Letter to the Editor

Development of A Reference Dose for BDE-47, 99, and 209 Using Benchmark Dose Methods*

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Eleven recently completed toxicological studies were critically reviewed to identify toxicologically significant endpoints and dose-response information. Dose-response data were compiled and entered into the USEPA's benchmark dose software (BMDS) for calculation of a benchmark dose (BMD) and a benchmark dose low (BMDL). After assessing 91 endpoints across the nine studies, a total of 23 of these endpoints were identified for BMD modeling, and BMDL estimates corresponding to various dose-response models were compiled for these separate endpoints. Thyroid, neurobehavior and reproductive endpoints for BDE-47, -99, -209 were quantitatively evaluated. According to methods and feature of each study, different uncertainty factor (UF) value was decided and subsequently reference doses (RfDs) were proposed. Consistent with USEPA, the lowest BMDLs of 2.10, 81.77, and 1698 μg/kg were used to develop RfDs for BDE-47, -99, and -209, respectively. RfDs for BDE-99 and BDE-209 were comparable to EPA results, and however, RfD of BDE-47 was much lower than that of EPA, which may result from that reproductive/developmental proves to be more sensitive than neurobehavior for BDE-47 and the principal study uses very-low-dose exposure.

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants, which have been widely applied in industrial products and house wears. There are 209 PBDEs homologs among which the most frequently ones in China include BDE-47, -99, -209.

PBDEs have increasingly captured the attention of scientists and policymakers. However, there are few recommendations for PBDEs intake and health risks may not be easily quantified. U.S. Environmental Protection Agency (USEPA) conducted health assessments on several PBDEs congeners after a comprehensive review of toxicity data and proposed oral Reference Dose (RfD) latest updated in 2008. Since all the EPA oral RfD were focused on neurobehavioral effects, this paper critically evaluated toxicological database on other endpoints along with recent neurotoxicity study results. USEPA's benchmark dose software (BMDS) Version 1.4.1 were used to identify benchmark dose (BMD) and benchmark dose low (BMDL) based on data from selected literature. According to methods and feature of each study, different uncertainty factor (UF) value was decided and subsequently RfD were proposed.

The selection of appropriate studies is based on animal studies whose route of exposure were similar to human, the quality of the studies, and the relevance and reporting adequacy of the endpoints. More specifically, the following requirements should be met:

1) Only studies in which animals are administered of oral exposure are included as this study addresses human oral exposure of PBDEs.

2) Studies should show a statistically or biologically significant graded monotonic dose-related trend in the selected endpoints.

As almost all toxicology studies of PBDEs are consisted of continuous data, when individual data are unavailable (which is usually the case in published reports), the number of subjects, means of response variable, and measure of response variability [e.g., standard deviation (SD), standard error (SE), or variance] are needed.

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For the nine selected studies, we used USEPA's benchmark dose software (BMDS) Version 1.4.1 to calculate BMD which following the six-step process for BMD analysis developed by USEPA, including: (1) choice of a BMR, (2) selecting a set of models, (3) assessing model fit, (4) model selection when BMDLs are divergent, (5) model selection when BMDLs are not divergent, and (6) data reporting.

A total of 91 endpoints across nine studies for three major PBDE congeners (BDE-47, -99, -209) were evaluated for applicability to BMD modeling (Table 1). Based on the nature of the significant endpoints, the following categories of endpoints were identified across the studies: (1) thyroid, (2) neurobehavior, and (3) reproductive and developmental. After conducting modeling for each of these 91 endpoints, 68 endpoints were eliminated from further consideration because of they could not be successfully modeled. For each of the remaining 23 endpoints, the BMD and BMDL values were chosen to represent these endpoints.

The studies considered for quantitative evaluation included all multi-dose studies discussed below: one reproductive/developmental study for BDE-47[1], three studies for BDE-99 two thyroid studies[2-3] and one reproductive study[4], and two neurobehavior studies for BDE-209[5-6]. For each of these studies, endpoints were selected for BMD modeling on the basis of toxicological significance, relevance to humans, whether effects were test-related, evidence of a dose-response, statistical significance, and severity of effect.

Because all the data are continuous measurements, we use 1 standard deviation as a benchmark response (BMR) in BMD modeling for each type of endpoint. BMDs and BMDLs for each of the 23 successfully modeled endpoints were identified and are presented in Table 2. A total of 4 reproductive/developmental endpoints of BDE-47 were compiled, along with 9 thyroid and 7 reproductive/developmental endpoints of BDE-99 across two generations and 3 neurobehavior endpoints of BDE-209. There is a widespread range of values across endpoints. Results of significant BMDLs are separately discussed by different PBDE congeners below and shown in Table 2, and the lowest BMDLs of each congener were selected to develop an RfD.

Table 1. Endpoints Evaluated Using the Benchmark dose Modeling Software

Critical Effect	Study Reference
BDE-47	
Reproductive and Developmental endpoints	
Testicle/body weight	He et al. (2011)[1]
Epididymis/body weight	He et al. (2011)[1]
Ovaries/body weight	He et al. (2011)[1]
Uterus/body weight	He et al. (2011)[1]
Thyroid/body weight	He et al. (2011)[1]
Cerebrum/body weight	He et al. (2011)[1]
BDE-99	
Thyroid endpoints	
Parent (F0) generation endpoints	
TSH	Alonso et al. (2010)[2]
T4	Alonso et al. (2010)[2]; Kuriyama et al. (2007)[3]
FT4	Alonso et al. (2010)[2]; Kuriyama et al. (2007)[3]
T3	Alonso et al. (2010)[2]; Kuriyama et al. (2007)[3]
FT3	Kuriyama et al. (2007)[3]
First (F1) generation endpoints (Male/Female)	
T3	Kuriyama et al. (2007)[3]
FT3	Kuriyama et al. (2007)[3]
T4	Kuriyama et al. (2007)[3]
FT4	Kuriyama et al. (2007)[3]
Neurobehavior endpoints	
Brain region volumes (HVC, RA, Mass)	Eng et al. (2012)[7]
Reproductive and Developmental endpoints	
Body weight	Kuriyama et al. (2005)[4]
Liver weight	Kuriyama et al. (2005)[4]
Thymus weight	Kuriyama et al. (2005)[4]
Thymus/body weight	Kuriyama et al. (2005)[4]
Spleen weight	Kuriyama et al. (2005)[4]
Spleen/body weight	Kuriyama et al. (2005)[4]

Continued

Critical Effect	Study Reference
Testis weight	Kuriyama et al. (2005)[4]
Testis/body weight	Kuriyama et al. (2005)[4]
Epididymis weight	Kuriyama et al. (2005)[4]
Epididymis/body weight	Kuriyama et al. (2005)[4]
Seminal vesicle weight empty	Kuriyama et al. (2005)[4]
Seminal vesicle/body weight	Kuriyama et al. (2005)[4]
Prostate weight	Kuriyama et al. (2005)[4]
Prostate/body weight	Kuriyama et al. (2005)[4]
Spermatid	Kuriyama et al. (2005)[4]
Daily sperm production	Kuriyama et al. (2005)[4]
Sperm number	Kuriyama et al. (2005)[4]
Abnormal sperm	Kuriyama et al. (2005)[4]
LH	Kuriyama et al. (2005)[4]
Testosterone	Kuriyama et al. (2005)[4]
Uterine weight	Kuriyama et al. (2005)[4]
Implantations per litter	Kuriyama et al. (2005)[4]
Viable fetuses per litter	Kuriyama et al. (2005)[4]
Fetal weight per litter	Kuriyama et al. (2005)[4]
Mounting latency	Kuriyama et al. (2005)[4]
Intromission latency	Kuriyama et al. (2005)[4]
Ejaculatory latency	Kuriyama et al. (2005)[4]
Intromission frequency	Kuriyama et al. (2005)[4]
Number of penetrations before the first ejaculation	Kuriyama et al. (2005)[4]
BDE-209	
Neurobehavior endpoints	
Fetal brain weight (g)	Chi et al. (2011)[5]
Fetal brain/body weight (g)	Chi et al. (2011)[5]
Hemisphere height (PND 72)	Biesemeier et al. (2011)[8]
Cortex vertical thickness (PND 72)	Biesemeier et al. (2011)[8]
Cortex radial thickness (PND 72)	Biesemeier et al. (2011)[8]
Vertical height between hippocampal pyramidal neuron layers (PND 72)	Biesemeier et al. (2011)[8]
Dentate hilus vertical height (PND 72)	Biesemeier et al. (2011)[8]
Length of ventral limb of dentate hilus (PND 72)	Biesemeier et al. (2011)[8]
Pons vertical thickness (PND 72)	Biesemeier et al. (2011)[8]
Habituation ratio locomotion (2-month-old and 4-month-old)	Johansson et al. (2008)[6]
Reproductive and Developmental endpoints	
Gestation length	Biesemeier et al. (2011)[8]
Number of implantation sites	Biesemeier et al. (2011)[8]
Number of unaccounted for sites	Biesemeier et al. (2011)[8]
Number born/litter	Biesemeier et al. (2011)[8]
Number live/litter	Biesemeier et al. (2011) [8]
Body weight	Kim et al. (2009)[9]; Tseng et al. (2008)[10]
Liver weight	Kim et al. (2009)[9]
Kidney weight	Kim et al. (2009)[9]
Testes (or ovary) weight	Kim et al. (2009)[9]
Epididymis (or uterus) weight	Kim et al. (2009)[9]
Prostate weight	Kim et al. (2009)[9]
Thyroid weight	Kim et al. (2009)[9]
Adrenal weight	Kim et al. (2009)[9]
Liver/body weight	Kim et al. (2009)[9]
Kidney/body weight	Kim et al. (2009)[9]
Testes/body weight	Kim et al. (2009) [9]
Epididymis/body weight	Kim et al. (2009)[9]
Prostate/body weight	Kim et al. (2009)[9]
Ovaries/body weight	Kim et al. (2009)[9]
Uterus/body weight	Kim et al. (2009)[9]
Thyroid/body weight	Kim et al. (2009)[9]
Adrenal/body weight	Kim et al. (2009)[9]
Gestational length	Tseng et al. (2008)[10]
Live pups per litter (PND 1, PND 4)	Tseng et al. (2008)[10]
Live pup weight (g) (at birth, at weaning)	Tseng et al. (2008)[10]

BDE-47 Reproductive and Developmental Endpoints

In this study, SD rats were exposed to a single oral dose of BDE-47 (1, 5, and 10 mg/g, three rats each group) on postnatal day (PND) 10. Organ-to-body weight ratios were measured in 2-month-old rats. This study found that some doses of BDE-47 decreased the organ (testicle, epididymis, ovaries and uterus)-to-body weight ratios of the thyroid and uterus, and increased the ratio of ovaries ($P<0.05$)[5].

Animal researches studying on toxicity of BDE-47 were rare. Reproductive endpoints are often the most sensitive endpoints of BDE-47, and provide important information regarding multi-generational effects of this chemical. He et al.[1] analyzed 6 reproductive and developmental endpoints for BDE-47, and only four of which seemed to be well modeled. Three of the four BMDLs for BDE-47 ranged from 2.1 to 5.13 μg/kg, and the lowest BMDL obtained for uterus/body weight is recommended for consideration in developing an RfD for BDE-47.

Table 2. Summary of BMD and BMDL estimates for PBDEs

Endpoint	Study Reference	Benchmark dose (BMD; μg/kg)	Benchmark dose low (BMDL; μg/kg)
BDE-47			
Reproductive/Developmental			
Testicle/body weight	He et al. (2011)[1]	13.94	5.13
Epididymis/body weight	He et al. (2011)[1]	435.36	69.53
Ovaries/body weight	He et al. (2011)[1]	3.47	2.46
Uterus/body weight	He et al. (2011)[1]	3.93	**2.10**
BDE-99			
Thyroid			
I. Parent (F0) generation			
TSH	Alonso et al. (2010)[2]	1,127.76	654.21
T4	Alonso et al. (2010)[2]	8,949.97	1,378.94
FT4	Alonso et al. (2010)[2]	3,273.13	1,084.64
FT3	Kuriyama et al. (2007)[3]	127.12	90.72
II. First (F1) generation			
Male			
FT3	Kuriyama et al. (2007)[3]	112.08	**81.77**
T4	Kuriyama et al. (2007)[3]	574.41	230.36
T3	Kuriyama et al. (2007)[3]	226.51	134.05
Female			
T3	Kuriyama et al. (2007)[3]	319.50	172.46
FT3	Kuriyama et al. (2007)[3]	202.66	129.10
Reproductive/Developmental			
Body weight	Kuriyama et al. (2005)[4]	104.96	80.90
Thymus/body weight	Kuriyama et al. (2005)[4]	164.24	117.37
Testis weight	Kuriyama et al. (2005)[4]	283.73	173.94
Seminal vesicle weight empty	Kuriyama et al. (2005)[4]	234.52	152.94
Prostate weight	Kuriyama et al. (2005)[4]	158.33	114.03
Intromission frequency	Kuriyama et al. (2005)[4]	108.61	83.34
No. of penetrations before the first ejaculation	Kuriyama et al. (2005)[4]	103.61	79.99
BDE-209			
Neurobehavior			
Fetal brain weight	Chi et al. (2011)[5]	609,749	496,873
Habituation ratio locomotion (2-month-old)	Johansson et al. (2008)[6]	2,352	1,990
Habituation ratio locomotion (4-month-old)	Johansson et al. (2008)[6]	2,000	**1,698**

Note. Bold values represent recommended BMDL for each type of endpoint.

BDE-99 Thyroid endpoints In Alonso et al.[2] study, ten adult male rats received BDE-99 by gavage at single doses of 0, 0.6, and 1.2 mg/kg, respectively. Forty-five days after exposure, serum samples were collected for thyroid-stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) analysis. A tendency to enhance TSH was noted in the BDE-99 exposed groups, while no significant differences were observed in serum T3 and T4 (total and free) in comparison to the control group.

In the other study, thyroid hormone concentrations were evaluated in Wistar rats (dams and offspring) after treatment by gavage on gestation day (GD) 6 with a single low dose of either 60 or 300 μg BDE-99/kg body weight (bw), respectively. The dose of 300 μg BDE-99/kg·bw reduced T4 concentration in dams at the beginning of lactation [post-gestational day (PGD) 1], and caused a slight reduction in T4 on PGD 22, although not statistically significant. In offspring, reduced T4 was observed only at PND 22, probably due to cumulative effects of BDE-99 during lactation. PBDEs have been shown to reduce T4 concentrations in several studies, but this is the first study demonstrating endocrine disruption at low doses[3].

Reproductive and Developmental Endpoints This study assessed the effect of developmental exposure to BDE-99 on adult male reproductive health. Wistar rat dams were treated by gavage on GD 6 with a single low dose of 60 or 300 μg BDE-99/kg·bw (12 rats each group). In offspring, reproductive performance was assessed in male pups at adulthood. The exposure to low-dose BDE-99 during development caused permanently impaired spermatogenesis by the means of reduced sperm and spermatid counts. The doses used in this study are relevant to human exposure levels, being approximately 6 and 29 times, respectively[4].

Reviewing modeling results presented in Table 2, the lowest BMDL obtained for thyroid endpoints of BDE-99 was 81.77 μg/kg, and 79.99 μg/kg for reproductive endpoints. Kuriyama et al.[3] conducted a two-generation study, analyzing total 12 thyroid endpoints for F0 and F1 generation, and obtained the lowest BMDL of 81.77 μg/kg for FT3 level of male offsping. Due to the reproducibility of this endpoint, the rigorous study protocol and thorough documentation[3], the BMDL of 81.77 μg/kg is recommended to represent the toxicity of BDE-99 for purposes of developing an RfD instead of the lower value obtained for reproductive endpoint. In view of the lack of mechanistic data on potential trans-generational effects of PBDEs, that use of data from the F1 generation was appropriate and protective.

BDE-209 Neurobehavior Endpoints In Chi et al.[5] study, four groups of pregnant C57 mice were administrated with BDE-209 in 20% fat emulsion at a dose of 150, 750, 1500, or 2500 mg/kg body weight via gastric intubation on gestation days (GD) 7 to 9, while a control group was given 20% fat emulsion (ten mice in each group). Maternal mice were euthanized on GD 16 and examined for external malformations of the fetus. Results indicated the weight of brain was decreased in the four dosed groups, of which the 2500 mg/kg group exhibited a statistically significant decline.

The other study was undertaken to explore the dose-response effects of BDE-209 on spontaneous behavior and habituation. Neonatal male NMRI mice were given 1.3, 2.2, 13.4, or 20.1 mg BDE-209/kg·bw (ten mice in each group) when 3 days old. The agent was administered as a single oral dose via a metal gastric tube. Spontaneous behavior was observed in adult mice at 2 and 4 months of age, showed reduced or lack of habituation, effects that worsen with age. This indicated that BDE-209 can be as potent as the lower brominated PBDEs in causing developmental neurotoxic defects[6].

The lowest BMDL based on the Johansson et al.[6] study was 1698 μg/kg for habituation ratio locomotion of adult mice at four months old. However, compared with the four-month study, BMDL of Chi et al. study[5] was too high to be taken into consideration in developing an RfD, as shown in Table 2, which may indicate the developmental effect is less sensitive than the neurobehavior endpoints, so that 1698 μg/kg for habituation ratio locomotion at 4-month-old was selected to represent toxicity of BDE-209.

The BMDLs recommended for each of the three major PBDE congeners (BDE-47, -99, -209) are shown in bold font in Table 2. We tested all the models (linear, Polynomial, power and hill) for continuous data, only to find that linear model was the optimal one. And UF was applied to extrapolate animal experimental results to human population, which means to derive RfD from BMDLs.

Uncertainties generally result from two sources. The first one is extrapolation, both from non-human species to humans and from general human population to sensitive individuals. For this study, species differences arise from differences in toxicokinetics and toxicodynamics of PBDEs and a

Table 3. Summary of RfDs for Three Types of Endpoints

PBDE congener	Type	RfD (mg/kg·day)	Reference
BDE-47	Reproductive/Developmental	2.1×10^{-6}	This study
	Neurobehavior	0.0001	USEPA
BDE-99	Thyroid	2.7×10^{-4}	This study
	Neurobehavior	0.0001	USEPA
BDE-209	Neurobehavior	1.7×10^{-3}	This study
	Neurobehavior	0.007	USEPA

10-fold default is assigned. Another 10-fold is intended to cover the difference in sensitivity between an average person in the population to a sensitive human in the population (WHO, 1994).

The other is uncertainties relating to deficiencies in the database, consisting of absence of a NOAEL, exposure route difference, exposure duration difference and insufficient database. In some studies NOAEL are not available and LOAEL may be used as an alternative and uncertainty needs to be accounted. While in this study, BMD is calculated and now recognized as more scientifically credible approach thus negates the need for an LOAEL to NOAEL UF. Meanwhile, selected studies and human exposure are via the same route, orally through the diet, indicating that no extra UF should be used here. A threefold UF was used to adjust for exposure duration for BDE-47 and BDE-209 as the selected studies used short-term administration. While the principal study for BDE-99 was two-generation study which can be recognized as chronic exposure, no extra UF was counted. Another factor called modifying factor (MF), generally no higher than 3, is typically also used to account for other considerations such as perceived adequacy of the scientific data. We assigned 1, 3, and 1 to BDE-47, -99, and -209, respectively. The UFs and the MFs are then multiplied together to provide a total UF, which is used to derive a chronic RfD. UFs figured out for BDE-47, -99, and -209 were 1000, 300, 1000, respectively.

Consistent with USEPA, the lowest BMDLs of 2.10, 81.77, and 1698 μg/kg were used to develop RfDs for BDE-47, -99, and -209, respectively. These BMDLs were derived from data on decreased uterus/body weight, FT3 (F1 generation) and habituation ratio locomotion (4-month-old) provided in animal experients[1,3,5]. These endpoints are relevant to humans, represent sensitive endpoints.

Due to the large toxicology data base, which encompasses varying stages of the life cycle, varying exposure periods, and studies on several rat strains, an uncertainty factor is not necessary to protect healthy population. Uncertainty factors are used to address lack of knowledge regarding the relative sensitivity of a chemical in humans versus animals. As shown in Table 3, acceptable RfD based on this study evaluating the entire PBDEs data for BDE-47, -99, and -209 were 2.1×10^{-6} mg/kg·day, 2.7×10^{-4} mg/kg·day, and 1.7×10^{-3} mg/kg·day, respectively. RfDs for BDE-99 and BDE-209 were comparable to EPA results. RfD of BDE-47 was much lower than that of EPA and there may be two reasons accounting for this: one is that reproductive/developmental proves to be more sensitive than neurobehavior for BDE-47; the other reason is that the principal study uses very-low-dose exposure and thus BMDL obtained is relatively low.

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