External Peer Review

Toxicological Review for Polybrominated Diphenyl Ethers (PBDEs) Human Health Assessment

FINAL REPORT

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Notice: EPA modified this report in August, 2007 to include only four of the five reviewers' comments. One reviewer's comments were excluded from the report and were not considered by EPA due to the perception of a potential conflict of interest.

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The ORISE IRIS Technical Assistance Team has neither altered nor edited these comments for grammatical or other errors.

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The U.S. EPA's National Center for Environmental Assessment (NCEA) has developed several draft Toxicological Reviews for Polybrominated diphenyl ethers. Specifically, toxicological review documents have been developed for tetra-, penta-, hexa-, and decabrominated diphenyl ethers. The charge questions below specifically address the polybrominated diphenyl ethers assessment.

GENERAL QUESTION

Are you aware of other published peer-reviewed toxicological studies not included in these Toxicological Reviews that could be of relevance to the health assessment of BDE-47, BDE-99, BDE-153 or BDE-209?

Ahmed Ahmed, Ph.D.

I have examined the available literature, for the past 10 years, for BDE-47, BDE-99, BDE-153, and BDE-209 by searching under their individual chemical name and chemical abstracts number (CASRN) and crossing the outcome with adverse effects, cancer and/or systemic or genetic toxicity,. I found about 200 citations and their abstracts which represent various types of studies on these compounds. The majority, if not all, of these references are included in various "Toxicological Review" document for one or the other of these chemicals. A detailed literature search analysis is included in this report. The authors of the document may need to verify the existence and use of the appropriate citations in the reviews using the reference manager software programs.

The Toxicological reviews for BDE-47, BDE-99, BDE-153, and BDE-209 are extremely detailed and commonly repetitive. The same information is repeated several times in the same document under different headings (subsection) and in documents for other congeners. Although these repetitions are distracting, I realize they are pluses rather than negatives, I believe that was done to emphasize particular point or effect, to establish the validity of a study and its results and to point out the drawbacks in experimental design or data analysis and interpretation. Therefore, I find the quality of these review documents in a "good to excellent" range. In some of these reviews however, the lack of scientific information makes the decision making processes difficult due to limited number of studies or narrow scientific objectives that disregarded other possible interpretations. This problem does not point to any shortcomings in the efforts of the authors of these documents. Rather it indicates the limitation and shortage of available information (studies).

In a general sense, these chemicals are congeners of each other and because each one is contaminated by one or more of the others. Also, they metabolically, under environmental and biological conditions, are inter-convertible to each other (compounds with higher bromine numbers are converted to compounds with lower bromine). Therefore, I believe that putting them all in one comprehensive review document would serve the purpose more adequately. They almost have the same toxic end points with varying degrees. The effects of these compounds, which came from limited number of studies, are repeatedly compared in each document for each individual compound.

Metabolic and distribution studies are extensively described in the document without deep scientific interpretation of the results nor how these results could be interpreted in the evaluation of human body burden. Furthermore, the purities of the radioactive chemicals used in most studies were not cited. The position of the label as well as the specific activities of the 14C compounds, used in these studies were not indicated in most sections. I am also troubled by the different ways used for the presentation of the doses and concentrations of the compounds and

their metabolites in biological tissues (umole, ug, percent of the dose). A unified parameter should be used to insure comparative analysis and easy follow up.

Richard Bull, Ph.D.

I am not aware of other peer-reviewed toxicological studies that would contribute to an understanding of risks associated with these PBDE's. However, some papers included in the review should be better integrated into the derivation of the reference doses.

Lucio Costa, Ph.D.

There are a number of publications that have not been included in the Reviews and that may be relevant to the health assessment of BDEs.
A few studies indicated that house dust is a major source of exposure to BDEs, and for toddlers in particular, dust has been estimated to account for 80% of BDE exposure.

- Jones-Ortazo HA et al., Environ. Sci. Technol. 39: 5121-5130, 2005
- Wilford BH et al., Environ. Sci. Technol. 39: 7027-7035, 2005
- Schecter A et al., J. Toxicol. Environ. Health Part A 68: 501-513, 2005

Contamination of food with BDEs has not been mentioned, yet diet is believed to contribute to the overall body burden of BDEs.

- Hites RA et al., Environ. Sci. Technol. 38: 4945-4949, 2004
- Schecter A et al., Environ. Health Perspect. 114: 1515-1520, 2006

High levels of BDEs were found in children (case study).

- Fischer D et al., Environ. Health Perspect. 114: 1581-1584, 2006

Levels of BDEs were recently determined in plasma of pregnant women in California.

- Bradman A et al., Environ. Health Perspect. 115: 71-74, 2007

Various studies have explored possible mechanisms of action of BDEs, including activation of protein kinase C and induction of oxidative stress.

- Kodavanti PRS, Derr-Yellin EC, Toxicol. Sci. 68: 451-457, 2002
- Kodavanti PRS et al., Toxicol. Sci. 88: 181-192, 2005
- Reistad T, Mariussen E, Toxicol. Sci. 87: 57-65, 2005
- Reistad T et al., Arch. Toxicol. 80 : 785-796, 2006

Ralph Kodell, Ph.D.

I am not aware of any other relevant studies.

1. QUESTIONS RELATED TO THE DERIVATION OF THE REFERENCE DOSE

FOR BDE-47, BDE-99, BDE-153 and BDE-209

1.1 Have the rationale and justification for deriving RfDs on the basis of the neurobehavioral toxicity studies been transparently and objectively described in the draft

Toxicological Reviews of BDE-47, BDE-99, BDE-153 and BDE-209? Are there additional studies that should be considered for deriving the RfDs for any of the four PBDE congeners?

Ahmed Ahmed, Ph.D.

In all the documents provided, except for BDE-209, no cancer related data was available. In each individual document for every compound there were several sporadic, non cancer related, referenced molecular and systemic toxicological studies. Most of the available and presented studies, unfortunately, toxicological and molecular (receptor binding etc.), were designed to look for the effect(s) of these modern environmental pollutants in light of the toxicological effects of traditional and well studied compounds such as PCB. The large numbers of the reported molecular studies on BDEs were tailored after the effect of poly chlorinated aromatic hydrocarbons. Although the chemicals under review (BDE) are halogenated (brominated) aromatic hydrocarbons, these tailored studies back fired. That is because we ended up with a number of negative or nonspecific effects and obtained limited information to use for the proper evaluation of the risk from BDEs.

The few available systemic toxicological studies are blemished by the fact that most of these studies came from the same laboratory. Although, the data seems to be academically solid, their validity and strength is debatable because it has not been verified in various other laboratories neither in the U.S. nor in other countries. This factor alone weakens but not hinders the derivation of the specific RFD. It makes the confidence in these RFD's low

The authors of the Toxicological Reviews for BDE-47, BDE-99, BDE-153, and BDE-209 have to base their derivation of RFD on continuous scientific information. They used those studies that have specific doses and defined time of exposure as well as a defined adverse effect(s). The only available studies that have dose response and time course were those described by the authors in the texts and were used for the derivation of RFDs. With this in mind, they have clearly and very transparently derived the rational for using these studies. They also took into consideration all drawbacks that weakness each particular study. I was impressed by the way they listed at least 6-10 points explaining their concerns for the use of particular data, in each selected study, in the derivation of RFD for each individual compound. Meanwhile they documented the rational for using the study in their derivation of RFD. There was very clear presentation of the pros and cons of each study utilized in the estimation of RFD.

Richard Bull, Ph.D.

The data available on most of these compounds is quite limited (BDE-209 being somewhat of an exception). In most cases, the risk assessment focuses solely on results from a non-conventional neurodevelopmental test. In one case, BDE-209, a conventional reproductive/developmental toxicity test found no effects at doses much higher than observed in the neurobehavioral study. This may reflect the insensitivity of conventional reproductive/developmental toxicity tests OR more likely, increasingly smaller fractions of the doses being absorbed as doses are increased. The doses utilized were huge, 100, 300 and 1000 mg/kg in corn oil. One would predict that the aqueous phase through which these compounds would have to diffuse to get to an absorptive surface to be exceeded at doses orders of magnitude lower than the lowest dose! Nevertheless, the discrepancies raise significant issues with the standard application of uncertainty factors to results that are clearly more sensitive than conventional methodologies. To put this starkly, either the UFs applied to the "standard" test data are way too small OR smaller UFs need to be applied to these data must be adjusted to account for their much greater sensitivity (and specificity).

There are particular UFs that appear completely inappropriate in the current derivation of reference doses. For example, defaulting to a database uncertainty factor when data of this relative sensitivity to conventional evaluations of reproductive/developmental results are available is very inappropriate.

A more central role also needs to be given to the neurochemical data that is provided, especially those that utilized essentially parallel study designs to the neurobehavioral studies. They are presented as supporting data. They are a lot more than that. They provide an organic measure of modified neural development. It may not be entirely clear how the neurobehavioral and neurochemical data are linked functionally, nevertheless, the two data types contribute as an independent measures that there is an apparently permanent alteration in development. This type of corroborating data should be taken as being much more critical than data from 'standard protocols'. Targeted data of this kind in developmental neurotoxicity would be useful if broadly available. It is simply too expensive and time consuming to identify "critical" endpoints for every compound subjected to safety assessments when the expectation is that such effects are relatively rare. The neurobehavioral data may still be the most appropriate to use for the dose-response assessment, but in a sense the neurochemical data are the critical information. Until I found that data in the documents, I was teetering to the point of saying neurobehavioral data was an inappropriate, unsupported, and poorly understood endpoint (despite what is said in the guidelines).

The more critical question with the apparent developmental impacts of the PBDEs is the apparent consistent developmental neurotoxicity that is seen within this class of compounds. This is observed despite very different patterns of metabolism, distribution, and persistence of these compounds in the body. Some effort should have been expended to see if the relative potency of the PBDEs could be rationalized considering the differences in the extent of metabolism.

On the other hand, one has to be concerned that very similar data have been generated by this same laboratory on a broader set of unrelated compounds. It is time to be concerned about the specificity of these data for developmental neurotoxicity. Independent confirmation is essential when such a apparently non-specific endpoint ends up flagging everything studied.

I most strenuously object to the treatment of these single-dose experiments as being equivalent to a subchronic exposure when applying uncertainty factors to arrive at an RfD. This is justified on the basis that brain development does not occur at an equivalent rate over a lifetime. This question is very relevant to whether of not a body burden approach should have been taken for this endpoint. Scientifically, the question is whether a body burden accumulated over time before the critical period would have the same impact as tissue levels that were achieved by the single dose protocol that was used. The available data do not allow this possibility to be evaluated. Therefore, the assumption that the acute dose at PND 10 is equivalent to a subchronic exposure is highly suspect.

Obviously, the data that are available are not sufficiently developed to allow the development of PK models. However, if evaluated critically (i.e. all the data are not equally informative as indicated in the editorial comments appended to the response to the formal questions) there may be enough absorption, distribution, and elimination data to make some ballpark estimates of consistent measures of how much of the parent compound and/or metabolites are present in tissues. The essential question is whether the effects of 1/10 the dose administered between PND 0 and PND 10 are the same as the full dose that is administered that is administered on PND 10. This experiment or an analogous one has not been done. Therefore, no conclusions can be made. Until such data are available, these studies have to be treated as a single dose studies, not a subchronic exposure.

There is some serious question related to whether the anti-thyroid effects of these compounds are of importance to the neurodevelopmental effects observed. The data of Zhou et al. (2002) establishes that the antithyroid effects are observed with DE-71, a formulation that might be reasonably linked to neurodevelopmental effects observed with BDE-47 and BDE-99. The Zhou et al. (2002) study was very well done and included not only good dose-response information, but a probable mechanism for the observed effects on thyroid hormone(induced metabolism of thyroid hormone metabolism). As pointed out in more detail in the editorial comments below, the thyroid effects seen in this study occur at doses that are higher than those that produce the neurodevelopmental effects of BDE-47 and BDE-99. As a result, the conclusion needs to be made that the neurodevelopmental effects cannot be linked to the anti-thyroid effects of these compounds.

Lucio Costa, Ph.D.

The rationale and justification for deriving RfDs on the basis of neurobehavioral toxicity studies have been well described and documented. The additional studies indicated above, particularly on body burden in infants and toddlers, would strengthen the health assessments, though they will not change the final determination of RfDs.

Ralph Kodell, Ph.D.

Limited human data do not indicate an association between exposure to PBDEs and adverse health outcomes. However, these data are insufficient to conclude that there is no potential for such effects. For BDE-47, BDE-99 and BDE-153, neurobehavioral effects are the only toxic effects that have been observed consistently in rodent studies of PBDEs, and therefore provide the only data for deriving RfDs. For BDE-209, the NTP rat and mouse bioassays (NTP, 1986) provide suitable data on noncancer endpoints other than neurobehavioral endpoints. The rationale for basing the RfD for BDE-209 on neurobehavioral effects instead of the other effects is that the neurobehavioral effects lead to the lowest point of departure. The rationale and justification for deriving RfDs on the basis of the neurobehavioral toxicity studies is transparent and appears to be objective. I do not know of additional studies that should be considered for any of the PBDEs. However, among the studies that have been considered, I think that greater consideration should be given to some of the studies other than the Eriksson/Viberg studies. Specifically, the studies of Branchi et al. (2002) and Kuriyama et al. (2005) provide data on BDE-99 that is suitable for deriving an RfD. The Branchi data have not been modeled while the Kuriyama data have been modeled but not used.

As noted in the documents, an association between neonatal exposures to BDE-47, BDE-99, BDE-153 or BDE-209 and neurobehavioral dysfunction in humans has not been established. Nevertheless, the behavioral studies appear to provide the most appropriate, dose-response data on adverse noncarcinogenic effects on which to base the health assessment of the four PBDE congeners.

1.2 Do you agree or disagree with EPA basing the health assessment of BDE-47, BDE-99, BDE-153 and BDE-209 to a large extent on the Eriksson/Viberg neurobehavioral studies?

Ahmed Ahmed, Ph.D.

As described earlier, the limitation of the availability of complete and comprehensive one or more studies that evaluate various types of adverse effects of these chemicals necessitated the utilization of what is available such as Eriksson & Virberg neurobehavioral studies. It is well known that there could be no one complete and comprehensive toxicological study. Every individual study will have its own limitation. In spite of some technical weaknesses in these studies, there use in the risk and RFD estimation is warranted.

The reason for the limitation of traditional toxicological evaluation on these newly detected environmental pollutants is because there are no extramural funds in NIH or EPA for this type of studies in the United States of America. That is why we ended up using studies from overseas to set up risk evaluations in the USA. This seems disturbing, specifically if we realize that the concentrations of these chemicals are estimated to be about 10-15 times higher in the biological fluids and tissues of American citizens as compared to European (specifically Swedish) citizens. I urge the responsible agents to encourage the classical toxicological evaluation of these compounds not in light of being similar in their structure to other compounds but in light of the fact that they are highly lipid soluble compounds that form depot in particular tissues of the ecological and mammalian biological systems. These depots create guaranteed life long exposure to these Xenobiotics, even after these chemicals are removed from the environment. The placental storage and transfer (if any) of these chemicals will turn them and their metabolites into trans-generational pollutants.

Richard Bull, Ph.D.

I do support the use of the neurodevelopmental studies for the purpose of deriving points of departure. As intimated above, however, considered alone, these endpoints have too little specificity to be of great concern. There are four fundamental issues: 1) The central importance of parallel neurochemical findings has not been used to advantage (or at least to the advantage it could be), 2) the interpretation of the neurobehavioral data as decreased habituation should be more critically evaluated, 3) the assessments of all the BPDEs depend upon results obtained from a single laboratory, and 4) the overwhelming application of uncertainty factors to such a subtle effect (i.e. relative to the usually accepted protocol) needs to be reconsidered. The first issue was addressed in question 1. The other three are discussed below:

It would have been better to present the actual behavioral data in the document rather than the habituation ratio. This ratio clearly is affected by both the depressed activity seen in the first period and the higher activity seen in the third period. The use of the habituation ratio forces an interpretation that may not be appropriate (or at least the reasons for selecting this parameter was not justified sufficiently in the documents or in the original papers). From the qualitative description of these data that was provided in these documents, they could be interpreted, with equal, if not greater, validity, as a depressed reaction to a novel environment and/or sustained hyperactivity.

Scientific findings need independent confirmation. Thus, EPA should be concerned about placing so much emphasis on set of studies that are generated completely within one laboratory, especially when the methodology and design are peculiar to that laboratory. Although it is not identified in the document, there is corroboration of the findings of Viberg and Eriksson with one compound (PBDE-99) in the study of Kuriyama et al. (2005). The experimental design was somewhat different. Nevertheless, the authors observed an increase in activity at PND 36 with a single dose of 300 µg/kg to the dam on GD 6. The measurement of activity after the animals had habituated to the test system, so the data are comparable to the activity observed in the 40-60 minute interval in the Eriksson et al. (2002) paper. Moreover, the lowest effective dose was somewhat lower than that reported by Eriksson and there is a clear NOAEL at 60 µg/kg.

It must be noted, however, that the implied relationship of the effects of PBDE-99 to altered thyroid function is not substantiated in Kuriyama et al. (2005). The doses producing the neurobehavioral changes occur at too low of a dose to be attributed to changes in thyroid hormone. In general, all the documents need to be more rigorous in their treatment of so-called "mode of action" data.

The application of group of uncertainty factors to a NOAEL despite the fact that the basis of this effect is not established and the apparent subtlety of the response relative to conventional developmental studies is not appropriate. This is discussed in some detail in the answer to question 1.5.

Lucio Costa, Ph.D.

Given the limited body of toxicological information available, use of the Eriksson/Viberg neurobehavioral studies as a basis for the health assessment is the proper approach.

Ralph Kodell, Ph.D.

The study of Eriksson et al. (2001) is the only available toxicity study with dose-response data on adverse (neurobehavioral) effects from exposure to BDE-47. The study of Viberg et al. (2004) has dose-response data on BDE-99. However, the studies of Branchi et al. (2002) and Kuriyama et al. (2005) also provide dose-response data. The data from Kuriyama were modeled and compared to the results from modeling the Viberg data, but the results from the Viberg study were selected for deriving the RfD. The reason for not using Branchi et al. (2002) for modeling is that there is an inversion at the highest dose. This does not seem to be a compelling reason to avoid modeling the data at the other doses. The study of Viberg et al. (2003a) is the only study with dose-response data on adverse effects for BDE-153. For BDE-209, the NTP rat and mouse bioassays (NTP, 1986) provide suitable data on noncancer endpoints as does the neurobehavioral study of Viberg et al. (2003b). Data from these studies have been evaluated and compared. There seems to be no alternative to basing the health assessment of the four PBDEs to a large extent on the Eriksson/Viberg studies, but the additional modeling for BDE-99 should be done and compared to Viberg (2004).

1.3 Are the Eriksson et al., 2001 (BDE-47), Viberg et al., 2004 (BDE-99), Viberg et al.,2003a (BDE-153) and the Viberg et al., 2003b (BDE-209) studies appropriate for determining the point of departure? Have the strengths and weaknesses of the Viberg and Eriksson studies been appropriately characterized and considered?

Ahmed Ahmed, Ph.D.

Following my review of the original reports, I found that Eriksson et al, 2001 (BDE 47), Virberg et al, 2004 (BDE-99), Virberg et al., 2003 (BDE-153) and Virberg et al, 2003b, 2003 (BDE-209) represent the most relatively complete studies on dose/response (neurobehavioral effects) relationships in animals. Most other available studies, discussed in these documents, could not directly correlate or tie the dose response and time course relationships. My main concern is that all these studies are coming from one group of scientists who pioneered the neurobehavioral / ADME (distribution in rodents) evaluation of these chemicals. With this information, come the problems of lack of validation of data coming from one laboratory with other data coming form other laboratories on the same scientific issues. Nevertheless, the authors of these reviews have

clearly and transparently judged these studies without biased. They expressed the weakness of each study as well as its strengths in their utilization for driving the RFDs. The authors should be commended on their unbiased evaluation.

Richard Bull, Ph.D.

It is my opinion that these data are appropriate as long as it is emphasized that the neurochemical data that have been collected using parallel protocols add significant support to the conclusion that a modification(s) of the normal developmental pattern occurs as a result of these treatments. Please note that other neurobehavioral data were identified in the PBDE-99 document that should have been more formally considered as indicated in the response to question 1.2. Moreover, the data sets on activity per se are much more appropriate for dose-response assessment than the habituation ratio, for reasons provided above. The habituation ratio is derived data, but the data provide very little support for the idea that loss of habituation is the basis of these results.

Lucio Costa, Ph.D.

The studies by Eriksson and Viberg are unusual, as indicated in the reports, but are the only available for most BDEs. At this stage, they are the only ones available for determination of point of departure. However, the study of Kuriyama et al. (2005) should be considered for determination of point of departure for BDE-99. Strengths and weakness of these studies have been described appropriately (but see comments below). A sentence may be added to indicate that further developmental neurotoxicity studies are needed for BDEs.

Ralph Kodell, Ph.D.

The Eriksson et al. (2001) study appears to be the only study available with data that might be used to determine the point of departure for BDE-47. For BDE-99, data from several candidate studies are modeled for determining a point of departure. The Viberg et al. (2004) study appears to have the best dose-response data and to provide the best model fits. However, the data from Kuriyama et al. (2005) also fits acceptably and gives lower BMDLs for some endpoints. Reasons were given for choosing the Viberg-derived BMD over the Kuriyama-derived BMD, but I think the Kuriyama-derived value is equally justifiable. The only well-designed dose-response study with dosing both pre-natally and post-natally (Branchi et al., 2002) was not modeled because of an inversion at the highest dose. Conceivably, these data could be modeled for BMD estimation, perhaps by omitting the highest dose, as is sometimes done with cancer incidence data. The Viberg et al. (2003a) study is the only study with dose-response data for determining a point of departure for BDE-153. Both the NTP bioassay in rats and mice (NTP, 1986) and the study of Viberg et al. (2003b) in mice provide data appropriate for determining a point of departure for BDE-209. Of these, the Viberg et al. (2003b) study gives the lowest point of departure, and is thus selected.

A clear weakness of the Eriksson/Viberg studies is that they did not conform to health effects test guidelines for a neurotoxicity screening battery or developmental toxicity studies. The dosing regimen did not include gestation and lactation exposure, and only single doses were administered. More than one pup per litter was used for behavioral testing, thus confounding dose effects with litter effects. (Although the authors reported doing a split-plot analysis of variance in all four primary papers, which would correctly recognize and use the litter as the experimental unit for testing treatment effects, their pair-wise testing for such effects appears to have treated each pup as an independent experimental unit.) In the cases of BDE-153 and BDE-

209, the dose-response data were not considered suitable for statistical modeling because only graphical information was available. These weaknesses have been characterized and considered, and offsetting strengths have been discussed. However, it is surprising that the data on BDE-153 and BDE-209 cannot be recovered from the authors to allow dose-response modeling and BMD estimation. One important weakness that was not emphasized for BDE-47, BDE-153 and BDE-209 is that the Eriksson/Viberg mouse studies were done in males only.

1.4 Have the most appropriate critical effect and point of departure been selected? And has the rationale for the point of departure been transparently and objectively described?

Ahmed Ahmed, Ph.D.

With all the above consideration the authors have critically and objectively selected the most critical effect (neurobehavioral) and established the most appropriate point of departure (dose response) for all chemicals under review. The authors have very clearly rationalized, in great detail, in each individual review, their selection of each particular point of departures.

Richard Bull, Ph.D.

Among the data that are available, the neurobehavioral data provide the best data for identifying a point of departure. There are some lingering questions that relate to effects of BDE-99 that are reflected in histologic changes that occurred in the ovaries and vaginal epithelium at very small doses (i.e. 0.06 mg/kg day). However, these data were not described with sufficient clarity in the document to determine if they should not be used (i.e. neither incidence or severity are described in the context of dose-response).

My review of the Talness et al. (2005) study does raise some issues both about design and interpretation that may have been the basis of the Agency's apparent reluctance to use the Kuriyama et al. (2005) data on PBDE-99 (although it was not stated, if that were the case). For example, it is difficult to understand the conclusion that necrosis was observed by electron microscopy (Fig 1 & 4), but were not observable by light microscopy (see p. 194, section 3.1.2). Second, the number of animals examined for changes in the vagina was very small. Further, the authors do not make clear the basis for their repeated assertion that the doses tested resulted in levels of 6-29 times greater than reported in human breast adipose tissue. Was a human study conducted that looked at single doses that gave rise to such levels? I could find no reference to such a study.

Lucio Costa, Ph.D.

The most appropriate critical effect and point of departure have been selected and the rationale has been adequately described.

Ralph Kodell, Ph.D.

Although the seriousness of decreased habituation capability as a manifestation of an adverse effect on spontaneous motor behavior is not discussed, it is a common measure of developmental toxicity across studies. Thus, it appears to be an appropriate critical effect for deriving a point of departure. However, the individual measures of decreased habituation might be as appropriate as

or more appropriate than the habituation-ratio as indicators of toxicity. For BDE-153 it is unfortunate that data on neither the habituation-ratio nor the swim-maze learning/memory test could be recovered for modeling. The dose-response for the swim-maze effect might be different from the habituation-ratio dose-response. (Unfortunately, swim maze data on BDE-99 (Eriksson et al., 2001) involved only a single high dose.) For BDE-209, too, it is unfortunate that dose-response data on the habituation ratio cannot be recovered for modeling and BMDL calculation. It's not clear why the actual data on BDE-153 and BDE-209 cannot be recovered from the studies' authors, given that the studies were published fairly recently (2003). In fact, it would be good if the dose-response data for all four PBDEs could be obtained so that litter effects could be handled properly in the BMD modeling. It would be far better if a BMDL rather than a NOAEL for habituation-ratio could be used for setting an RfD for neurobehavioral effects of BDE-153 and BDE-209.

For BDE-47 and BDE-99 and BDE-209, the rationale for selecting the point of departure in terms of the BMD and associated BMR are clearly spelled out. Selecting the BMD as the dose that corresponds to a shift of 1 standard deviation in the mean (BDE-47 and BDE-99) corresponds approximately to a 10% BMD with a critical cut-off defined by assuming 1% background risk for a normal distribution with constant variance. The use of a BMR of 10% for the BDE-209 quantal noncancer endpoints is explained. I think it ought to be stated explicitly that the BMDL rather than the BMD is used for these endpoints in order to account for experimental uncertainty (variation), which is a good practice and is related to the use of uncertainty factors. (However, the uncertainty may be understated because of treating littermates as independent experimental units.) Generally, for each endpoint, the BMD and BMDL resulting from the best-fitting model (or an average of the best-fitting models: BDE-209) and/or the model fitted to the most dose groups are selected. Then, the lowest BMDL among those is selected as the point of departure. The sequential likelihood-ratio testing approach for selecting the fitted model for neurobehavioral effects of BDE-47 and BDE-99 is very sound and is explained very clearly. However, I do not understand why, in the case of BDE-99, it must be assumed that 8 animals of each sex were tested behaviorally. Can't this be clarified by contacting the study's authors?

To the extent that data are available for dose-response modeling, I believe that the selection of critical effects and points of departure are appropriate, and that the rationale for the points of departure has been transparently and objectively described. However, I think a strong effort should be made to recover neurobehavioral toxicity data from the authors (Viberg et al., 2003a, 2003b) to allow dose-response modeling and BMD/BMDL calculations for neurobehavioral effects of BDE-153 and BDE-209, and to refine the modeling for BMD-47 and BMD-99.

1.5 Have the rationale and justification for each uncertainty factor (UF) selected in the draft 2 Toxicological Reviews of BDE-47, BDE-99, BDE-153 and BDE-209 been transparently described? If the selected UFs are not appropriate, what alternative UFs would you suggest and what are the scientific rationales for those suggested? Does the database support the determinations of the RfDs for BDE-47, BDE-99, BDE-153, and BDE-209?

<u>Ahmed Ahmed, Ph.D.</u>

In their calculation of each individual RFD for each PBDPE, the authors have clearly classified several uncertainty factors for each compound based on the available information in the studies utilized. I found that their selection of these uncertainty factors to be well rationalized and the objectivity of each uncertainty factor to be very clear. It was apparent that the number assignment was very uncertain during the panel meeting on Feb 22,2007. It seemed very uncertain whether several UFs should be assigned a10 or 3.

In considering acute versus chronic uncertainty factor, I feel that the accumulation of BDEs, specifically the lower brominated compounds, tetra (BDE-47 and penta (BDE-99) should be considered in the calculation of their RFDs. These compounds detected in various human tissues and biological fluids and have very long half lives in human tissues; the adipose tissues in vital organs and biological fluids; breast milk. Thus, for the highly susceptible human groups such as fetuses and infants there will be continuous internal dosing that is coming from storage depots. Accordingly, the body burden for infants will not be only dependant on exposure to external dosing from the environment. It will be dependant on a combination of external (environmental) and internal factors (the depot of biological tissues and fluids). This complexity should be considered in the estimation of RFDs. Specifically for the U.S. population. In the American population, the levels of most of these chemicals in blood, tissues and biological fluids are about 10 or more times higher than that detected in biological fluids from other (European) countries. Such facts should be considered in the calculation of RFDs here in U.S.A. Finally I believe that available limited data base should support the determination of the RFDs for BDE 47, BDE 99, BDE 153, and BDE 209.

Richard Bull, Ph.D.

The rational and justification of each UF has been transparently described. However, I believe that they have been inappropriately applied to these chemicals.

There was no consideration in the document related to the specificity and sensitivity of the neurobehavioral and neurochemical measures that have been applied vs. more standard endpoints. In particular, it is inappropriate to apply the UF relative to data base inadequacies. One might consider modifying UFA and UFH as well.

Given the nature of the available data, the rationale for using an adjustment from subchronic to chronic exposures (UFS) for these data is also not sustainable scientifically. In this case, the critical issue is whether the body burden of parent compound is important. Thus, the adjustment of 3 that is made on the UFS is not appropriate. Rather one needs to consider the extent to which the mother's pre-pregnancy accumulated body burden would influence the developmental outcome. As I see no way of even making a judgment on this issue based on the available data, I suggest that UFS be raised to 10.

There is a more general problem with the database uncertainty factor when it is applied to the development of RfDs. The traditional across species and within species uncertainty factors relate to the critical endpoint being addressed in the risk assessment. The database uncertainty factor addresses questions that go beyond this endpoint and focus on risks that might occur, but there are no relevant data. Thus, it does not reflect on the accuracy of the assessment of the endpoint being evaluated. This uncertainty factor is more appropriately applied at the point that risk management decisions are being made (e.g. at the point that MCLs are being developed). This concern should be introduced at the point where other parallel uncertainties are entertained (e.g. can remediation efforts reduce the risk or whether the MCL is within a range that can be measured).

Lucio Costa, Ph.D.

The selection of UFs has been described in detail, though I do not fully agree with some choices (see comments below for individual BDEs). The database on which RfDs are determined is in most cases very poor, particularly in case of BDE-47 and BDE-209. Perhaps RfDs should be acknowledged as "temporary", while waiting for additional studies that may increase confidence.

Ralph Kodell, Ph.D.

Default uncertainty factors of 10 for UFA and UFH are used for BDE-47, BDE-99 and BDE-153 without much explanation or justification. For BDE-209, the UFA default (but not the UFH default) has been explained satisfactorily. Taken together as a product, values of 10 each for UFA and UFH may be overprotective, but there is no alternative methodology that is currently accepted for expressing these factors as a smaller unitary factor, as might eventually be done via hierarchical modeling.

Looking at these factors separately, a UFA of 10 might at first seem conservatively large given that no evidence of an association between neonatal exposure to PBDEs and neurobehavioral effects in humans exists. On the other hand, there is no evidence to the contrary, and the mode of action is not yet understood. Because the potential adversity to humans is present, a value of 10 seems justifiable in all four cases. For UFH, I believe there is justification for deviating from the default value for all four congeners. Because the neurobehavioral effects seen in rodent studies result from exposures at critical points in early development, I believe it can be argued that the most sensitive members of the population for these effects (neonates) are already accounted for. Because the default value of 10 is used to account for differences in sensitivity among all members of a population, and because it seems hard to justify a factor that large for variation just among neonates, a smaller value of UFH appears justified. I recommend a value of 3 for UFH for all four PBDEs.

The choice of 3 for UFS for all four congeners appears justifiable to me, recognizing that timing of exposure is more critical than duration of exposure for the neurobehavioral effects observed. However, I regard this factor as accounting more for uncertainty arising from a lack of pre-natal exposure in the studies used to derive the RfDs than for uncertainty regarding potential effects of chronic exposure.

Using a value of 10 for UFD for BDE-47, BDE-99 and BDE-153 does not seem justifiable. In my opinion, if the database is so uncertain as to require a UFD value of 10, then the database is too limited to allow the derivation of meaningful RfDs. The primary neurobehavioral endpoint appears to be quite sensitive, and it is consistent across congeners. For BDE-47, I recommend a UFD value of 1. Although there is only a single study, the neurobehavioral endpoint is the same as for the other congeners, and dose-response data are available for basing the point of departure on a BMDL. For BDE-99, I also recommend a UFD value of 1. There are several studies and dose-response data are available for basing the point of departure on a BMDL. For BDE-153, I recommend a UFD value of 3. There is only a single study, and dose-response data could not be recovered for BMDL calculation. If those data can be obtained from the study's authors and used for BMDL calculation, then this factor could be reduced to 1. For BDE-209, I recommend raising the factor from 1 to 3. Granted, there are data from more than one study, and BMD modeling was done along with model averaging for non-neurobehavioral effects. However, a NOAEL for neurobehavioral effects rather than a BMDL was selected for the point of departure, because of the inability to recover the neurobehavioral dose-response data. To me, the absence of definitive data for dose-response modeling for neurobehavioral effects reflects an inadequacy in the database, and in fact, inadequacy in the very data ultimately used for deriving the RfD. If data can be obtained from the study's authors and used for BMDL calculation, then this factor could be reduced to 1.

One factor that is not mentioned is UFL for going from a LOAEL to a NOAEL, because LOAEL-to-NOAEL extrapolation is not done here. However, I think this factor should also apply when using a BMDL10 as a point of departure. A 10 % benchmark response is not negligible. A BMD10 (BMDL10) is more in line with a LOAEL than with a NOAEL. I recommend applying a UFL factor of 3 to each of the BMDL10 values to account for uncertainty in extrapolating from a dose of non-negligible toxicity to a dose of negligible toxicity.

I believe the database supports the determinations of RfDs, but I agree with the statements in the documents that the overall confidence in the RfD assessment of BDE-47, BDE-99 and BDE-153 are low. I feel the same way about BDE-209.

2. BODY BURDEN APPROACH

2.1 Are there adequate data for considering body burden as an alternative dose metric to administered doses in any of the RfD derivations?

Ahmed Ahmed, Ph.D.

The current available animal and human data regarding the body burden of BDE's are sporadically designed and extremely limited. Although there are several studies under each compound that describe the ADME or body burden concept (absorption, distribution and tissue accumulation and excretion), the kinetics (t1/2, volume of distribution, rates of absorption, metabolism and excretion) of the absorption, accumulation and removal processes of each individual compound have not been established. Similarly the identity, type and structure of the accumulated chemical species have not been recognized. In natural environmental exposure setups, there is no single (exposure) dose. Humans are continuously exposed, continuously storing, distributing, metabolizing and excreting the compounds or their metabolites. In this regard even the year or more long half life, described for some congeners, is in reality not a true kinetic factor, because the parent molecules or its metabolites are primarily stored in certain target tissues and continuously released into the blood for circulation, metabolism and excretion. These tissue stores are continuously replenished by absorbing more from the external surrounding environment. All these events should be taken when considering body burden as an alternative dose metric in the derivation of any of the RFDs. Such information is currently scarce (McDonald, 2005).

Richard Bull, Ph.D.

There may be a reason for considering body burden. As indicated in the response to questions 1.1 and 1.5, the question of whether increased body burden would contribute to the critical neurobehavioral endpoints evaluated has simply not been tested. It is for this reason that treating a single dose experiment as if it were a subchronic exposure is inappropriate. In addition, it is not clear whether the body burden or daily dose is the best dose metric for the chronic effects of those members of the PBDE class that have been evaluated in chronic studies. Therefore, this question also depends upon the endpoint that is being evaluated and the documents have not provided a basis for either approach. There are huge inconsistencies in the ADME among the PBDEs. Chronic exposure would appear to lead to large body burdens with some of the PBDEs, but not others. Furthermore, simply showing that the compound, a metabolite, or a radiolabel derived from that compound reaches its target site does not establish a causal link. For the developmental effects, in particular, the question of whether levels of the parent compound or a metabolite is the most appropriate dosimeter has to be resolved experimentally, not guessed at. As a start, the variability in the extent of metabolism across these four PBDEs needs to be evaluated to see whether the neurodevelopmental effects appear to occur at similar body burdens of the parent compounds. That is about as far as an analysis could be taken given the data that are available.

The above points are critical as the only compound for which there are cancer data is BDE-209, which appears to be metabolized and excreted much more rapidly than the PBDE's with lower bromine substitution. It does not bioaccumulate to the extent of the other compounds. Therefore, even this chronic effect may not be a function of the body burden. Part of this may have resulted from the lack of context provided for the metabolism & kinetic data that were provided. There are several aspects of the write-ups that suggest that data to this effect may exist, but were simply not critically and/or creatively interpreted or used by the authors of the document. Some of these issues are discussed in greater detail in the editorial comments provided below. To make proper use of the available metabolism and kinetic data that are available requires an integrated assessment of all four compounds. Therefore, one questions why these chemicals are addressed in four separate documents.

Lucio Costa, Ph.D.

Body burden would be an alternative dose metric to derive RfDs, however, current available data are still too limited, in my opinion.

Ralph Kodell, Ph.D.

There are not adequate data for considering body burden as an alternative dose metric to administered dose.

2.2 Do you agree with the rationale described in the Toxicological Review of BDE-99 that the data on the window of susceptibility of the cholinergic receptors to BDE-99 tend to minimize body burden concerns?

Ahmed Ahmed, Ph.D.

Even though I don't completely agree and don't understand clearly the question, I have to accept the concept. That is because any experimental design has to have an assumption that is based on the available information. We know from the literature that the fetal and neonatal brain developments are different in rodents and humans. Therefore, fetal and neonatal cholinergic receptors regulation (up or down) could also be different. While brain development in rodents occurs during postnatal periods, it occurs in utero in humans. Both conditions have extremely different manifestations regarding nutritional, environmental exposure and other toxicokinetic parameters. Therefore, to focus on a specific and certain particular time for susceptibly (PND 3, 7 or 10) for cholinergic receptors in neonates may increase the uncertainty factor in the RFDs' calculation.

Richard Bull, Ph.D.

I am not sure that I understand this question. There is no direct evidence provided that BDE-99 treatment directly affected cholinergic receptors that I could identify in the document. What was presented was an indication that animals treated neonatally with nicotine were responsive to the "loss of habituation" following dosing with BDE-99 at 8 mg/kg at 5 months of age. The effect was not produced by nicotine pretreatment alone, or by only administering BDE-99 at 5 months of age. This is not evidence of an interaction at cholinergic receptors! On the surface, the data

obtained with preadministration of nicotine appears to actually separate the mechanism of nicotine from that of BDE-99. The nicotine effects are actually inverted (i.e. suppressed activity) with nicotine at higher doses. Why do neither BDE-99 or nicotine, administered alone, produce the effect when administered at 5 months? The mechanism of the interaction must be complex and indirect.

The second part of the story is that nicotine treatment at PND 10 caused decreases in binding sites for bungarotoxin and QNB, that specifically bind to nicotinic and muscarinic subtypes. A similar effect was observed with BDE-99 administered at PND 10. It is unlikely that the effects of BDE-99 are an expression of an interaction at nicotinic receptors, per se. Binding to such receptors has structural requirements not remotely met by BDE-99 or any of its likely metabolites. Whatever BDE-99 does must involve a less direct mechanism. That could be an inhibition of the expression of these receptors, but just as likely, it could mean that there is a deficit of neurons that express these receptors. If the document goes into mode of action questions, it is imperative that it clearly distinguishes among alternative mechanisms that are possible. One of those criteria clearly has to be a consistency in the doses required to produce the effect and that needed to activate a particular mechanism thought to be a key event in the mode of action. If one mechanism is considered to be more probable, the reasons for this conclusion need to be explicitly stated. Otherwise the document has to conclude that the mechanism of these effects have not been established.

Lucio Costa, Ph.D.

Question is unclear.

Ralph Kodell, Ph.D.

The hypothesis of impaired development of the cholinergic system during the postnatal "brain growth spurt" period as an explanation of the neurobehavioral effects seen in the Eriksson/Viberg studies is plausible. Staskal et al. (2006b) showed that brain levels of BDE-47 administered to neonatal mice on PND 10 were higher than levels in adult animals. Branchi et al. (2002) showed significantly elevated brain levels of BDE-99 in mice administered a high dose from GD 6 to PND 21. Eriksson et al. (2002) also showed that radiolabeled BDE-99 can be taken up and retained in the neonatal mouse brain, although the amount of radioactivity in the brain was only between 0.4% and 0.5% of the administered dose. Viberg et al. (2003b) reported a similar result for BDE-209. The limited data available on BDE-99 indicate that the effects on habituation were seen only at doses that also cause decreased binding of the cholinergic receptor antagonists. For BDE-153, Viberg et al. (2003a) showed that the density of nicotinic acetylcholine receptors in the hippocampus of 6-month old mice treated on PND 10 was significantly decreased at 9.0 mg/kg, a dose at which mice showed significant defects in learning and memory. However, there are no definitive data on mode-of-action. PBDEs have some structural similarity to the thyroid hormone T4. It has been suggested that they may interfere with thyroid hormone transport by competitively binding with one of the transport proteins in plasma. Although it is known that thyroid hormones are essential for normal brain development in humans and that decreases in thyroid hormone levels during fetal and early neonatal life may have profound adverse effects on the developing brain, thyroid hormone levels and behavioral activity were not co-measured in any of the developmental toxicity studies in mice or rats. Although the data on the window of susceptibility of the cholinergic receptors to the PBDEs (PND 3 – PND 10) are suggestive, I believe there are too many other possibilities for mode of action for this rationale to minimize body-burden concerns.

3. QUESTIONS RELATED TO THE CARCINOGENICITY ASSESSMENT OF BDE-209

3.1 Is the weight of evidence for the carcinogenicity of BDE-209 in the draft Toxicological Review appropriately described? Are there additional studies that should be included?

Ahmed Ahmed, Ph.D.

The weight of evidence on the carcinogenicity of BDE 209 in the draft toxicological review is based on a carcinogenicity studies that were conducted more that quarter of a century ago. Although the authors of the draft review document have provide a methodical and complete description of the studies, the study itself and its results leave a lot to be desired. Modern, molecular and more sensitive biomarkers of cancer should be implemented in future carcinogenesis studies for BDE-209 and other congeners. The current available studies had shed some doubts regarding the pre-carcinogenic effects of BDE 209. The studies utilized very high doses which lead to some toxic effects and increased animal deaths.

Currently I am not aware of any recent study that addressed the carcinogenicity of BDE-209.

Richard Bull, Ph.D.

Yes.

Lucio Costa, Ph.D.

The weight of evidence for carcinogenicity of BDE-209 is appropriately described. I am not aware of any additional studies.

Ralph Kodell, Ph.D.

The weight of evidence for the carcinogenicity of BDE-209 is appropriately described. I do not know of additional studies that should be included.

3.2 Do the data support estimation of a cancer slope factor for BDE-209? If yes, is the rationale for the quantitative analysis objectively and transparently described, considering the uncertainty in the data and the suggestive nature of the weight of evidence? Have the rationale and justification for the use of linear low-dose extrapolation been objectively and transparently presented?

Ahmed Ahmed, Ph.D.

The available data supports the estimation of a cancer slope factor for BDE-209. The rationale for the qualitative analysis was objectively and transparently described. Similarly the justifications for the use of linear low dose extrapolation have been objectively presented.

Richard Bull, Ph.D.

After reading the public comments directed at treatment of the carcinogenesis data that is available on BDE-209, it is apparent that there are a variety of problems with the NTP data. Comments from industry focused on the fact that the responses were fairly modest and that the criteria have changed for classifying liver lesions in the rat since this study was completed. I am less concerned with the scoring nodules as tumors. Nodules generated in initiation promotion protocols are known to regress with suspension of treatment. This is much less likely to happen with chronic studies of a single compound.

However, I question the appropriateness of the original NTP study for developing risk estimates in general. The very high concentrations were provided to the animals in the diet make it almost certain that the external dose is not a reliable indication of the systemic dose. The extremely low solubility of BDE-209 in aqueous media (<0.1 ug/L) suggests that the transfer rate from food to aqueous phase would sharply limit to absorption proportional to external dose to much lower concentrations than the 25,000 and 25,000 ppm in the diets used in the NTP study. The document contains data indicating that absorption is sharply curtailed at much lower concentrations in the diet (see quotation of studies of El Dareer et al., 1987) and the fact that much higher percentages of absorption were derived from the gavage dosing of much smaller doses of PBDE-209 (see Sandholm et al. 2003; Morck et al. 2003). These observations are supported by the more uncertain studies of the absorption of the labeled compound. While there were some evidence of tumor induction in both mouse and rat studies, there was little indication of dose-response, suggesting that the response was limited by absorption. If this is the case, the true point of departure for estimating cancer risk could be order of magnitude lower than is suggested by the doses administered. For this reason, revisiting the results of the NTP studies will be a waste of time and money. I would strongly suggest that a new bioassay be conducted that can be more easily related to environmental exposures. The doses administered in such studies should be established based on better designed studies of absorption, distribution and metabolism than have been conducted to date and development of a first generation PBPK model.

Lucio Costa, Ph.D.

Data are indeed suggestive of a carcinogenic potential of BDE-209. BDE-209 does not appear to be genotoxic (though database is limited), but no clear indication of possible epigenetic mechanism is available. On the other hand, chronic toxicity studies suggest the induction of non neoplastic or pre-neoplastic lesions in target organs. The linear low dose extrapolation as a default, conservative approach appears justified in this case, as is the classification of BDE-209 as a possible human carcinogen.

Ralph Kodell, Ph.D.

The data appear to support estimation of a cancer slope factor for BDE-209 based on neoplastic nodules or carcinomas (combined) in the livers of male rats (NTP, 1986). This conclusion assumes that any change in the classification of neoplastic nodules that might have taken place subsequently would not change the dose-response appreciably. The rationale for the quantitative analysis is transparently described. The cancer data are characterized as providing "suggestive evidence of carcinogenic potential," in keeping with the EPA cancer guidelines (EPA, 2005a). The modeling of several tumorigenic endpoints leads to selection of the cancer slope factor corresponding to the most sensitive endpoint (liver nodules/carcinomas). I did not see a rationale or justification for the use of linear low-dose extrapolation, except for a reference (page 62) to two EPA guidelines documents for cancer assessment (EPA, 2005a, 2005b).

3.3 Are there alternative modeling approaches that should have been considered instead of or in addition to the linear low-dose extrapolation approach?

Ahmed Ahmed, Ph.D.

To my knowledge the authors have explained all possible modeling approaches. They selected the best fit model for their interpretation of the experimental data. I commend the authors' consciousness for siting the drawbacks of these studies and for the thorough analysis of the available data.

Richard Bull, Ph.D.

There are no data that would permit departure from linear low-dose extrapolation. As indicated above, I am concerned about the very high doses that were used in these studies. It is not clear that absorption would be linear with dose because of the solubility problems that are discussed in more detail in the editorial comments that have been appended to these responses to EPA's specific questions.

Lucio Costa, Ph.D.

Data are indeed suggestive of a carcinogenic potential of BDE-209. BDE-209 does not appear to be genotoxic (though database is limited), but no clear indication of possible epigenetic mechanism is available. On the other hand, chronic toxicity studies suggest the induction of non neoplastic or pre-neoplastic lesions in target organs. The linear low dose extrapolation as a default, conservative approach appears justified in this case, as is the classification of BDE-209 as a possible human carcinogen.

Ralph Kodell, Ph.D.

Other modeling approaches could have been considered, but I don't think that they necessarily should have been considered. The liver dose-response data are quite linear and there are only two nonzero doses plus a zero-dose control. The linear model fits quite well. It seems likely that other dose-response models would give similar fits. It's possible that such models would extrapolate differently from the linear model below the data, but it is my opinion that the data themselves are not informative enough to justify a different, say, sub-linear, extrapolation approach.

MISCELLANEOUS REVIEWER COMMENTS

Ahmed Ahmed, Ph.D.

Comments on Each Document:

BDE 47:

1. Page 7; 3.2.1: Current Human data regarding distribution and disposition of BDE 47 is spotty, sporadic with low number of samples and uncontrolled health and environmental conditions, with no regard to geographical or ecological circumstances.

2. Human data should be summarized and presented in a comprehensive table with references in this and in all other documents.

3. Page 13; 3.2.2: Animal data on the disposition of BDE-47 focused on several 14C labeled compounds. In most of these studies, the position of the label was not mentioned, the purity of the 14C-compound was not known, the specific activity of the compound was not given and the ADME studies were based on following the radioactivity in various tissues and biological media. No pharmacokinetic parameters were estimated from these studies.

4. The identity of the detected radio labeled species in tissues and biological fluids is very important to clarify whether this species is the parent molecule, a metabolite or even a carbon dioxide molecule.

5. The levels of radioactivity should be uniformly expressed throughout this document and other documents. That is essential to be able to do reasonable comparisons among various congeners. Concentrations as mg-equivalent / g tissue/g lipid weight should be used across the document.

6. I suggest that the authors to put some proposed metabolic pathways for this compound. It could be extracted from other brominated congeners such as BDE-99. The proposed metabolic pathway similar to that presented for BDE-99 could be used here too. This will strengthen the fact that this and other lower brominated congeners could be obtained from higher brominated ones.

Mice are known to have higher levels of various CYT P450 enzyme activities than rats. This should be reflected in the discussion of the differences in the disposition, metabolism and excretion of this compound and other congeners.

8. Extensive explanation of receptor site interaction is somewhat confusing due to the lackluster effects of these compounds on various receptors. It would be beneficial if a summary table is presented either in the beginning or at the end of this section. Relative potency of various congeners on specific receptors should be indicated.

9. A table, in page 39, that could summarize the neurobehavioral, developmental and reproductive effects of BDE-47 should be added. It will show how Eriksson et al represent as complete a study to be used in RFD determination. It will be helpful if in each document a similar table is inserted before the selection of the principal study as in case of the document of BDE-99.

10. The Uncertainty Factors for population at risk (fetuses and newborn) and for conversion from single dose to multiple and continuous routes of exposure should be adjusted for, as described before.

11. The neurobehavioral studies that are utilized in RFD evaluation have limited end points and were conducted in one Swedish research location. Due to the lack of complete dose response/ time course studies, I have to agree on using them in the determination of the corresponding RFD. My agreement is based on the fact that these studies have statistically significant end points for neurobehavioral adverse effects within the framework of the experimental design.

12. A major concern about the experimental design, described in these studies, is ignoring the “letter effect”. The authors of the study should have used offspring from multiple letters rather than from one letter. Randomization was based on only two letter and randomization approach was not described.

13. Although radioactivity was detected in brain, in the studies, there was no explanation in which brain areas radio activity was detected. Anatomical or histological localization of the radioactivity would have added significant weight to the mechanisms of the observed neurobehavioral studies.

14. There seems to be no correlation between the postnatal day of exposure and the concentration in the brain. Exposure at PN Day 10 provided the brain with the highest concentration while had no increase in neurobehavioral adverse effect.

BDE-99

1. The table in page 58 is an excellent summary to the developmental and reproductive effects of several BDEs. It shows how Viberg et al (2004a) represent as complete a study to be used in RFD determination. It will be helpful if in each document a similar table is inserted before the selection of the principal study for RFD estimation.

2. Study of Kuriyama et al (2005) showed some good dose-response relationship for neurobehavioral as well as male reproductive effects. This study should be considered in the determination of RFD for BDE-99.

3. ADME evaluations in animals are un-objectively presented. They were presented as several summaries of several studies but with no connection and with limited interpretations of the data. The proposed metabolic pathway presented in page19 is very helpful. Similar pathway should be presented for other congeners.

4. Due to the metabolic relationship between higher and lower congeners of BDE, a comprehensive metabolic profile for all of these compounds should be presented in every toxicological review for each individual BDE.

5. Comments on the radioactive compound, its purity and specific activity that are described before, will also apply for this and every other congener.

6. There are large number of receptor studies that are presented in the document. A comparative summary with references will be helpful for the reader to easily conclude which is the most sensitive receptor for this and other congeners.

7. Concerns regarding Uncertainty Factors described before, apply here, too, for BDE-99.

BDE-153

1. Human distribution studies are somewhat sporadic and most of them were designed for the detection of other chemicals. The data in table 5 should be expanded to include BDE- 209. This table should be presented in the document of every congener.

2. I would suggest that the authors of the document should create a summary of values for the concentration of various PBDs in human tissues in various geographic locations. Schecter et al (2003) showed that the mean median concentrations of BDE in Texas are much higher than any other places in USA or Europe.

3. A summary table for receptor studies is needed.

4. Comments about 14C ADME studies described before apply here too.

5. Need to insert comprehensive metabolic pathway similar to that in BDE-99 document.

6. In most of the in vitro and in vivo animal studies that are presented in these documents, oily vehicles were used (corn oil etc.). I found this problematic. That is because large number of bromine atoms on a phenyl ring imparts several electrophillic and lipophilic properties to the aromatic ring. The increased lipophilicity will significantly alters the distribution and tissue uptake of BDE between the oily vehicle and the biological system with its hydrophilic / lipophilic properties. These conditions lead to decreased absorption and distribution with subsequent alteration in metabolism and excretion with little correlation to real occupational and environmental conditions.

7. Rational for driving RDF and Conclusions are appropriate and transparently presented. A summary table for all available studies that could be used for the estimation of RFDs should be included. This table will clarify why a particular study was the most appropriate for the estimation of RFD.

BDE-209

1. The cancer risk derived for BDE-209 is based on very high doses utilized in a two and half decade old study. These doses could not be occupationally or environmentally possible except in accidental conditions.

2. Like other toxicological reviews of other BDEs, ADME evaluations in animals are un-objectively presented. There are several summaries of several studies but no connection and limited interpretations of the data. The proposed metabolic pathway presented in for BDE-99 is very helpful. Similar pathway should be presented for this and other congeners.

3. Due to the conversion of higher molecular weight BDEs (those with larger numbers of bromine atoms) metabolic relationship between higher and lower congeners of BDE exists. Therefore, a comprehensive metabolic profile of all these compound should be presented in every toxicological review for each individual BDE.

4. Comments on the radioactive compound, its purity and specific activity that are described before, apply for this and every other congener.

5. Extensive number of receptor studies is presented in the document. A comparative summary with references will be helpful for the reader to easily conclude which is the most sensitive receptor for this congener (and other ones).

6. Concerns regarding Uncertainty Factors described before, apply here for BDE-209, too.

<u>Richard Bull, Ph.D.</u>

Editorial comments:

1. The Agency should reconsider the traditional organization of these review documents. The old organization is becoming very cumbersome as more and more data become available that bear on the issue of mode of action or corroborating data that support the documents overall

conclusions. In this particular set of documents, the artificial separation of the neurobehavioral studies and neurochemical data over three categories (reproductive/developmental studies, neurotoxicity and Other studies) really made these difficult documents to review. I suggest that all of the data that contribute to a cohesive understanding of a particular toxicological effect need to be included in the same section. To pick a specific case, all data relevant to the neurodevelopmental effects of BDE-99 should be presented together, whether the data relate to neurobehavioral, neurochemical, or gene expression data in the CNS.

Related to this point, it is also suggested that conclusions that are derived from explorations of alternative modes of action for a particular effect be expressed more categorically. For example, there have been some investigation of contribution of thyroid hormones changes to the neurodevelopmental effects of the BPDEs. The only clear results with respect to the thyroid hormones is that of Zhou et al (2002), where effects of a different mixture (DE-71, a mixture of tetra and penta PBDEs) on thyroid hormone levels were described. The doses required to produce such effects were not far off those that produced neurodevelopmental effects with BDE-47, but were much higher that the doses of PDE-99 that produced neurodevelopmental effects. Thyroid hormone levels were examined in a study of adults treated with BDE-47 by Hallgren and Darnerud (2002), but only FT4 was reduced, not thyroxine, and the dose required to produce this effect was much too high. Other studies with the PBDEs appear to be negative, but in some cases the actual doses utilized were not provided in the document (e.g. the Hakk et al. 2002 study cited in the BDE-99 document). To this reviewer there is no basis for suggesting that the neurodevelopmental effects observed with the PBDEs are related to thyroid hormone-related effects. This actually raises concerns, rather than lowering them. If a thyroid hormone-based mode of action were identified, there would be less of concern because the risks associated with this mode of action are reasonably well understood. Therefore, a clear conclusion should be drawn that says it appears unlikely that modified thyroid function plays a role in the neurodevelopmental effects observed with this class of compounds. Similar statements should address other modes of action that have been found to be consistent with or inconsistent with respect to particular toxicological effects. In the absence of a particular toxicological effect, these data are of little use. It is really important to begin to make these distinctions as the "presumed mode of action data" may frequently form the bulk of the available data on a compound and this information needs to be placed into better context.

2. The data on PBDE occurrence in biological samples involve relatively small populations and may not be representative of either the U.S. or the World populations. Nevertheless, there are observations that imply an evolution of exposures that are somewhat different in the U.S. and other parts of the world. Similarly, there appears to be a pattern of exposures, reflected in tissue measurements, that is increasing in recent years (presumably because of the recent introduction of these compounds in new industries). These are important issues and need to be presented more proactively – i.e. in a way that supports or denies these apparent trends in exposure and providing a specific a basis for these observations, if true. As the section now stands, it simply iterates studies in a more or less sequential way, providing some casual notion of the existence of these patterns, but not really determining whether the databases are strong and representative enough to support these conjectures or not. A few specific comments, drawn largely from the BDE-47 document as much of this information is repeated in the subsequent documents, which illustrate the problematic nature of some the discussions that were provided in the document are provided below:

a. There are several places in the discussion of data obtained with 14C that need to be clarified. Either the document poorly explains the nature of the data that is summarized or it has been misinterpreted. One example of a problem in interpretations of greater concentrations of 14C in tissues following a series of doses of non-radiolabeled compound. Such observations are frequently the result of isotope effects (e.g. the prior exposure results in a tissue pool of compound in tissues that is significantly larger than in control animals. As a result more isotope remains in the tissue pools as it becomes diluted in specific activity). The document suggests that these data reflect is a magnification of amounts of chemical that is retained with subsequent

doses. This is unlikely. The only way this can be dealt with quantitatively is if the specific activity of the chemical is determined in each tissue for the single dose without prior treatment vs. that observed with a dose after prior treatment with non-labeled chemical.

b. The frequent suggestion that a smaller fraction of dose was absorbed with increasing dose as evidence of saturable absorption may also be inappropriate. There are other possibilities that have to be entertained. In the case of compounds with chemicals of such limited water solubility, such observations may well be explained by limited access to membranes because of low solubility and, more important, the limited solubility in body fluids to carry away the compound from its site of application. The solubilities of these compounds in water are listed as BDE-47 = 11 µg/L, BDE-99 =2.4 µg/L, BDE-209 = <0.1 µg/L, BDE = 0.9 µg/L. The Hughes et al. 2001 study of passage of BDE-209 through mouse skin in vitro would be plagued by this problem unless a perfusion system was used (not stated in the document). Incidentally, the doses applied in this preparation were given in amounts, not concentrations. It is essential to know concentrations to interpret these in vitro studies. The limited solubility could even affect the data obtained with 14C-labeled compounds (e.g. Viberg et al.) because these generally much smaller doses (e.g. 2.2 mg/kg) still exceed the solubility in water by a 4 orders of magnitude in the case of BDE-209. The authors of the papers reviewed may have addressed these issues, but it did not come through in the Toxicological Reviews.

The in vivo studies that employed huge concentrations in the diet would certainly have the same problem. In fact, one wonders whether the lack of clear dose-response in the NTP study of BDE-209 might reflect this problem. Equivalent responses may have been observed at much lower doses. In my view this is a real concern for estimating cancer risk from these data.

3. It is nice to have a reasonably complete discussion of the metabolism of the PDBEs in the IRIS documents. Nevertheless, there is a need to address some confusing aspects of the discussions of uptake, metabolism, distribution, and elimination of compounds in these documents rather than simply reiterating the results. One way of reducing the confusion might be a summary at the beginning of this section that lays out what appears to be happening, even to providing the conclusions of the section up front. This would avoid being surprised by the apparent finding that urinary elimination in mice, for example, appears dependent upon binding of the parent compound to a urinary protein at the end. As the descriptions are laid out, the reader is continuously struck by what appears to be results that appear contrary to general rules (e.g. that non-polar compounds are not efficiently eliminated in the urine).

There are differences among studies related to the apparent extent of metabolism of the compounds and the extent to which metabolites occur in the excreta that are presented without comment. If at all possible, this issue should be driven to conclusions that are useful in making judgments related to how harmful these compounds are. If the information is not useful or conclusions cannot be drawn, this should be stated and the presentation of this material substantially shortened.

Part of the confusion arises from the apparent reliance on measurement of 14C in these studies. These measurements are intermixed with some direct chemical measures, but that is frequently not made clear in the Toxicological Reviews. While these sections read well and are unlikely to be questioned by a casual reader, the informed reader immediately gets distracted by the methodological questions and feels that the apparent conclusions require challenge. Furthermore, questions of metabolism, covalent binding, and distribution are not possible simply by measuring the 14C.

Some specific points in the text of the BDE-47 document are referred to below where the interpretation of ADME data is ambiguous are provided below:

p. 14, 3rd para. This is an example where the legitimacy of the interpretation that there are different amounts absorbed from a dose of radio-labeled compound was administered on day 1 vs. after 10 days of administration of non-labeled compound needs to be questioned.

p. 17, second para in after Metabolism title. The last two sentences appear contradictory.

p. 17, last para. How is "covalently bound" defined (or measured)?

P. 18, 2nd full para. These results for the last day are likely to reflect accumulated metabolites from the prior exposures. Therefore, it is inappropriate to compare the amount of metabolite observed after day 1 to day 10.

p.22, 2nd par. Fifth line. It is not clear where the half-life is being measured. Was it actually in the perirenal fat or was it in the blood? The writing implies that it is in the perirenal fat but the wording is not definitive. This needs to be very clearly stated as most half-life measures are made in blood and that half-life is used to predict turnover in tissues that are sinks (e.g. fat).

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Additional comments

BDE-47

p. 9 Discussion on levels in human milk could be expanded.

p. 17 It is unclear why uptake of BDE-47 in cultured neuronal cells in the presence of 10% horse serum "is likely to be more representative of in vivo conditions".

p. 39 It is unclear why data on exposure to BDE-47 in relationship to thyroid hormone levels cannot be used for deriving RfD. The study of Hallgren and Darnerud provides a NOAEL of 6 mg/kg/day and a LOAEL of 18 mg/kg/day. Changes in mixed function oxidase system that were observed at 6 mg/kg/day may be discounted, as suggested.

p. 40 The important issue of pup vs. litter as the statistical unit is discussed, with the indication that the use of more than one pup/litter in the Eriksson et al., 2002 study may bias the results. Yet, in the footnote, a study by Eriksson et al., 2005 (only in abstract form) with BDE-99 is discussed, in which this same issue was shown not to influence the outcome. Perhaps this should be integrated in the main text.

p. 41 An UF of 3 for single vs. chronic exposure may not be necessary. The concept of "windows of susceptibility" during brain development would argue that chronic exposure may not necessarily result in greater adverse effects, but rather that even a single exposure at a specific sensitive time-point may elicit the highest effect. Eriksson et al. have shown with other compounds (e.g. BDE-99) that a single exposure on PND10 is the most sensitive time point. This comment would apply to other BDEs as well.

BDE-99

p. 29 Data on nicotinic receptor changes from the Viberg et al. 2004b study could be presented here. Refer here to data on p. 49.

p. 46 Here and elsewhere, when referring to Casarett and Doull's Toxicology textbook, the most recent edition (2001) could be used.

p. 48 Better references than Klaassen, 1996 or Ankenberg, 2003 could be used to discuss distribution and functions of cholinergic receptors.

p. 48 Discussion of the Ankenberg, 2003 study could be shortened, as it is not of much relevance. It is also presented unclearly.

p. 49 Choose reference other than Klaassen 1996, to indicate effects of atropine. Also, specify % decrease of 3H-QNB binding.

p. 51 Section 4.4.3 (neurotoxicity) should be renamed, as several previous sections (e.g 4.3.1, 4.3.2) already deal with developmental neurotoxicity. Eriksson is also a co-author of the proteomics paper by Alm et al. discussed here.

p. 53 Additional in vitro mechanistic experiments (see references above) may be added here and in section 4.5.3 on mode of action.

p. 56 Data on oxidative stress and BDEs (see references above) may be added here.

p. 57 In section 4.7.2 the reference Branchi et al. 2002 may be added to indicate lack of gender-specific effects.

p. 65 Use of an UF of 3 for extrapolation from subchronic to chronic may not be necessary (see comment for BDE-47). Also, given the availability of several developmental neurotoxicity data on BDE-99, an UF of 10 for database uncertainty seems excessive.

p. 68 The overall confidence in the RfD determined for BDE-99 is listed as low, same as in case of BDE-47. However, given the existence of multiple studies corroborating the main conclusions, confidence level may be raised to moderate.

BDE-153

p. 18 The % decrease of nicotinic receptors in the study by Viberg et al. 2003a could be indicated (see p. 23).

p. 28 UF of 3 for subchronic to chronic may not be necessary (see comments for BDE-47).

BDE-209

p. 12 The possible debromination of BDE-209 to lower bromine congeners may be better discussed, if possible. Should also environmental debromination be mentioned?

p. 16 Half-life may not be necessarily directly linked to rapid metabolism.

p. 38 Content of section 4.5.1 is not in tune with title of section.

p. 58 An UF (e.g. 3) for deficient available database may be added. While, differently from other BDEs, studies are available on subchronic, chronic and developmental toxicity (all yielding NOAELs or BMDs substantially higher than that from the stuies of Viberg et al), no other developmental neurotoxicity evidence is available.

- Report is at times repetitive, and could be shortened. For example, results of the Viberg et al. 2003a study are described on p. 10, 33-34, 41-42, 50 and 53.

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Miscellaneous comments

General

The presentation of the data and the derivation of RfDs might benefit from a unified presentation in a single document, instead of four separate documents. In particular, similarities and differences in ADME could be addressed in a cohesive manner.

Specific

BDE-47
Section 3.1, last sentence: It seems that the relative absorption rates of rats and mice are not qualitatively the same in the two studies compared, contrary to what is stated.

Page A-2: The AIC is highest for the selected linear model, which fits best in terms of the p-value. For BDE-209, page A-10, low AIC seems to be one of the selection criteria, along with high p-value. I believe the selection criteria should be consistent. AIC is sometimes used as a weight for averaging the results of several models. It might be a better criterion than p-value.

Page A-2: The indication is that modeling was done for both males and females. But, there were no female data.

BDE-99
Page 81: The Hill model fits best in terms of its having the highest p-value. However, it also has the highest AIC. This isn't consistent with BDE-209, where low AIC is one of the criteria for model selection. AIC should be used consistently.

BDE-209
Page 21: How can 99 mg/kg-day be characterized as a NOAEL in the study by Carlson (1980) when it was associated with a statistically significant increase in the liver-to-body-weight ratio? (Same in Table 2, page 19)

Page 32, lines 5-8: I strongly disagree with the conclusion by Eriksson et al. (2005) regarding litter-based studies. This sentence should be re-stated so that it doesn't sound like this conclusion is accepted as fact by EPA. In general, this conclusion is erroneous.

Page 34, lines 13-15: There is something wrong with the sentence. A decrease in disruption of habituation is not an adverse effect.

Page 38, lines 6-3 from bottom: There is something wrong with this sentence. Thyroid tumors were seen in females as well as males, but they were not significant in either sex. Why is NTP (1984) cited?

Page 39, lines 12-11 from bottom: There is the same problem regarding the statement about thyroid tumo