External Peer Review

Summary for the DiButyl Phthalate Human Health Assessment

FINAL REPORT

Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Prepared by
ORISE IRIS Technical Assistance Team
Oak Ridge Institute for Science and Education
Oak Ridge Associated Universities

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EXTERNAL PEER REVIEWERS

Elizabeth Anderson, Ph.D., Chair
Exponent, Inc.
Alexandria, VA

Paul Foster, Ph.D.
National Institute of Environmental Health Sciences
Research Triangle Park, NC

Edwin Garner, Ph.D.
RTI International
Research Triangle Park, NC

Bill Lasley, Ph.D.
University of California
Davis, CA

Suresh Sikka, Ph.D.
Tulane University Health Sciences Center
New Orleans, LA

PEER REVIEW PROJECT

Jamie Strong, Project Manager
U. S. Environmental Protection Agency

Leslie Shapard, Peer Review Manager
Oak Ridge Institute for Science and Education

IRIS Toxicological Review for Di-n-Butyl Phthalate

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Charge to External Reviewers for the IRIS Toxicological Review for Di-n-Butyl Phthalate

The U.S. EPA is conducting a peer review of the scientific basis supporting the human health risk assessment of dibutyl phthalate that will appear on the Agency's online database, the Integrated Risk Information System (IRIS).

The draft documents for the external peer review contain a description of the oral reference dose, inhalation database, and a qualitative cancer assessment. Please provide detailed responses to the charge questions below.

General

Question 1 - ***Are there additional key published studies or publicly available scientific reports that are missing from the draft document that might be useful for the discussion of the hazards of dibutyl phthalate?***

Elizabeth L. Anderson, Ph.D.
I know of no key published studies or publicly available scientific reports that are missing from the draft document.

Paul Foster, Ph.D.
I am not aware of any other major, key articles or reports that would impinge on the current discussions on dibutyl phthalate.

C. Edwin Garner, II, Ph.D.
The document is thorough and comprehensive.

Bill L. Lasley, Ph.D.
Yes, the report of Gray et al (2006) indicates the in-utero effect on gonadal steroidogenesis may be similar in males and female (rats) and the report of Reddy et al. (2006) indicates an association between phthalate exposure and endometriosis in women. This report, at best, only indirectly relates to dibutyl phthalate, as is; exposures were not limited to this compound. Lee et al. (2006) suggest a direct effect of pthtalate on hypothalamic gene expression that could have long-lasting effects on sexual behavior while, Mahood et al. (2006) provide additional insight into the cellular basis of testicular toxicity of dibutyl phthalate in rats. These studies tend to confirm reports already included in the report and serve to take the mechanistic possibility more specific at the cellular and molecular levels. Finally, the NRT study (1995) shows a LOAEL at 80 mg/kg-day, but did not test lower. It seems that either this study should be repeated at a lower does to exclude the possibility that fetal loss could be a result of doses lower than 80 mg/kg-day or an augmentation to the text be added to indicate why the NRT study should not influence the consideration of the LOAEL.

Gray LE, Laskey J, Ostby J. Chronic di-n-butyl phthalate exposure of rats reduced fertility and alters ovarian function during pregnancy in female Long-Evans hooded rats. Toxicol. Sci. In Press, 2006.

Reddy BS, Rozati R, Reddy BV, Rama NV, Association of phthalate esters with endometriosis in Indian women. BJOG 113: 515-520, 2006

Lee HC, Yamanouchi K, Nishihara M. J reprodct Dev. In press 2006 (web preprint)

Mahood IK, McKinnell C, Walker M, Hallmark N, Scott H, Fisher JS, Rivas A, Hartung S, Ivell R, Mason JI, Sharpe RM. Cellular origins of testicular dysgenesis in rats exposed in utero to d-n-butyl phthalate. Int J Androl 29(1):148-154, 2006.

Suresh Sikka, Ph.D.
Yes, the following additional key publications should be addressed in the draft document.

Brock, J. W.; Caudill, S. P.; Silva, M. J.; Needham, L. L.; Hillborn, E.D.,Phthalate monoesters levels in the urine of young children. Bull Environ Contam Toxicol 2002, 68 (3), 309-14.

Hauser, R.; Duty, S.; Godfrey-Bailey, L.; Calafat, A., Medications as a source of human exposure to phthalates: a case report. Environ Health Perspect 2004, doi:10.1289/ehp.6804.

Harris, C. A., Henttu, P.; Parker, M. G.; Sumpter, J. P., The estrogenic activity of phthalate esters in vitro. Environ Health Perspect 1997, 105 (8), 802-11.

Arcadi, F. A.; Costa, C.; Impatore, C.; Marchese, A.; Rapisarda, A.; Salemi, M.; Trimarchi, G. R.; Costa, G., Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the Long-Evans rat. Food Chem Toxicol 1998, 36, (11), 963-70.

Moore, R. W.; Rudy, T. A.; Lin, T. M.; Ko, K.; Peterson, R. E., Abnormalities of sexual development in male rats with in utero and lactational exposure to the anti-androgenic plasticizer Di(2-ethylhexyl) phthalate. Environ Health Perspect 2001, 109, (3), 229-37.

Nagao, T.; Ohta, R.; Marumo, H.; Shindo, T.; Yoshimura, S.; Ono, H., Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study. Reprod Toxicol 2000, 14, (6), 513-32.

Sjoberg, P.; Lindqvist, N. G.; Ploen, L., Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. Environ Health Perspect 1986, 65, 237-42.

Akingbemi, B. T.; Ge, R.; Klinefelter, G. R.; Zirkin, B. R.; Hardy, M. P., Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc Natl Acad Sci U S A 2004, 101, (3), 775-80.

In addition, the following internet site published by a non-profit organization "Environmental Working Group" that is available to the public should be of great interest and concern to the EPA as can affect the EPA policy to release certain information through this document.
http://www.health-report.co.uk/phathalates.html

Question 2 – Does the hazard characterization discussion for dibutyl phthalate provide a scientifically-balanced, objective, and complete description that synthesizes the human and laboratory animal evidence for a human developmental hazard?

Elizabeth L. Anderson, Ph.D.
I believe that the hazard characterizations discussion does provide a balanced description that synthesizes the human and animal evidence concerning a human developmental hazard. Of importance is the discussion that points to the public health protective nature of the evaluation and the limited but consistent evidence in humans.

Paul Foster, Ph.D.
In general this is a well-written document that does provide a balanced description and uses appropriately the animal and human data in describing the proposed mode of action. It provides necessary information on linking the adverse findings after exposure developmentally, with key biochemical and morphological events *in utero* that may not be manifest as malformations until some time after birth, or even at adulthood.

Minor improvements might be afforded by providing some additional human information (from reviews etc or case reports of known genetic issues with e.g. AR or steroidogenic enzymes) on the critical role of androgens in the normal development of the human male reproductive tract. This seems to be a relatively easy task to "complete the circle" on why a decrease in testosterone in the fetus at a critical stage of development (during sexual differentiation) could have a major impact on normal development of the male reproductive tract. The relationship between the decreased fetal testosterone levels in the rat and issues with development of the tract that are known to be androgen-dependent are well presented. So we could (and do) have parallel biology between humans and the experimental species, but we do not know the relative sensitivity of the development of the human system, but there are examples of problems with androgen signaling in humans that clearly produce similar patterns of reproductive tract malformations to those seen with DBP (e.g. cryptorchidism, hypospadias etc).

C. Edwin Garner, II, Ph.D.
Yes.

Bill L. Lasley, Ph.D.
No. Due to the species specificity of reproductive function, the limitation of exposure times for most laboratory animal models and differences in sensitivity to teratogens the report leaves open the possibility that human sensitivity to dibutyl phthalate may not yet be appropriately-defined. In particular, the well-established adverse effect on steroidogenesis, indicates that dibutyl phthalate could target androgen production from the human adrenal through the same mechanism(s) are identified in the rat testes…. i.e., the steroidogenic machinery. Examples of anomalies in human neonates could also be presented to indicate what would be expected to be seen as a result of parallel developmental alterations. Additional studies focusing on potential adverse effects on adrenal steroid production still need to be conducted. As indicated in response to question #1, the 1995 NRT study does not adequately define a NOAEL (see response to question #1) on fetal loss which could be through a suppression of fetal-placental androgen production. However, it also seems that the NRT study may not have been presented as clearly as it could have been in this report. This reviewer was left with the impression that this study

deserves more explanation and,but less emphases in determining the LOAEL. On close examination and discussion with other reviewers it seems that this study may not provide significant or instructive information. Again, a more complete discussion of this paper seems warranted.

Suresh Sikka, Ph.D.
Yes, it does provide balanced discussion that is scientifically objective and provides sufficient experimental evidence for a human development hazard. Additional description, especially based upon above mentioned key references should be included.

Consideration of Human Epidemiological Studies

Question 3 – ***Have the epidemiological data (Murature et al., 1987; Duty et al., 2003a, 2003b; Swan et al., 2005; Main et al., 2006) been objectively characterized and used transparently in the assessment?***

Elizabeth L. Anderson, Ph.D.
The listed epidemiological studies have been objectively characterized and relied upon. Potential differences in sensitivity between the responses observed in laboratory animal studies and in humans remains an important issue. The document should clearly recognize this fact and recommend that, as more studies emerge that they be included in an updated evaluation.

Paul Foster, Ph.D.
I think this is a balanced presentation. The epidemiology studies do have weaknesses, as the review authors point out, and would be considered preliminary evidence, but in the case of DBP, they do show significant correlations with end points that have been shown to respond and be sensitive in rodents exposed to this agent. The human data are thus consistent with the animal findings and one might expect the most sensitive end points (changes in AGD and T) found in rodents to be the ones most likely to show changes in exposed humans. Another weakness of the human publications, not illustrated in the review, are the data for diethyl phthalate (which is not part of this review) which also show a positive statistical relationship with AGD and T, but for which there are no indications of adverse effects on reproductive development in rodents. Similarly for DEHP, which has shown effects in rodents, there are no correlations in the human data

C. Edwin Garner, II, Ph.D.
Treatment of this data is fairly balanced and comprehensive. Limitations of data are made clear, thus it is generally agreed that the epidemiological data has been objectively characterized and used transparently in the assessment.

Bill L. Lasley, Ph.D.
Yes, as far as this investigator can determine. All epidemiologic studies suffer from a lack of control of the exposure. It is likely that the exposure to most humans is actually mixtures which makes drawing conclusion regarding a single agent impossible. In addition, the sensitive period of human fetuses to dibutyl phthalate is not known and therefore the exposure during the sensitive period could not be known. In the final analysis critical exposures cannot be established.

Suresh Sikka, Ph.D.
Yes, the referred epidemiological data does provide objective characterization of the assessment of toxicity of DBP. However, the clinical studies related to long term low dose exposure are still not sufficient. Other additional issues mainly the critical role of cosmetic and pharmaceutical industries and exposure through their popular products including entero-coating of pills (e.g., Hauser et al, 2004) need to be referenced and may impact EPA's decision. Additional reference to the above mentioned internet site published by "Environmental Working Group" is needed for further information even though not enough scientific data is available on the effect of DBP in reducing testosterone production by such exposure.

Mode of Action for Effects on Male Reproductive Tract

Question 4 – ***Does the Toxicological Review provide sufficient information to support a conclusion that there is a relationship between lower testosterone levels in the fetal rat testis and structural anomalies and functional deficits in the male reproductive system? Do you agree that this mode of action is applicable regardless of the duration of exposure?***

Elizabeth L. Anderson, Ph.D.
While sufficient information is provided to support a public health protective conclusion that there is a relationship between lower testosterone levels in the fetal rat testis and deficits in the male reproductive system, a clear causal link to humans remains uncertain.

Paul Foster, Ph.D.
Yes. I think the case is well made to illustrate this relationship for the androgen-dependent reproductive tract malformations and androgen-mediated developmental landmarks, which can be permanently altered by DBP. The cryptorchidism related to decreased expression of insl3 is also discussed, and clearly normal formation of the gubernaculum and testicular descent is dependent on insl3 and for scrotal descent, on androgen. It would appear that a much higher dose level of DBP is required to inhibit insl3 gene expression, than to significantly reduce testosterone production/levels. The effects of DBP on fetal gonocytes are not necessarily due to a decreased testosterone level – it could be related to the published changes in c-kit, for example, but this is not detrimental to the proposal, changes in gonocytes tend to occur later than the morphological changes in Leydig cells – the origin of fetal testosterone.

Clearly the exposure of DBP has to occur during a window of susceptibility to produce the series of irreversible changes noted. This could occur with one dose, if at the critical time, has been shown clearly with two daily doses (in defining the critical exposure windows for rats), for 10 daily doses and is noted with exposure throughout pregnancy. Since the development of the male reproductive tract can only occur within a specific window, it does not matter if exposure occurs long after this time; it is not likely to make a significant contribution to the induction of the effect(s). It is exposure during the window that is critical. Thus, even for a human, an exposure of a male after birth to an agent interfering with androgen action will not result in a hypospadias; the penis has already been formed!

Support for the MOA could be garnered from studies with other active phthalate esters (eg DEHP) which produce similar malformations and decreases in fetal testicular testosterone production. Moreover, data on fetal testicular gene expression (Liu et al. 2005) indicate that those esters with activity have similar gene expresson profiles whereas those esters that do not

induce reproductive and developmental toxicity do not have equivalent gene expression changes. Inclusion of this evidence strengthens the proposed mode of action, but also indicates that a similar mode of action may apply for these structurally related compounds. Since human exposure has indicated that women of child bearing age are exposed to multiple phthalate esters with activity in rodents this does raise the issue of whether a cumulative RfD is required.

C. Edwin Garner, II, Ph.D.
The literature supports a relationship between lower testosterone levels in the fetal rat testis and structural/functional effects in the male reproductive system. The document comprehensively reviews this data. It is agreed that this mode of action is likely independent of duration of exposure.

Bill L. Lasley, Ph.D.
Yes. As long as the conclusions are limited to the rat, this is appropriate from the data available. It would be potentially rewarding to attempt to replace testosterone and determine if exogenous testosterone support can prevent all testicular adverse effects of dibutyl phthalate. This reviewer recognizes the difficulty in replacing testosterone to fetuses and pharmacologic doses would be required. However, this would be the most direct approach and provide the most compelling information.

4b) *Is this applicable regardless of the duration of exposure?* No. It seems that the timing of exposure is critical for developmental adverse effects in rate and it is likely that a similar kind window of sensitivity would be found for all other mammals including humans. Exposures in human pregnancies before or after this window would not be expected to induce these adverse effects.

Suresh Sikka, Ph.D.
The review does provide sufficient information to support such conclusion related to lower testosterone level in the fetal rat testis that affect male reproductive system. This is considered to be the major mode of action. However, DBP also act through other non-testosterone dependent paracrine and/or autocrine mechanisms (e.g., insulin-like peptide3 mRNA down regulation) that are responsible for sexual differentiation. Functional end-point of male reproductive system is fertilizing capacity of mature spermatozoa. It is not clear whether DBP has any direct effect on this sperm fertilization capacity that may be prevalent in off-springs that are exposed to DBP in-utero.

Question 5 – *Has EPA provided a cogent and objective analysis of existing information to justify the conclusion that the mode of action based on the decrease in testosterone concentration in the fetal rat testis is relevant for humans? If not, what information should be added?*

Elizabeth L. Anderson, Ph.D.
With statements of uncertainty, the EPA has presented sufficient information to justify a public health protective conclusion that the decrease in testosterone concentration in the fetal rat testis is relevant for humans. Limited but consistent signals in humans have been noted. The document notes abstracts of emerging data that could be important to further clarification in the near future. The document should recommend that these studies be closely followed and reviewed for relevance when they become available.

Paul Foster, Ph.D.
Yes, although see comments above about providing some extra information on human syndromes that result from impaired androgen signaling, to provide some context for the proposed MOA in animals. However there are other potential modes of action involved in the developmental toxicity to the fetal reproductive system including effects on *insl3* and changes in gonocytes (see below)

C. Edwin Garner, II, Ph.D.
No comment.

Bill L. Lasley, Ph.D.
Yes, but other targets, i.e., the adrenal and other possible modes of action should be addressed. It seems possible that dibutyl phthalate could interfere with fetal adrenal steroidogenesis. Since fetal adrenal androgens are essential for the placental production of estrogens, it is possible that dibutyl phthalates could reduce estrogen production in human pregnancies (see response to #2). The nature of fetal adrenal steroid production in the human fetus is such that either direct targeting of the steroidogenic machinery of the adrenal or even targeting of hypothalamic/pituitary targets could adversely affect human pregnancies. The potential of a central (hypothalamic-pituitary) effect should also be mentioned.

Suresh Sikka, Ph.D.
Although the above mentioned modes of action based upon animal studies are convincing and physiologically relevant, the validity to human situation still needs further discussion. Animal studies are normally done under controlled environment with single or multiple doses of exposure and fixed duration. That usually does not apply to human situation. In the absence of sufficient information on specific exposure, the only indicators are certain end-points, e.g., evaluation of DBP metabolites in urine or other fluids. The linear assessment of such metabolites and their correlation to the extent of exposure is not easy and further tools and information is needed for such validation. Such metabolites may appear in milk in lactating mothers and thus can affect the young ones (Moore et al, 2001).

Oral Reference Dose (RfD) for Dibutyl phthalate

A. Selection of Principal Study and Endpoint

Question 6 – ***Has the most appropriate principal study, critical effect, and method of analysis been chosen? The issues to be considered include:***

- ***a. Is it appropriate to consider the results from Lehmann et al. (2004) as the principal study for all durations of exposure (acute, short term, subchronic, and chronic)?***
- ***b. Is the number of animals examined in the study sufficient to support the scientific conclusion that the decrease in testosterone concentration is the critical effect?***
- ***c. Is the statistically significant decrease in testosterone concentration of 61% at 50 mg/kg-day, called a LOEL by Lehmann et al. (2004), an adverse effect and a LOAEL as described by EPA?***

d. Do the available data and discussion support the use of a biochemical change (decrease in concentration of testosterone in the fetus) as the point of departure of 30 mg/kg-day as a NOAEL for deriving the reference values for all durations of exposure? Do you agree that this biochemical change is a no observed adverse effect level?

e. Is it appropriate to use the NOAEL/LOAEL approach rather the Benchmark Dose approach on the decrease in testosterone concentration (Lehmann et al., 2004) to derive the RfD?

f. Is it scientifically appropriate to assume that preventing the decrease in testosterone concentration in the fetus will prevent all developmental effects and other effects in children and adults?

g. Has the decision not to use the exposure-response results from Salazar et al. (2004) been sufficiently justified?

h. Lee et al. (2004) reported several biological changes at an exposure below that which is associated with the decrease in fetal testosterone (Lehmann et al., 2004). These include the changes in relative pituitary weight in males at postnatal week 11 and in females at postnatal week 20; the decrease in percentage of FSH producing cells in the anterior pituitary in females at postnatal week 20; the changes in the mammary gland of females at postnatal day 21; and the changes in the mammary gland of males at postnatal week 11 and 20. Do the available data and discussion adequately support EPAs conclusion that these effects should not be used to derive the RfD?

i. Is there sufficient information to support the conclusion that monobutyl phthalate is the toxic metabolite?

Elizabeth L. Anderson, Ph.D.

For public health protective purposes, the analyses has chosen the most protective interpretation of the existing studies. The choice of study and end point of concern is consistent with the data and the objectives of the assessment. The rational for using the results of the Lehmann study for durations of exposure is a matter of public policy, because the uncertainties in the science can not provide a factual basis for this decision. Conservatively the interpretation of 61% decrease in testosterone concentration at 50 mg/kg-day as an adverse effect is supportable as a LOAEL.

For the same reasons noted above, the biochemical change noted as the basis for a NOAEL is supportable. Further, given the limitations of the Lehmann study, the justification for not using the Benchmark Dose approach is understandable.

Since the choice of a NOAEL for a decrease in testosterone concentration in the fetus is the most sensitive endpoint in the laboratory animal studies, and there is no evidence that humans are more sensitive, it is reasonable to assume that this level will also be protective against other effects. No other studies exist on which to base an alternative decision. However this choice is a matter of public policy and can not be definitively supported with scientific certainty; consequently the resulting guidance may be either too conservative or not not protective enough, although no existing data would suggest the latter.

The decision not to use the exposure-response from Salazar et al. (2004) has been justified. EPA's conclusion not to use the Lee et al. (2004) study to derive the RfD is supportable. There appears to be sufficient information to support the conclusion that monobutyl phthalate is the toxic metabolite.

Paul Foster, Ph.D.

a. For the adverse developmental changes, this would seem to be the underpinning study providing the key biochemical information over an extended dose range. As noted above, this could have occurred after one, two or many daily doses as long as the exposure was during the critical developmental window for sexual differentiation.

b. On reflection, just about sufficient animals were used for these purposes. Also used, in my consideration, was the number of other studies from different laboratories, showing similar deficits in fetal testicular testosterone levels (at least at higher dose levels) indicating that this is a robust and reproducible early experimental finding. It would be difficult to argue that a statistically significant decrease in testosterone at 50 mg/kg/d was not "real" and by chance, given the dose response data presented and the limited number of animals used.

c. Testosterone levels would not seem to offer a "bright line" in the assessment of adversity, unlike the incidence of permanent changes in reproductive tract end points and developmental landmarks, for example. However, based on the EPA's arguments and precedents of using a biochemical change as an index of adversity, I think this value can be justified as being adverse and a LOAEL. However does this mean that a 60% decrease in another study is not adverse? Or, why is the 26% change at 30 mg/kg/d also not adverse? A higher powered study with a smaller variance may have shown this magnitude of change to be statistically significant. As the data currently stand, the 61% change being statistically significantly reduced from control and having a reasonable, biologically meaningful, magnitude of response, it is appropriate. If this is the first time that the use of a biochemical measure for setting an LOAEL and NOAEL has been used in an IRIS review, it would be worth spending some more time discussing the reasoning for selection, and consistency across numerous studies and with different phthalates.

d. Yes, but see discussion above.

e. The arguments proposed by the Agency on the quality and quantity of data available would better justify the NOAEL approach than the BMD for derivation of the RfD.

f. No. The T production should be the lowest dose level with a statistically significant biochemical effect, consistent with a MOA, for this to be true. The Lehmann paper also had statistically significant decreases in gene expression below the 30 mg/kg/d level (in c-kit production at 0.1 and 1 mg/kg/d), so I am not convinced that this can be fully justified. In the context of the reduction in T as a MOA, then this is correct. It is potentially possible that there are other MOA's involved in the induction of the gallery of developmentally-induced reproductive effects induced by DBP. I would agree that the developmental effects are the ones that occur at the lowest dose levels and therefore using this end point would also be protective for adults. The pup loss in the NTP study at also does not seem to be related to the decrease in fetal T.

g. Yes. There appear to be numerous problems with this study and with such a consistent database available, this does seem to be an outlier. The lack of response from the authors also supports the EPA action.

h. The EPA explanation is reasonable based on the lack of internal concordance of findings at different ages of animals. The male breast findings are interesting, but the magnitude of change is small and it does not seem to coincide with the known antiandrogenic effects of DBP on the nipple anlagen in utero. The report suggests an increase in alveolar atrophy compared to the

control males (who would not have nipples). These control males would have the nipple anlagen undergoing apoptosis under androgen
action in utero, that could be blocked by DBP, yet the degree of alveolar degeneration was greater in the DBP treated animals, although the effects was very small and probably not of toxicological significance. This seems to be a finding not consistent with previous studies.

i. Yes. It would be definitive to have treated pregnant rats in utero with MBP and followed the offspring until adulthood, but this would be "gilding the lily" based on the other TK and toxicological information already available.

C. Edwin Garner, II, Ph.D.

a.-b. Yes. Duration of exposure is likely not a significant issue given the proposed mechanism(though single dose exposure is not a realistic model of likely typical human exposure). Lehmann (2004) study did address repeat exposure during critical window of vulnerability and was sufficiently long to cover accumulation of metaboites. Animal number is sufficient in the study of Lehman et al. (2004)

c.-d. Yes. Since this mechanism has sufficient support in the literature, then it can serve as a basis for NOAEL assessment.

e. Yes. I have issue with making an assessment of the magnitude of change in testosterone concentration which should be the benchmark response.

f. There is indeed a threshold to this effect, thus if all testosterone effects were reduced to zero, it would be likely that risk would be significantly reduced.

g. Yes.

h. Yes, the available data and discussion adequately support EPA's conclusion that the effects observed by Lee et al (2004) should not be used to derive the RfD. The contributon of these effects to the plausible mechanism of effect is not as well understood, thus its basis for NOAEL assessment is questionable.

Bill L. Lasley, Ph.D.

a. Yes. The Lehman study provides the most comprehensive data in terms of dose range during the sensitive period.

b. Probably there are. This study alone, however, is not completely comprehensive. If 20-30 animals per group had been used it would have been more compelling. However the scientific conclusions in this report are well supported when the data from all other studies are considered as well.

c. Yes. While the use of a biochemical end point may be unusual, in this case it seems appropriate as down-stream effect of testosterone (such and masculinization of the neural substrate) may offer no physical change.

d. Yes. This seems to be the best defined lower limit if the testis target is considered to be the primary target of toxicity.

e. Yes, based on the available data.

f. No. While this may be true for the rat model, the mechanism for developmental toxicity cannot be extrapolated to other species particularly the human. As explained in response to question #5, perturbing fetal adrenal androgen during the human pregnancy could occur at lower levels and have more significant developmental affects. In addition perturbation of adrenal androgen production in pre-adolescents could delay or prevent adrenarche and/or some aspects of puberty.

g. Yes, there are clear limitations of the Salazar et al. study that EPA has clearly indicated.

h. No. The pituitary is responsible for the maintenance of pregnancy on the rat while the feto-placental unit is responsible for pregnancy in higher primates.

There remains an open question as to whether the effect on the rat fetal testes is appropriate for determining what may be the effect on the higher primate feto-placental unit. There also seems to be a lack of understanding that nipple and breast development is a process shared by both estrogen and androgen. One need not invoke and "estrogenic action" to explain the persistence of breast or nipple tissues in males.

i. Yes. The evidence is compelling and could only be more so if a study were done using only MBP.

Suresh Sikka, Ph.D.

a. Results from Lehmann et al (2004) as the principal study for all durations of exposure, seem appropriate as related to animal modeling.

b. The scientific conclusions suggesting lower testosterone (T) as the critical effect should have been based upon larger group of animals.

c. Thus, the NOAEL and LOAEL as suggested by EPA based upon this study alone need more validation in larger group of animals.

d. None of the studies take into consideration the presence of feto-placental barrier in the mother and blood-brain and blood-testicular barrier in the fetus and young ones suckling on mother's milk that may alter the extent of damage to cellular components in the testis to affect testosterone production. Thus, establishing 30 mg/kg-day as NOAEL reference value need further evaluations. Duration of exposure also needs to be considered in recommending such RfD's.

e. NOAEL/LOAEL approach of EPA to derive RfD is appropriate.

f. Scientifically, preventing a decrease in T in fetus does not imply that the fetal will be normal with completely functional reproductive system.

g. There is ample justification at this time not to consider exposure-response results from the study of Salazar et al (2004) which is comparatively different from the study of Lehmann et al. and also uses a different rat species than other studies.

h. The available data and discussion from study by Lee et al (2004) has many methodological and statistical issues. There is a lack of information on alternate effect on HPA-axis (alternate source of adrenal steroid production) as well as the role of chronological exposure. Thus EPA's decision not to use the information to derive at RfD is appropriate.

i. Yes, monobutyl phthalate is the major toxic metabolite, but there are many other metabolites, and some are detectable in urine samples (at least 10 esters have been described in literature) that are critical and need to be considered as well.

B. Application of Uncertainty Factors

Question 7 – ***Has the rationale for the selection of uncertainty factors been objectively and transparently described in the draft document? Does the science support the selection of uncertainty factors?***

Elizabeth L. Anderson, Ph.D.
The discussion of the choice of uncertainty factors is clearly presented. Selection of uncertainty factors has always been a matter of public policy rather than one expected to be scientifically supported. The rational presented for the choices is consistent with the policy default choices.

Paul Foster, Ph.D.
On balance, I think the Agency has made reasonable proposals that are scientifically justified. It does seem odd however, that even with the relative wealth of information on PK and PD for this agent, including human studies, that we cannot make any adjustments to the default values for interspecies response.

C. Edwin Garner, II, Ph.D.
Yes.

Bill L. Lasley, Ph.D.
Yes, it is clear which and why specific uncertainty factors were selected. Yes, but those selected seem exceptionally conservative since there are some existing data relating to estimating the potential of placental transfer.

Suresh Sikka, Ph.D.
Yes, the selection and expression of uncertainty factors (UF) is a great idea and has significant scientific importance. The value quoted in the draft document needs validation and further discussion.

Question 8 – ***EPA concluded that there are insufficient data to support reducing the pharmacokinetic portion of the interspecies uncertainty factor. Are there additional data that could be used to justify changing the pharmacokinetic portion of the interspecies uncertainty factor?***

Elizabeth L. Anderson, Ph.D.
There appear to be emerging data to support the use of pharmacokinetic data that could be used to provide a better' scientific basis for interspecies extrapolation. When available, these model results should be used in the place of default uncertainty factors.

Paul Foster, Ph.D.
I was wondering why more emphasis had not been made of the levels of metabolites in amniotic fluid seen in rats and humans (Calafat *et al.* 2006; Silva *et al.* 2004)? Since amniotic fluid is the closest that we are likely to get to fetal levels in human studies (and the fetus is the critical target and amniotic fluid is essentially fetal urine) and we have some human data, why have we made no direct comparisons to the rat information produced by Calafat et al? In the Calafat study, we have dose levels selected for their ability to induce adverse reproductive tract changes in rats, the timing of the measurements in amniotic fluid is exactly what we want, based on the critical window experiments, and we have this very interesting difference between what the fetus sees (primarily free MBP) and the mother (urine containing predominantly MBP-glucuronide). Surely some direct comparison is possible? I also believe the fetus swallowing the amniotic fluid it produces containing MBP, would explain the slower clearance from the fetal, compared to the maternal, compartment that is not used in current PBPK models. My understanding is that the placenta contains β-glucuronidase that would hydrolyze MBP-glucuronide from the mother, but at this stage of development the fetus is not capable of the correct UDP-glucuronidation to restore the MBP-glucuronide. Since MBP is the toxic metabolite this seems to be an important consideration for fetal dosimetry and why this lifestage may be especially at risk.

C. Edwin Garner, II, Ph.D.
Agreed that there are insufficient data to support reducing the pharmacokinetic portion of the interspecies uncertainty factor.

Bill L. Lasley, Ph.D.
Possibly there are. A careful investigation of amniotic fluid levels may provide additional information and support for lowering uncertainty factors.

Suresh Sikka, Ph.D.
Yes the data for expression of interspecies UF is insufficient at this time. More controlled studies are needed to validate this. Also, it is very difficult to do such studies in human.

Question 9 – *EPA concluded that there are insufficient data to support reducing the pharmacodynamic portion of the interspecies uncertainty factor. Is the role of testosterone in the development of the male reproductive tract sufficiently understood in all species to justify reducing the pharmacodynamic portion of the interspecies uncertainty factor?*

Elizabeth L. Anderson, Ph.D.
The role of testosterone in the development of the male reproductive tract across species is a matter of considerable scientific uncertainty.

Paul Foster, Ph.D.
I think the role of androgen in the normal development of the human male reproductive tract is fairly well understood, so it should be possible to reduce uncertainty here. The relative sensitivity of the human tract to the removal of androgens, of course, is not known. Most reported human

syndromes have produced drastic reduction in T levels or AR expression, that are then associated with impaired reproductive tract development.

C. Edwin Garner, II, Ph.D.
Agreed that there are insufficient data to support reducing the pharmacodynamic portion of the interspecies uncertainty factor.

Bill L. Lasley, Ph.D.
No. The identification of steroidogenic targets of toxicity indicate that steroid products other than testosterone could be adversely affected. For example, DHEA/DHEAS, which would also be adversely affected, could be inhibited in the human fetus and reduce the substrate necessary for placental aromatase to produce estrogens.

Suresh Sikka, Ph.D.
Testosterone role in development of male reproductive tract in-utero is well established in all species. Human situation is rather more complicated and other complex mechanisms are involved as well. How it affects pharmacodynamics and interspecies UF on DBP exposure still needs to be established.

C. Alternative Derivation of the Acute RfD

Question 10 – ***An alternative to using Lehmann et al. (2004) with exposure on GDs 12 - 19 to 30 mg/kg-day to derive the acute RfD is to use Thompson et al. (2005, 2004) with a single exposure on GD 19 at 500 mg/kg-day. This approach would require a uncertainty factor of 10 for LOAEL to NOAEL extrapolation as this was the only exposure tested in the study. Is this approach preferable to using Lehmann et al. to derive the acute RfD?***

Elizabeth L. Anderson, Ph.D.
The decision not to rely on the Thompson et al.(2005) study is supported by the discussion. There is considerable scientific uncertainty in either the choice of the Lehmann study or the Thompson study.

Paul Foster, Ph.D.
No. This provides no better information and is only at one dose level. You could even use Mylchreest et al (2002) or Mahood et al. (2005), but these have the same issues as the Thompson papers.

C. Edwin Garner, II, Ph.D.
No. Dose and duration of exposure in Lehman et al. (2004) study moe sufficiently addresses conditions that are closer to human environmental exposures and also better covers the window of vulnerability of male reproductive tract development.

Bill L. Lasley, Ph.D.
No. It is likely that the Thompson study may have missed a large portion of the sensitive period and this explains the higher exposure levels.

Suresh Sikka, Ph.D.
No, the approach to derive acute RfD using Thompson et al studies, which focuses more on timing with a single high dose, is not a good idea. Their data is more based upon gene expressions rather than the end-point (T) measurements and show a significant decrease in a single day (from GD 17 to GD18). The study of Akingbemi et al (published in PNAS) should also be considered in this respect.

C. Alternative Derivation of the Chronic RfD

Question 10 – ***An alternative to using the development toxicity study of Lehmann et al. (2004) to derive the chronic RfD is to derive the chronic RfD from a subchronic study showing hepatic toxicity from perinatal, lactational, and adult exposure with a NOAEL of 138 mg/kg-day (NTP 1995). Would it be appropriate to use an additional uncertainty factor of 10 for extrapolation from subchronic to chronic exposure on the hepatic toxicity from this study?***

Elizabeth L. Anderson, Ph.D.
Generally the choice of an additional safety factor of 10 for subchronic to chronic exposure has been supported as a policy matter.

Paul Foster, Ph.D.
See 10a.

C. Edwin Garner, II, Ph.D.
No. *Is it appropriate to use hepatotoxicity related to potential peroxisome proliferation mechanism when there is data to suggest that such effects are rodent specific?*

Bill L. Lasley, Ph.D.
No, this does not seem appropriate.

Suresh Sikka, Ph.D.
Deriving at acute, chronic, sub-chronic RfDs by considering different studies (e.g., the NTP 1995 with NOAEL of 138 mg/kg-day) with different design, doses, and parameters is not a good idea and adds to more confusion in establishing certain guidelines.

Inhalation Reference Concentration (RfC) for Dibutyl phthalate

Question 11 – ***Has the rationale and justification for not deriving an RfC been transparently described? Is the rationale scientifically justified and appropriate?***

Elizabeth L. Anderson, Ph.D.
Yes. I agree with the rational presented for not deriving an RfC. Should this derivation be carried out, the result would be highly uncertain.

Paul Foster, Ph.D.
Yes, self-evident based on the lack of data.

C. Edwin Garner, II, Ph.D.
Yes. The lack of studies is clearly communicated and the insufficiencies of the only study, Walseth and Nilsen (1984) are evident from the text.

Bill L. Lasley, Ph.D.
Yes, there is simply not enough scientific information to do so.

Suresh Sikka, Ph.D.
Yes, the rationale for not deriving an RfC is justified and appropriate.

Carcinogenicity of Dibutyl phthalate

Question 12 – ***Has the appropriate cancer descriptor been chosen? Has the rationale and justification for not deriving a quantitative cancer assessment been transparently described? Do you agree with EPA's rationale, justification and conclusion?***

Elizabeth L. Anderson, Ph.D.
I agree with EPA's rational for reaching the conclusion that the data base is inadequate for evaluating the carcinogenicity of dibutyl phthalate. The discussion could be enhanced by including a brief discussion of the in vitro studies that might indicate whether there appears to be a concern for carcinogenicity.

Paul Foster, Ph.D.
While there is no formal cancer study to evaluate, there have been DBP developmental exposures yielding Leydig cell tumors (LCT) of the testis (Mylchreest et al. 1999). Since these can be seen at 3 months of age from an in utero exposure (significantly shorter latency than seen in routine cancer bioassays) should these at least be mentioned in the cancer section? This might imply the potential for transplacental carcinogenesis. As the review points out, the morphological characteristics for the diagnosis of Leydig cell tumors are broad (based on size of the proliferative lesion compared to 3 seminiferous tubules according to the STP). The dysgenetic areas seen in the testis after in utero exposure to DBP, while not typical of the LCT's seen in 2-year rat bioassays, do meet these STP size criteria (Barlow et al. 2004). Since other phthalates (e.g. DEHP) have been shown to cause LCT's in conventional bioassays, a two year study with DBP, but commencing exposure in utero would fill an important data gap and is currently being considered by the NTP.

C. Edwin Garner, II, Ph.D.
Yes. The lack of studies is clearly communicated.

Bill L. Lasley, Ph.D.
12a) Have the appropriate cancer descriptors been chosen? Yes.

12b) Have the rationale and justification for not deriving a quantitative cancer assessment been transparently described? Yes.

12c) Do you agree with EPA's rationale, justification and conclusions? Yes, with the exception that the fetal testis is the only potential target of consideration in the human. A lack of evidence that fetal adrenal steroids are affected does not permit this potential target to be ruled out.

Suresh Sikka, Ph.D.

Yes, this document does justify for not deriving a quantitative cancer assessment at this time. There is lack of enough scientific evidence regards to the role of DBP in carcinogenicity. However, there have been many recent public reports (mainly by many Environmental Working Groups) that warrant assessment of DBP in etiology of cancer especially considering a low dose chronic exposure through many consumer products.

In conclusions, EPA has done a great job and thorough investigation in reassessing and collecting this voluminous report on DBP toxicity. Overall, this draft review does present a scientifically balanced and objective appraisal of animal and some human data. Comparison of available animal data does support the reference to reports by Lehmann et al as the primary studies for determination of the NOAEL for RfD derivation and relevance to human situation. However, there are still many inconclusive debates regards to reliability and validity of testosterone measurements (total vs free) in human situation. Proper sampling is another issue considering a vast variation in blood testosterone levels in samples whether collected in the morning or evening. The chronic low dose long-term exposure makes it difficult to recommend appropriate RfD. Issue of carcinogenicity as a result of DBP toxicity although important makes it difficult to completely assess the situation at this time.