature and data:*
The primary strengths of this study were (a) the animal environment (e.g., caging materials) but not diet was well controlled for contamination with estrogenic substances; (b) multiple doses of BPA were employed; and (c) the food wafer method of BPA delivery avoided confounding effects of more stressful drug dosing measures. There were several limitations to the methodology, and only some will be listed here: (a) in vivo exposures were not verified through analysis of blood or urine for levels of BPA; (b) it is generally accepted that one pup per litter should be included per assay to remove litter bias. In this paper, the authors noted that “[f]our males and four females per litter were used to generate the data,” so it is not at all clear that the litter is the unit of analyses; (c) in terms of

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36 *Id.* at 49.

37 *Id.* at 92.
tissue preparation, it is not clear if brain slices were processed in batches containing samples from both sexes and several treatment groups, in order to control for bias in tissue processing and immunolabeling; (d) although it was previously reported that SDN-POA structures can be bilaterally asymmetrical (He et al., 2012), the investigators did not discuss how differences in asymmetry were addressed; and (e) the source and purity of BPA was not specified. **This study has no utility for HI and no utility for RA.**

- **Fetal Growth and Prenatal Exposure to Bisphenol A: The Generation X Study** (Snijder et al., 2013).
  
  **FDA conclusion in 2014 updated review of literature and data:** Although the results of this study suggest that increasing the number of urinary BPA measurements per subject during pregnancy may result in better exposure-response estimates, the study is not conclusive in demonstrating that higher BPA levels in prenatal urine may result in lower fetal growth rate, since the findings were only positive in a subgroup of 80 women with all three urinary BPA measurements. Other limitations of the study include a relatively small sample size and the generalizability of study results. **The findings on the BPA-associated lower fetal growth rate in a subgroup of 80 women with triplicate measurement needs to be further confirmed in a well-designed prospective study. This study has limited utility for HI and no utility for RA.**

- **Bisphenol A differentially activates protein kinase C isoforms in murine placental tissue** (Tan et al., 2013).
  
  **FDA conclusion in 2014 updated review of literature and data:** **This study has no utility for HI and no utility for RA.** Sample size was small, 3-5 animals per analysis. Potential sources of contamination in caging, water, and food were not evaluated. In the statistical analysis, the standard deviations are small and uniform in each analysis, which is unexpected for the endpoints measured.

- **Associations of prenatal exposure to phenols with birth outcomes** (Tang et al., 2013).
  
  **FDA conclusion in 2014 updated review of literature and data:**

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38 Id. at 51.

39 Id. at 167.

40 Id. at 97.
The study sample is a convenience sample recruited at NMU-affiliated hospitals in a metropolitan city in China with questionable generalizability to other populations. Of the confounders taken into account, most were self-reported and not otherwise verified, except for BMI for which measurements were taken at delivery. Exposure measurement methods were validated, but BPA concentration was measured from urine samples taken at only one time point (at admission for delivery, introducing the inconsistency of different times of day), and it is unknown how that measurement relates to exposure during the rest of the pregnancy. Also, it is not clear how storage time affects measured BPA concentrations. This study has limited utility for HI and no utility for RA. Non-linearity in effect was observed. There is no clear dose-response relationship found in this study for BPA and decreased time of gestation in the population of pregnant women from Nanjing, China.41

Developmental programming: gestational bisphenol-A treatment alters trajectory of fetal ovarian gene expression (Veiga-Lopez et al., 2013). [NOTE: This study is also included in the Peretz 2014 review.]

FDA conclusion in 2014 updated review of literature and data:
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 downregulate 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.42

Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age (Weinberger B, et al., 2014).

FDA conclusion in 2014 updated review of literature and data:

41 Id. at 170.

42 Id. at 107.
This study has no utility for HI and no utility for RA. The findings of this study are consistent with some previous publications (Latini et al., 2003; Meeker et al., 2009), which found a relationship between higher concentrations of certain phthalate/BPA metabolites and shorter gestation. It should be noted that the metabolite(s) associated with shorter gestation was not the same across studies, however. Other studies have reported opposite results (Adibi et al., 2009), showing women with concentrations of DEHP metabolites at the 75th percentile had a longer gestation than women with concentrations at the 25th percentile.43

Available Data Do Not Support the Conclusion that BPA Causes Female Reproductive Toxicity

As noted on the OEHHA website, DARTIC is charged with determining whether BPA "has been clearly shown through scientifically valid testing according to generally accepted principles to cause female reproductive toxicity" (emphasis added). While there have been numerous studies conducted, many have serious limitations, as highlighted in FDA’s conclusions listed above. Thus, while there may be some potential indicators, the available data simply do not meet the criteria of “clearly showing” female reproductive toxicity. This view is reflected in the 2015 EFSA conclusion on BPA reproductive toxicity, which states:

There are indications from prospective studies that BPA exposure during pregnancy may be associated with disturbed fetal growth, and weak indications that BPA exposure during pregnancy may be associated with maternal and infant decreased thyroid function, but it cannot be ruled out that the results are confounded by diet or concurrent exposure factors. The associations found in the human studies are not sufficient to infer a causal link between BPA exposure and reproductive effects in humans. Potential effects are considered to be as likely as not (emphasis added).44

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43 Id. at 179.

Ms. Monet Vela  
April 20, 2015  
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Thank you for this opportunity. If you or your staff has any questions regarding this letter, please do not hesitate to contact me. I can be reached at kroberts@metal-pack.org or 443-964-4653.

Respectfully submitted,

[Signature]

Kathleen M. Roberts  
Executive Director