Critique of the U.S. Environmental Protection Agency's Draft Toxicological Review of Decabromodiphenyl Ether (BDE-209) (CASRN 1163-19-5)

Prepared for The Dow Chemical Company

Catherine E. Campbell, PhD
John M. DeSesso PhD, DABFM, DABFE, Fellow ATS

Mitretek Systems
3150 Fairview Park Drive
Falls Church, Virginia 22042

29 January 2007

This critique of the U.S. Environmental Protection Agency's (EPA) Toxicological Review of Decabromodiphenyl Ether (BDE-209) (Draft; CASRN 1163-19-5) was prepared for The Dow Chemical Company. This assessment is organized to address the EPA's review of the literature pertaining to the neurotoxic effects of this compound (BDE-209) and the agency's recommendations for establishment of an oral reference dose (RfD) for BDE-209. Major comments concerning the scientific relevance of selected studies and calculation of RfD are addressed below.

Selected Studies

The EPA has used four studies to assess the toxic effects of BDE-209 on the development of rodents. Of these studies only two address the potential neurotoxic effects of BDE-209 during development, both of which were performed by the same researchers using the same experimental protocols, one in rats and one in mice (Viberg et al. 2006; Viberg et al., 2003). There are some critical flaws in these studies which should raise serious questions regarding the ability of these studies to be used as an assessment of the toxicity of BDE-209 or to set an oral RfD.

Tests used to assess behavior. There are many tests commonly used to assess behavioral changes in rodents, including elevated plus mazes, open field tests, and rotarods to name a few (Ulbrich et al., 1996). These tests have been utilized for many years using both rat and mouse models. The behavioral test utilized by Viberg et al. (2003, 2006) has not been validated either within or outside of their research group, and the details of the controls for this test are not well described. For example, the animals should be run through the test, randomized by treatment, so that all of the animals exposed to a given dose are not run through the test sequentially. The test platform should be adequately cleaned so that pheromones and other stress hormones are not detectable by subsequent animals. Additionally, the test should be validated with a large number of control animals to assess the normal distribution of behaviors in both rats and mice. More practically, behavioral changes should be assessed using more than one assay. This is addressed in the EPA document, but could be emphasized.

The selection of individual animals from within a treatment cohort should be randomized and blinded from the assessor, and animals should be randomly selected from within each litter for treatment, rather than treating entire litters with a single dose. This would more adequately control for litter effect. The issue of litter effect and cohort randomization has been raised previously (DeSesso and Mavis, 2003; see Appendix A)

Statistics. The statistics chosen for the Viberg experiments are adequate for the measures taken. However, at each individual measurement time point, there are 27 pairwise statistical comparisons made. A correction for multiple comparisons (a Bonferroni correction or false discovery rate) should be applied, to ensure that the potential for false positives is accounted for. Applying a Bonferroni correction to these data results in a p-value threshold of significance of 0.002, much lower than that used in this study, and lower than any of the p-values observed. Additionally, the three time point measurements (2 months, 4 months, and 6 months) are not independent variables and this should be accounted for as well.

One additional concern is that significant effects were measured over time in the control group. This concern has also been addressed by DeSesso and Mavis (2003; see Appendix A). This effect was seen concerning the habituation ratio of animals as they age. Both control and high dose animals showed significant increases in this ratio over time, yet the EPA report clearly uses this information as evidence that the toxic effect of PDBE-209 gets worse over time (pp. 34, 53). Until inconsistencies such as these are clarified, it is difficult to draw conclusions from the Viberg studies.

Concentration in Brain. The dosage of PDBE-209 used in the ^{14}C labeled radioactive experiments was unclear in the original Viberg experiment (2003). In this reference, the dose is referred to in μCi/kg body weight. In a published critique of Viberg, Vijverberg et al. (2004) estimate that this can be calculated as 13.8 nmol, but are unclear whether that is per animal or per kg body weight. The EPA report inaccurately states that 2.22 μg/kg of ^{14}C labeled BDE-209 was used (p. 34). The actual dose used is not clear in the original methods, and this confounds the amount that is measured in various organs. The dose measured in the brain is expressed in parts per thousand of the total dose given, although the EPA assessment (p. 10) describes this as an exception and as the second highest amount seen in any organ, although only three organs were

measured, and in liver up to 13% of the total dose was measured. This deceiving description of preferential distribution in brain tissue has several flaws.

- The blood brain barrier in rats begins to form around day 6 postpartum, with only 30% of the capillary surface area formed by day 20 and over 80% of the barrier formed by day 40 (Sibbons et al., 1996). Development of the blood brain barrier follows a similar schedule in other rodents. In mice, most maturation occurs during post natal days 12 – 24 (see discussion in Watson et al [2006]). Therefore, many molecules that would not cross this barrier in adult mice could cross in neonates. In humans, this barrier begins to form by the end of the first prenatal trimester. Consequently, the brain of the human infant is far better protected than that of the neonatal mouse.
- In mice, the dose observed in the brain was highest when the animals were dosed at day 10 rather than at days 3 or 19, yet the behavioral effects observed were maximal when the dose was administered at day 3. This discrepancy is not explained. Additionally, the argument is made that the day 3 dose is acting during the brain growth spurt which occurs around day 10, yet even though it takes less that 24 hours for BDE-209 to reach the brain, no changes were observed when the dose was administered at day 10.
- The authors have not analytically identified the source of radioactivity in the brain, so they are unable to discern whether this is due to parent compound or a metabolite. To state that it must be a metabolite ignores the striking developmental changes that occur in the mouse brain between days 3 and 10, which could alter the sensitivity of the mouse brain to these effects. It is not reported how the radiolabeled material was synthesized and chromatographic or other characterization of the test material was not supplied (see also comments in DeSesso and Mavis, 2003; Appendix A). Given that the rodent brain is altricial relative to a newborn human infant (Rodier, 1980, Bayer et al., 1993; Vidair et al., 2004) and that the human blood-brain barrier has greater integrity in infants than neonatal rodents (Adinolfi and Haddad, 1977; Bonati et al., 1981), it is difficult to extrapolate these effects to humans. Therefore, there is no guarantee that the differences seen are real or caused by BDE-209 entering the brain.
- The majority of human studies have looked at the concentrations of BDE in serum. It would be preferable to measure this sample in animals as a direct comparison to the amounts observed in other organs and in humans.

RfD Calculations

The EPA has used the Viberg (2003) study as its principal study to determine the RfD of BDE-209. There are several plausible reasons for this.

- This is the only study to look at developmental toxicity and behavior.
- The NOAEL is lower than that observed in other studies.
- Data from similar rat studies support the mouse data (albeit with similar flaws in analysis).

However, this study has several critical flaws (discussed above) and the reliance on a single study that results in an RfD orders of magnitude below what would be calculated from other studies is a significant problem.

In addition to the reliance on a single study, the assumptions made to calculate the multipliers and arrive at an RfD are also questionable. There is a multiplier of 10-fold for translating this research to humans, and one of 3-fold for translating this single-dose study to a more chronic exposure model. These two multipliers are sound, but the third multiplier (10-fold) to account for sensitive groups, is neither founded by any of the models tested nor by the epidemiological studies in humans. There is no evidence that groups exist that are sensitive to this chemical. It is understandable that the EPA would rather err on the side of caution, but the RfD that they have derived (7 μg/ kg/ day) is lower than the concentration observed in the sera of workers exposed to BDE-209 on a routine basis (up to 10 μg/ kg). Considering that the exposure of these individuals would be expected to be at least an order of magnitude higher than this and the concentrations in the serum in low-exposure individuals is approximately 1 μg/ kg , the RfD seems out of synch with the amounts of BDE-209 currently present in the general population, with no ill effects.

In addition, the relevance of these studies to human exposure during development is unclear. The period of development analogous to that studied in mice occurs *in utero* in humans. It is unclear whether this molecule crosses the placenta, whether it could be absorbed by the fetus, or whether it could be transmitted to the fetus via the umbilical cord. Further studies are needed to translate the effects observed in mice and rats to humans.

Other Comments

The primary basis for the oral RfD calculations are based on the Viberg studies. Neither of these studies provided assurance that they were Good Laboratory Practices-compliant. Beyond the previously detailed scientific issues, the studies lacked guarantees regarding the training and oversight of technical personnel; the methods of collecting, storing and auditing of raw data; the characterization and archiving of the test material; as well as the use of new and unvalidated model for collecting data. Basing the calculation of an oral RfD on such a poorly documented, weak data set is highly questionable.

There are a few other inconsistencies in the EPA document that should be addressed. The document refers to BDE-209 as having a high molecular mass and that this high mass is inconsistent with its bioavailability in humans. Although the mass of BDE-209 is sufficient whereby it may not diffuse across lipid membranes, at 989 Daltons it is just small enough that its bioavailability (based solely on size) would not necessarily be expected to be zero.

In the discussion of the critical dose necessary for an observed effect, BDE-209 is alternately referred to as having a long half-life and a short half-life. Since approximately 90% of BDE-209 seems to be eliminated within 72 hours of oral exposure, and BDE-209 does not preferentially accumulate in adipose tissue (Morck et al. 2003), it seems unlikely that BDE-209 would significantly bioaccumulate in tissues. In addition, serum studies in Swedish workers exposed to BDE-209 (Sjodin et al., 1999) were not correlated with age and showed significant decreases in levels of BDE-209 after 30 days without exposure.

Throughout toxicological review, concentrations are expressed as mol/kg or as g/kg with the denominator being either total weight or lipid weight. Additionally, some of these concentrations from radioactive studies have been converted from curies/ kg to g/kg with no discussion of the basis for the conversion (e.g. the specific activity of the label). Consistent measures of concentration should be used throughout the document, and where conversions have been made, a discussion of the logic behind the conversion should be included.

Conclusions

The EPA assessment of BDE-209 neurotoxicity points out some of the flaws of the Viberg experiments, however, EPA's choice to use this study as their principle study is problematic. Although the EPA is clear about the weak points of the studies it has chosen, it has missed several points in its assessment and more time is spent discussing uncertain results of the studies. RfDs should be derived from sound data, as arbitrary multipliers are generally added to experimental doses as a part of the RfD calculation. As such, the decision to base the RfD exclusively on a single study is weak and further more conclusive results are needed to determine a more reliable RfD.

References

Adinolfi, M, SA Haddad. 1977. Levels of plasma proteins in human and rat fetal CSF and the development of the blood-CSF barrier. *Neuropadiatrie* 8: 345-353.

Bayer, SA, J Altman, RJ Russo, X Zhang. 1993. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* 14: 83-144.

Bonati, M, R Latini, G Marra, BM Assael, and R Parini. 1981. Theophylline distribution in the premature neonate. *Dev Pharmacol Ther* 3: 65-73.

DeSesso, J., RD Mavis. 2003. *Critique of neurobehavioral derangements in adult mice receiving decabrominated diphenyl ethers (PBDE 209) during a defined period of neonatal brain growth.* Mitretek Systems, Falls Church VA.

Morck, A, H Hakk, U Orn. 2003. Decabromodiphenyl ether in the rat: absorption, distribution, metabolism and excretion. *Drug Metab Disp* 31:900-907.

Rodier, PM. 1980. Chronology of neuron development: animal studies and their clinical implications. *Develop Med Child Neurol* 22: 525-545.

Sibbons, PD, GL Aylward, CV Howard, D van Velzen. 1996. A quantitative immunocytochemical analysis of total surface area of the blood brain barrier in developing rat brain. *Comp Haematol Int* 6: 214:220

Sjodin, A, L Hagmar, E Klasson-Wehler. 1999. Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. *Environ Health Perspec* 107: 643-648.

Ulbrech, B, AK Palmer. 1996. Neurobehavioral aspects of developmental toxicity testing. *Environ Health Perspec* 104: 407-411.

Viberg H, A Frederiksson, E Jakobssen. 2003. Neurobehavioral derangements in adult mice receiving decabromodiphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol Sci* 76:112-120.

Viberg H, A Frederiksson, E Jakobssen. 2006. Changes in spontaneous behavior and altered response to nicotine in the adult rat, after neonatal exposure to brominated flame retardant, decabrominated diphenyl ether (PDBE 209). *NeuroToxicology* (Aug 23; epub ahead of print)

Vidair, CA. 2004. Age dependence of organophosphate and carbamate neurotoxicity in the postnatal rat: extrapolation to the human. *Toxicol Appl Pharmacol* 196: 287-302.

Vijverberg, HPM, M van den Berg. 2004. Letter to the Editor. *Toxicol Sci* 79: 205-206.

Watson, R. E., J. M. DeSesso, M. E. Hurtt, and G. D. Cappon, (2006) "Postnatal Growth and Morphological Development of the Brain: A Species Comparison," *Birth Def Res B Develop Reprod Toxicol*, **77**: 471-484.

APPENDIX A

Critique of
Neurobehavioural Derangements in Adult Mice Receiving Decabrominated Diphenyl Ethers (PBDE 209) during a Defined Period of Neonatal Brain Growth

by

H. Viberg, A. Fredriksson, E. Jakobson, U. Orn and P. Eriksson

(*Toxicological Sciences* **76**: 112-120 [2003])

John M. DeSesso, PhD, DABFM, DABFE, Fellow ATS
Richard D. Mavis, PhD, DABT

Mitretek Systems
Falls Church, Virginia 22042

24 October 2003

Executive Summary

The results of this study do not support any significant conclusions about the uptake of PBDE 209 by the neonatal mouse brain. The radiolabeled PBDE 209 administered to mice was not characterized with respect to purity nor was the radioactivity that was found in tissues characterized with regard to its chemical identity. The small amounts of radioactivity measured in brain, heart, and liver (tenths of a percentage of the dose) could have been contaminants of the starting radiolabeled PBDE 209. In addition, the form of the data presented does not allow comparison among uptake by the three tissues studied. It is clear that the total radioactivity present in the brain in two groups of mice dosed at different times does not correlate with the behavioral effects measured. The authors have attempted to explain this finding by suggesting, without supporting data, that a metabolic product of PBDE 209 could be the active component. An explanation of greater feasibility is that there is no correlation, and therefore no causality, between the brain content of radioactivity and the measured behavioral parameters. This latter explanation is supported by the observation of a significant behavioral effect in control animals that received no PBDE 209.

Potential conclusions regarding neurobehavioral effects of PBDE 209 on the neonatal mouse based on this study are weak because of a number of flaws in the experimental design and the isolation of the experimental findings. Study weaknesses include ignoring the possibility of a litter effect, unspecified procedures for random selection of test subjects, and limited behavioral testing, and ignoring a statistically significant effect in control (untreated) animals. Such an effect in control animals raises questions about the credibility of the experimental system and negates the significance of that effect in the treated animal groups.

Finally, the use of extremely large doses compared to any feasible human exposure and the study of a phase of rapid brain growth in neonatal mice that does not occur in humans *ex utero* raise serious questions about extrapolating the results of this study to human health risk assessment.

Introduction

The research article entitled "Neurobehavioural Derangements in Adult Mice Receiving Decabrominated Diphenyl Ethers (PBDE 209) during a Defined Period of Neonatal Brain Growth" by Viberg et al. suggests that PBDE 209 administered to mouse pups on post natal day 3, and highly brominated PBDEs in general, are "possible developmental neurotoxic agents." The authors administered 2.22 or 20.1 mg PBDE 209/kg body weight on one of postnatal days 3, 10, or 19 and examined spontaneous behavior at 2, 4 and 6 months of age. Additionally, ^{14}C-labeled PBDE 209 was administered on each of the 3 postnatal days and distribution of radiolabel was determined at 24 hours and 7 days post administration. Based on the presence of radiolabel in the brain at 24 hours and 7 days and significant differences in habituation ratios for spontaneous behaviors between high dose PBDE 209-treated mice and controls at 2, 4 and 6 months, the authors assert that PBDE 209 is a persistent developmental neurotoxicant. This document is a critical analysis of that paper; emphasis is given to the study design, methods, quality of the data and interpretation and conclusions.

The research can be divided into two phases. The first phase deals with the distribution and fate of PBDE 209 within the exposed pups. The second phase investigates the impact on adolescent and adult behavior of male mice exposure to high doses of PBDE 209 during the early postnatal life. The following paragraphs will discuss the quality of research with respect to general considerations and comments on each of the two phases.

General Considerations

An important potential confounding factor in developmental and reproductive toxicology studies is known as the "litter effect." This term refers to the tendency of the offspring in the litter of a given female to respond more similarly to a chemical or physical challenge than offspring born of other females. In order to minimize potential biasing of the results of these types of studies, special attention should be given to ensuring that (to the extent possible) tests/observations are made on offspring from multiple litters. A major weakness in the present study is that not only were no such precautions taken, but also measurements of ^{14}C-PBDE 209 uptake were taken from only two complete litters per time period. If a litter effect is present, it could have a large impact on the results of the study.

The authors mention several times that animals were selected "randomly" for various aspects of the study (e.g., behavioral tests at various months). It is important to know how the randomization has been achieved. Most experienced laboratories have procedures to ensure that there is no investigator bias. These procedures often use random numbers programs. The procedures followed by the authors were not described.

Distribution and Fate (Radiolabeled PBDE 209)

There are numerous uncertainties surrounding the data in this phase of the study. The findings mentioned when taken together pose significant problems for the interpretation of the data.

A major question relates to the purity of the radiolabeled PBDE 209. Previous publications by these authors report that the purity of the radiolabeled preparation exceeded 98%. If a preparation of the same purity were used here, there would be about 1.5% impurities. The amount of radioactivity discerned in the brain samples is quite small. The authors' assumption about the identity of the relatively tiny amounts of radioactivity in the whole organ preparations is unsubstantiated. Because the amounts of radioactivity found in the brain are so small (only tenths of a percent of the dose), the radioactivity could potentially be an impurity (which made up ~1.5% of the labeled PBDE preparation). A credible study would have reported not only the radiochemical purity of the injection material (that is, a figure showing the results of a chromatograph), but also the chemical identity of the radioactivity found in tissues after administration to the animals (also by means of chromatography). Consequently, it is not possible to state with certainty the identity of the radiolabeled chemical in brain. The only conclusion that can be made from these studies is that the brain contains ^{14}C.

It is well established that PBDE 209 is lipid soluble. If one assumes that the counts in tissues are PBDE 209, one would expect PBDE 209 (and therefore the radioactivity) to partition into tissues in proportion to (1) the lipid content and (2) the mass of the tissue or size of the organ. This is important to note because the authors express their results as counts per organ. This leads to some interesting issues. For instance, the size of a mouse brain on postnatal day

(PND) 3 is considerably smaller than that of a PND 10 mouse. If PBDE 209 is evenly distributed in brain tissue, the sample from a PND 10 brain would be expected to have a higher number of counts based on size alone. This makes interpretation of Figure 6, wherein the data are expressed as radioactive counts per whole organ, impossible.

Inspection of the reported data reveals some other surprises. An example is the similar amounts of radioactivity in the brain and heart despite the fact that the heart has much less fat than the brain. At face value, this argues against selective uptake of PBDE 209 by the brain. The point of this discussion is that one needs to consider the sizes and lipid contents of the organs studied to interpret these data in any way that would shed any light on the authors' hypothesis that there is selective uptake of PBDE 209 by the developing brain.

To summarize these points, the radioactive uptake study would have been more informative (1) if the counts were expressed per gram of tissue wet weight, per gram of tissue lipid, per gram of tissue protein, or (preferably) all three, and (2) if the chemical identity of the radioactivity in tissues were determined. The data expressed are suggestive but not conclusive. The increasing number of counts in the brain during the intervals studied could simply result from an increase in the size of the brain from day 1 to day 7 after administration.

Because the authors contend that the effects seen are neurotoxic, it should be required that a target tissue be specified. The brain is a large histologically and anatomically diverse organ. Had at least one brain been prepared for radioautographic examination, the authors could have determined the location of the radiolabel with respect to compartments such as the gray matter (where the neurons are located), the white matter (which is dominated by lipid-rich myelin), the vascular bed (i.e., the arteries, veins and blood) and the capacious ventricular system (the cavities within the brain that are filled with cerebrospinal fluid). This information would bear directly on the authors' hypothesis and could shed light on the plausibility of any potential direct effect on the nervous system. In the absence of this type of data, statements about neurotoxicity are circumstantial and speculative.

Behavioral Findings

For the measured behavioral parameters, only the mice dosed at 3 days showed any significant behavioral changes. The behavioral measurements are hard to interpret, but in the absence of more complete data it seems premature to identify them as “neurotoxic effects.” While there appears to be a statistically significant difference in the habituation capacity of the mice treated at 3 days with the high dose of PBDE 209, there is also a decrease in habituation capacity in the control mice over time. The calculation of habituation ratio is a derived value based on the recorded mean values for locomotion, rearing and total activity. By expressing habituation rate as a ratio, the authors have likely diminished the impact of variability across animals. This could contribute to the apparent increase in effect at 6 months of age. Furthermore, there appears to be a shift in the response curve for the control animals as they age (e.g., in the 0-20 min interval, rearings were ~1600, ~2000 and ~ 2500 in 2-month old, 4-month old and 6-month old control animals, respectively, whereas rearings were virtually undetectable in each of these groups at 40-60 minutes. Aside from rearings, the authors acknowledge the change in total activity with age. Thus, it appears that the response curves for the control animals are not parallel across these ages. It is difficult to interpret the impact of treatment on responses that change with age in a nonlinear manner in control animals. Additionally, as mentioned above, the “random” selection of 10 subjects out of 50 for testing was not described. It is possible that the selection methodology could have resulted in the choosing of the least active animals or of animals from the same litters.

It is important to note that habituation can be measured in variety of behavioral tests (e.g., acoustic startle, cognitive function using mazes) in addition to the locomotor activity tests used by the authors. It would be more convincing if the habituation effects had been seen in a variety of tests. This information seems to be a missing key supporting element in the authors’ proposed concept. In addition, the use of the term "habituation" with respect to the decreased activity seen during a motor activity session is controversial. Habituation occurs when an organism ceases to respond to an essentially irrelevant stimulus after several exposures. In the case of motor activity, the activity measured is spontaneous without any applied stimuli. In this case, the term "adaptation" is more accurate.

Discussion

The authors contend that the purpose of their investigation was "to see whether the tissue distribution and/or a defined critical phase of brain development is the underlying cause of developmental effects, a study of the uptake and retention of ^{14}C-labelled PBDE 209 at different stages of neonatal mouse brain development was conducted" (page 6, paragraph 1). This objective is certainly not accomplished by this study. As mentioned above, selectivity of uptake by the brain has not been demonstrated. Furthermore, the radioactivity found in the brain clearly does not correlate with the alleged behavioral effects. In the Discussion (on page 19 of the manuscript), the authors admit this but then attempt to use this inconsistency as evidence that a metabolite of PBDE could be the active agent in the mice exposed at 3 days. Without information about (1) the metabolism of PBDE 209 in neonatal animals and (2) the identity of the ^{14}C-labeled chemical found in the tissues, such an explanation is merely conjecture. Other hypothetical explanations could be developed to explain such an inconsistency. Perhaps the most obvious alternative explanation is that there is a lack of correlation, and therefore lack of causality, between the brain content of radioactivity and the measured behavioral parameters. This latter explanation is supported by the observation of a significant decrease in habituation capacity in control animals that had not been exposed to PBDE 209.

Any conclusions regarding the neurobehavioral effects observed in this study are weak due to shortcomings in experimental design and because only a single experimental system was used. Shortcomings include failure to consider the existence of a litter effect, undescribed methods for randomization of test subjects, limited behavioral testing, and failure to address the statistically significant neurobehavioral effect observed in untreated animals. The existence of such an effect in control animals degrades the credibility of the experimental system and negates the significance of that effect in the treated animal groups.

There are two issues that bear on the application of this study to the assessment of risk to human health. First, the doses used in this study are many times larger than any reasonably expected human exposure. Even at these extreme doses, behavioral effects were seen only for the high (20.1 mg/kg) dose. At the lower (2.22 mg/kg) dose there were no differences from control values. Thus, no effects of this type could be expected in humans as a result of exposure

to these chemicals. Second, the time of rapid brain growth in mice begins postnatally and persists for only about 21 days; in humans, rapid brain growth begins during intrauterine life during the 5th month of gestation, and continues until about the age of 2½ years. Thus, there are major differences with regard to exposure routes. Human exposure is via the placenta for the early phase of rapid brain growth, which occurs during the last 4-5 months of gestation. The occurrence of the corresponding early phase of rapid brain growth in mice after birth (*ex utero*) required that exposure in this mouse study was by gastrointestinal absorption through an immature intestinal tract in the mouse. These differences between the pathway of human exposure and the experimental exposure of mice in this study raise serious questions about extrapolating the results of this study to human health risk assessment.

Concluding Remarks and Recommendations

The conclusions of this study are weakened by its inadequate experimental design. Study weaknesses include ignoring the litter effect, unspecified procedures for random selection of test subjects, limited behavioral testing, failure to characterize both the purity of the radiolabeled starting compound and the chemical identity of the radioactivity found in tissues.

Similar studies have been published previously by this same group of investigators. The compounds tested previously included other PBDEs, PCBs, organophosphates, and pyrethroids. At first glance, the amount of research seems overwhelming in terms of the number of studies and the consistency of their findings. A brief look at the other papers, however, reveals the same shortcomings leading to significant uncertainties in the data as in the present paper. A closer look shows a surprising similarity of rather specific neurobehavioral effects for several disparate classes of chemicals (PCBs, pyrethroids, organophosphates, and PBDEs).

It is possible that the experimental system used to test these agents is nondiscriminative. That is to say it exhibits similar behavioral changes in response to many or all chemical challenges during a phase of rapid brain growth in mice that does not occur at a corresponding phase of development in humans. Consequently, these results may not be relevant for human health risk assessment. The scientific community would be well served to have a more robust data set from which to make decisions regarding human risks. Such a data set would be

generated utilizing a more sophisticated experimental design and measurement of a more complete set of experimental parameters, as mentioned above.

With regard to the present study, there are statistically significant differences in a specific behavioral test in mice treated with extremely high doses. However, there are so many weaknesses in design that the findings should not be used for risk assessment. They should only be considered as the basis for designing robust, hypothesis-driven experiments to determine whether PBDE 209 poses any risk at environmentally relevant exposure levels.

With respect to the body of literature upon which the authors rely for the importance of their findings, it is suggested that an extensive analysis of the whole series of papers be analyzed, critiqued, and published. If the entire body of data does indeed suffer from the same flaws as the present paper, it is in the public interest to communicate the limitations of the database to the scientists in the regulatory community.