

February 3, 2006

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Dr. Michael D. Shelby  
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Dear Dr. Shelby:

The American Chemistry Council Phthalate Esters Panel (PE Panel) is submitting the attached comments on the November 2005 *Expert Panel Update on the Reproductive and Developmental Toxicity of Di(2-ethylhexyl) Phthalate* (Final Update) to assist the NTP-CERHR in its review of the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate (DEHP) and in response to NTP's request for comments on the Final Update. 70 Fed. Reg. 69567 (Nov. 16, 2005). The PE Panel includes the major domestic manufacturers of phthalate esters and some users.

The PE Panel appreciates the Expert Panel's work in preparing the Final Update and believes that the Final Update, in general, provides a reasonable summary of the new information that has become available on DEHP since the first Expert Panel review in 2000. The PE Panel believes that the Final Update suffers from several shortcomings due to some aspects of the process by which the Final Update was produced, and that some of the scientific conclusions in the Final Update are not supported by the scientific data for DEHP. In particular, the PE Panel has obtained opinions from two experts in marmoset toxicology, which indicate the Final Report understates the value of recent marmoset data for evaluating human male testicular development, while it overstates the value of the marmoset data for evaluating human female reproductive development.

For the reasons discussed in the PE Panel's comments on the August 2005 Draft Update, and the comments presented here, the PE Panel believes that the available information for DEHP supports a conclusion that the overall concern for risk to human reproduction from DEHP exposure is minimal.

If you have any questions, or if you need any further information, please call Marian K. Stanley, Senior Director and Manager of the Phthalate Esters Panel, at (703) 741-5623, email her at [marian\\_stanley@americanchemistry.com](mailto:marian_stanley@americanchemistry.com), or write her at the address below.

Sincerely yours,



HasmuKh C. Shah  
Acting Vice President, CHEMSTAR

Attachments



**Comments of The  
American Chemistry Council Phthalate Esters Panel  
On the Final NTP-CERHR Expert Panel Update On The  
Reproductive And Developmental Toxicity Of Di(2-Ethylhexyl) Phthalate**

**February 3, 2005**

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## INTRODUCTION AND SUMMARY

The American Chemistry Council Phthalate Esters Panel (PE Panel) submits these comments on the November 2005 *Expert Panel Update on the Reproductive and Developmental Toxicity of Di(2-ethylhexyl) Phthalate* (Final Update) to assist the NTP-CERHR in its review of the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate (DEHP) and in response to NTP's request for comments on the Final Update. 70 Fed. Reg. 69567 (Nov. 16, 2005).<sup>1</sup> The PE Panel includes the major domestic manufacturers of phthalate esters and some users.<sup>2</sup>

The PE Panel believes that the Final Update, in general, provides a good summary of the new information that has become available on DEHP since the first Expert Panel review in 1999-2000. However, the PE Panel also believes that the Final Update suffers from several shortcomings that NTP-CERHR should take into account while reviewing the reproductive and developmental toxicity of DEHP. The following comments and suggestions are intended to assist NTP-CERHR in its review and use of the Final Update and to enhance the robustness of NTP-CERHR's conclusions. Attached to these comments are expert opinions by Professor Stefan Schlatt and Dr. Suzette Tardif, both scientists with extensive experience in the use of marmosets for toxicology. Those opinions provide perspectives that differ from those of the Final Update and which should be seriously considered by the NTP-CERHR.

These comments make the following points:

- The Expert Panel review process, while well-executed in many respects, suffered in several critical aspects, such that the Final Update contains some scientific conclusions which have not been fully deliberated or subjected to public comment. Key process concerns include:
  - The Expert Panel relied heavily on a study that was published on the last day of the public comment period, and there was no prior public notice that the study would even be discussed at the public meeting. The study was highly important to the deliberations, because it questioned the suitability of marmoset studies for assessing potential human health hazards, and was used by the Expert Panel as a primary rationale for largely disregarding a key DEHP study in marmosets that showed no male reproductive effects following very high exposure. Stakeholders were given inadequate opportunity to provide scientific comment on this important publication. As a consequence, the Expert Panel appeared to adopt the critical positions in this publication with no meaningful reflection on other information supporting the use of marmosets as an experimental model for testicular toxicity. The PE Panel is providing with these comments the opinions of two experts with extensive experience in marmoset research. These opinions rebut many of the hasty conclusions adopted during the Expert Panel deliberations.

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<sup>1</sup> The Final Update is available at [http://cerhr.niehs.nih.gov/chemicals/dehp/DEHP\\_\\_Report\\_final.pdf](http://cerhr.niehs.nih.gov/chemicals/dehp/DEHP__Report_final.pdf).

<sup>2</sup> The Panel members are: BASF Corporation, Eastman Chemical Company, ExxonMobil Chemical Company, Ferro Corporation and Teknor Apex Inc.

- The Update was conducted in a manner that prevented reaching conclusions based on an integrated view of the entire database for DEHP. For example, the Expert Panel discussed the marmoset data in the Mitsubishi (2003) study virtually independent of several other studies which also indicate primate insensitivity to testicular effects from DEHP. As a consequence, the Expert Panel's conclusions regarding concern for male reproductive toxicity were unduly influenced by its unfavorable view of the Mitsubishi marmoset study, and insufficiently influenced by the total weight of the evidence from all primate studies.
- Several significant scientific issues were raised by the Expert Panel for the first time in the final minutes of the public meeting, and thus were not fully deliberated, but were nonetheless included in the Final Update. This, combined with the inability of the public to comment on these issues at the meeting, led to several hastily- and scantily-considered conclusions being adopted as the consensus opinion of the Expert Panel.
- The PE Panel believes that these procedural flaws compromised the ability of the Expert Panel to render an objective and thoughtful opinion as to the reproductive toxicity of DEHP. The PE Panel makes recommendations to enhance the Expert Panel review process in the future, including:
  - Being more cautious about relying on information that becomes available at the last minute, and where the information is significant to the deliberations, adjusting the process to allow adequate scientific input from stakeholders, including allowing additional comments and expert opinions after the close of the public meeting for consideration by the Expert Panel, and even reconvening the Expert Panel where necessary; and
  - Being more flexible about accepting stakeholder scientific input during the Expert Panel deliberations, as critical issues arise.
- The marmoset, despite some unique aspects of its biology, is a valuable model for male human reproductive toxicity – particularly for the evaluation of developmental and toxicological aspects of the testis and spermatogenesis.
  - The review of the Mitsubishi study relied upon by the Expert Panel exhibits an unwarranted bias against the marmoset as a model, focusing on negative information and ignoring data that indicate the marmoset is a good model.
  - The marmoset has several similarities to human reproductive biology, in particular Sertoli cell development – a primary concern of the Expert Panel – that makes it an excellent model for male reproductive toxicity.
  - There is no evidence that the generalized steroid hormone resistance in the marmoset applies to sex steroids.

- As discussed in previous comments to NTP-CERHR, the marmosets' requirement of dietary Vitamin C and lack of luteinizing hormone are not significant disadvantages of the marmoset model.
- Additional criticisms of the marmoset model have been addressed in other comments, which are provided as an attachment to these comments.
- The Mitsubishi marmoset study, while presenting some concerns about the health of the animals, nonetheless provides valuable information regarding the sensitivity of primates to DEHP. The study provides strong evidence that DEHP had no major effect on testicular development even after long-term DEHP exposure at very high concentrations that would have profound adverse effects on rodent testes.
- In its comments on the August 2005 Draft Update, the PE Panel summarized an extensive body of literature indicating that differences between rodents and primates in the absorption, distribution, metabolism and excretion (ADME) of DEHP can explain in large part the lower sensitivity of primates to the reproductive effects of DEHP. Because most, but not all, of the primate ADME data came from the marmoset, the Expert Panel apparently chose to disregard these data due to perceived significant limitations with the Mitsubishi study. At the very least, the ADME data should provide the Expert Panel with sufficient information to acknowledge that the use of rat data for human risk assessment is likely to be very conservative (i.e., health protective).
- While the PE Panel applauds the Expert Panel for considering the PE Panel's recommendation in its comments on the Draft Update to calculate exposures based on available biomonitoring data, the Final Update also could have used available biomonitoring data to show that DEHP exposures to children ages 12-18 months are *not* several fold higher than adults.
- The Final Update erroneously indicated that cosmetics and breast milk pumps are significant sources of human exposure to DEHP. To the contrary, the data on DEHP in cosmetics and pumped breast milk indicate that DEHP exposures from these sources are minute or non-existent.
- The data reviewed in the final update do not support an oral developmental NOAEL as low as 3-5 mg/kg/day. The Expert Panel failed to include a significant number of individuals (F1 and F2 breeding males and F2 non-breeding males) in the overall number of individuals examined in the NTP multi-generation study (NTP 2004), which caused the Expert Panel to significantly overstate the incidence rate of "small" organs and, in turn, significantly overstate the magnitude of the effect. In addition, no laboratory historical control data were made available for review in these studies, which makes it difficult to adequately evaluate the statistical significance of the very low reported incidence rate of treatment effects.
- The data from the Mitsubishi study showing increased uterine and ovarian weights in female marmosets do not support the conclusion that DEHP exposure resulted in precocious puberty in marmosets. The increased uterine and ovarian weights correlate to

body weights which were higher in the higher dose females, versus lower body weights in controls and low dose females (probably the result of an unhealthy marmoset colony). Those higher body weights likely reflected healthier animals that were able to reach sexual maturity, with its accompanying increase in ovarian weight, rather than an effect of DEHP.

- The studies by Akingbemi et al. (2001; 2004) reviewed in the Final Update are flawed and do not support a NOEL of 1 mg/kg/day. The studies: 1) rely on single-point measures of serum hormones to support the report of abnormal changes in testosterone production in response to DEHP exposure; and 2) fail to account for differences in Leydig cell density between treatments and controls, which leads to the authors analyzing only a subset of the Leydig cells present in the treatment group.

Based on the complete database for DEHP, for the reasons discussed in the PE Panel's comments on the August 2005 Draft Update and the comments presented here, the PE Panel believes that the available information for DEHP supports a conclusion that the overall concern for risk to human reproduction from DEHP exposure is minimal.

## **I. THE FINAL UPDATE CONTAINS SCIENTIFIC CONCLUSIONS THAT WERE NOT FULLY DELIBERATED OR SUBJECT TO PUBLIC COMMENT**

The NTP-CERHR intended that the Expert Panel's review "provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with [DEHP] exposures."<sup>3</sup> Toward that end, CERHR solicited scientific information on DEHP and nominations for the Expert Panel (*see* 70 Fed. Reg. 6024 (Feb. 4, 2005)), provided a draft of the Report for public comment, and held a public meeting for the Expert Panel deliberations. Given the considerable amount of information that had become available after the first Expert Panel Report was published in October 2000,<sup>4</sup> the PE Panel agrees that an "objective and scientifically thorough" update is needed. However, we believe that in several respects the Final Update falls short of the mark, and that the shortcomings reflect at least in part inadequacies in the assessment process.

### **A. Stakeholders Were Not Given Adequate Time to Analyze and Comment on Late-Arising Information**

Public comments on the Draft DEHP Update were due September 28, 2005.<sup>5</sup> At the public meeting on October 11-12, 2005, the Expert Panel relied heavily on a paper by Li et al. (2005) that only became available as an online preprint on September 28 – the day public comments were due. Even assuming the PE Panel became aware of that paper the day it was released, it clearly could not have commented on the paper within the comment deadline. Input

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<sup>3</sup> Final Update Preface, page ii.

<sup>4</sup> NTP-CERHR Expert Panel Report on Di(2-ethylhexyl) Phthalate, October 2000. Available at: <http://cerhr.niehs.nih.gov/chemicals/dehp/DEHP-final.pdf>.

<sup>5</sup> *See* 70 Fed. Reg. 43870 (Jul. 29, 2005).

is now being provided with these comments, but it is too late to be reflected in the Expert Panel's deliberations.

The Li et al. paper critiqued the suitability of the marmoset as a human reproductive model. The Li paper was critical to the Expert Panel's deliberations, because it raised doubts about reliance on a key study for DEHP in marmosets that showed no effects on male reproductive development, even at very high doses (Mitsubishi, 2003). The PE Panel was not made aware that the Expert Panel would be discussing the Li et al. paper and relying on it to critique the DEHP study until the second day of the public meeting. When the lack of opportunity for public comment was pointed out, an opportunity for brief oral comment to the Expert Panel was provided, but this clearly was an unsatisfactory situation. Stakeholders had a very limited amount of time to review and comment on the study, and the Expert Panel had extremely limited time in which to consider those undoubtedly incomplete comments.

As detailed in Section II.A below, a critique of Li et al. by an expert in marmoset reproductive biology shows that the Li et al. paper overstates the case against using marmosets as a model, and fails to acknowledge factors that make marmosets good models for human testes development. Without the benefit of that input from a scientist who has done extensive research in marmosets and is expert in marmoset reproductive biology, the Expert Panel adopted the positions in Li et al. with little or no discussion. The net result, as explained more fully later in these comments, is that the Expert Panel received a one-sided and overly-critical view of the scientific value of DEHP marmoset studies, and inappropriately discounted the results of those studies.

The PE Panel realizes that its more robust comments on the Li et al. review will now be considered by the NTP-CERHR as it prepares its final brief on DEHP. However, the Expert Panel report, as a consensus document of an independent group of experts, carries great weight. Further, until the NTP-CERHR brief is public, the Expert Panel report is the "last word" on DEHP reproductive toxicity. Therefore, it is important that the Expert Panel report reflect truly fair and robust consideration of all key studies cited in its report. That did not happen in the case of the paper by Li et al., because the process did not allow it to occur.

There were other instances of the Expert Panel considering last minute information for which the public had no meaningful opportunity for comment. These include a summary of raw data from a key multi-generation study (see Section IV.A) and speculation on the potential contribution to exposure from use of breast milk pumps (see Section III.B).

In the future, the PE Panel strongly recommends that NTP-CERHR exercise caution in using or relying on last minute information, and that it adjust its process so that stakeholders have adequate opportunity to provide comment and scientific input concerning the late-arising information, including statements by independent experts where appropriate. For highly significant information, NTP-CERHR should allow a post-public meeting comment period with an opportunity for the Expert Panel to review and consider those comments. In some cases, it may be necessary to reconvene the Expert Panel to entertain other expert scientific input relevant to the new information. Otherwise, there is a risk that the Expert Panel report will appear up-to-date but in fact be the product of hasty and premature conclusions, as the PE Panel believes occurred in this case.

B. The Update Report Does Not Adequately Reflect the Complete Weight of Evidence for DEHP, Because the Expert Panel Reviewed Post-2000 Data in Isolation from Other DEHP Data

The PE Panel considers that a major failing of the update process was that the post-2000 data was reviewed almost in complete isolation from the pre-existing database for DEHP. Thus, the Update Report conclusions essentially reflect a view of DEHP based on the post-2000 set of data, rather than on the complete weight of evidence for DEHP.

A prime example of the imbalance created by this approach is the Expert Panel's consideration of and conclusions relating to primate data. In drawing its conclusions about the significance of primate data for assessing potential risks of DEHP, the Expert Panel focused only on the recent Mitsubishi (2003) marmoset study and its limitations. However, as discussed in Section II.C, below, several pre-2001 studies in both old world and new world primates show that primates are generally much less sensitive to testicular effects from DEHP exposure than are rodents. Consideration of these data in addition to the Mitsubishi study might well have modified the Expert Panel's conclusions. To complete a comprehensive review, the Expert Panel should have considered the post-2000 data as an integrated package with previous data, both while drafting the Final Update and during the public meeting. However, there was no mechanism in the review process for doing so. For its final brief of DEHP, NTP-CERHR should itself consider the full body of data for DEHP. The PE Panel also urges that the process for future updates to provide a mechanism for integrating previous and update information.

C. Several Significant Issues Were Raised for the First Time in the Final Minutes of the Public Meeting, and Thus Were Not Fully Deliberated, but Were Nonetheless Included in the Final Update

Several significant issues were raised in the final minutes of the public meeting and, despite not being fully deliberated, were included in the Final Update and represented to be the Expert Panel's consensus opinion. One such issue, as pointed out in an October 12, 2005 letter from the PE Panel to NTP-CERHR,<sup>6</sup> concerns the first paragraph of Section 5.3 (Overall Conclusions) of the Final Update. This paragraph states that "[t]he combined effects of multiple phthalate exposures have implications for exposure and risk assessment."<sup>7</sup> There were absolutely no deliberations concerning additive effects of DEHP and any other chemical by the Expert Panel, and no opportunity for public comment on this paragraph, which was written at the very end of the meeting. As stated in the PE Panel's letter, the only study available on this issue, Foster et al. (2002), found no additive effect from a combination of DBP and DEHP. Foster et al. concluded "[t]his study did not indicate an additivity of response or an interaction of the two phthalates in combination. Aggregation of risk of these doses would not be appropriate."<sup>8</sup> Thus, the paragraph suggesting a concern for additivity of effects is presented as the consensus of the Expert Panel after receiving no deliberation or opportunity for comment, and in direct opposition

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<sup>6</sup> Letter from Marian K. Stanley, Manager PE Panel, to Dr. Michael Shelby, CERHR Director, Re: DEHP Update Expert Panel Report, dated October 12, 2005.

<sup>7</sup> Final Update at page 171.

<sup>8</sup> *Id.*

to the only available evidence about additivity. For these reasons, this paragraph should not have been included in the Final Update.

In addition, the Final Update concludes that “[t]here is sufficient evidence in female marmosets to conclude that DEHP causes reproductive toxicity (increased ovary weight and uterine weight) when exposure is by oral gavage at 500 mg/kg bw/day for ~15 months in the peripubertal period . . . .”<sup>9</sup> The Final Report interprets this observation by stating that “[t]he Expert Panel found these data consistent with precocious puberty . . . .”<sup>10</sup> However, this conclusion was not the result of any true deliberation by the Expert Panel. In the closing minutes of the public meeting, a comment was made that the observation of increase in ovarian weight should be accompanied by a statement about the implications of that observation. A single panel member, almost off-handedly, asserted that the data were indicative of precocious puberty. This interpretation was accepted by the Expert Panel with no discussion and no opportunity for public comment. For this assertion, which has very significant implications for concern about potential DEHP effects,<sup>11</sup> there was absolutely no deliberation by the Expert Panel or opportunity for public comment about the interpretation of the data. In fact, as discussed in Section V.A, below, the increased ovarian and uterine weights may simply correlate with increased female body weights, and therefore represent no adverse effect at all. Yet as a result of there being no deliberation or opportunity for public comment, the conclusion that DEHP causes precocious puberty in marmosets is now represented as the consensus opinion of the Expert Panel.

As a final example, Table 23 of the Final Update, which was compiled at the last minute with no real deliberation or quality checking, arguably contains errors in the total numbers of animals observed at each dose (see Section IV.A, below). These errors likely affected the Panel’s interpretation of the data, and consequently its conclusions regarding the toxicity of DEHP.

Due to the lack of any deliberation or opportunity for public comment, the PE Panel believes it is highly inappropriate that the above statements are included in the Final Update as the Expert Panel’s consensus opinion. In denying the PE Panel’s request that the paragraph suggesting additivity of effects be removed from the Final Update, NTP-CERHR disclaimed that “the conclusions reached by CERHR expert panels are their own” and “should not be construed to represent the views of the National Toxicology Program.”<sup>12</sup> Despite this disclaimer, it is likely that the Final Update will nonetheless be pointed to by interested parties as the consensus view of an ostensibly objective “eleven-member panel of government and non-

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<sup>9</sup> Final Update at page 163.

<sup>10</sup> *Id.*

<sup>11</sup> Given current debate about endocrine disruption, “precocious puberty” is a highly charged term.

<sup>12</sup> Letter from Dr. Michael D. Shelby, Director CERHR, to Marian K. Stanley, Manager PE Panel, dated October 28, 2005. NTP-CERHR also stated that “because the expert panel report is not a government document, the NTP is unable to [remove the paragraph].” *Id.* It seems somewhat disingenuous, however, to suggest that NTP-CERHR is powerless to correct a documented flaw in the report. If nothing else, NTP-CERHR could have shared the PE Panel’s letter with the Expert Panel to receive direction on whether to remove the offending paragraph.

government scientists.”<sup>13</sup> Consequently, it is not sufficient merely to state that the conclusions of the Expert Panel are their own, and not those of NTP-CERHR. NTP-CERHR should take a hard look at its Expert Panel process and implement safeguards to ensure that the “consensus opinions” put forth in panel reports are indeed the fully-deliberated consensus view of the panel, and not last-minute, hastily accepted but substantively important add-ons.

The need for such a deliberative period is illustrated by the note included in the Preface to the Final Update which states that “[w]hile the expert panel reached consensus on all conclusions during the panel meeting, following the meeting . . . three panel members reconsidered their position on one conclusion. Upon reconsideration, they did not concur with [the conclusion arrived at the public meeting].”<sup>14</sup> The mere existence of this note shows that a hastily-arrived-at “consensus” is really no consensus at all. The Final Update should reflect a true consensus, in which case there would be no need of a note such as this. Allowing an additional period of reflection and comment would help ensure that panel reports reflect a true consensus of the expert panel.

#### D. NTP-CERHR Should Be More Flexible In Allowing Audience Participation

The negative impact of the process flaws identified above might have been *partially* mitigated had the public meeting been run in a similar manner as the first phthalate Expert Panel meetings in 1999 and 2000. At those meetings, members of the public were allowed to raise their hands during the meeting to comment and provide clarification on various scientific issues or specific data. These public comments were presented in a respectful and orderly fashion, by highly qualified scientists, and contributed to, rather than detracted from, the Expert Panel’s deliberations. Indeed, the value of that scientific input was acknowledged by many participants in the public meetings. In the 2005 meeting, however, public comments appeared to be more discouraged than encouraged. They were limited to 15 minute presentations and there was no opportunity to comment during the Expert Panel’s discussion.<sup>15</sup> Without adequate opportunity for the public to comment during the deliberations, there was no effective way to address or even identify many of the issues described above. Indeed, there was effectively little or no way to address issues that had not first surfaced in the draft Expert Panel report.

The issues that are addressed by an NTP-CERHR Expert Panel frequently are cutting-edge scientific issues that benefit from robust discussion. That discussion, of course, is centered around the Expert Panel, which hopefully will have the requisite expertise and be free of conflict or bias. However, industry scientists or other stakeholder scientists often will have important points to contribute, and may in some cases be able to offer the perspective of expertise that is lacking on the Expert Panel. The PE Panel believes NTP-CERHR should not

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<sup>13</sup> See Final Update at page ii.

<sup>14</sup> *Id.* at page iii.

<sup>15</sup> Comments could be given to the CERHR director to be passed on to the Expert Panel chair, but this was very ineffective. It is not clear that all comments were given to the chair, or that all comments were presented to the full Expert Panel, and even when comments were passed on, they often “lost something in the translation.”

manage the deliberations in a way that shuts out that input, but instead should encourage participation in a reasonable and respectful manner, as occurred with the initial phthalate Expert Panel assessments.

If NTP-CERHR's goal truly is an "objective and scientifically thorough assessment," then NTP-CERHR should be flexible in the way it manages the public meetings, and give scientists in the audience some credit for being able to exercise discretion in offering scientific input during the deliberations. An overly rigid approach can only serve to deny Expert Panel members access to relevant scientific input. A more flexible approach can only enhance the quality and objectivity of the final work product.

## **II. THE MARMOSET IS A VALUABLE MODEL FOR MALE HUMAN REPRODUCTIVE TOXICITY**

The Final Update lists several aspects of marmoset biology that differ from human biology and, based largely on a review of marmoset reproductive biology by Li et al. (2005), ultimately concludes that these differences "significantly limit our reliance on this species as a surrogate for humans."<sup>16</sup> While the marmoset, like all animal models, is not a perfect model for all aspects of human toxicity, the PE Panel believes strongly that marmoset is a good model for male reproductive development. In particular, the marmoset is a particularly good model for the reproductive endpoints investigated in the Mitsubishi (2003) study.

To assess the usefulness of the marmoset as a model for human reproductive toxicity, the PE Panel engaged Professor Stefan Schlatt to render his opinion on the value of the marmoset model. Dr. Schlatt is an Assistant Professor in the Department of Cell Biology and Physiology at the University of Pittsburgh School of Medicine, and is an expert in mammalian reproductive biology. Dr. Schlatt has more than 13 years of experience researching reproductive function and endocrine activities in animal models, including rats, hamsters, and various nonhuman primates, including marmosets, and has authored more than 50 refereed articles on various aspects of this field. In Dr. Schlatt's opinion (provided as Attachment 1 to these comments), "there is no doubt that the marmoset is a useful model to explore developmental and toxicological aspects of the testis," and "many similarities in regard to testicular organization, general developmental pattern and hormonal regulation render the marmoset a much more useful model when compared to rodents."<sup>17</sup>

### **A. The Review Relied Upon by the Expert Panel, Li et al. (2005), Exhibits an Unwarranted Bias Against the Marmoset as a Model for Male Reproductive Toxicity**

In discussing the validity of the marmoset model, the Final Update relies heavily on the review by Li et al. (2005), which points out several differences between marmoset and human reproductive biology. This review, however, ignores information or data that indicate the marmoset is a good model, and focuses on negative information. As stated by Dr. Schlatt, the Li

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<sup>16</sup> *Id.* at page 141.

<sup>17</sup> Opinion of Dr. Schlatt, Attachment 1, at page 4.

et al. “interpretation of the suitability and validity of the [marmoset] model carries an unjustified negative bias.”<sup>18</sup> As discussed above, the merits of the Li et al. criticisms were not sufficiently evaluated by the Expert Panel, due to the timing of its publishing versus the public comment period.

B. The Marmoset Has Similarities to Human Reproductive Biology that Make it a Valuable Model

As discussed in Dr. Schlatt’s opinion (Attachment 1), several features of marmoset reproductive biology make it a valuable model for human reproductive toxicity.

1. The Similarity of Human and Marmoset Sertoli Cell Development Make the Marmoset a Good Model for Studying Effects on Germ Cell Development – a Primary Concern of the Expert Panel

One of the key concerns of the Expert Panel was the effect of DEHP exposure on Sertoli cells (vacuolation and reduced proliferation) in developing animals. This concern is based on data from rats, in which Sertoli cell effects are seen at relatively low oral doses. However, the data in Mitsubishi (2003) indicate that extremely high oral doses of DEHP, up to 2500 mg/kg/day, had no effects on marmoset testes even at the cellular level. Because of this disparity in effects, the value of the marmoset as a model of human reproduction becomes an essential question.

As stated by Dr. Schlatt, there are “striking similarities of organization of the marmoset and human spermatogenic epithelium (both species have a multistage organization of spermatogenic stages per tubular crosssection),” and “quite similar mechanisms of germ cell development and clonal expansion of germ cells in marmosets and man.”<sup>19</sup> Also, while Li et al. point out an unusual uniformity of Sertoli cell morphology throughout the marmoset spermatogenic cycle, Dr. Schlatt explains that it is not yet known whether humans and marmosets differ with respect to this observation, and that “it appears likely that the human and marmoset Sertoli cell show a high degree of similarity in this respect”.<sup>20</sup> Dr. Schlatt concludes:

With choice of the correct timepoints [the marmoset] should be highly useful and informative for exploring the effects on Sertoli cell differentiation, testicular growth and effects of FSH on the testis. The striking similarities to man make it an excellent model for studying effects on germ cell development, the organization of the seminiferous epithelium and changes to the kinetics of spermatogenesis.<sup>21</sup>

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<sup>18</sup> *Id.*

<sup>19</sup> Opinion of Dr. Schlatt, Attachment 1, at page 2.

<sup>20</sup> *Id.*

<sup>21</sup> *Id.* at page 4.

Thus, similarities in biology indicate that the marmoset is a good model for determining the potential effects of DEHP on human Sertoli cells, and available data indicate that the marmoset is far less sensitive to oral DEHP exposure than rodents. Despite concerns with the health of the animals in the Mitsubishi study (see discussion in Section II.C below), the extreme insensitivity of marmosets to oral DEHP exposure, at the very least, provides information that puts the rodent model into perspective as being much more sensitive to testicular effects of DEHP than primates. Therefore, the marmoset data should not have been completely disregarded for purposes of evaluating male reproductive toxicity.

2. There Is No Evidence that the Generalized Steroid Hormone Resistance in the Marmoset Mentioned by Li et al. Applies to Sex Steroids

Based primarily on the review of Li et al. (2005), the Expert Panel concludes that marmosets have a general end-organ steroid resistance relative to humans, as indicated by their high serum levels of steroids, which limits their value as a human reproductive model. However, as explained by Dr. Schlatt (see Attachment 1), while a general insensitivity has been proposed for the mineralocorticoid and glucocorticoid hormones, it is unknown whether such steroid resistance exists for the gonadal (sex) steroids. Marmosets, but not mice, have sex hormone binding globulin that separates the serum testosterone into active free (biologically active) and bound (biologically inactive) fractions. Due to the binding affinities of these proteins, marmosets appear to have high levels of unbound testosterone in the circulation which is similar to their unusually high levels of glucocorticoids. However, these higher testosterone levels may have little or no biological significance since the kinetics of testosterone-receptor binding and post-receptor binding events is unknown. Therefore, high levels of serum testosterone in marmosets are not necessarily indicative of sex steroid resistance. As stated by Dr. Schlatt, “in the absence of solid data on sex steroids it appears poorly justified and premature to transfer the conclusion of high sex steroid resistance from the glucocorticoid and mineralocorticoid system to the sex steroid system.”<sup>22</sup>

3. The Marmoset’s Requirement of Dietary Vitamin C and Lack of Luteinizing Hormone Are Not Significant Disadvantages of the Marmoset Model

The Final Update, citing the review of Li et al. (2005), mentions, as additional disadvantages of the marmoset model, the potentially protective action of Vitamin C in the marmosets’ diet and the marmosets’ lack of Luteinizing Hormone (LH). Each of these concerns was addressed in the PE Panel’s April 2005 submission to NTP-CERHR<sup>23</sup> and the Vitamin C issue again in the PE Panel’s September 2005 Comments on the Draft Update,<sup>24</sup> and has been

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<sup>22</sup> *Id.* at page 3.

<sup>23</sup> Recent Information on Exposure to and Toxicology of Di(2-ethylhexyl) Phthalate (DEHP), American Chemistry Council Phthalate Esters Panel, submitted to NTP-CERHR April 21, 2005. (April 2005 Comments.)

<sup>24</sup> See Section IV.B. of: Comments of The American Chemistry Council Phthalate Esters Panel On the Draft NTP-CERHR Expert Panel Update On The Reproductive And Developmental Toxicity

shown to be of no substantial importance in assessing the validity of the marmoset model. As discussed in the PE Panel's previous submissions, the marmosets' dietary Vitamin C requirement should not be of concern because: 1) the levels of Vitamin C used in the Mitsubishi study are not high relative to the marmoset's requirements, and 2) based on the available science, it is not clear that Vitamin C affords any protection to primates from DEHP exposure. Moreover, if the level of Vitamin C in the marmosets' diet in the Mitsubishi study in fact provided the degree of protection necessary to be responsible for the observed lack of effects, then the level of Vitamin C in the average human diet would be protective of any likely exposure to DEHP. In other words, the Vitamin C levels in the marmoset diet in the Mitsubishi study were similar to normal levels in the human diet and, consequently, whether Vitamin C had a protective effect in this study is not directly relevant to a risk assessment.

As for the marmoset's lack of LH, the data cited in the PE Panel's earlier submission show that this difference in the hormone that initiates testosterone synthesis between the common marmoset and humans does not provide a sufficient basis for rejecting the marmoset as a model for human testicular development and function.<sup>25</sup> This opinion is shared by Dr. Schlatt who states: "Despite . . . the exchange of LH by CG the marmoset shows many similarities to man. The function and regulation of FSH and CG and their feedback mechanisms resemble other primates."<sup>26</sup> Even with this hormonal difference, Dr. Schlatt concludes that the marmoset is a valuable model, particularly for the investigation of effects of DEHP exposure on Sertoli cell proliferation, which is regulated primarily by Follicle Stimulating Hormone in marmosets and other primates, but not rodents.

4. Other Criticisms in the Li et al. Review Are Insufficient to Invalidate Use of the Marmoset Model to Evaluate Potential Effects of DEHP on Human Reproductive Development

The Li et al. review includes some additional criticisms of the marmoset model, not explicitly discussed in the Final Update. Both these and some of the foregoing criticisms have been addressed in the context of comments by the PE Panel to the California Office of Environmental Health Hazard Assessment (OEHHA). A copy of those comments is provided as Attachment 2. As NTP-CERHR prepares the final brief for DEHP, to the extent it considers the Li et al. review paper, it also should consider Section I of the comments to OEHHA.

C. The Mitsubishi Study Provides Valuable Information Regarding the Sensitivity of Primates to DEHP

The Final Update notes concern about the body weights of some of the animals in the Mitsubishi marmoset study, as does Dr. Tardif in her independent review (See Attachment 3). However, as pointed out in the opinion of Dr. Schlatt, notwithstanding these concerns, the Mitsubishi study nonetheless provides "strong evidence that DEHP had **no major** effect on

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Of Di(2-Ethylhexyl) Phthalate, Submitted to NTP-CERHR September 28, 2005. (Comments on Draft Update.)

<sup>25</sup> See Section II.B.2.d.2. of April 2005 submission.

<sup>26</sup> Opinion of Dr. Schlatt, Attachment 1, at page 3.

testicular development even after very long and intense DEHP exposure.”<sup>27</sup> Dr. Schlatt concludes that the Mitsubishi data “should be carefully and critically considered for evaluating the risk of gonadotoxic effects in humans after exposure to DEHP.”

Indeed, the Mitsubishi data should not be completely disregarded with respect to implications for the degree of concern for male reproductive effects in humans. The shortcomings of the study might limit its usefulness on a quantitative level, but it still provides important qualitative information about the relative toxicity of DEHP to primates versus rodents, specifically that *very high concentrations of DEHP that would have profound adverse effects on rodent testes had no adverse effects on the testes of marmosets*. Moreover, despite it being considered essentially in isolation in the Final Update, the Mitsubishi study results are supported by several other primate studies. In other studies of both old world and new world primates, no testicular effects have been observed at doses up to 2500 mg/kg/day (Pugh et al., 2000; Kurata et al., 1998; Rhodes et al., 1986; Short et al., 1987). Combined with the Mitsubishi study, the clear conclusion is that primates are much less sensitive than rodents to the effects of DEHP. Even if primates are not used to establish the NOAEL for male reproductive effects, the PE Panel strongly believes that these data should be used to modify the degree of concern for effects in human testicular development from DEHP.

Due to the limitations in the process discussed above, the Expert Panel essentially ignored these older studies and instead focused only on the Mitsubishi data and its limitations. The Panel should consider the Mitsubishi data in light of these other primate studies, which together provide substantial evidence that primates are less sensitive to DEHP than rodents. The Expert Panel’s lack of consideration of Mitsubishi in the context of this additional primate data might have been avoided had the Public Meeting allowed for public comments beyond the 15 minute presentations allowed.

In summary, substantial evidence, not all of which appears to have been adequately considered by the Expert Panel, indicates that marmosets are a valuable model for the investigation of human reproductive toxicity. Like any other non-human model, the marmoset is useful for some comparisons but not others. In this case, the parameters measured in the Mitsubishi study, particularly the lack of Sertoli cell effects, were those for which a marmoset model would be appropriate, and in fact superior to the rodent model. The use of these data should not be precluded by the fact that the marmoset may not be a good model in other respects not germane to the issues at hand.

#### D. Toxicokinetic Data Support the Lower Sensitivity of Primates to the Effects of DEHP

In Section II of its comments on the August 2005 Draft Update, the PE Panel summarized an extensive body of literature indicating that differences between rodents and primates in the absorption, distribution, metabolism and excretion (ADME) of DEHP can explain in large part the lower sensitivity of primates to the reproductive effects of DEHP. The ADME data provide a consistent explanation both among mammals (i.e., rodents vs. primates) and within primates (i.e., marmoset vs. cynomolgus monkey) as to why primates absorb less of the

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<sup>27</sup> *Id.* at page 6 (emphasis in original).

toxicologically relevant metabolite (MEHP) and exhibit lower MEHP / DEHP levels in the blood and target tissues than rodents. The Expert Panel does acknowledge some of the ADME data that have appeared since the first Expert Panel review in 2000 but in its conclusions seems unwilling to make the connection between lower systemic doses of MEHP in primates and the lower sensitivity of primates to DEHP toxicity. Because most, but not all, of the primate ADME data came from the marmoset, the Expert Panel apparently chose to disregard these data due to perceived significant limitations with the Mitsubishi study. At the very least, however, the ADME data should provide the Expert Panel with sufficient information to acknowledge that the use of rat data for human risk assessment is likely to be very conservative (i.e., health protective).

### III. COMMENTS ON SECTION I: USE AND HUMAN EXPOSURE

- A. The Final Update Could Have Used Available Biomonitoring Data to Show That DEHP Exposures to Children Ages 12-18 Months Are Not Several Fold Higher than Adults

The PE Panel applauds the Expert Panel for considering the PE Panel's recommendation in its comments on the Draft Update to calculate exposures based on available biomonitoring data. It was the PE Panel's recommendation that "[t]he section. . . on human exposures to DEHP could be significantly improved by including conversions of urinary metabolite levels to estimates of environmental exposure," and that "[t]he CDC biomonitoring data are the most comprehensive and accurate estimates available of exposures of the U.S. population, including children, to DEHP."<sup>28</sup> Using this approach, the PE Panel developed exposure estimates from the biomonitoring data that were consistent with probabilistic estimates based on sources of exposure. Although the Expert Panel articulated the uncertainties associated with each method, the PE Panel agrees that combining, or at least comparing, the two methods, as done in the Final Update, provides the best overall approach.

However, the Expert Panel did not extend the process of exposure estimation from biomonitoring data to children ages 12-18 months (Brock et al., 2002), a subpopulation that was considered at potentially greater risk. Instead, the Expert Panel indicated that children 1-6 years old could have exposures several-fold higher than the population estimates based on the study by Koch et al. (2004) of German children ages 2.5-6.5, which showed higher levels of excreted phthalate per gram creatinine than adults in the same household.<sup>29</sup> However, the Expert Panel could instead have used the same approach of calculating exposures on the Koch et al. study, and combined it with the study by Brock et al., to show that exposures of this age bracket are in fact not several fold higher than adults (see Table 1 below). The PE Panel urges NTP-CERHR to take this approach in assessing DEHP exposures for its final brief.

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<sup>28</sup> Comments on Draft Update at page 7.

<sup>29</sup> As stated in the PE Panel's September 2005 comments on the Draft Update, there are significant differences in creatinine excretion between adults and children – children generally excreting half the level of creatinine excreted by adults).

Table 1: DEHP Exposures to Children Calculated from Biomonitoring Data in Koch et al. (2004) and Brock et al. (2002).

Source	Mean/median DEHP metabolite/g creatinine	Exposure estimate (µg/kg/d)
Koch et al.	55.8 (5-OH-MEHP) <sup>a</sup>	5.52
Brock et al.	26.14 (MEHP) <sup>b</sup>	2.57

a = calculated using the method of David (2000) and the excretion ratio of Koch et al. (2004).

b = calculated using the method of David (2000) and the excretion ratio of Anderson et al. (2001).

**B. The Final Update Erroneously Indicates that Cosmetics and Breast Milk Pumps Are Significant Sources of Human Exposure to DEHP**

Section 1.0 of the Final Update states: “Phthalates are used in a variety of products including . . . perfumes, hairsprays, and cosmetics . . .”, implying that DEHP is so used.<sup>30</sup> The Final Update conclusions also imply that cosmetics are a significant source of human exposure to DEHP.<sup>31</sup> However, information in the Final Update indicates that DEHP use in cosmetics and personal products is extremely limited or non-existent. As discussed in Section 1.1.1, a survey of 42 perfumes, 8 deodorants, 21 nail polishes, and 31 hair care products marketed in Korea found DEHP in only 2 of the perfumes and 2 of the nail polishes, and none of the deodorants or hair products (Koo and Lee, 2004). The maximum level of DEHP detected was 25 mg/L (25 ppm). Similarly, a 2002 Environmental Working Group report found only 3 products out of 72 tested that contained DEHP, again with a maximum concentration of 25 ppm.<sup>32</sup> That is a concentration of only 0.0025%. It is unlikely that DEHP would have functionality and therefore be intentionally added to a formulation at such a low level. More likely, the DEHP was a trace contaminant in the formulation or was an artifact of laboratory contamination. In fact, the Cosmetic, Toiletry and Fragrance Association has indicated that no cosmetic products currently manufactured in the US contain DEHP.

In an exceedingly large number of places, often particularly prominent places such as boxed language, the Final Update speculates that breast milk expressed into breast pumps may be a source of DEHP exposure, and uses concern about the potential for such exposure as a basis for asserting uncertainty about exposures of infants to DEHP. See pages 7, 33-34, 54, 55, 93, 97, 171, and 175 of the Final Update. Yet the Final Update cites not one analytical result demonstrating that breast milk pumps contribute any DEHP to breast milk, much less significant amounts that would warrant the great deal of attention given to this

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<sup>30</sup> Final Update at 2.

<sup>31</sup> See Final Update at page 169, which states: “DEHP is ubiquitous in the environment. Humans can be exposed to DEHP through many routes including ingestion (food, infant formula, and breast milk), contact with contaminated household dust and consumer products (cosmetics and toys). . . .”

<sup>32</sup> Environmental Working Group (2002). Not too pretty: Phthalates, beauty products and the FDA., page 2. Available at: [http://www.ewg.org/reports\\_content/nottoopretty/nottoopretty\\_final.pdf](http://www.ewg.org/reports_content/nottoopretty/nottoopretty_final.pdf)

speculative source. In fact, the available data cited in the Final Update suggests breast milk pumps do not affect DEHP levels in breast milk.<sup>33</sup>

The PE Panel's knowledge of breast pumps indicates that it is highly unlikely breast milk pumps would be a significant contributor to DEHP exposures. To the PE Panel's knowledge, no milk container portion of any pump is made of vinyl that would contain DEHP (or any other phthalate), and therefore there would be no DEHP available to migrate into the milk during storage. Some breast milk pumps have flexible vinyl tubing that may contain DEHP; however, the tubing is used to pull air away from the container (creating the vacuum that pumps the milk); the milk does not come into contact with the tubing. The cup that is placed against the breast might be made of flexible vinyl that might contain DEHP, and it is possible some breast milk might come into contact with the cup, but any such contact would be fleeting and would allow for very little migration of DEHP into the milk.<sup>34</sup>

For these reasons, the rather extreme concern about potential infant DEHP exposures to DEHP from breast milk pumps is spurious. The PE Panel requests that, in its final brief, the NTP-CERHR clarify the potential role of breast milk pumps in DEHP exposures. To the extent that the Expert Panel expressed concern due to uncertainty over infant exposures because of the speculative contribution of breast milk pumps, NTP-CERHR should express a lower level of concern.

#### **IV. COMMENTS ON SECTION 3: DEVELOPMENTAL TOXICITY DATA**

##### **A. The Data Reviewed in the Final Update Do Not Support an Oral Developmental NOAEL of 3-5 mg/kg/day**

The first CERHR Expert Panel Report did not identify a firm NOAEL for developmental toxicity on the developing male reproductive tract.<sup>35</sup> In the Final Update, the Expert Panel cited the multigeneration continuous breeding study conducted by NTP (2004) as providing a developmental NOAEL of 3-5 mg/kg/day. This NOAEL is based on the incidence of gross observations of small reproductive organs (with no change in organ weights) observed in a few animals in the 14-23 mg/kg/day and the 46-77 mg/kg/day groups. In deriving this NOAEL, the Expert Panel stated that it evaluated the combined incidence of small male

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<sup>33</sup> "The authors tested milk samples in 1 common Danish pump system and found no effect on phthalate monoester levels." Final Update at 7. "Women who used a breast pump in Denmark had significantly higher levels of monoethyl and monobutyl phthalate. Breast pump-associated levels of other phthalates were not significantly different . . ." Final Update at 55.

<sup>34</sup> Like the Li et al. paper, the concern about breast milk pumps is an example of information newly raised at the Expert Panel meeting for which there was no adequate opportunity for public comment (see Section I.A, above). The PE Panel attempted to provide an explanation such as given herein to the Expert Panel, but it is not clear that the explanation was conveyed to the Expert Panel Chair, much less the rest of the Expert Panel, a failing which could have been overcome by a more flexible approach to audience participation (see Section I.D., above).

<sup>35</sup> The Expert Panel stated "The Panel is not confident that the lowest dose has been established at which developmental toxicity (the development of the male reproductive system) occurs." First Expert Panel Report at 88.

reproductive organs in the F1 and F2 non-breeding males.<sup>36</sup> However, the total number of examined organs listed in Table 23 of the Final Update does not reflect this, as the table contains only the number the F1 non-breeding males examined and omits the number of F2 non-breeding males.

Counting the F2 non-breeding males, the total combined number of organs examined should have been 59, 61, 64, 61, 66, 68, 50 for the 1.5, 10, 30, 100, 300, 1000, and 7500 ppm dose levels, respectively. In addition, the PE Panel believes that the number of breeding males in the F1 and F2 generations should also be included in the combined incidence. While the breeding males were older than non-breeding males at the time of sacrifice, the effect of small reproductive organs, if present, would not be expected to disappear over this time period. Including the breeding males would increase the total number of each group by 20. By not including in the total number of examined organs the number of F2 non-breeding males and breeding males, the Expert Panel significantly overstates the incidence rate of small organs, which in turn significantly overstates the magnitude of the effect. The PE Panel provides below what it believes to be the correct version of Table 23.

Table 23. Reproductive Organ Abnormalities in Combined F<sub>1</sub> + F<sub>2</sub> Non-breeding Males in NTP Multigeneration Study

Organ	DEHP dose level, ppm in feed (number of organs examined)						
	1.5 (79)	10 (81)	30 (84)	100 (81)	300 (86)	1000 (88)	7500 (70)
Testis	0	0	0	0	4	3	21
Epididymis	0	0	0	0	3	3	7
Seminal vesicles	0	1	0	0	2	0	0
Prostate	0	0	0	0	0	4	1
Any reproductive organ	0	1(1)*	0	0	5(4)*	7(5)*	22(14)*

\*Data expressed as number of animals (litters) affected. From NTP (114)

In addition, although the NTP study reported that the finding of small reproductive organs in the 300 and 1000 ppm groups was at a significantly higher incidence rate than laboratory historical control data, the historical data were not available for review. The PE Panel questions whether sufficient historical control data for small reproductive organs have been evaluated to ascertain whether the limited incidence of small organs seen in the NTP study can be definitively determined to be treatment related. For the low incidence of small organs observed in the treatment groups to be statistically significant, the control incidence of this effect would have to be zero, or very close to zero. As mentioned in the PE Panel's comments to the Draft Update, historical data from contract laboratories indicate that there is a 2-3% incidence of testicular atrophy at necropsy in control populations of sexually mature Sprague-Dawley rats from Charles River Laboratories. In the absence of reduced organ weight (individual or group mean) or evidence of lowered reproductive success, which cannot be assessed because the effects were only reported for non-mating males, the small organs reported at 1000 and 300 ppm should

<sup>36</sup> Final Update at 66 and 94.

not be considered toxicologically significant. Therefore, the PE Panel believes it is necessary to evaluate historical control data for this strain of rat to evaluate the statistical significance of the incidence of the small reproductive organs. Without such information, the PE Panel questions the validity of making the determination of a NOAEL in this study based on this finding.

Moreover, at the Expert Panel public meeting, Dr. Robert Chapin, the Expert Panel member who most closely reviewed the NTP data, stated that the 300 ppm (about 14-23 mg/kg/day) LOAEL was at the “very tail end of the response”<sup>37</sup> and that the Expert Panel was “flying along in the weeds” at this level. This raises the issue of whether the effects reported in the NTP study at the 300 ppm LOAEL were in fact treatment related, or were non-treatment related noise. In other words, if 14 mg/kg/day is the LOAEL, then the NOAEL is likely much closer to this value than the value chosen by the Expert Panel, the lowest tested dose of 100 ppm (about 3-5 mg/kg/day).

Thus, the PE Panel believes that the NTP (2004) data as reviewed by the Expert Panel do not support a NOAEL as low as 3-5 mg/kg/day. For the foregoing reasons, the PE Panel believes NTP-CERHR should consider 14-23 mg/kg/day as a NOAEL, or at the least should find the NOAEL to be near that level (e.g., 12 mg/kg/day).

## **V. SECTION 4: REPRODUCTIVE TOXICITY DATA**

### **A. The Data From the Mitsubishi Study Showing Increased Uterine and Ovarian Weights in Female Marmosets Do Not Support the Conclusion that DEHP Exposure Resulted in Precocious Puberty in Marmosets**

After rejecting the Mitsubishi (2003) data for assessing male reproductive toxicity, due in part to concerns about the reliability of the study, the Expert Panel used data from that same study to conclude that increased uterine and ovarian weights in female marmosets were “consistent with precocious puberty in the 2 highest dose DEHP-exposed groups (500 and 2500 mg/kg bw/day).”<sup>38</sup> However, a review of the Mitsubishi study by Dr. Suzette Tardif, the Associate Director of the Southwest National Primate Research Center and an expert in female marmoset reproductive biology with over twenty years experience raising primates, determined that these data are inconclusive and do not support the conclusion that DEHP exposure causes precocious puberty in female marmosets.

Dr. Tardif’s analysis of Mitsubishi (2003) (see Attachment 3 to these comments) shows that the increased uterine and ovarian weights correlate to body weights that were higher in the higher dose females, versus lower body weights in controls and low dose females. As Dr. Tardif explains, the females in the Mitsubishi study, particularly the controls and the low dose group, were of extremely low weight for their age at the end of the study (about 17 months), compared to a healthy marmoset colony. Dr. Tardif attributed this to the study procedures, which involved daily gavage for many weeks in a row and resulted in many “basically unhealthy

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<sup>37</sup> This comment is acknowledged in the Final Update, which states at page 151: “The Expert Panel considers 300 ppm and 1000 ppm to represent the tail of the dose-response curve in this study....”

<sup>38</sup> Final Update at page 163.

animals with impaired growth.”<sup>39</sup> While there was no significant difference in average weight across the treatment groups, there was a trend for the females in the two highest dose groups to have the highest body weights, and these were the groups which also had increased uterine and ovarian weights. Specifically, only three out of six subjects in the control and low dose groups had a 17-month-old body weight that Dr. Tardif would consider suitable for research (275 g), five of six and six of six of the animals in the higher dose groups would have been suitable.

Moreover, based on measured estradiol concentrations, many of the animals in fact appeared to be pre-pubescent throughout the length of the study, which was terminated when the animals were at an age at which all should have been adults. The failure of many individuals to enter puberty was correlated with the abnormally low body weights.

Dr. Tardif found that the higher ovarian weights in the higher dose groups were generally associated with higher body weights and the occurrence of ovulation and corpus luteum formation that accompanies sexual maturity. In Dr. Tardif’s opinion, the higher ovarian weights seen in the highest dose group were simply due to the higher weight group having more normal weight animals which were more likely to have ovulated. Normal ovarian function in marmosets includes the development and maintenance of a large, steroidogenic interstitial gland, and the persistent presence of this gland, along with the cyclical presence of corpus lutea, leads to the heavier, sexually mature females having higher ovarian weights. Thus, the higher ovarian and uterine weights in the two high dose groups is an artifact of those groups having higher weight females, and is not an effect of DEHP dose. Accordingly, the PE Panel disagrees with the Expert Panel’s conclusion that the increased female ovarian and uterine weights in the Mitsubishi study are indicative of precocious puberty.<sup>40</sup>

**B. The Studies by Akingbemi et al. Reviewed in the Final Update Are Flawed and Do Not Support a NOEL of 1 mg/kg/day**

The Expert Panel concluded that the results from two Akingbemi et al. (2001; 2004) studies reporting a NOEL of 1 mg/kg/day based on data obtained from purified Leydig cells were adequate for the evaluation process.<sup>41</sup> The effects reported by Akingbemi et al. at the LOEL (10 mg/kg/day) were abnormal changes in testosterone production and altered Leydig cell proliferation in the testes of prepubertal rats. However, because these studies suffer from a methodological deficiency – they rely on single-point measures of serum LH and testosterone – the PE Panel has significant concerns regarding the suitability of these studies for deriving the NOEL.

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<sup>39</sup> Opinion of Dr. Tardif, Attachment 2, at page 1.

<sup>40</sup> An equally plausible interpretation of the data is that high doses of DEHP enhance the health of females, thus enabling high dose females to mature in the usual time frame, with resulting increases in ovarian weights over the less healthy controls and low dose females. The PE Panel does not advance this hypothesis – it would be far too speculative a conclusion based on this one study. The PE Panel believes that the assertion of precocious puberty is equally speculative.

<sup>41</sup> Final Update at 168.

Several papers and texts (Culler, 1998; Levine and Duffy, 1988; Creasy, 1999) provide excellent descriptions as to why single point measures of isolated or combined gonadotropin and gonadosteroid measurements do not allow for identifying toxicity from chemical exposures. The methodological problem arises from the fact that pulsatile releases of gonadotropin releasing hormone (GnRH) control LH release and pulsatile LH levels control testosterone production and release from Leydig cells. These pulsatile releases are superimposed on a circadian rhythm pattern for the secretion of these releasing factors and hormones. If multiple samples are not collected and analyzed from each animal over several hours during the peak phase of the circadian cycle, then the pulsatile releases may be missed. To ensure accurate comparisons among individuals, care must be taken to collect samples from all of the animals at precisely the same time relative to the circadian pattern described above.

The Akingbemi et al. studies do not meet these criteria. The Materials and Methods Sections in both Akingbemi et al. papers simply state that the blood was taken within 24 hours of the last DEHP exposure; there is no mention of any attempt on the part of the authors to control for the time of day (circadian pattern) between treatment groups. The lack of control for these circadian patterns and pulsatile releases of these factors and hormones is a critical flaw in the study design. For example, if all the control animals were sacrificed first, followed by the low dose, mid dose, and high dose animals, the investigators would be introducing a systematic sampling bias to their measurements because the animals may have been in different stages of the circadian pattern and have been experiencing varied pulsatile release of these factors/hormones.

Another significant flaw in the Akingbemi et al. studies lies in the method by which the authors isolated the Leydig cells from these animals and how they interpreted the data from endpoints derived from these cells. Leydig cells in immature rats are relatively quiescent and small and will elute/migrate through a Percoll gradient with a different density than mature Leydig cells. As the authors describe in the 2001 paper, PND 21 (progenitor) Leydig cells elute/migrate within the Percoll gradient at a band representing a density of 1.062 – 1.070 g/ml. PND 35 control rats have immature Leydig cells which elute/migrate through the same gel at a band representing a density of 1.070 to 1.088 g/ml and the Leydig cells obtained from PND 49 and 90 (mature) animals have densities greater than 1.070 g/ml. In addition, the authors used an enzyme critical for testosterone biosynthesis, 3 $\beta$  hydroxysteroid dehydrogenase (3 $\beta$ -HSD), as a biomarker for whether these cells were able to produce testosterone. PND 21 Leydig cells stain lightly or not at all for 3 $\beta$ -HSD indicating that they are quiescent in terms of testosterone biosynthesis. The Leydig cells from PND 35 and greater stain intensely for 3 $\beta$ -HSD indicating that testosterone biosynthesis can occur in those cells. Therefore, in control rat testes, as the Leydig cells mature and gain the ability to synthesize testosterone, their cellular density (a product of cell mass and size) changes and, correspondingly, the distance they elute/migrate within the Percoll gradient changes.

Typically, one of the first alterations noted in cells undergoing abnormal cell division (e.g., hyperplasia) or reacting to a toxic insult, is a change in cell mass and size. These changes, which are well described in textbooks of toxicological pathology, are changes that pathologists look for microscopically. However, measuring changes in cell mass or size (i.e., density) is very difficult using histological techniques, as a three dimensional object (the cell) is

only represented in two dimensions under a microscope. Consequently, it is not difficult to miss changes in cell density using microscopic histological techniques.

In mature rat testes, Leydig cells make up a significant portion of the total weight, about 20 – 25% (Creasy, 1999), and any change in Leydig cell number should cause a change in that percentage. However, in the Akingbemi et al. papers, the authors note a lack of change in either testes weight or size. If there was Leydig cell hyperplasia (as proposed by these authors), but no corresponding increase in testicular mass, then it stands to reason that the Leydig cells present are of different size, shape, and density than those found in control testes. The authors, therefore, have made a fundamental error in these experiments; they assumed that the Leydig cells from the treated animals would have the same density as those from the control animals. Because they assumed that the control and treated animals would have the same Leydig cell density, the authors only collected the Leydig cells that migrated within the band of the Percoll gradient that matched the control Leydig cells' migration. They did not consider that the Leydig cells from the treated animals may have a different density and therefore would migrate into a different band within the Percoll gradient.

As a consequence, all of the reported measures from the isolated fraction of Leydig cells simply represent the remaining Leydig cells within the treated testes that have the same density as those of control Leydig cell populations at that developmental stage. The authors did not collect other density bands within the Percoll gradient and examine whether the Leydig cells within those bands could also synthesize testosterone or have other characteristics of mature Leydig cells. As described above, cells that are hyperplastic or that have undergone toxic insult can have a different density. These cells were never detected or collected in these experiments.

Therefore, all the endpoints in Akingbemi et al. that make use of isolated Leydig cell preparations, (the ability of the Leydig cells to produce testosterone at either a basal level or in response to LH stimulation, the indicators of cell cycle stage used to investigate hyperplasia, the counting of the cells, aromatase levels, etc.) are flawed due to the assumption made by the investigators that the Leydig cells from the DEHP treated animals would migrate/elute in the same density band as those from the control animals.

In addition to the above flaws, at least one member of the Expert Panel questioned the reliability of the data in the Akingbemi et al. studies at the Public Meeting, stating that the data were “messy and perplexing” and that “I would never ask this group to do toxicological work again.” However, rather than finding these data unsuitable for its review, the Expert Panel instead cherry-picked results from the studies which suggested DEHP toxicity. This is yet another example of procedural inequities in the review process described above, and is unacceptable in a review process that is supposed to be objective and deliberative.

Because of the serious flaws in the Akingbemi et al. studies described above, the PE Panel does not agree with the Expert Panel that these studies “are sufficient to conclude that DEHP is a reproductive toxicant in male rats at the indicated dose levels.”<sup>42</sup>

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<sup>42</sup> *Id.*

## CONCLUSION

For the reasons stated herein, the PE Panel has significant concerns regarding both the process by which the Final Update was produced and some of the scientific conclusions present in the Final Update. The PE Panel believes that because of significant procedural flaws, the Final Update does not adequately represent an objective and fully-deliberated consensus opinion. In addition, the PE Panel believes that the Expert Panel incorrectly disregarded scientific data which show that very high concentrations of DEHP that would cause profound adverse effects in rodent testes had no adverse effects on the testes of marmosets. The marmoset is a useful model for human male reproductive toxicity and the Mitsubishi study, while not perfect, provides valuable information about the toxicity of DEHP to primates, with importance implications for the degree of concern for human health. In addition, several aspects of the Expert Panel's review of reproductive and developmental toxicity do not, in fact, support the low NOAELs chosen, and there is less uncertainty about infant exposures than indicated by the Final Report. The PE Panel requests that the NTP-CERHR keep these points in mind as it considers the conclusions of the Expert Panel in the Final Update.

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## **ATTACHMENTS**

1. Curriculum Vitae and Opinion of Dr. Stefan Schlatt entitled: Evaluation of the marmoset (*Callithrix jacchus*) as a model for reproductive toxicity.
2. Comments of the Phthalate Esters Panel of the American Chemistry Council on Notice of Intent to List Chemicals, submitted to the California Office of Environmental Health Hazard Assessment, May 24, 2005.
3. Curriculum Vitae and Opinion of Dr. Suzette Tardif entitled: Findings regarding female reproductive physiology from the Mitsubishi Study #B000496, "Sixty-five week repeated oral dose toxicity study of DEHP in juvenile common marmosets."

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