Neurotoxicology and Teratology 34 (2012) 20–26

Contents lists available at SciVerse ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Acute postnatal exposure to the pentaBDE commercial mixture DE-71 at 5 or 15 mg/kg/day does not produce learning or attention deficits in rats

Lori L. Driscoll *, Joshua Kaplan, Eleni Bucuvalas, Hunter Allen, Joshua Kraut, Jesse Fitzpatrick

Laboratory of Behavioral Neurotoxicology, Department of Psychology, The Colorado College, Colorado Springs, CO 80903, USA

ARTICLE INFO

Article history:
Received 25 May 2011
Received in revised form 30 September 2011
Accepted 3 October 2011
Available online 17 October 2011

Keywords:
Polybrominated diphenyl ethers
Postnatal exposure
Cognition
Learning
Attention

ABSTRACT

Polybrominated diphenyl ethers (PBDEs), flame retardant chemicals added to polymer products, have become ubiquitous in the environment, and they are bioaccumulating in humans and wildlife. Therefore, understanding their biological effects is important for public health. We have previously observed learning deficits in rats exposed to DE-71, a commercial PBDE mixture consisting primarily of pentabrominated diphenyl ethers, at a dose of 30 mg/kg/day from postnatal day (PND) 6 to 12. The purpose of the current study was to determine if this effect could be seen with lower doses of DE-71. Long–Evans rats were administered daily oral doses of corn oil alone or DE-71, 5 or 15 mg/kg/day, dissolved in corn oil, from PND 6 to 12. As young adults, the rats were administered a series of five-choice visual learning and attention tasks. No effects of DE-71 were found on learning, attention, or inhibitory control. Given that developmental DE-71 exposure at similar doses and for shorter time periods has been shown in other laboratories to affect locomotion and hyperactivity, the current results suggest that cognitive functions may not be as sensitive as neuromotor functions to the effects of acute DE-71 exposure.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are synthetic flame retardant additives used in polymers such as hard plastics, foam furniture, and carpets. They are easily released from these products into the environment and are commonly detected in house dust (Jones-Otazo et al., 2005), sediment (Moon et al., 2007; Pan et al., 2007), surface waters (Ueno et al., 2008), and sewage sludge (Knoth et al., 2007; Wang et al., 2007) throughout the world. Environmental PBDE contamination originates from commercial PBDE mixtures, each of which contains several PBDE congeners, or molecular variants that differ by the number and position of bromine atoms attached to the two aromatic rings of the PBDE molecule. For example, the pentaBDE mixture DE-71, which was produced in the United States, contains primarily tetra- and pentaBDEs (i.e., molecules with four and five bromines, respectively); the decaBDE mixture contains primarily the sole decaBDE, PBDE209 (La Guardia et al., 2006).

The molecular structure of PBDEs resembles that of polychlorinated biphenyls (PCBs), which are known persistent organic pollutants. As is the case with PCBs, PBDEs are lipophilic and bioaccumulate in wildlife (Chen et al., 2007; Kelly et al., 2008) and humans (Petreas et al., 2003; Schecter et al., 2003; Sjodin et al., 2004). Humans are mainly exposed to PBDEs through inhalation of house dust (Zota et al., 2008; Wu et al., 2007) and consumption of contaminated fish and meat (Schecter et al., 2006a,b; Voorspoels et al., 2007). Although the lower-brominated DE-71 mixture is no longer produced, the congener profile of PBDEs found in samples of human blood, breast milk, and fatty tissue closely matches that of DE-71, with the most heavily represented congeners being the tetraBDE PBDE47, the pentaBDEs PBDE99 and PBDE100, and the hexaBDE PBDE153 (Johnson-Restrepo et al., 2005; Schecter et al., 2006a,b). Given the stability of these flame retardant chemicals and the durable nature of the products in which they have been used, PBDEs will be present in the environment and in biota for decades to come. Therefore, it is important to determine the health effects of these chemicals and the levels at which such effects appear.

PBDE congeners, hydroxylated PBDEs (OH-PBDEs, which are metabolites of PBDEs), and PBDE commercial mixtures appear to interact with several target proteins, many of which play vital roles in nervous system development or functioning. In vitro, the pentaBDE mixture DE-71 increases the translocation of protein kinase C and inhibits Ca^{2+} uptake by mitochondria and microsomes (Coburn et al., 2008; Kodavanti and Ward, 2005) in turn disrupting downstream signaling pathways (Fan et al., 2010). Depolarization-evoked Ca^{2+} release is inhibited by OH-PBDEs (Dingemans et al., 2010). Anti-thyroid activity has also been observed in vitro; in cultured human neural progenitor cells, PBDE 47 and PBDE 99, the most predominant congeners found in biota, suppress differentiation through antagonism of thryoid hormone receptors (Schreiber et al., 2010). In vivo, oral exposure to PBDEs in rodents results in temporary suppression of serum total thyroxine (T4) levels (Hallgren et al., 2001; Kodavanti et al., 2010; Zhou et al., 2002). Recent work has also demonstrated that PBDEs can induce oxidative damage in

* Corresponding author at: Department of Psychology, The Colorado College, 14 E. Cache La Poudre, Colorado Springs, CO 80903, USA. Tel.: +1 719 227 8201; fax: +1 719 389 6284.
E-mail address: Ldriscoll@coloradocollege.edu (L.L. Driscoll).

0892-0362/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ntt.2011.10.002

cells (Gao et al., 2009; Huang et al., 2010) and modulate DNA methylation (Chen et al., 2010) and gene expression (Alm et al., 2010).

Taken together, these and other mechanisms of biological disruption by PBDEs could have particularly devastating consequences for neural development in humans and non-human animals alike. Developing humans readily absorb PBDEs through the placental barrier (Schecter et al., 2007) and consume them in high levels in mothers' milk (Johnson-Restrepo et al., 2007; Schecter et al., 2003), in addition to ingesting and inhaling them in house dust (Jones-Otazo et al., 2005; Wilford et al., 2005). Exposure levels have been shown to be higher in young children than in adults who live in the same household (Fischer et al., 2006). The perinatal and early postnatal periods, when this increased exposure takes place, are times of increased growth and plasticity of the nervous system. As a result, disruptions of these processes by chemicals such as PBDEs could result in lasting alterations in neural structure and function.

Animal models have, until very recently, been the exclusive source of information regarding the neurobehavioral effects of developmental PBDE exposure. Hyperactivity is the most commonly reported effect of exposure to commercial PBDE mixtures, regardless of whether the exposure is brief and during the perinatal period (Gee and Moser, 2008; Viberg et al., 2003b, 2006) or more prolonged and spanning from gestation through weaning (Kodavanti et al., 2010; Kuriyama et al., 2005; Suvorov et al., 2009). However, very little is known about the cognitive effects of developmental PBDE exposure. The few studies that have explored cognitive endpoints have reported learning impairments in the Morris water maze after acute exposure to individual congeners (Viberg et al., 2003b; Viberg et al., 2006), slowed learning of a light–dark discrimination task after acute exposure to PBDE209 (Rice et al., 2009), and slowed learning of a food-motivated visual discrimination task after exposure to DE-71 (Dufault et al., 2005). In contrast to the effects on learning, effects on attention appear to be dependent upon whether the exposure is brief or chronic. No deficits in visual sustained attention were observed in animals exposed briefly to relatively high levels of DE-71 (Dufault et al., 2005), but lower-level chronic exposure to DE-71 did produce deficits in the same task (Driscoll et al., 2009), suggesting that for attentional function, exposure concurrent with testing, or total PBDE body burden, has more of an impact on performance than does the level of exposure during development.

Fortunately, epidemiological investigations of the cognitive effects of early PBDE exposure have recently been published, enabling a comparison with the findings in animal models and a broader characterization of the impact of these compounds on cognitive function. In the Netherlands, children who presented with higher PBDE cord blood levels at birth demonstrated impaired attention and fine motor function compared to children with lower levels, but they showed no deficits in global intelligence measures (Roze et al., 2009). In the United States, children who typically presented with 2–5 times higher cord PBDE levels than did the children in Roze et al., demonstrated significant decrements in both psychomotor and mental subscales of the Bayley Scales of Infant Development and the Weschler Preschool and Primary Scale of Intelligence (Herbstman et al., 2010). Therefore, it appears that the nature of effects associated with developmental PBDE exposure varies depending on the level of exposure. More work needs to be done to determine the levels of PBDEs that produce harmful cognitive effects in developing animals and children.

The current experiment was designed to address gaps in the animal work by exploring the effects of early postnatal DE-71 exposure on learning and attention using the same acute exposure paradigm employed in our lab previously (Dufault et al., 2005), but with lower doses of DE-71 (5 and 15 mg/kg/day as opposed to 30 mg/kg/day). The window of exposure, from PND 6 to 12, marks a period of pronounced synaptogenesis (Sutor and Luhmann, 1995) and gene expression (Stead et al., 2006) in the rodent brain, and encompasses the developmental window of sensitivity to PBDE exposure reported by other laboratories (e.g., Gee and Moser, 2008; Viberg et al., 2004a,b). Given that PBDE mixtures and individual congeners produce effects on motor behavior and learning at or below the dose range of 15 mg/kg/day, it was hypothesized that the higher dose of DE-71 would produce significant effects on learning, but that the lower 5 mg/kg/day dose would have no effects on performance. No effects on sustained attention were expected, given that the higher dose of 30 mg/kg/day did not produce attention deficits previously (Dufault et al., 2005).

2. Methods

2.1. Animals and DE-71 exposure

The current study was conducted in two replications of equal size across two consecutive summers. Only males ($n = 24$ per treatment across replications) were tested in this study in order to obtain sufficient statistical power with the available resources. Nulliparous female Long–Evans rats (Blue Spruce stock; Harlan Sprague–Dawley, Indianapolis, IN) were bred with 24 male Long–Evans rats that had been born in the colony at Colorado College. Dams were housed singly in polycarbonate cages and given unlimited access to tap water and standard laboratory chow (LabDiet 5001; PMI Nutrition International, Richmond, IN). Twenty-four hours following parturition, on postnatal day 1 (PND 1), each litter was culled to nine pups, with 4–5 males per litter.

Three male pups per litter were used for the current study; the remaining pups in each litter were reserved for breeding and pedagogical laboratory exercises in the department. For each litter of three pups, two were fostered to other litters such that each new litter contained one biological pup and two non-littermates. Each pup in the foster litter received the same treatment, to avoid the potential for cross-contamination between treatments. Allocation of fostering and treatment assignments was such that each original biological litter contributed one pup to each of the three DE-71 treatment conditions (i.e., each of the three littermates were allocated to litters receiving one of the three treatments). To retain the birth litter information, pups were color-coded on the tips of their tails using Sharpie markers. However, in the first replication, an error in tail marking resulted in confusion between two of the tail marking colors. As a result, although every biological litter was represented once in every treatment group, the matched litter information for individual pups was not retained, which influenced the statistics that could be performed (litter was a balanced variable, but could not be used as unit of analysis).

From PND 6 to PND 12, the three male pups in each litter earmarked for the study were daily administered the commercial PBDE mixture DE-71 dissolved in corn oil, or corn oil alone. The DE-71 (lot 7550OK20A), generously donated by Dr. Kevin Crofton of the U.S. EPA, contains approximately 25% tetraBDE, 50–60% pentaBDE, and 4–8% hexaBDE (Sjodin, 2000). The DE-71 stock solution (300 mg/ml) was prepared by sonicating the DE-71 with corn oil for 30 min at 40 °C. The dosing solutions were prepared by diluting the stock solution with corn oil to the desired concentrations and vortexing for 30 s. The control and experimental solutions were administered orally via a metal gastric tube at a volume of 3 ml/kg of body weight, resulting in a daily DE-71 dose of 15 mg/kg of body weight per day for the high dose pups, and a dose of 5 mg/kg of body weight per day for the low dose pups.

At weaning (PND 21), the pups were ear punched for identification and housed in same-treatment pairs. Pups were gradually food restricted to 12 g of chow per day (24 g of chow per cage) on PND 25 and 10 g of chow per day (20 g per cage) on PND 35. From the onset of behavioral testing (PND 40) through the end of the experiment (ranging from PND 82 to PND 95), pups were housed individually, and daily chow allotments for each animal were tailored based on trial completion rates to maintain motivation while still allowing for growth of at least 2 g per day. If an animal did not complete its session of 100 trials for two consecutive days, its daily food allotment was decreased by 1 g. Conversely,

if it did not gain weight for 2 days in a row (regardless of motivation level), the daily food allotment was increased by 1 g. By the end of testing, the mean daily allotment was 12.1 g and did not differ between treatment groups (data not shown). This food restriction procedure resulted in body weights that were approximately 85% of ad libitum weights. All animal care and experimental procedures were conducted in compliance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996).

2.2. Apparatus

The four Plexiglas chambers used for behavioral testing (ENV-008, Med Associates, Inc.; St. Albans, VT) were modeled after the five-choice serial reaction time chambers originally described in Carli et al. (1983). Each 30.5×24×34.5 cm chamber was enclosed in a sound-attenuating exterior box. Embedded in one wall of the main chamber, 2.5 cm above the floor, was a square alcove (5 cm tall×5 cm wide), into which the animal made a nosepoke to initiate each trial, and into which a sweetened 45 mg reward pellet (Noyes formula AIN-76A; Research Diets, Inc.; Lancaster, PA) was dispensed after each correct port choice. An automated guillotine-type door limited alcove access to specific parts of each trial. On the concave wall opposite the alcove, five square ports (2.5×2.5 cm) served as the choice points into which nosepoke responses were made. The ports were at a height of 2.5 cm from the floor and spaced 1 cm apart. Yellow light-emitting diodes (LED), outfitted into the back wall of each port, and produced the visual cues to which the animals were trained to respond. Nosepokes into the alcove and the ports were detected by infrared photodiodes. A 4 W house light, situated above and to the left of the alcove, was illuminated for most of the session but was extinguished for a 5 s "timeout" upon the commission of an error (see error types below). If the rat made a nosepoke in a port during a timeout, the 5 s timer was reset, the house light remained extinguished, and no rewards were delivered.

2.3. Behavioral testing

The subjects were tested 6 days per week; the duration of each daily session was 100 trials or 60 min, whichever came first. First the rats were administered a series of four training tasks to shape the eventual sequence of responses that would constitute a trial in the learning and attention tasks: opening of the guillotine door and initiation of the trial with a nosepoke into the alcove; a 3 s "turnaround" period in which the animal was required to reorient his attention to the ports on the wall; a nosepoke into an illuminated port; and a timeout (if a correct response was not made) or receipt of reward in the alcove (if a correct response was made). The criterion to proceed from one training task to the next was the attainment of 100 reward pellets within a single 60-minute session. In Training Task 1 (alcove training), the alcove door remained open, and the rat received one pellet for each nosepoke into the alcove. Training Task 2 (door training) was similar to the previous task, but the alcove door closed 3 s after each pellet was obtained and reopened 2 s later for the beginning of the next trial. For Training Task 3 (turnaround training), the animal was required to make a non-rewarded nosepoke (hereafter referred to as "trial initiation") into the alcove, turn around, then make a nosepoke response into any of the five ports, all of which were illuminated, in order to receive a reward. Training Task 4 (equal port exposure), which was designed to minimize response port biases, was five sessions in duration. For each session, access to four of the five ports was blocked by a metal plate, and the animal was required to make 100 nosepoke responses to the one, illuminated port available. A different port was uncovered on each of the five sessions.

Once each rat completed training, it was administered a series of five-choice visual tasks designed to assess learning and sustained attention. These tasks incorporated the same basic trial events as above, but the rats were now required to make a nosepoke only into the one illuminated port and avoid the four non-illuminated ports. Presentation of the visual cue followed trial initiation and the 3 s turnaround delay. The location of the visual cue on a given trial was pseudorandomized. To receive a reward, the rat was required to poke in the illuminated port (i.e., make a *correct response*) sometime between the cue onset and 5 s after the cue offset (the "limited hold" period). A nosepoke made prior to cue onset was recorded as a *premature response*; a failure to respond to any port within 5 s after cue offset was scored as an *omission error*. Premature responses, omission errors, and *inaccurate responses* (i.e., responses made at the appropriate time but to the incorrect port) were all considered to be errors and resulted in a five-second timeout, in which the house light was illuminated and access to the alcove was prevented. A nosepoke to a response port during the timeout resulted in a resetting of the timeout clock.

Visual Task 1 assessed subjects' ability to learn the basic rules of the visual tasks. On each trial, the cue appeared in one of the five ports for 15 s or until a response was made, whichever came first. Criterion performance to advance to the next task was at least 80% correct responses for two out of three sessions of 100 trials each. Visual Tasks 2 and 3 were similar to Visual Task 1 but featured briefer cue durations of 5 s and 1 s. The rats received one and three sessions on each of these tasks, respectively. These two tasks were designed to prepare the subjects for the briefer cues in the Sustained Attention tasks.

In the subsequent task, Sustained Attention Task 1, the 1 s visual cue occurred after a pseudorandomized pre-cue delay of 0, 3, or 6 s (in addition to the 3 s turnaround period), thus requiring the animal to sustain attention across the five ports for an indeterminate period of time. The animals were administered ten sessions on this task. The final task, Sustained Attention Task 2, included the same variable pre-cue delays but also incorporated shorter and variable cue durations of 200 ms, 500 ms, and 800 ms in order to more extensively tap attentional resources.

2.4. Performance measures

For each of the three tasks reported here (Visual Task 1 and Sustained Attention Tasks 1 and 2), *premature responses*, *omission errors*, and *correct responses* were tallied and expressed as a percentage of all responses. *Accuracy* was defined as the percentage of correct responses made within the allotted time window. This measure was less influenced by motivational issues and impulsivity than was the more global measure of *percentage correct*, which took into account all response types (i.e., omission errors, premature responses, accurate responses, and inaccurate responses). Percent premature responses were used as an index of impulsivity. Failures to respond to any port within 5 s after cue offset were scored as omission errors. In addition to the above measures, total trials and errors to criterion were calculated for Visual Task 1 to reflect learning rate.

Three latency measures were also recorded. Initiation (alcove) latency, the elapsed time between the opening of the alcove door and the rat's initiation of a new trial, was indicative of the animal's motivation and motor function, as was reward latency, the time between the animal's correct response and its retrieval of the reward pellet. The elapsed time between cue onset and the animal's nosepoke in the correct port, the correct response latency, reflected the animal's information processing speed in addition to motivation and motor function. For the sake of brevity, and because they did not vary as a function of treatment, the latency data are not presented in this report.

2.5. Statistical analyses

The Statistical Package for the Social Sciences (version 16 for Windows; SPSS Inc., Chicago, IL) was used to analyze the data. As mentioned above, the design was balanced for litter, but the loss of matched littermate information precluded using litter as the unit of analysis.

Performance and latency measures were subjected to mixed ANOVA, with treatment as a between-subjects factor and task variables (i.e., session for Visual Task 1, pre-cue delay for Attention Task 1, and pre-cue delay and cue duration for Attention Task 2) as within-subjects factors. Effect sizes (partial eta squared or partial η^2) were calculated in addition to traditional F and p values and are presented for statistically significant effects. One-way ANOVA was used to analyze treatment differences in body weights and criterion measures (mean number of errors and trials to criterion) for Visual Task 1.

Because the study was run in two replications, the first set of analyses was conducted with replication as an additional between-subjects factor. However, because no significant main effects of replication were found on any of the dependent measures, nor were there significant interactions between cohort and the other independent variables, the analyses were run again without replication as a factor. The results presented below are based on the latter analyses.

3. Results

3.1. Body weights

DE-71 treatment did not significantly affect body weights during treatment (PND 12), at weaning (PND 21), or at any point during behavioral testing (weights compared once per week from PND 40 through PND 105, all $p > .05$; see Fig. 1 for mean weights at weaning, the beginning of each food restriction change, and during each of the three tasks).

3.2. Visual Tasks 1, 2, and 3

The total number of trials required to reach criterion on the visual discrimination task (2 out of 3 sessions with at least 80% correct) did not differ significantly between the three treatment groups (Fig. 2A), $F(2, 71) = 0.221$, $p > .05$, nor did the number of errors to criterion (Fig. 2B), $F(2,71) = 0.019$, $p > .05$.

All subjects reached criterial performance in Visual Task 1 in 10 or fewer sessions ($M = 5.95$ sessions; $SD = 2.61$; see Fig. 2C). In order to best capture performance during the portion of the task when most rats were still participating, performance measures were analyzed for the first five sessions only. Performance improved steadily during acquisition, as evidenced by a significant increase across sessions for percentage correct, $F(4, 313) = 8.33$, $p < .001$, partial $\eta^2 = .10$, and response accuracy, $F(4, 313) = 9.57$, $p < .001$ partial $\eta^2 = .11$. Percent premature responses and percent omission errors tended to decrease across sessions, but the decrease was not significant in either case due to high inter-individual variability and relative infrequency of these types of errors (percent omission errors, $F(4, 313) = 1.93$, $p = .11$; percent premature responses, $F(4, 313) = 1.69$, $p = .15$). There were no significant main treatment effects or treatment by session interactions for percent correct, accuracy, percent premature responses, percent omission errors, initiation latency, correct response latency, or reward latency (all $p > .05$).

Visual Task 2, which employed visual cues of 5 s in duration as opposed to the 15 s cues in Visual Task 1, resulted in overall performance levels that were similar to those in Visual Task 1 (e.g., mean percentage correct was 81% and mean percent omission errors was 14%). Although mean percentage correct (63%) and percent omission errors (20%) increased when the cue duration was shortened to 1 s in Visual Task 3, there was no significant effect of treatment on any performance or latency measure in this task (all $p > .05$).

3.3. Attention Task 1

In Attention Task 1, the imposition of unpredictable pre-cue delays (0, 3, or 6 s) produced significant impairments in the ability to wait for, detect, and respond accurately to the brief visual cues. Declines in percent correct, $F(2, 132) = 180.62$, $p < .001$, partial $\eta^2 = .73$, and accuracy, $F(2, 132) = 52.14$, $p < .001$, partial $\eta^2 = .44$, and increases in percent premature responses, $F(2, 132) = 38.70$, $p < .001$, partial $\eta^2 = .48$, and percent omission errors, $F(2, 132) = 63.54$, $p < .001$, partial $\eta^2 = .49$, were seen with increasing pre-cue delay (see Fig. 3 for effect of delay on percent correct). There were no main effects of treatment on any performance or latency measure in this task, nor were there any interactions between treatment and delay (all $p > .05$).

3.4. Attention Task 2

As was the case with Attention Task 1, performance measures in Attention Task 2 showed decrements with increasing pre-cue delay (main effects of delay: all $p < .05$ for percent correct, accuracy, percent premature responses, and percent omission errors). This task was more challenging than Attention Task 1 in that it utilized briefer visual cues that also varied unpredictably in duration (200, 500, or

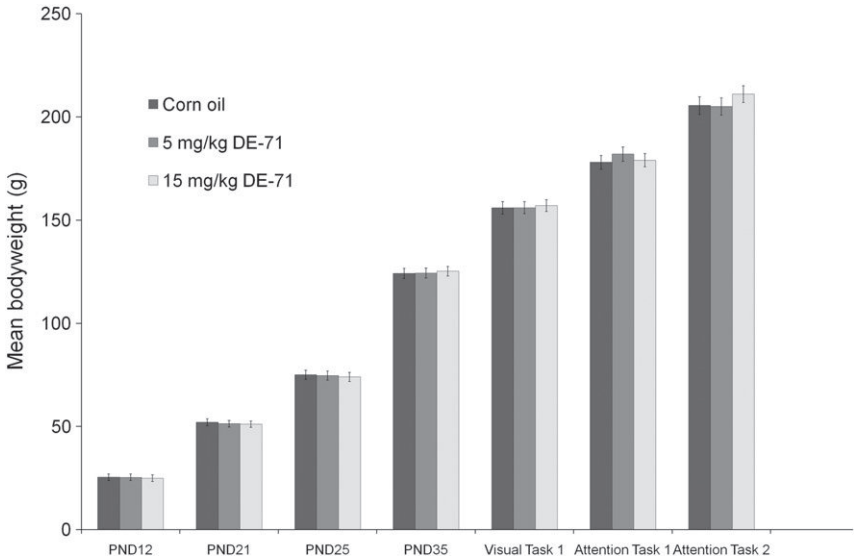

Fig. 1. Mean body weights of control rats (n = 24) and rats exposed to 5 mg/kg/day (n = 24) or 15 mg/kg/day (n = 24) DE-71 at cessation of treatment (PND 12), weaning (PND 21), and several points throughout the experiment. Error bars = ±2 SEM.

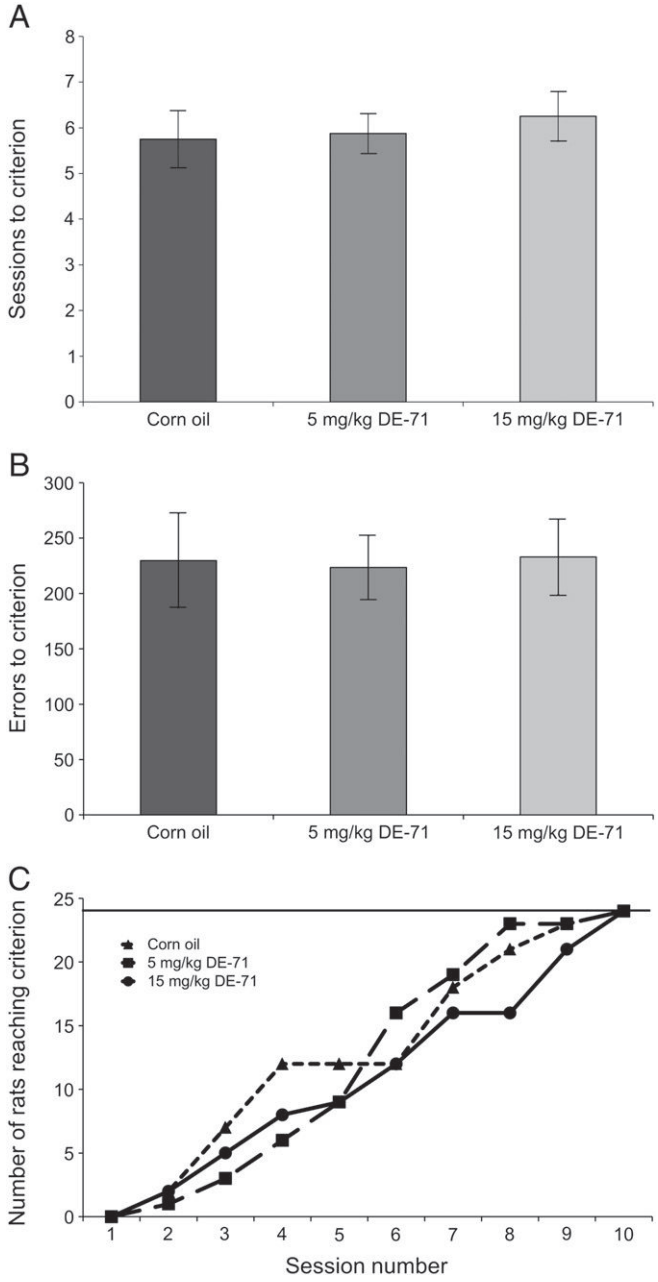

Fig. 2. Acquisition of Visual Task 1 as a function of DE-71 exposure (0, 5, or 15 mg/kg/day). (A) Mean number of sessions to criterion; (B) mean number of errors to criterion; (C) number of animals remaining on task (i.e., performing at below criterion) as a function of session number. For (A) and (B), error bars = ±2 SEM.

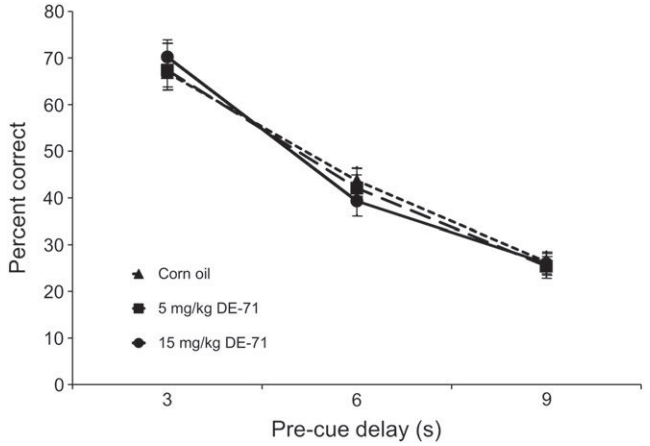

Fig. 3. Percentage of correct responses in Attention Task 1 as a function of the delay between trial onset and 1 s cue presentation. Although overall performance decreased as the delay between trial initiation and cue onset increased (from 3 to 9 s), there was no significant effect of DE-71 exposure (0, 5, or 15 mg/kg/day) on performance in this task, nor was there an interaction between treatment and pre-cue delay.

800 ms). As cue duration decreased, percent correct decreased, $F(2, 264) = 467.53$, $p < .001$, partial $\eta^2 = .88$, percent accuracy decreased, $F(2, 264) = 505.04$, $p < .001$, partial $\eta^2 = .91$, and percent omission errors increased, $F(2, 264) = 44.58$, $p < .001$, partial $\eta^2 = .40$ (see Fig. 4 for effect of cue duration on percent correct), indicating that the briefer cues were more difficult to detect than were the longer cues. As with Attention Task 1, there were no significant differences between the DE-71-exposed and control groups on any of the performance or latency measures, nor were there any interactions between treatment and delay or duration (all $p > .05$).

4. Discussion

Male rats exposed orally to DE-71 from postnatal day 6 to 12, at doses of 5 and 15 mg/kg/day, demonstrated no impairments in learning, attention, or impulsivity when compared to controls. The failure to find effects on attention and impulsivity is consistent with our previous findings using an acute DE-71 exposure of 30 mg/kg/day (Dufault et al., 2005). However, whereas we had previously observed slowed acquisition of Visual Task 1 at the higher dose of 30 mg/kg/day, we found no effects on acquisition at the lower DE-71 doses of 5 or 15 mg/kg/day in this study.

The lack of significant findings in this study suggests that a higher dose of DE-71 than 15 mg/kg/day may be required in order to impact performance in these automated tasks. However, because there was no dose at which behavioral effects were seen in this study, we can make no conclusions regarding the level at which such a threshold dose may be. Ideally, the former 30 mg/kg/day dose would have been included as a positive control in the current study. However, we did not choose this dose because we expected that the 15 mg/kg/day dose would be sufficient to produce effects. Several additional possibilities have to be considered in light of these negative findings. Lack of power is not a likely culprit, as we used 24 animals per treatment group. Protocols between the current and previous (Dufault et al., 2005) studies were identical, with the exception of the individuals who tested the animals. Using different testers may have had an effect; however, this seems unlikely given the automated assessment. Another possibility is that the DE-71/corn oil dosing solutions degraded prior to use (they were between 1 and 2 years old at the time of dosing). To explore this possibility, the original solutions, which were now 3 years old, were subjected to gas chromatogram–mass spectrometry for determination of the presence and amounts of the three most common congeners in DE-71 (BDE 47, 99, and 100). The analysis verified that these congeners were still present in amounts consistent with the amounts that were originally added to the solution, and did not differ from amounts found in newly prepared dosing solutions (data not shown).

In contrast to the lack of effects on cognitive endpoints in the current study, significant effects of developmental PBDE exposure (both commercial mixtures and individual congeners) have often been found on measures of motor activity (Gee and Moser, 2008; Rice et al., 2009; Viberg et al., 2003a,b, 2004a,b, 2007). For example, mice exposed to PND 10 to a single oral dose of BDE47 as low as 1 mg/kg showed increased vertical activity compared to controls when they were tested at 4 months of age (Gee and Moser, 2008). Other laboratories have also reported increased hyperactivity in animals exposed to a variety of PBDE congeners (BDE47: Suvorov, 2009; BDE99: Kuriyama et al., 2005; BDE209: Rice et al., 2007). Motor alterations of a different sort (typically hypoactivity compared to controls when the animals are

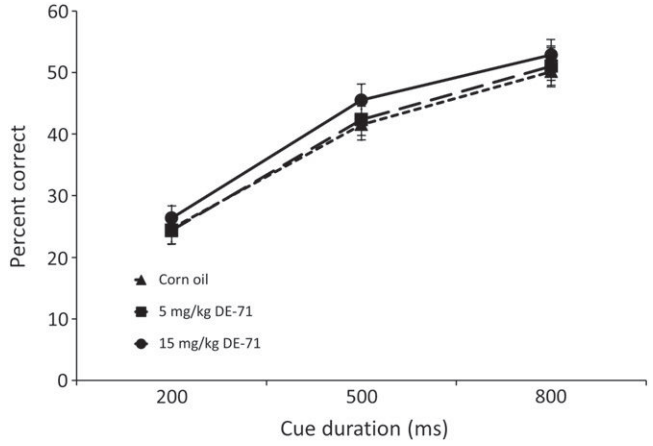

Fig. 4. Percentage of correct responses in Attention Task 2 as a function of visual cue duration. Although overall performance showed the expected decrements when cue duration was shortened (from 800 to 500 to 200 ms), there was no significant effect of DE-71 exposure (0, 5, or 15 mg/kg/day) on performance in this task, nor was there and interaction between treatment and cue duration.

first placed in an open field, followed by relative hyperactivity after control animals have habituated) have been reported in rats and mice exposed to single doses of individual PBDE congeners on PND 10 (BDE209 in mice, Viberg et al., 2003b; BDE209 in rats, Viberg et al., 2007; BDE99 in mice, Viberg et al., 2004a,b). It is possible that locomotor functions are more sensitive to the effects of developmental PBDE exposure than are cognitive functions. However, if this is the case, not all motor functions are equally sensitive, as we have never found significant effects of DE-71 exposure on latency measures (initiation latency, response latency, reward latency) in any of our tasks, whether exposure is brief (Dufault et al., 2005; current study) or chronic (Driscoll et al., 2009). In our task, subjects' nosepoke responses are made in the context of standing and turning around in a small chamber, so minimal locomotion is required. This may be the crucial difference between our task and tasks that have revealed motor effects of PBDE exposure.

It is also possible that some behavioral effects of early postnatal PBDE exposure do not become manifest until later in life or are exacerbated with age. For example, locomotor effects of PBDE exposure are often reported to be greater in older than in younger animals (Gee and Moser, 2008; Rice et al., 2009; Viberg et al., 2003a, 2004a,b). This form of delayed neurotoxicity might also hold true for cognitive performance, as one recent experiment suggests (Rice et al., 2007). When mice were exposed to daily doses of BDE209 for 2 weeks (from PND 2 through 15), no effects on fixed-interval responding and only minimal effects on a light–dark discrimination task were observed if the animals were tested as young adults. However, learning deficits, increased perseverative responding, and slowed response latencies were observed when the testing was conducted in aged (16 month old) mice (Rice et al., 2007). Our animals were only 2–4 months old at the time of testing, so it is unknown whether cognitive effects would have expressed themselves had we delayed testing until later in life. Delayed neurotoxicity is a phenomenon that has been observed in animals exposed to methylmercury (Rice, 1996). Although the mechanisms of such effects are unclear, they may be a result of latent damage to systems that do not normally reach their full expression until later in development. The possibility of delayed neurotoxicity as a result of developmental exposure to PBDEs should be explored in more detail.

In summary, early postnatal exposure to DE-71 at doses of 5 or 15 mg/kg/day for 1 week produced no effects on learning, attention, or inhibitory control in the current study. However, effects have previously been found on these tasks with acute exposure at higher doses of DE-71 (30 mg/kg/day; Dufault et al., 2005), or with chronic low-level exposure (Driscoll et al., 2009). The magnitude and nature of the effects varies depending upon the exposure paradigm, with learning deficits being more pronounced after higher-level acute exposure and attention deficits appearing when the exposure is continued into adulthood. Understanding the circumstances under which cognitive effects of exposure to PBDEs can be found, as well as when in adulthood these effects become manifest, is of great importance for understanding the course of neurotoxicity in humans. It is particularly important to continue to explore the effects of developmental exposure to PBDEs, as children have been shown to carry larger burdens of these toxins than do adults living in the same environment (Lunder et al., 2010).

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank the Colorado College for providing funding for this study in the form of Research and Development grants to L.L. Driscoll, a Dean's Summer Research Grant to L.L. Driscoll and E. Bucuvalas, and Venture Grants to J. Kaplan and H. Allen.

References

Alm H, Scholz B, Kultima K, Nilsson A, Andrén PE, Savitski MM, et al. In vitro neurotoxicity of PBDE-99: immediate and concentration-dependent effects on protein expression in cerebral cortex cells. J Proteome Res 2010;9:1226–35.
Carli M, Robbins TW, Evenden JL, Everitt BJ. Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. Behav Brain Res 1983;9:361–80.
Chen D, Mai BX, Song J, Sun QH, Luo Y, Luo XJ, et al. Polybrominated diphenyl ethers in birds of prey from Northern China. Environ Sci Technol 2007;41:1828–33.
Chen J, Liufu C, Sun W, Sun X, Chen D. Assessment of the neurotoxic mechanisms of decabrominated diphenyl ether (PBDE-209) in primary cultured neonatal rat hippocampal neurons includes alterations in second messenger signaling and oxidative stress. Toxicol Lett 2010;192:431–9.
Coburn C, Currás-Collazo M, Kodavanti P. In vitro effects of environmentally relevant polybrominated diphenyl ether (PBDE) congeners on calcium buffering mechanisms in rat brain. Neurochem Res 2008;33:355–64.
Dingemans MML, van den Berg M, Bergman A, Westerink RHS. Calcium-related processes involved in the inhibition of depolarization-evoked calcium increase by hydroxylated PBDEs in PC12 cells. Toxicol Sci 2010;114:302–9.
Driscoll LL, Gibson AM, Hieb A. Chronic postnatal DE-71 exposure: effects on learning, attention and thyroxine levels. Neurotoxicol Teratol 2009;31:76–84.
Dufault C, Poles G, Driscoll LL. Brief postnatal PBDE exposure alters learning and the cholinergic modulation of attention in rats. Toxicol Sci 2005;88:172–80.
Fan C, Besas J, Kodavanti PRS. Changes in mitogen-activated protein kinase in cerebellar granule neurons by polybrominated diphenyl ethers and polychlorinated biphenyls. Toxicol Appl Pharmacol 2010;245:1–8.
Fischer D, Hooper K, Athanasiadou M, Athanassiadis I, Bergman A. Children show highest levels of polybrominated diphenyl ethers in a California family of four: a case study. Environ Health Perspect 2006;114:1581–4.
Gao P, He P, Wang A, Xia T, Xu B, Xu Z, et al. Influence of PCB153 on oxidative DNA damage and DNA repair-related gene expression induced by PBDE-47 in human neuroblastoma cells in vitro. Toxicol Sci 2009;107:165–70.
Gee JR, Moser VC. Acute postnatal exposure to brominated diphenylether 47 delays neuromotor ontogeny and alters motor activity in mice. Neurotoxicol Teratol 2008;30:79–87.
Hallgren S, Sinjari T, Hakansson H, Darnerud PO. Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. Arch Toxicol 2001;75:200–8.
Herbstman JB, Sjödin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect 2010;118:712–9.
Huang SC, Giordano G, Costa LG. Comparative cytotoxicity and intracellular accumulation of five polybrominated diphenyl ether congeners in mouse cerebellar granule neurons. Toxicol Sci 2010;114:124–32.
Johnson-Restrepo B, Kannan K, Rapaport DP, Rodan BD. Polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue from New York. Environ Sci Technol 2005;39:5177–82.
Johnson-Restrepo B, Addink R, Wong C, Arcaro K, Kannan K. Polybrominated diphenyl ethers and organochlorine pesticides in human breast milk from Massachusetts, USA. J Environ Monit 2007;9:1205–12.
Jones-Otazo HA, Clarke JP, Diamond ML, Archbold JA, Ferguson G, Harner T, et al. Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. Environ Sci Technol 2005;39:5121–30.
Kelly BC, Ikonomou MG, Blair JD, Gobas FAPC. Bioaccumulation behaviour of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. Sci Total Environ 2008;401:60–72.

Knoth W, Mann W, Meyer R, Nebhuth J. Polybrominated diphenyl ether in sewage sludge in Germany. Chemosphere 2007;67:1831–7.
Kodavanti PRS, Ward TR. Differential effects of commercial polybrominated diphenyl ether and polychlorinated biphenyl mixtures on intracellular signaling in rat brain in vitro. Toxicol Sci 2005;85:952–62.
Kodavanti PRS, Coburn CG, Moser VC, MacPhail RC, Fenton SE, Stoker TE, et al. Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. Toxicol Sci 2010;116:297–312.
Kuriyama SN, Talsness CE, Grote K, Chahoud I. Developmental exposure to low-dose PBDE-99: effects on male fertility and neurobehavior in rat offspring. Environ Health Perspect 2005;113:149–54.
La Guardia MJ, Hale RC, Harvey E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. Environ Sci Technol 2006;40:6247–54.
Lunder S, Hovander L, Athanassiadis I, Bergman Ã. Significantly higher polybrominated diphenyl ether levels in young U.S. children than in their mothers. Environ Sci Technol 2010;44:5256–62.
Moon H, Kannan K, Lee S, Choi M. Polybrominated diphenyl ethers (PBDEs) in sediment and bivalves from Korean coastal waters. Chemosphere 2007;66:243–51.
Pan J, Yang Y, Xu Q, Chen D, Xi D. PCBs, PCNs and PBDEs in sediments and mussels from Qingdao coastal sea in the frame of current circulations and influence of sewage sludge. Chemosphere 2007;66:1971–82.
Petreas M, She JW, Brown FR, Winkler J, Windham G, Rogers E, et al. High body burdens of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in California women. Environ Health Perspect 2003;111:1175–9.
Rice DC. Evidence for delayed neurotoxicity produced by methylmercury. Neurotoxicology 1996;17:583–96.
Rice DC, Reeve EA, Herlihy A, Zoeller RT, Thompson WD, Markowski VP. Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully brominated PBDE, decabromodiphenyl ether. Neurotoxicol Teratol 2007;29:511–20.
Rice DC, Thompson WD, Reeve EA, Onos KD, Assadollahzadeh M, Markowski VP. Behavioral changes in aging but not young mice after neonatal exposure to the polybrominated flame retardant decaBDE. Environ Health Perspect 2009;117:1903–11.
Roze E, Meijer L, Bakker A, Van Braeckel, Koenraad NJA, Sauer PJJ, et al. Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. Environ Health Perspect 2009;117:1953–8.
Schecter A, Pavuk M, Papke O, Ryan JJ, Birnbaum L, Rosen R. Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. Environ Health Perspect 2003;111:1723–9.
Schecter A, Päpke O, Harris TR, Tung KC. Partitioning of polybrominated diphenyl ether (PBDE) congeners in human blood and milk. Toxicol Environ Chem 2006a;88:319–24.
Schecter A, Papke O, Harris TR, Tung KC, Musumba A, Olson J, et al. Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of US food and estimated PBDE dietary intake by age and sex. Environ Health Perspect 2006b;114:1515–20.
Schecter A, Johnson-Welch S, Tung KC, Harris TR, Papke O, Rosen R. Polybrominated diphenyl ether (PBDE) levels in livers of US human fetuses and newborns. J Toxicol Environ Health A 2007;70:1–6.
Schreiber T, Gassmann K, Gotz C, Hubenthal U, Moors M, Krause G, et al. Polybrominated diphenyl ethers induce developmental neurotoxicity in a human in vitro model: evidence for endocrine disruption. Environ Health Perspect 2010;118:572–8.
Sjodin A. Occupational and dietary exposure to organohalogen substances, with special emphasis on polybrominated diphenyl ethers. Doctoral dissertation, Stockholm University 2000.
Sjodin A, Jones RS, Focant JF, Lapeza C, Wang RY, McGahee EE, et al. Retrospective time-trend study of polybrominated diphenyl ether and polybrominated and polychlorinated biphenyl levels in human serum from the United States. Environ Health Perspect 2004;112:654–8.
Stead JDH, Neal C, Meng F, Wang Y, Evans S, Vazquez DM, et al. Transcriptional profiling of the developing rat brain reveals that the most dramatic regional differentiation in gene expression occurs postpartum. J Neurosci 2006;26:345–53.
Sutor B, Luhmann HJ. Development of excitatory and inhibitory postsynaptic potentials in the rat neocortex. Perspect Dev Neurobiol 1995;2:409–41.
Suvorov A, Girard S, Lachapelle S, Abdelouahab N, Sebire G, Takser L. Perinatal exposure to low-dose BDE-47, an emergent environmental contaminant, causes hyperactivity in rat offspring. Neonatology 2009;95:203–9.
Ueno D, Darling C, Alaee M, Pacepavicius G, Teixeira C, Campbell L, et al. Hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in the abiotic environment: surface water and precipitation from Ontario, Canada. Environ Sci Technol 2008;42:1657–64.
Viberg H, Fredriksson A, Eriksson P. Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. Toxicol Appl Pharmacol 2003a;192:95-106.
Viberg H, Fredriksson A, Jakobsson E, Orn U, Eriksson P. Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. Toxicol Sci 2003b;76:112–20.
Viberg H, Fredriksson A, Eriksson P. Neonatal exposure to the brominated flame-retardant, 2,2′,4,4′,5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. Environ Toxicol Pharmacol 2004a;17:61–5.
Viberg H, Fredriksson A, Eriksson P. Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl ethers in mice. Toxicol Sci 2004b;81:344–53.
Viberg H, Johansson N, Fredriksson A, Eriksson J, Marsh G, Eriksson P. Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. Toxicol Sci 2006;92:211–8.
Viberg H, Fredriksson A, Eriksson P. Changes in spontaneous behaviour and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). Neurotoxicology 2007;28:136–42.
Voorspoels S, Covaci A, Neels H, Schepens P. Dietary PBDE intake: a market-basket study in Belgium. Environ Int 2007;33:93–7.
Wang Y, Zhang Q, Lv J, Li A, Liu H, Li G, et al. Polybrominated diphenyl ethers and organochlorine pesticides in sewage sludge of wastewater treatment plants in China. Chemosphere 2007;68:1683–91.
Wilford BH, Shoeib M, Harner T, Zhu JP, Jones KC. Polybrominated diphenyl ethers in indoor dust in Ottawa, Canada: Implications for sources and exposure. Environ Sci Technol 2005;39:7027–35.
Wu N, Herrmann T, Paepke O, Tickner J, Hale R, Harvey E, et al. Human exposure to PBDEs: associations of PBDE body burdens with food consumption and house dust concentrations. Environ Sci Technol 2007;41:1584–9.
Zhou T, Taylor MM, DeVito MJ, Crofton KM. Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. Toxicol Sci 2002;66:105–16.
Zota AR, Rudel RA, Morello-Frosch R, Brody JG. Elevated house dust and serum concentrations of PBDEs in California: unintended consequences of furniture flammability standards? Environ Sci Technol 2008;42:8158–64.