Critique of the Final Draft of the CERHR Bisphenol A Report

January 25, 2008
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1. THE DESIGNATION OF DEVELOPMENTAL NON-ORAL ADMINISTRATION STUDIES AS BEING OF "LIMITED UTILITY" WAS MADE IN DIRECT CONTRADICTION TO THE PUBLISHED SCIENTIFIC LITERATURE

FROM P 122 of the CERHR REPORT

> "In addition, the Panel carefully considered the value of studies where Bisphenol A was administered anywhere other than to the mouth or stomach of the experimental animal. Human exposure is overwhelmingly oral, and oral exposure produces an internal metabolite profile, which is overwhelmingly dominated by the (inactive) glucuronide in both rats and humans. Subcutaneous or parenteral injections result in blood levels of active parent compound which are much higher than those seen after oral exposure. **In light of these pharmacokinetic differences, the Panel concluded that injection studies, unless they proved otherwise, would produce irrelevantly high internal doses of the active parent compound, and would tend to produce "false positive" effects from the point of view of the human oral situation**. Thus, the Panel viewed those otherwise adequate studies which injected bisphenol A as providing "supplemental" information (i.e., of **limited utility**), unless they also analyzed the levels of parent compound and metabolites after the injection. **The intent of this approach is limit the impact of those studies which produced an unrealistic and irrelevant internal metabolite profile (i.e., one which is significantly different from that experienced by humans).** Thus, the closer any given study came to replicating the human situation, the more weight it had in the final analysis."

As discussed extensively in Vandenberg et al. 2007, published in *Reproductive Toxicology*, and our recently published findings (Taylor et al., 2008), which is discussed below, the above conclusions by the ÇERHR panel are without merit or any support what-so-ever from the scientific literature. The predicted pharmacokinetic difference due to route of administration in adults is about 10-20 fold in the adult. Given this expectation, there was no scientific justification for the panel to state that "irrelevantly high internal doses" would be achieved after administering doses 2-million times lower (used by Rubin et al. 2006) than the LOAEL of 50 mg/kg/day used to set the current reference dose; findings showing effects below this obviously invalid LOAEL would alter calculation of the reference dose. The consequence of being categorized as being of "limited utility" was that the findings were not used in determining the potential for BPA to affect human health; in other words, the studies were in fact rejected as unimportant. The conclusions reached make that clear.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to

bisphenol A (BPA). Reprod Toxicol. 2007;24:139-77.

There is nothing in the published literature to predict that a fetus or newborn rat or mouse can rapidly metabolize BPA. It is well known that the UDP-glucuronidase that metabolizes BPA, as well as the estrogenic drug DES, is not found in fetuses (Matsumoto et al., 2002) and has very low activity in the neonate (Fischer et al., 1972; Matsumoto et al., 2002). While route of administration does lead to differences in pharmacokinetics of BPA in adults, this does not apply to neonatal mice or rats. It is well recognized that rapid passage of BPA from the mother to fetus occurs, and regardless of how the mother is exposed, once BPA is in the fetus, it will not be rapidly metabolized. The decision of the panel to not consider non-oral routes of administration as relevant to human health is invalid for studies conducted during development. It appears that the CERHR assembled a panel to review the health effects of one of the highest volume chemicals in commerce, that is a known endocrine disruptor, that did not have anyone with the appropriate expertise to know that this decision was directly contradicted by a literature that has been published over the last 30 years.

Fischer LJ, Weissinger JL. Development in the newborn rat of the conjugation and de-conjugation processes involved in the enterohepatic circulation of diethylstilboestrol. Xenobiotica. 1972;2(4):399-412.

The data in Taylor et al. 2008 now clearly demonstrates that the rationale stated above by the committee was, in fact, not valid. However, in an article published on January 23 in the Milwaukee Journal Sentinel, the chair of the CERHR panel, Robert Chapin, was quoted as stating referring to Taylor et al: "the new research 'stands in contrast to a number of other studies that show the opposite.' He said it was those other studies that 'led us to the logical conclusion we reached.' When asked to supply the citations for those studies, he said he could not remember them offhand."

The following is the abstract from the article by Julia Taylor et al. 2008.
Reproductive Toxicology, Online: January 17, 2008, doi:10.1016/j.reprotox.2008.01.001

Title: **No Effect of Route of Exposure (Oral; Subcutaneous Injection) on Plasma Bisphenol A throughout 24 hr after Administration in Neonatal Female Mice**

Abstract. Route of administration of chemicals in adults is an important factor in pharmacokinetics of chemicals such as bisphenol A (BPA), the monomer with estrogenic activity used to make polycarbonate plastic products and to line food and beverage cans. Based on findings in adults it has been proposed (CERHR, 2007) that non-oral routes of administration in newborn rodents would also lead to high exposure relative to oral administration. However, in fetuses and neonates, the enzyme that conjugates BPA (UDP-glucuronosyltransferase) is expressed at low levels, suggesting that there may be no differences in pharmacokinetics between oral and non-oral dosing. We thus conducted an analysis of plasma concentrations of unconjugated 3H-BPA after HPLC separation in postnatal day 3 female mice throughout the 24 hr after administering 3H-BPA orally or via subcutaneous injection at doses above and below the current EPA reference dose. We found no significant difference in plasma BPA based on route of administration in

neonatal mice at either dose. However, compared to data from other studies conducted with adults, there was a markedly higher plasma BPA level after oral administration of BPA in newborn mice. This finding sets aside the belief that non-oral administration of BPA renders data as not suitable for consideration of the hazard posed by low-dose exposure to BPA during neonatal life. Therefore the large numbers of BPA studies that used non-oral administration at very low doses during the neonatal period should not be dismissed by scientists or the regulatory community based on route of administration.

In the discussion section of this article by Taylor et al we state the following:

Our findings are important in that they add to a large literature showing that the maxim in pediatric medicine that "babies are not little adults" has to be recognized by scientists and regulators who are making determinations about the potential for chemicals in the environment to adversely impact the health of fetuses, infants and children. A key factor in categorizing developmental studies as being of "limited utility" in assessing concern for human health by the CERHR BPA panel was administration of BPA by sc injection. A recently published study showed a number of adverse effects of BPA on the female mouse reproductive system (Newbold et al., 2007) and another study (reviewed by the CERHR panel) showed prostate interepithelial neoplasia (PIN) lesions in male rats (Ho et al. 2006); these reported adverse effects were due to exposure during the first 5 days after birth via sc injection, and in each case the dose of BPA was 10 μg/kg/day. This dose is 5,000 times lower than the 50 mg/kg/day dose used by the US EPA to estimate the "safe" daily human exposure dose of 50 μg/kg/day. Other "limited utility" studies involved continuous release of extremely low doses of BPA (0.025 – 25 μg/kg/day) from sc-implanted capsules (for example: Rubin et al. 2006; Susiarjo et al. 2007). Importantly, published studies reviewed above suggest that the expected difference based on route of administration in an adult would only be 10-20 fold, while in the neonate our data show that there is no difference at all. The prediction of the CERHR panel was that sc administration during development: "would produce irrelevantly high internal doses of the active parent compound" (CERHR, 2007; p 122)**,** and this prediction is not supported by our findings or any other published data. Evidence that humans are most likely continuously exposed to BPA, which is best approximated by use of continuous-release capsules, has been previously reviewed (Vandenberg et al. 2007).

The references cited by Taylor et al. follow below:

Newbold RR, Jefferson WN, Padilla-Banks E. Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. Reprod Toxicol. 2007;24(2):253-8.

Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res. 2006;66(11):5624-32.

Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinol. 2006;147(8):3681-91.

Susiarjo M, Hassold TJ, Freeman E, Hunt PA. Bisphenol A exposure in utero disrupts early oogenesis in the mouse. PLoS Genet. 2007;3(1):63-

The chair of the CERHR panel, Robert Chapin, who in public session strongly pushed the non-toxicologists on the committee to accept the decision to declare non-oral administration studies to be of "limited utility" now states (to the Milwaukee Journal Sentinel) that he has no recollection of any study that supports that position.

Another toxicologist on the CERHR panel who argued to reject non-oral administration studies in public session was L Earl Gray. After Dr. Chapin failed to remember any study that would support his position, Dr. Gray sent the Milwaukee Journal Sentinel reporter, Suzanne Rust, an article published by Domoradski et al. 2004, and included the following statement:

> "The paper [by Domoradski] demonstrates that "the half-lives for the elimination of BPA-glucuronide in plasma were more rapid in neonatal animals than in adults". Pups have higher liver glucurondation activity than adults so BPA is more quickly converted to inactive BPAG and excreted in the urine. The reduced enterohepatic recirculation and reduced microfloral glucuronidase activity in the pup versus the adult means that less BPA is excreted as BPAG into the gut and even less of that is converted by glucuronidase that converts BPAG to BPA and readsorbed into the circulation. Enterohepatic recirculation extends the half life in the adult. Therefore pups excrete BPA more quickly as BPAG and the half life is less than adults. Humans have much lower levels of enterohepatic recirculation of BPAG than rats, so the half life is less in humans than rats."

Domoradzki JY, Thornton CM, Pottenger LH, Hansen SC, Card TL, Markham DA, et al. Age and dose dependency of the pharmacokinetics and metabolism of bisphenol A in neonatal sprague-dawley rats following oral administration. Toxicol Sci. 2004;77(2):230-42.

The conclusion by Earl Gray in his email that: "Pups have higher liver glucurondation activity than adults so BPA is more quickly converted to inactive BPAG and excreted in the urine" is <u>false</u> in that it is directly contradicted by the findings reported by Domoradski et al. 2004 as well as in other published articles showing that the levels of the liver enzyme that glucuronidates (inactivates) BPA is significantly lower in neonates than in adults (Matsumoto et al. 2002).

Matsumoto J, Yokota H, Yuasa A. Developmental increases in rat hepatic microsomal UDP-glucuronosyltransferase activities toward xenoestrogens and decreases during pregnancy. Environ Health Perspect. 2002;110(2):193-6.

In stating that: "the half-lives for the elimination of BPA-glucuronide in plasma were more rapid in neonatal animals than in adults", Dr. Gray is not discussing the metabolism of BPA to BPA glucuronide, which is what really matters since BPA glucuronide is know to have no estrogenic activity. The conclusion by Dr. Gray that "**Pups have higher liver glucurondation activity than adults so BPA is more quickly converted to inactive BPAG**" does not follow logically from his prior statement about the half life for elimination of the biologically inactive molecule

BPA-glucuronide.

On page 240, Domoradski et al 2004 state:

> Matsumoto *et al*. (2002) studied the ontogeny and activity toward BPA of GT in developing Wistar rats and found an age dependency in liver microsomal activity, with activity increasing as the age of the neonate increased. Consistent with the observations of Matsumoto *et al.* (2002), the data from this study clearly demonstrated an age dependency in BPA metabolism, likely due to the ontogeny of GT in neonates. Age-related differences were observed in the plasma metabolite profiles as well in the pharmacokinetics of BPA and BPA-glucuronide.
>
> An age dependency in the half-lives for the elimination of BPA from the plasma was also observed. BPA in the plasma of adult rats was barely detectable and reached concentrations that were nondetectable by 0.75 h postdosing. Plasma concentrations of BPA in neonates declined to the levels found in adults in 12 h or less but did not reach nondetectable concentrations until 12–24 h postdosing

At the last meeting of the CERHR BPA panel in August, I presented the following statement to the panel in public session, however, the panel ignored my comments.

> The decision by the CERHR – BPA panel to declare that studies that did not involved oral administration are of no value is based on studies comparing metabolism in adults, because the adult liver rapidly metabolizes orally administered BPA and this first-pass metabolism is bypassed by injection or capsule implant.
>
> The maxim in pediatric medicine is that babies are not little adults.
>
> Thus, the application of adult metabolism to the fetus and newborn is inappropriate.
>
> Fetuses do not eat. It makes no difference how BPA gets into the mothers blood. The published literature shows that BPA rapidly passes across the placenta from the mother following oral administration, subcutaneous or intraperitoneal injection.
>
> Metabolism of BPA is significantly reduced in the pregnant rat, and fetuses do not metabolize BPA.
>
> Newborn rats show very limited metabolism of BPA, and there is a 10-fold increase in capacity to metabolize BPA between birth and weaning.
>
> The decision by the CERHR – BPA panel to discount studies that did not involved feeding pregnant females or newborns is not supported by the published scientific literature. This report will undergo peer review. This decision should be reconsidered by the panel, since the conclusions will not pass peer review.

It is clear that the NTP is now faced with the task that I predicted: the panel's decision to categorize studies as being of "limited utility" based on route of administration needs to be overturned. The studies considered supplemental are listed as "limited utility" in tables at the end of sections 3 and 4. My position is that the panel was making irrational decisions in their categorization of these studies that directly contradicted the published scientific literature.

The few panel members that were responsible for pushing in public session the rest of the panel members to make this decision should be compelled to justify this decision, since it has major public health implications. Simply saying "I can not remember" or providing an article to a reporter as support for this position that, in fact, provides data that directly contradicts the position, is unacceptable.

2. THERE ARE MANY FACTUAL ERRORS IN THE REPORT THAT CONTRIBUTED TO THE PANEL CATEGORIZING STUDIES OF LOWER UTILITY BASED ON INACCURATE INFORMATION

There are many instances of factual errors.

The panel had a discussion during the public panel meeting about setting arbitrary numbers for sample sizes for a study to be considered acceptable. This discussion was stunning in that such an approach would violate NIH Guidelines concerning the use of the least number of animals required for statistical significance based on power analysis. The idea that an arbitrary number of animals is needed for statistical significance is a statistical fallacy and should be removed from the final report as the basis for criticizing studies.

Ref 241 (Rubin et al 2006) was published in *Endocrinology*, the flagship journal of the Endocrine Society. I was invited by the editor of Endocrinology to write an editorial accompanying this publication, due to the decision by the editor that this was important enough, and of high enough quality, to warrant special attention. On p 3684 in the Rubin paper they state: "A total of 94 animals were tested in the open field, including 14–17 males and females from each of the three treatment groups. Only a single male and female from each litter were examined in these studies to eliminate potential litter effects." They also state that 7-8 animals were examined for neuroanatomical differences. The criticisms of this study are without merit, and the panel's decision to disregard this publication should not be sustained on re-review.

The CERHR panel proposed to reject any study that used under 6 animals per group. How did the Rubin study get labeled as inadequate based on sample size?

In addition, the argument about DMSO administered by Rubin et al. 2006 at a 50% concentration via Alzet pumps is invalid given the extremely small amounts released from the Alzet pumps in relation to amounts that have been shown to cause harm.

THE ISSUE OF STRAIN VARIABILITY

96-37 Inter-strain variability in rats has been evaluated as a source of variability in estrogenicity assays. Inspection of Table 53 does not suggest large sensitivity differences between Sprague Dawley, Wistar, and Long Evans rats.

CRITIQUE: This is an invalid conclusion that is contradicted by studies that directly compare SD rats with F344 rats. This is true for phytoestrogens as well (Thigpen et al. 2007 Environ. Health Perspect.). This was identified as a major issue by the NTP Low Dose Peer Review Panel Report in 2001. It is unfortunate that none of the participants on the CERHR panel were familiar with this prior NTP review that directly contradicts the conclusions here. This issue led to the following statements in the executive summary of the Low Dose Report:

> Executive summary: "Because of clear species and strain differences in sensitivity, animal model selection should be based on responsiveness to endocrine active agents of concern (i.e. responsive to positive controls), not on convenience and familiarity."

This issue was discussed extensively in an article that alsso included a discussion of the issue of lack of appropriate consideration of positive controls in studies reporting no effect of BPA (vom Saal and Welshons, 2006).

> vom Saal, F.S. and Welshons, W.V. Large effects from small exposures: II. The importance of positive controls in low-dose research on bisphenol A. Environmental Research 100:50-76, 2006.

Vom Saal et al 1998 Ref 392

189 – (6-8). Information about number of males per litter used is cited as a personal communication.
RESPONSE: We state in the methods that detailed methods are presented in the Nagel et al. 1997 EHP article. On p 72 at the top of the second paragraph in the Nagel article it states that one male per litter was used to control for litter effects. This should not be cited as a "personal communication", since the information about controlling for litter is published in the companion paper.

189 – (22-28; 30-36). Strengths and Weaknesses: Statistical weaknesses are cited.
RESPONSE: The criticism about the statistics is unwarranted. There is no requirement to use Dunnett's test rather than Fischers LSD test, which we routinely use.

189-(30-32) "Weakness are the inability to assume the genetic comparability and responsiveness of CF-1 mice maintained in a closed colony for almost 20 years is comparable to other sources of CF-1 mice)"
RESPONSE: This is a ridiculous criticism. There are many different animal models used in endocrine disruptor and other types of research. The objective in toxicology is to choose an appropriate animal model that is sensitive to the class of chemical being studied (as stated by the Low Dose Peer Review Panel in 2001). This strain is sensitive, as opposed to other strains that are not, such as the CD-SD rat, which are inappropriate for use as model animals in BPA experiments.

189-33 "the lack of information on testis weight (which is needed for consideration of daily sperm production)."
RESPONSE: Attached below is figure 1 from this publication. If anyone had read this article and looked at this figure, how could they make the criticism that we did not take testis weight into account in determining daily sperm production. The correction for testis weight is carefully described in the results as well identified in the heading for the figure.

189-34 "[a weakness is] small sample size for sperm production measurement, and the questions about the statistical analysis."
RESPONSE: The idea that you generate false positives when you achieve high precision and find statistical significance without testing some arbitrary number of animals is simply false!

189-35 “An additional weakness is the unusual/unexplained findings of low dose only effect on weights.”
RESPONSE: There are 20 published studies showing responses to BPA at a low dose and not at higher doses. This statement demonstrates that lack of knowledge of whomever is responsible for this criticism with the published BPA literature. This type of finding may violate the false assumption in toxicology that all dose response curves are monotonic, but even a superficial knowledge of endocrinology would have resulted in the reviewer encountering this type of finding throughout the endocrine literature, in addition to the BPA literature.

189-38 **“Utility (Adequacy) for CERHR Evaluation Process:** The body weight data contained in this paper are adequate for the evaluation process, however overall utility is limited because of sample size and statistical Developmental Toxicity Data concerns. Data on reproductive organ weights and sperm production are considered inadequate for the evaluation.”

RESPONSE: This critique is without merit. The sample size was adequate to reveal statistical significance. We corrected organs for body weight and sperm production for testicular weight. **There are no valid statistical concerns and the data are clearly adequate for the evaluation.**

Figure 1 from vom Saal et al. 1998 REF 392

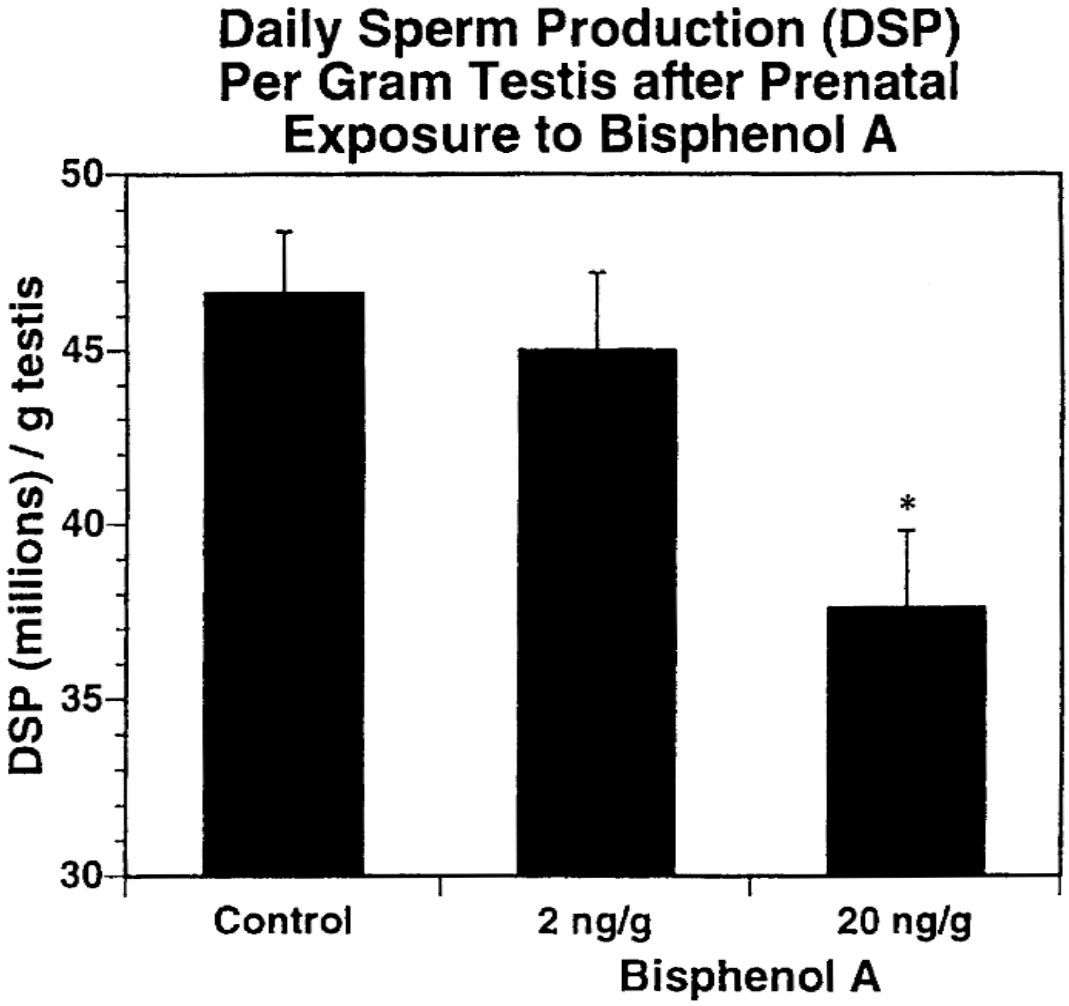

FIGURE 1. The mean (± SEM) daily production (in millions) of sperm per g testis (efficiency of sperm production) in control adult male mice and the male offspring of pregnant females fed bisphenol A at 2 or 20 ng/g body weight. * indicates $p < 0.05$ relative to controls.

Howdeshell et al. 1999 Ref 396

194-(4-5). [The NTP Statistics Subpanel (340) requested the Howdeshell et al. (396) dataset for reanalysis, but it was not provided by study authors.]

RESPONSE: This information was offered to the statistics sub-panel chair, Dr. Haseman at the NIEHS, but it was not in electronic form, since vaginal smear data were recorded on paper and not entered into a computer. Dr. Haseman indicated that only data in excel spread sheets could be accepted for review. Also, he indicated that even if we sent it in, they were already overwhelmed and would not be able to analyze the data. This was indicated on page A-91 of the Low Dose Peer Review Report. The statement made here is misleading in that we were told not to send the data to the review panel because it was not in an electronic file.

Palanza et al. (REF *403*)

198-30. "The use of a diet high in soy isoflavones is an additional weakness."

RESPONSE: **Anywhere in this report that this criticism appears, it should be deleted for the reasons stated below.**

This is a very strange criticism given that a member of the panel (Earl Gray) attended a NIEHS-sponsored meeting on effects of components of animal feed at which information contradicting this statement was presented. Specifically, there are two publications (Cederroth et al., 2007; Ruhlen et al., 2007) showing that there is a marked adverse effect (obesity, early puberty, abnormal reproductive organs, and disruption of many other physiological processes) in mice as a result of removing all phytoestrogens from feed, including a paradoxical significant elevation in fetal estradiol, resulting in the "fetal estrogenization syndrome". Since these papers are published in the NIEHS journal *Environmental Health Perspectives*, and a member of the panel was aware of these findings prior to their publication, it is surprising that this panel was not made aware that it is the absence soy phytoestrogens in feed that should be considered a problem, not the presence.

This misconception comes from studies conducted by Julius Thigpen (2003) in which he weaned mice at a very early age (1 week prior to normal age at weaning) and gave them feed with different levels of phytoestrogens; the high phytoestrogen-containing feed advanced puberty. This finding raises concerns with directly feeding babies phytoestrogen-containing formula at a time in life that they would normally be nursing and only getting the very small amount of phytoestrogens that is present in milk produced by a lactating female eating phytoestrogen-containing feed. However, these findings are not what is found when mice are weaned at the normal age at weaning and put on a phytoestrogen rich or phytoestrogen free feed (Ruhlen et al, 2007). In summary, the concern with phytoestrogens stated by the panel is really only directed at an event (direct exposure of pre-weanling animals) that is not relevant to any study concerning the effects of BPA.

References

Cederroth CR, Vinciguerra M, Kühne F, Madani R, Klein M, James RW, et al. 2007. A phytoestrogen-rich diet increases energy expenditure and decreases adiposity in mice. Environ Health Perspect 115(10):1467-1473.

Ruhlen, R.L., Howdeshell, K.L., Mao, J., Taylor, J.A., Bronson, F.H., Newbold, R.R., Welshons, W.V. and vom Saal, F.S. Low phytoestrogen levels in feed increase fetal serum estradiol resulting in the "fetal estrogenization syndrome" and obesity in CD-1 mice. Environ Health Perspect, online (doi:10.1289/ehp.10448), 2007.

Thigpen JE, Haseman JK, Saunders HE, Setchell KDR, Grant MG, Forsythe DB. 2003. Dietary phytoestrogens accelerate the time of vaginal opening in immature CD-1 mice. Comp Med 53:477-485.

Zsarnovsky et al. REF 391
187- (31-36)
Strengths/Weaknesses: The use of 17β-estradiol as a positive control is a strength of this study. Weaknesses are the intracerebellar injection and the administration on a per pup basis.
Utility (Adequacy) for CERHR Evaluation Process: This paper is inadequate for the evaluation process due uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal injection to human exposures).
RESPONSE: These criticisms are bizarre given that effects were reported at 0.23 parts per trillion. It is irrelevant how that dose is administered given that median human blood levels of unconjugated BPA are 2- 3 parts per billion, or 10,000-times higher than this amount, and BPA is not inhibited from entering cells by plasma binding proteins to the degree that they inhibit estradiol (Nagel et al. 1997 REF 275).

253-131 – If Timms et al. (2005) is a concern, why was this not considered in relation to the findings reported by Gupta (2000), which was also deemed adequate and of high utility, since the findings are virtually identical, and why did the panel not connect these findings to the results reported by Richter et al. 2007, which further extended these findings, which was not considered by the panel. This inability to "connect the dots" is a characteristic of this report.

Richter, C.A., Taylor, J.A., Ruhlen, R.R., Welshons, W.V. and vom Saal, F.S. Estradiol and bisphenol A stimulate androgen receptor and estrogen receptor gene expression in fetal mouse prostate cells. Environ. Health Perspect. 115:902-908, 2007.