June 20, 2007

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

Re: Natural Resources Defense Council (NRDC) Comments on the Expert Panel Interim Draft on the Reproductive and Developmental Toxicity of Bisphenol A, April 2007

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 ensure a safe and healthy environment for all living things. NRDC has no financial interest in bisphenol A (BPA).

NRDC appreciates the significant amount of time spent by the expert panel in the preparation of this interim draft report. We also appreciate that the expert panel has attempted to address some of the concerns raised by NRDC on the first draft report. However, we feel there are still significant problems with this draft report and welcome the opportunity to be able to comment on them. We encourage the expert panel to consider them in their final evaluation of the reproductive and developmental toxicity of BPA.

Summary of comments.

A. **There are inconsistencies in the use of evaluation criteria when determining the utility of studies.** Evaluation criteria were not uniformly addressed or applied to studies for the designation of a study as "high utility", "low utility" or "inadequate" for the CERHR evaluation process. This is particularly evident when comparing the decisions to include or exclude studies between sections of the document. Although not a comprehensive list, some specific examples are listed in detailed comments below.

B. **References are not listed in the interim draft and it appears some studies determined to be of utility for the evaluation process are unpublished**. Unpublished research studies have not been peer-reviewed and have not been subjected to a rigorous analysis of study design, statistical analysis, or data interpretation. These studies should not be utilized unless the original data is available and has been thoroughly reviewed by the expert panel. Furthermore, evaluation criteria should be uniformly applied when determining the adequacy of the study for evaluation by the expert panel regardless of whether or not a study has been peer-reviewed.

C. **Inappropriate designation of non-oral routes of exposure as being of limited utility or inadequate for utilization by expert panel.** Although we agree there should be consistent evaluation criteria for inclusion or exclusion of studies, the exclusion or limited utility designation for subcutaneous routes of exposure is overly stringent and inappropriate for evaluation of the developmental studies of immature animals.

D. **There are significant typographical errors in the interim draft.** These errors are outlined below and make interpretation of the interim draft analysis difficult and confusing.

NRDC Comments on NTP CERHR interim draft expert panel report on BPA.

A. There are inconsistencies within the interim draft in the use of evaluation criteria for determining the utility of studies.

The expert panel has outlined their criteria for evaluating the utility of individual studies in the Developmental Toxicity section on p. 130. It is assumed that similar criteria were applied to inclusion of studies in the Reproductive Toxicology section 4, however this is not explicitly stated in the document.

The evaluation criteria for determination of an "adequate" study include statistical analysis by litter, n values >7-8 (for in vivo studies), oral/gavage route of exposure, consideration of vehicle for administration of BPA, and a study design that includes appropriate controls. In addition, condition of animal care were considered including cage type, food source, type of water vessel, and type of bedding material. These criteria were used when evaluating the strength or weakness of individual studies and for the final determination of the utility of the study in the CERHR evaluation process.

Unfortunately, these evaluation criteria were not uniformly addressed or applied in the study summaries or in the determination of whether a study was of "high utility", "low utility" or "inadequate" for the CERHR evaluation process. This is particularly evident when comparing the decisions to include or exclude studies between sections of the document (presumably reviewed by different panel members) and when comparisons are made between industry-funded and independent studies. These inconsistencies result in an unfair and unbalanced evaluation of the science. The expert panel should ensure that significant weaknesses and strengths are consistently and uniformly identified in each study, especially those that are considered of high utility and in those studies where the analysis has significantly changed from the first draft document.

Some specific examples include:

1) Many studies were scrutinized for whether the litter or the offspring were used as the statistical unit. Studies were characterized as having significant weaknesses if they did not use the litter as the statistical unit or did not identify whether the litter or offspring were the statistical unit. This characterization was not uniformly applied in the evaluation of all studies and was identified as a significant weakness of many independent studies not considered for evaluation (for example Talsness 2000; Schonfelder 2004; Yoshino 2002; and Iida 2002) and while other studies were identified as being "high utility" but that did not identify the statistical unit (Tyl 2000 and 2002).

2) Several studies (Facciolo 2005; Aloisi 2002; Farabollini 2002; Porrini 2005) were determined to be inadequate because there was cross-fostering of litters which according to the expert panel "confounded litter of origin". However other studies that had cross-fostering of pups were included in Table 85 as being of "high utility" (Ceccarelli 2007; Della Santa 2006) or limited utility (Atanassova 2000; Williams 2001; and Rivas 2002). These inconsistencies in how studies were evaluated suggest that criteria were not uniformly applied when deciding the utility of the study for evaluation by the panel and that more careful attention should be paid to including or excluding a study.

3) The expert panel on p. 131 of the interim draft states, *"Inadequate challenge by the positive control, resulting in no response, leaves the reader uncertain whether the lack of response is due to the selection of too low a dose, or whether the experimental model is incapable of responding to a sufficient challenge. Even though the Panel, based on its own scientific experience, might conclude that inappropriately low doses had been selected and thus a lack of response is not surprising, the Panel was left with little choice in such situations but to give much less weight to such studies where non-effective control doses were used."*

Yet, there are several studies that were designated as "high utility" for the evaluation process despite having no positive control or no effect when a positive control was used. (examples include Cagen 1999; Ema 2001; Tyl 2002; and Tyl 2000.)

4) There is an inconsistent evaluation of animal housing conditions. For some but not all studies, the type of feed and soy or phyto-estrogen content is identified. Some studies note what type of material was used for bedding, cage material or drinking water. Other studies do not evaluate this. This creates a problem and inconsistency in how studies are evaluated when significant weaknesses are pointed out based on animal housing conditions. For example, the evaluation of Nagel 1997 identified corn cob bedding as a source of potential anti-estrogenic activity. Yet, no other evaluation of other studies noted this as a weakness but many used corn cob bedding.

B. **References are not listed in the interim draft and some studies determined to be of utility for the evaluation process are not published**.

The interim draft contains links to references in a bibliographic database and does not contain a list of references. This does not allow the reader to know the source of the reference and requires either searching through the first draft of December 2006 or conducting a literature search to find the manuscript. Using this approach, it is evident that new references have been added to the interim draft report most of which are industry-funded and appear to be unpublished and not peer-reviewed.

Such references should not be utilized for the evaluation process without careful consideration of the original data (including figures) by the expert panel. If the panel considers unpublished studies in the final evaluation of BPA, panel members should assure themselves that study

design, statistical analysis, and interpretation are supportable and conform to both recognized standards and the evaluation criteria outlined by the panel.

In particular, the studies by General Electric (1976 and 1978) and Fukumori (2003) do not adhere to panel's stated evaluation criteria and should not be considered adequate for inclusion.

The General Electric studies (1976 and 1978) were unpublished, did not include negative or positive controls in their study design, and did not specify whether the statistical unit of analysis was the litter or the offspring. Furthermore, in the 1978 study the expert analysis reads "*It was not clear how long before mating that the dosing was started or if dosing was continued through the gestation and lactation periods."* On the 1976 study the expert panel makes the following statement, *"Ages at the start of dosing were not reported, but based on body weight ranges reported (64–138 g for males and 57–118 grams for females) it appears that rats were different ages at the start of dosing."* Finally, in this study the final interpretation is that there were "no adverse effects reported" but it is noted that "some significant organ weight changes were noted by the study authors". Given these substantial and significant weaknesses in study design coupled with the lack of an adequate and thorough peer-review process, these studies should not be considered for even limited utility by the expert panel.

The Fukumori study (2003), was translated from Japanese to English and was provided to the expert committee by the American Plastics Council. It did not include any of the original figures. Furthermore, this study did meet a number of the criteria as outlined by the expert panel for utilization in their review. Specifically, this study dosed animals by a sc injection of BPA dissolved in DMSO. Also, neither the numbers of animals treated nor the numbers of litters was reported. Based on these significant weaknesses, this study should not be considered adequate for evaluation by the committee.

Finally, a new study has been added to the interim draft, Masutomi (2004) but it is not clear this study has been published or peer-reviewed. An on-line literature search through the National Library of Medicine did not find this manuscript. Given its designation as a "high utility" study, whether or not this study has been published and peer-reviewed should be clarified.

C. **Routes of exposure other than oral or gavage should be considered of utility for the evaluation process, in particular for prenatal and immature animal studies.**

The expert panel acknowledges that immature animals (fetuses and neonates) are susceptible sub-populations because they are unable to de-toxify BPA by glucuronidation. However, studies that administer BPA through non-oral routes and thereby bypass intestinal metabolism are given less weight in this interim draft. This represents an inconsistency in reasoning since intestinal metabolism will not occur in immature species anyway and for reasons of experiment design, exposure via non-oral routes (for example subcutaneous or sc) might be the most practical and will result in realistic exposures in immature animals. As noted by the expert panel, because of the evaluation criteria utilized, there are a relatively small number of studies that are considered to be of utility for evaluation by the expert panel. Incorporating more studies that use subcutaneous routes of exposure appropriately would strengthen the analysis of effects on immature animals and allow for inclusion of many more well-designed studies.

D. There are significant typographical errors in the interim draft.
These errors are outlined below and make interpretation of the interim draft analysis difficult and confusing.

p. 127, lines 40-43 reads: *"[Based on one comprehensive study of the effecs of bisphenol A orally delivered from 60 to 1000 mg/kg for 3 to 7 days, the Expert Panel concludes that the uterotrophic responses were only found at higher does (Kanno, 2003 #1642; Ashby, 2002 #?) whereas sc dosing produced consistent uterine weith increases at higher doses.]".*

In addition to numerous spelling mistakes, the conclusion of this statement makes it sound as if oral routes of exposure require lower dosing than sc routes to produce a uterotrophic response. This doesn't agree with other statements made in the document.

Table 84, outcomes in prostate tissue are identified under the reference from Tinwell but outcomes are more consistent with Ramos (2001 or 2003). This should be corrected for the proper reference.

Table 84, Naciff 2002 and 2005 are designated as inadequate for study but still appear under limited utility.

E. **In several instances throughout the interim draft is it noted that "because the effect was not dose-related it is unlikely to be of biological significance."**

This statement should be struck from all evaluations. There is a substantial amount of peer-reviewed scientific literature which indicates that for many endpoints, BPA has non-monotonic dose related effects. Even if the panel has not placed a high utility on these studies, there is not enough evidence to indicate that this is not true.

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

Respectfully,

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