February 2, 2007

To: National Toxicology Program (NTP); Center for the Evaluation of Risks to Human Reproduction (CERHR)

From: Natural Resources Defense Council

These comments are submitted by Natural Resources Defense Council (NRDC), who on behalf of our 1.2 million members and online activists, uses law and science to protect the planet's wildlife and wild places and to ensure a safe and healthy environment for all living things. NRDC has no financial interest in bisphenol A.

The CERHR has prepared a draft report on bisphenol A and has asked for public comments on their draft. NRDC feels strongly that bisphenol A, at environmentally relevant doses experienced by the majority of the human population on a daily basis, is a hazard to human development and reproduction.

Background.

Bisphenol A (BPA) is an endocrine disrupting chemical used in wide variety of consumer products, including polycarbonate plastics, the lining of food cans and dental sealants. It is a high production volume chemical with approximately ~2.3 billion pounds produced in the US in 2004. This large production volume and use in common consumer products results in widespread human exposure. BPA has been detected in 95% of nearly 400 urine samples collected by the Centers for Disease Control.[1] Furthermore, the fetus is a susceptible population exposed to BPA during critical periods of neuro- and reproductive development. BPA has been measured in human biological fluids, including blood, urine, breast milk, amniotic fluid, and follicular fluid. Laboratory experiments have suggested that for doses within the range of human exposures, *in utero* exposure to BPA causes developmental and reproductive harm including changes in circulating levels of hormones, lower sperm counts, mammary cancer, abnormal development of the prostate gland, increased susceptibility to prostate cancer, and changes in sex-differentiated behaviors.

Comments on NTP CERHR draft expert panel report on BPA.

NTP has failed to include some important studies in their draft report.

NTP's CERHR has done a very thorough review of the studies published on toxicity of BPA. There are a few studies which were not included in this draft that should be evaluated by the committee.

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[1] Calafat, A. M., Kuklenyik, Z., Reidy, J. A., Caudill, S. P., Ekong, J. and Needham, L. L. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perspect 2005; 113: 391-5.

These include studies previously published that highlight the potential of BPA to mimic estrogen at extremely low doses:

1. Walsh DE, Dockery P, Doolan CM. Mol Cell Endocrinol. 2005 **Estrogen receptor independent rapid non-genomic effects of environmental estrogens on [Ca2+]^{i} in human breast cancer cells.** 230(1-2):23-30.

This study found rapid, non-genomic effects of BPA on calcium influx into MCF-7 cells with concentrations as low as 0.1 nM. This effect was not mediated through the estrogen receptor and suggests a possible alternative pathway for modes of action of BPA.

2. Wozniak AL, Bulayeva NN, Watson CS. Environ Health Perspect. 2005 **Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca2+ fluxes and prolactin release in GH3/B6 pituitary tumor cells**. 113(4):431-9.

This study also found rapid, non-genomic effects of BPA on calcium influx into rat pituitary cells at extremely low concentrations of 1 pM (0.23 ppt). At this dose, there was a doubling in prolactin secretion by the cells.

There has been another study published since the release of the draft expert panel review which is also important to include in the evaluation of the potential for BPA to cause developmental toxicity.

1. Susiarjo, M, TJ Hassold, E Freeman and PA Hunt. 2007. **Bisphenol A Exposure *In Utero* Disrupts Early Oogenesis in the Mouse.** PLoS Genet. 2007 Jan 12;3(1):e5 [Epub ahead of print: doi: *10.1371/journal.pgen.0030005*.]

This study was done to assess the effect of BPA on the developing ovary. The study was done in C57BL/6 mice housed in rack caging with drinking water from glass bottles and Purina 5010 mouse chow. Estrogen receptor α and β knock-out mice also were utilized to determine whether BPA was acting primarily through one receptor. BPA was administered through pellets designed to release 400 ng of BPA daily and were implanted into pregnant mice at 11.5 days gestation. This is equivalent to 20 ug/kg of body weight per day and is within the range of exposure in the general population. Fetuses were exposed for 7 days to this dose of BPA.

This study found disruptions in several phases of meiosis. First, oocytes in meiotic prophase were found to have synaptic defects and increased levels of recombination. Second, when the females were allowed to mature and oocytes evaluated at metaphase, there continued to be evidence of increased recombination frequency and disruptions in exchange frequency. Third, when the *in utero* exposed females were allowed to mature into adulthood, there was a significant increase in the level of hyperploid eggs from 1.8% to 21.4%. Assuming that hyperploidy represents one-half of all non-disjunction, this data led the authors to estimate that as many as 40% of oocytes in exposed females would be chromosomally abnormal. Finally, when 4-5 week old exposed females were

superovulated and mated to wild-type males, there was a non-statistically significant increase in the level of hyperploidy in 2-cell embryos (0/13 in controls v. 4/19 in exposed). When estrogen receptor knockout mice were used, the ER-β knockouts were found to have similar defects to the BPA exposed wild-type animals. This suggests that BPA interferes with the ER-β receptor.

NTP should consider the choice of experimental animal when evaluating studies.
It has been noted by other investigators that the Charles-River Sprague-Dawley (CD-SD) rat is relatively insensitive to exogenously administered estrogen, including potent estrogenic drugs.[2] Several of the studies under consideration by NTP's CERHR used this animal strain in their experimental design and found either no effect of BPA or effects only at high doses [NTP references (#293 Tyl et al.); (#297 Kwon et al.); (#411 Tyl et al.); and (#438 Ashby et al.)].

In the study conducted by Ashby et al. (438); the NTP CERHR panel concluded,

> "Given the robustness and comprehensiveness of this study, it is highly useful. It strongly suggests that the NOAEL for potential bisphenol A-mediated effects on the adult rat reproductive system exceeds 200 mg/kg/day."

And in the study by Tyl et al. (411), the NTP CERHR panel concluded,

> "This study is highly valuable for human risk assessment for oral exposure to bisphenol A. This study identified a NOAEL of 75 ppm (for general toxicity) and 750 ppm (for reproductive toxicity)."

The other two studies by Tyl (293) and Kwon (297) were considered by the committee to be "adequate" for evaluation by the committee.

It is critical that use of an appropriate animal model is determined when evaluating the strength of a study. There was no discussion by the committee of the relative insensitivity of the CD-SD strain to exogenous estrogen treatment in their reviews of these studies. Further, there were no positive controls in the studies by Tyl (293 and 411) and Ashby (433). The study by Kwon (297) found no male reproductive effects with DES treatment, which would be expected at the dose administered. The lack of a positive control in these studies coupled with the documented estrogen insensitivity of this strain indicates that these studies should not be considered to be "highly valuable" or "highly useful" in the committee's evaluation but rather should be considered to have significant weaknesses that call into question they usefulness for evaluating the toxicity of BPA.

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[2] vom Saal FS, Welshons WV. Environ Res. 2006 Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. 100(1):50-76.

NTP has emphasized the importance of this issue in a previous Low-Dose Peer Review panel.[3]

NTP states:

> "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."

When evaluating studies for adequacy in the CERHR process, the committee must take into consideration the importance of strain differences and consider those studies using insensitive strains to be of lesser importance.

NTP should consider whether an adequate positive control was included when evaluating the strength of studies.

Establishment of a positive control is essential for determining the quality of a research study and its ability to predict differences in treatment outcomes. In addition to the studies described previously [NTP references (#293 Tyl et al.); (#297 Kwon et al.); (#411 Tyl et al.); and (#438 Ashby et al.)] that did not have adequate positive controls, there were other studies determined to be adequate for analysis by the committee that did not have positive controls (#294 Cagen et al., #342 Cagen et al.).

NTP has addressed this issue previously by stating:

> "… (in) a study in which the positive control does not produce the expected positive response. The prudent course of action in such cases may be to declare the study inadequate and repeat it, regardless of the experimental outcome in the test group."[3]

Based on these conclusions, NTP should justify how studies lacking adequate positive controls are determined to be appropriate for evaluation by the CERHR.

NTP should consider the impact of bias in industry-funded research.

A recently published study demonstrated that the source of funding had significant impact on whether or not adverse effects were found with low-dose BPA treatment.[2] Of a total 130 studies, 119 were done by government funded entities and 11 were done by chemical corporations. Ninety-two percent (109/130) of the government funded studies found evidence of adverse effects after low dose BPA treatment while only 8% found no adverse outcome. Some of the government funded studies finding no effect used the previously described estrogen insensitive strain of CD-SD rats. In contrast, 100% (11/11) industry funded studies found no evidence of harm from low dose BPA treatment.

The bias of industry-funded research has been well documented for many products such as tobacco, pharmaceuticals, and pesticides. NTP should carefully consider the source of funding when evaluating the adequacy of studies. A bias towards no-effect has already been demonstrated for low dose effects of BPA and several of those studies reviewed by the NTP CERHR committee have been determined to be adequate for evaluation.

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[3] NTP, 2001. Final report of the endocrine disruptors low dose peer review panel. http://ntp-server.niehs.nih.gov/ntp/htdocs/liason/LowDosePeerFinalRpt.pdf

Based on the existing data and comments made above, we strongly feel that BPA should be considered a hazard to human development and reproduction. BPA has been demonstrated to have multiple developmental and reproductive toxicities at low and environmentally relevant doses in a number of well designed government funded studies. In addition, significant new data indicates that *in utero* exposure to BPA causes aneuploidy in developing oocytes, which could contribute to miscarriage and birth defects.

NRDC appreciates the opportunity to make comments on the expert panel committee review of bisphenol A.

Respectfully,

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