TOXICOLOGICAL SCIENCES **88(1)**, 172–180 (2005)
doi:10.1093/toxsci/kfi285
Advance Access publication August 17, 2005

Brief Postnatal PBDE Exposure Alters Learning and the Cholinergic Modulation of Attention in Rats

Caitlin Dufault, Gabriela Poles, and Lori L. Driscoll[1]

Department of Psychology, The Colorado College, Colorado Springs, Colorado 80903

Received July 11, 2005; accepted August 10, 2005

Polybrominated diphenyl ethers (PBDEs), chemicals commonly used as flame retardants, are ubiquitous in the environment and bioaccumulate in humans and wildlife. However, little is known about their potential toxicological properties. In the present study, male Long-Evans rats orally administered the commercial PBDE mixture DE-71 or corn oil for 1 week, beginning at postnatal day (PND) 6, were tested on a visual discrimination task and two sustained attention tasks. After completion of these tasks, the rats were administered a drug challenge with the muscarinic antagonist scopolamine (0, 0.01, 0.03, 0.05 mg/kg), which was injected subcutaneously 30 min prior to testing on the second sustained attention task. The DE-71–exposed rats demonstrated deficits in learning but not in sustained attention when compared to controls. Scopolamine impaired the animals' ability to detect the brief visual cues in controls, as evidenced by decreases in accuracy and increases in omission errors. However, the DE-71–exposed rats were subsensitive to the effects of scopolamine on omission errors, particularly on trials in which a long delay preceded the cue, suggesting alterations in the cholinergic modulation of sustained attention. For the DE-71–exposed rats, the lack of sustained attention deficits in the absence of the drug, coupled with the subsensitivity to scopolamine's effects on sustained attention, suggest that although this PBDE mixture produced lasting alterations in cholinergic functioning, either (1) these alterations were not of sufficient magnitude to be behaviorally relevant, or (2) behavioral deficits resulting from these alterations were overcome by the development of compensatory neural mechanisms or response strategies in adulthood.

Key Words: **PBDE; learning; attention; scopolamine; cholinergic system.**

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are flame retardants commonly added to polymers for the manufacture of electrical appliances, carpets, and polyurethane foam. Their use has increased sharply in the last 20 years (Hale *et al.*, 2003), and they now account for nearly 40% of worldwide flame retardant production (Darnerud *et al.*, 2001). Because PBDEs are easily released into the environment, they are commonly detected in sediment, surface waters, sewage sludge, house dust, air, and on computers and electronics (*e.g.,* Oros *et al.*, 2005; Schecter *et al.*, 2005). Because they are lipophilic, they also accumulate in fatty tissue and have been found in high concentrations in blood, milk, and adipose tissue in humans (Schecter *et al.*, 2003; for a review, see Domingo, 2004).

Polybrominated diphenyl ether congeners differ according to the number (1–10) and position of bromine atoms attached to their aromatic rings. Commercial PBDE mixtures consist of several of the congeners. For example, the penta mixture DE-71 contains primarily tetra-, penta-, and hexa-BDEs; the hexa mixture DE-79 contains primarily hexa- and octa-BDEs; and the deca mixture DE-83R contains primarily deca-BDEs. However, although deca-BDEs are the most highly produced, congeners with lower molecular weights (*e.g.,* the tetra-, penta-, and hexa-BDE congeners BDEs 47, 99, 100, and 153) are the most commonly detected forms of PBDEs in human tissue (Mazdai *et al.*, 2003). Because over 95% of the worldwide use of penta-BDEs occurs in the United States (Hites, 2004), it is not surprising that breast milk in North American women harbors PBDE levels from 3 to one 100 times higher than that tested elsewhere in the world (She *et al.*, 2002).

Polybrominated diphenyl ethers are structurally similar to polychlorinated biphenyls (PCBs) and have been found to share some of their toxicological properties. Commercial PBDE mixtures induce phase I (EROD and PROD) and phase II (UDGPT) hepatic enzyme activities (Fowles *et al.*, 1994; Stoker *et al.*, 2004), increase protein kinase C translocation, inhibit microsomal and mitochondrial Ca^{2+} uptake (Kodavanti and Ward, 2005), and bind to both androgen and estrogen receptors (Meerts *et al.*, 2001; Stoker *et al.*, 2005). Also, like PCBs, they have been shown to disrupt the activity of thyroid hormones. Rodents exposed to PBDEs for a brief period in postnatal development demonstrate significant decreases in plasma thyroxine (T4) levels (Hallgren *et al.*, 2001; Zhou *et al.*, 2001). The decrease may be due in large part to the ability of PBDEs to induce certain phases of hepatic enzyme

Portions of this research were presented at the Neurobehavioral Teratology Society meeting, June 2005, and the Society for Neuroscience meeting, November 2005.

[1] To whom correspondence should be addressed at Department of Psychology, The Colorado College, 14 E. Cache La Poudre, Colorado Springs, CO 80903. Fax: 719–389–6284. E-mail: Ldriscoll@coloradocollege.edu.

activity, leading to an increased catabolism of T4 (Zhou *et al.*, 2002).

The disruption of thyroid homeostasis induced by PBDE exposure can potentially have serious consequences for nervous system development. Thyroid hormones serve an important role in neural development by regulating many other hormones and growth factors in the brain, in effect modulating the developmental timing of neuronal and glial proliferation, migration, and differentiation (*e.g.*, Auso *et al.*, 2004). As such, rodents with experimentally induced hypothyroidism during development exhibit general neural abnormalities such as delayed myelination and decreased synaptic connectivity (Figueiredo *et al.*, 1993), as well as aberrations in the development of the dopaminergic (Vaccari *et al.*, 1990) and cholinergic (Hayashi and Patel, 1987) systems. The cholinergic system, which reaches peak maturation levels during the first few postnatal weeks in rodents (Gould *et al.*, 1991; Oh *et al.*, 1991), is particularly sensitive to thyroid hormone levels, possibly because of the thyroid hormone regulation of nerve growth factor and the TrkA and p75 nerve growth factor receptors (Alvarez-Dolado *et al.*, 1994). Neonatal hypothyroidism in rodents decreases choline acetyltransferase (ChAT) activity in the striatum and prefrontal cortex (Patel *et al.*, 1987; Sawin *et al.*, 1998) of the brain, retards cholinergic cell body growth (Gould and Butcher, 1989), and possibly leads to a permanent decrease in cholinergic neuron density (Sawin *et al.*, 1998).

Given the thyroid hormone–disrupting effects of PBDEs in the developing brain, as well as the known effects of hypothyroidism on development of the cholinergic system, it is not surprising that lasting effects of early PBDE exposure have been found on nicotinic cholinergic receptor numbers in the hippocampus (Viberg *et al.*, 2003) and in locomotor responses to nicotine in mice (Viberg *et al.*, 2002). In addition, PBDE-induced cholinergic dysfunction may underlie the slowed Morris water maze acquisition observed in mice neonatally exposed to the hexa-BDE PBDE 153 (Viberg *et al.*, 2003). However, to date behavioral studies of the effects of PBDEs have focused primarily on general activity measures (Branchi *et al.*, 2002; Eriksson *et al.*, 2002; Viberg *et al.*, 2003, 2004) rather than using tests specific for functions that uniquely depend upon intact cholinergic functioning.

The present study was designed to provide new information about the lasting effects of brief postnatal PBDE exposure on cognitive functions modulated by the cholinergic system, including the detection, sustained attention to, and processing of visual stimuli (Bucci *et al.*, 1998; McGaughy *et al.*, 1994), and the ability to learn visuospatial discrimination problems (Ridley *et al.*, 1999). Rodents were administered the penta mixture DE-71 at a dose of 30 mg/kg (a dose that had previously been shown to temporarily suppress T4 levels; Zhou *et al.*, 2002) dissolved in corn oil, or corn oil alone, for 1 week during peak cholinergic system development (postnatal day 6 through postnatal day 12), and visual discrimination learning and visual attention were assessed in adulthood. In addition, because attentional orienting and sustained attention are sensitive to alterations in muscarinic cholinergic function (Jones and Higgins, 1995; Ruotsalainen *et al.*, 2000), a drug challenge study with the muscarinic antagonist scopolamine was conducted to provide a dynamic assessment of PBDE-induced alterations in the cholinergic modulation of attention.

MATERIALS AND METHODS

Subjects/DE-71 exposure. Sixteen timed-pregnant Long-Evans rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were housed singly in polycarbonate cages. The animals were given unlimited access to tap water and standard laboratory chow (LabDiet 5001; PMI Nutrition International, Richmond, IN) and were kept on a 9:15 light-dark cycle, with lights on at 0900. At parturition, assigned postnatal day (PND) 0, the litters were culled to nine pups, with an attempt to maintain a fairly equal sex ratio. No litter containing fewer than six pups was used.

Two males were randomly selected from each litter for behavioral testing. From PND 6 to PND 12, one of the pups was daily administered the commercial PBDE mixture DE-71, whereas the control pup was administered corn oil. The DE-71(lot 7550OK20A), generously donated by Dr. Kevin Crofton of the U.S. Environmental Protection Agency, contains approximately 25% tetra-BDE, 50–60% penta-BDE, and 4–8% hexa-BDE (Sjodin, 2000). The DE-71 stock solution (300 mg/ml) was prepared by mixing the compound with corn oil and sonicating for 30 min at 40°C. The dosing solution was prepared by diluting the stock solution with corn oil to a concentration of 10 ml/mg. 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 30 mg/kg of body weight for the experimental pup.

Upon weaning (PND 21), each pup was housed in a pair with its littermate from the opposite treatment group. Pups were gradually food restricted to 15 g of chow per day on PND 25 and 10 g of chow per day on PND 28. From this point forward, all pups were housed singly. From the onset of behavioral testing (PND 30) through the end of the experiment (ranging from PND 70 to PND 83), daily chow allotments were adjusted individually based on trial completion rates to maintain the animals' motivation while still allowing for normal growth (at least 2 g per day). If an animal did not complete all 100 trials within 60 min for 2 days in a row, the daily food allotment was decreased by 1 g. If, on the other hand, the animal did not gain at least 2 g of weight per day for 2 days in a row (regardless of motivation level), the daily food allotment was increased by 1 g. This food restriction procedure resulted in body weights that were $\geq$ 85% of *ad libitum* weights. All animal care and experimental procedures were conducted in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85–23, revised 1996).

Apparatus. Testing was conducted in four Plexiglas chambers (ENV-008, Med Associates, Inc., St. Albans, VT), each measuring 30.5 $\times$ 24 $\times$ 34.5 cm and enclosed in a sound-attenuating exterior box. The chambers were modeled after the 5-choice serial reaction time task chambers described elsewhere (Carli *et al.*, 1983). Embedded in one wall of the main chamber, 2.5 cm above the floor, was a square alcove (5 cm wide $\times$ 5 cm tall). A motorized guillotine-type door controlled nosepokes into the alcove, where a sweetened 45-mg reward pellet (Noyes formula AIN-76A; Research Diets, Inc., Lancaster, PA) was dispensed for each trial in which a correct response (a port nosepoke) was made. An automated pellet dispenser (ENV-203-451R, Med Associates, Inc.) delivered the pellets to the alcove from outside the chamber. Extending 1 cm deep from the curved wall opposite the alcove were five square ports (2.5 $\times$ 2.5 cm), 2.5 cm above the floor. Nosepokes into the alcove and the ports were detected by infrared photodiodes positioned just inside the openings of each. Each port was outfitted with a yellow light-emitting diode (LED) embedded in

the back wall. Illumination of these LEDs constituted the visual cue to which animals were trained to respond. A 4W house light, situated above and to the left of the alcove, was illuminated for most of the session but was extinguished for 5 s upon the commission of an error (an event termed a ''timeout''). 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. An infrared video camera was located above each chamber to monitor behavior.

Behavioral testing. Testing took place 6 days per week, with each testing session lasting 100 trials or 60 min, whichever came first. Each trial began with the opening of the guillotine door and the rat's nosepoke into the alcove (initiation) and ended with either a timeout or the rat's retreat from the alcove after reward delivery.

Each rat was first given a series of four training tasks to shape the sequence of responses that constituted a trial: initiation of the trial by poking into the alcove, followed by turning around and making a nosepoke into one of the ports, followed by receipt of a reward in the alcove. The criterion to proceed from one step to the next was the attainment of 100 reward pellets within a single 60-minute session. In the first task, the animal was trained to poke its head into the alcove to obtain a reward, with the door remaining open throughout the session. In the second task, the animal learned that the opening of the door signaled availability of reward. The door closed 3 s after the pellet was dispensed and reopened 2 s later for the beginning of the next trial. The third shaping task trained the animal to initiate a trial at the alcove, turn around, and make a nosepoke into any of the five ports before being rewarded. The purpose of the fourth and last shaping task was to give the rat equal experience with each of the five ports, so that port biases (*i.e.,* responding preferentially to some ports over others) could be minimized. Four of the five ports were covered during each session, forcing the rat to poke into the same port for all 100 trials. A different port was uncovered on each subsequent training session until the rat reached the performance criterion of 100 total responses for each of the five ports.

Once reliable nosepoke responding had been established, the rats progressed through a series of 5-choice visual discrimination tasks. For these and subsequent tasks, initiation at the alcove was followed by a 2-s delay before any visual cues were presented; this allowed the animal time to turn around and orient itself toward the ports. For the first discrimination task, the light cue appeared inside one of the five ports for 15 s or until a response was made, whichever came first. To receive a reward, the rat was required to poke in the illuminated port sometime between the cue onset and within 5 s after the cue offset (the ''limited hold'' period). The location of the visual cue on a given trial was pseudo-randomized such that each port was chosen randomly without replacement until all five ports had been chosen; the cycle was then reset for the next five trials. The animals were required to obtain a performance criterion of at least 80% correct for two out of three sessions of 100 trials each before advancing. The two subsequent tasks were similar to the visual discrimination task but had briefer cue durations of 5 s and 1 s. The rat received one and three sessions on each of these tasks, respectively.

In the sustained attention task that followed (Sustained Attention Task 1), the 1-s visual cue occurred unpredictably, with a pre-cue delay of 0, 3, or 6 s (in addition to the 2-s ''turnaround'' period), thus requiring the animal to sustain attention across the five ports for an indeterminate period of time. The delay on a given trial varied pseudo-randomly, as did the location of the cue. The animals were given 10 sessions on this task. The final sustained attention task, Sustained Attention Task 2, included the same variable pre-cue delays but also incorporated variable cue durations of 200 ms, 500 ms, and 800 ms. After 10 sessions on this task, the rats were administered the drug challenge.

Drug challenge. For the drug challenge phase, the rats were administered Sustained Attention Task 2 only 4 days per week (Monday, Tuesday, Thursday, and Friday) in order to minimize overpractice in the task. Scopolamine hydrochloride (Sigma Aldrich, Inc., St. Louis, MO) was dissolved in sterile water at concentrations of 0, 0.01, 0.03, and 0.05 ml/mg and filtered through a .2-μm filter. Each animal received one of these doses subcutaneously in a volume of 1 ml/kg body weight 30 min prior to testing to allow time for peak plasma levels to be achieved (Ali-Melkkila *et al.*, 1993). Each animal received each of the four doses twice; the dosing order was assigned according to a Latin-square design, which assured that each rat received each of the four doses once in a random order, followed by the same four doses in a different randomized order. Because receptor upregulation can occur with closely spaced scopolamine administrations (Sutin *et al.*, 1986), drug administrations took place only twice per week (on Tuesday and Friday). Animals completed one testing session without drug administration the day before each drug testing session. Differences in the response to scopolamine between control and DE-71–exposed rats were interpreted as evidence for DE-71–induced alterations in the cholinergic modulation of performance.

Dependent measures. For each session, the rates of specific error types were calculated. A premature response was a response made before the onset of the cue light, indicating a failure of inhibitory control. A response made after the onset of the cue light but at a non-illuminated port was deemed an inaccurate response. Finally, a failure to make a nosepoke response into any port within 5 s after cue onset was scored as an omission error. Both omission errors and inaccurate responses reflected lapses of attention. Percent accuracy was defined as the number of correct responses over the number of all ''timely'' responses (*i.e.*, responses made after cue onset and before 5 s following cue offset).

Three latency measures were also recorded. Initiation (alcove) latency, the time between the end of the previous trial and the rat's initiation of a new trial, reflected the animal's motivation, as did the reward latency, the time between the animal's correct response and its retrieval of the reward pellet. The time between cue onset and the animal's nosepoke in the correct port, the response latency, revealed the animal's information processing speed, in addition to its motivation.

Statistical analyses. The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL), version 12 for Windows, was used to analyze the data. For all tasks, the unit of analysis was litter, with one DE-71/control pair per litter. Paired *t*-tests with a Bonferroni correction were used to analyze dependent measures (mean errors to criterion, trials to criterion, percent accuracy, percent omission errors, percent premature responses, and mean latency measures) for the visual discrimination task. Repeated measures analyses of variance (ANOVAs) were used to analyze the dependent measures for sustained attention tasks 1 and 2 and the drug challenge, with drug dose, pre-cue delay, and cue duration treated as within-subjects factors.

RESULTS

Body weights did not differ significantly between PBDE (DE-71) rats and control rats at PND 6, 12, 21, or 30, or at three randomly selected intervals during the visual discrimination and sustained attention tasks (all $p > .05$). However, by the time the drug challenge began, the DE-71 rats weighed significantly more than the controls, $t(13) = 2.92$, $p < .05$.

For the sake of brevity, only the results for the visual discrimination task, Sustained Attention Tasks 1 and 2, and the drug challenge are presented below.

Visual Discrimination Task

The PBDE animals required significantly more trials to reach criterion (2 sessions out of 3 with 80% correct responses or better) than did control animals, $t(13) = 2.68$, $p < .05$, and they committed more errors than controls before reaching criterion, $t(13) = 2.27$, $p < .05$. These treatment differences persisted after removal of one outlier from the data set,

a DE-71–exposed rat that required a large number of sessions to meet criterion. Analyses of the first 5 days of the task alone revealed treatment by day interactions for accuracy, $F(4,8) = 4.09$, $p < .05$, and omission error rate, $F(4,8) = 4.76$, $p < .05$. Pairwise comparisons revealed that the impaired performance exhibited by the DE-71 rats was due to increased omission error rates compared to controls on days 2 through 4 of the task (all $p < .05$; see Fig. 1a), and to decreased percent accuracy of responses on days 4 and 5 of the task (both $p < .05$; see Fig. 1b).

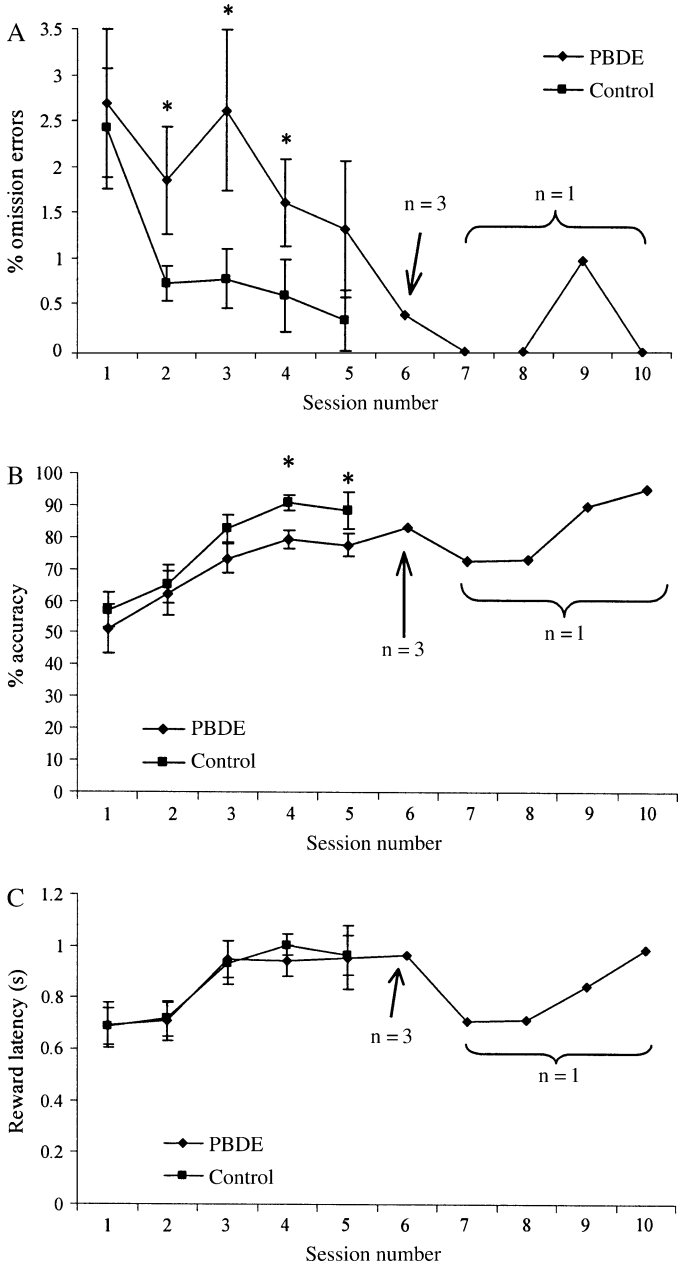

FIG. 1. Performance and latency measures for PBDE and control rats as a function of days spent on the visual discrimination task. (A) Percent omission errors. (B) Percent accurate responses. (C) Average reward latency. PBDE vs. control: * = $p < .05$.

Despite these treatment differences in performance variables, the DE-71–exposed rats did not differ from controls on any latency measure (all $p > .05$; see Figure 1c for reward latency).

Sustained Attention Task 1

For this task, the imposition of the variable pre-cue delays affected performance. As the delay increased, percent accuracy decreased, $F(2,26) = 59.34$, $p < .05$, and frequency of omission errors and premature responses increased, $F(2,26) = 6.60$, $p < .05$, and $F(2,26) = 140.22$, $p < .05$, respectively (data not shown).

There were no main effects of treatment on performance in this task (all $p > .05$), nor were there treatment by delay interactions for accuracy, omission errors, or premature responses. Furthermore, the DE-71–exposed and control rats did not differ on any latency measure.

Sustained Attention Task 2

The addition of both unpredictable pre-cue delays and unpredictable cue durations had a significant effect on performance. For most measures, as the delay increased, performance declined (all $p < .05$), and frequency of premature responses increased, $F(2,26) = 146.96$, $p < .05$. The exception to this pattern was percent omission errors, which declined with increasing delay, $F(2,26) = 20.27$, $p < .05$. For all measures, performance was impaired with shorter cue durations. A main effect of duration was found for percent accuracy, $F(2,26) = 251.03$, $p < .05$, and omission errors, $F(2,26) = 61.55$, $p < .05$. There were significant interactions between delay and duration for accuracy and omission errors (see Fig. 2 for omission errors), $F(4,52) = 22.93$, $p < .05$, and $F(4,52) = 24.51$, $p < .05$, respectively. The shorter cue durations impaired performance more at the 0-s delay than at the 3- and 6-s delays.

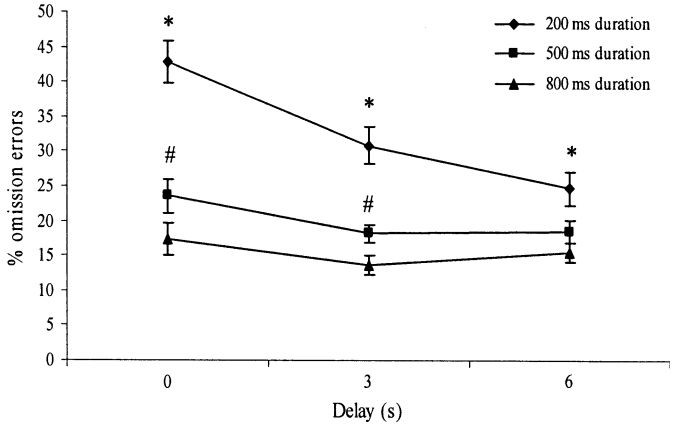

FIG. 2. Percent omission errors as a function of pre-cue delay and duration of the cue light in Sustained Attention Task 2. * = $p < .05$, 200 ms vs. 500 ms and 200 ms vs. 800 ms; # = $p < .05$, 500 ms vs. 800 ms.

DE-71–exposed and control rats did not differ on performance or latency measures, nor were there any interactions between treatment and delay or duration for any measure in this task (all $p > .05$).

Sustained Attention Task 2 with Drug Challenge

Scopolamine dose-dependently increased omission error rate in Sustained Attention Task 2, as evidenced by a main effect of drug dose, $F(3,39) = 11.18$, $p < .05$. A main effect of scopolamine on accuracy was also observed, $F(3,39) = 10.85$, $p < .05$, although the only significant impairment in performance (compared to vehicle) was at the highest dose. Premature response rate was unaffected by the drug. Although there were significant main effects of dose for alcove latency, $F(3,39) = 2.86$, $p < .05$, correct response latency, $F(3,39) = 2.94$, $p < .05$, and reward latency, $F(3,39) = 3.21$, $p < .05$, none of the scopolamine doses differed significantly from the vehicle dose for any measure.

A significant dose by delay interaction was observed for accuracy, $F(6,78) = 2.81$, $p < .05$ (Fig. 3a). Performance on trials with a 0-s delay was impaired by the highest dose of scopolamine ($p = .014$ for vehicle vs. high dose); trials with a 3-s delay were unaffected by any dose of the drug; and trials with a 6-s delay were improved by the medium dose ($p = .038$ for vehicle vs. medium dose). There were no dose by delay or dose by duration interactions for any dependent measure.

The DE-71–exposed rats were subsensitive, when compared to controls, to the effects of scopolamine on omission errors (treatment by dose interaction, $F(3,39) = 2.73$, $p = .05$; see Fig. 3b). For the control rats, all scopolamine doses, when compared to the vehicle dose, increased the frequency with which they missed the visual cue (all $p < .05$), but for the DE-71 rats, none of the scopolamine doses differed significantly from the vehicle dose. When directly comparing the two treatment groups, the DE-71–exposed rats demonstrated fewer omission errors than controls at the middle ($p = .031$) and high ($p = .019$) doses. The nature of the DE-71–exposed rats' subsensitivity to scopolamine was further elucidated by a three-way interaction between treatment, dose, and delay, $F(6,78) = 3.75$, $p < .05$: the subsensitivity was specific to the longer delays, particularly for the highest scopolamine dose (see Fig. 3c for pairwise comparisons).

The DE-71–exposed and control rats did not show differential sensitivity to scopolamine (no treatment by dose interactions) in terms of accuracy, premature responses, or latency measures.

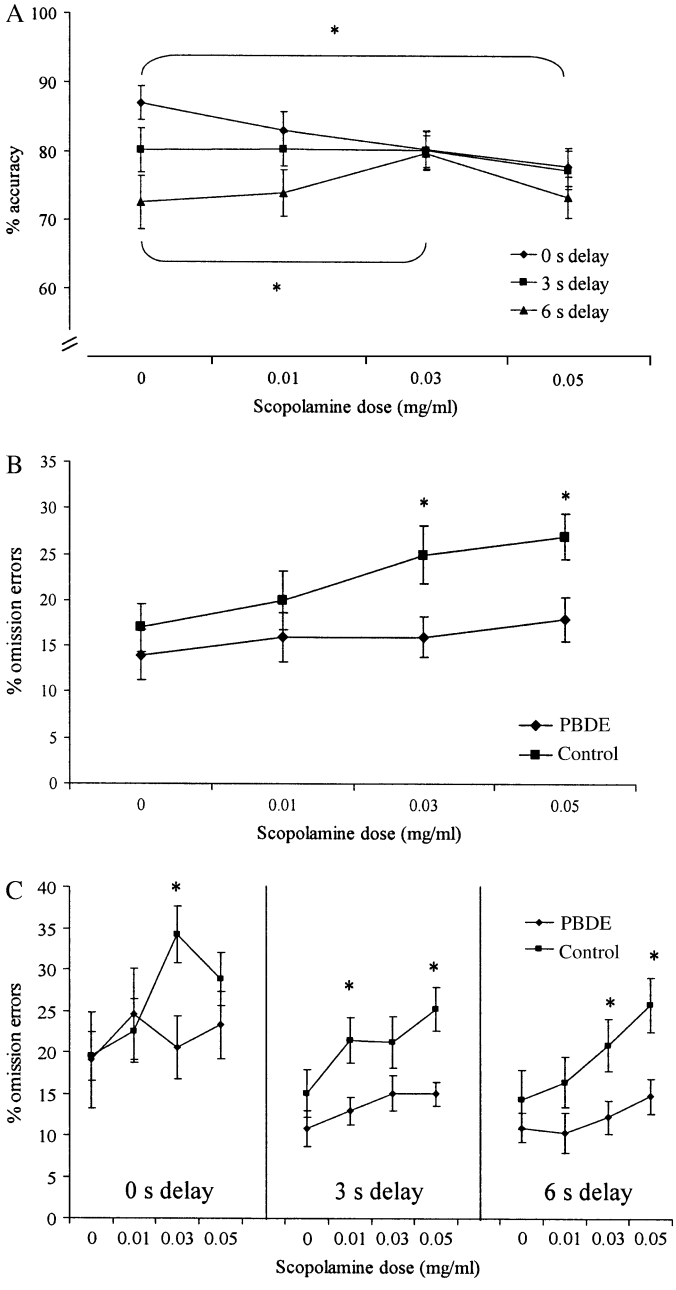

FIG. 3. Performance measures for PBDE and control rats in Sustained Attention Task 2. (A) Percent accurate responses as a function of scopolamine dose and pre-cue delay, collapsed across treatment, and cue duration. * = $p < .05$, vehicle vs. high dose at 0-s delay and vehicle vs. medium dose at 6-s delay. (B) Percent omission errors as a function of scopolamine dose and treatment, collapsed across pre-cue delay and cue duration. * = $p < .05$, PBDE vs. control. (C) Percent omission errors by PBDE-exposed and control rats as a function of scopolamine dose and pre-cue delay, collapsed across pre-cue delay and cue duration. * = $p < .05$, PBDE vs. control.

DISCUSSION

In the present study, brief exposure to DE-71 in the second postnatal week of life impaired learning of the visual discrimination task but did not alter sustained attention or inhibitory control. Despite the lack of a treatment effect on attention in the absence of the drug, the DE-71–exposed animals showed a blunted response to the effects of scopolamine on omission errors in the drug challenge. Each of these effects will be discussed below.

Effects of PBDE Exposure on Visual Discrimination Learning

As adults, rats exposed to DE-71 for 1 week postnatally required more trials and errors to learn the visual discrimination task than did the control animals. Analyses of response types revealed increased rates of both omission errors and inaccurate responses in the DE-71–exposed rats. This finding is consistent with learning impairments in the Morris water maze previously reported in rats orally exposed to 0.9 or 9.0 mg BDE 153/kg on PND 10 (Viberg *et al.*, 2003). However, PBDE exposure has not always been reported to impair learning. Eriksson *et al.* (2001) found no learning deficit during the acquisition phase of the Morris water maze test in mice administered a single dose of BDE 99 neonatally. Although the nature of these inconsistent findings is unknown, the discrepancy may be due to differences in species or exposure paradigms between studies. For example, mice excrete BDE 47, the most common PBDE congener found in animal tissue, more rapidly than do rats (Orn and Klasson-Wehler, 1998). Also, the PBDE exposure in the present study differed in two ways: (1) the compound was a commercial mixture of several PBDE congeners, so the observed effects cannot be attributed to a particular congener (or even to possible contaminants that the mixture may contain); and (2) administration occurred over the period of 1 week, compared to a single administration in the earlier study (Eriksson *et al.*, 2001).

The specificity of treatment differences in this task provide evidence for lasting effects of DE-71 on learning and rule out potential differences in motivation and information-processing speed. On sessions 2 through 5, error rates were higher in the DE-71–exposed rats than in controls: the exposed animals responded less accurately and made more omission errors, suggesting that it took them longer to learn the rules of the task. In contrast, the lack of treatment differences in alcove latency, correct response latency, and reward latency measures reflects that the DE-71–exposed animals were adequately motivated. In addition, they responded as quickly as controls on trials in which a response was made, suggesting that information processing was not slowed.

Effects of PBDE Exposure on Attention

Attention consists of overlapping processes such as attentional orientation and engagement, selective attention, and sustained attention (Behrmann and Haimson, 1999; Berger and Posner, 2000; Bushnell *et al.*, 2000). The two sustained attention tasks used in the present study were designed to assess rapid attentional orienting and the ability to sustain spatial attention divided among many locations over many trials (Robbins, 2002). As was evidenced by significant effects of pre-cue delay and cue duration on performance measures (accuracy, omission error rate, premature response rate), the tasks successfully tapped attentional function. It is notable, therefore, that early DE-71 exposure did not alter performance in either task. There are three possible explanations for this result, the most obvious of which is that the neural systems that mediate attentional performance were not damaged by early DE-71 exposure. Another possibility is that because the rats were administered a large number of trials for each task, the DE-71–exposed animals may have had the opportunity to compensate for attentional deficits through the adoption of response strategies. However, this is unlikely, because analyses of the first session of each of the tasks alone revealed no performance differences between DE-71 and control rats (data not shown). Finally, it is possible that early DE-71 exposure did produce damage to one or more neural systems mediating attention, but that redundant, undamaged systems compensated for the lost function (see below for additional discussion on this issue). Additional research is needed to elucidate the most likely of these possibilities.

Effects of Scopolamine on Visual Attention

Forebrain cholinergic activity, particularly that arising from the cholinergic nucleus basalis projections to neocortex, is essential for optimal attentional performance. Excitotoxic (Robbins *et al.*, 1989) and immunochemical (Risbrough *et al.*, 2002) lesions of the cholinergic basal forebrain, or blockage of the high-affinity choline uptake transporter (Muir *et al.*, 1992) dramatically reduce cortical cholinergic activity and impair performance in tasks similar to the ones used in the present study. Therefore, as was expected, scopolamine, a muscarinic cholinergic antagonist, dose-dependently increased omission error rate in the second sustained attention task. Evidence for the effect of scopolamine on attentional orienting was seen with response accuracy, which declined the most at the shortest pre-cue delay. In addition, because the drug significantly increased omission errors at both short and long delays, sustained attention may also have been impaired. These findings are consistent with previous reports of scopolamine's effects on attention (Jones and Higgins, 1995; Ruotsalainen *et al.*, 2000). However, unlike the animals in those studies, the rats in the present study showed no increase in premature response rate in response to scopolamine. The reason for this discrepancy is unknown.

PBDE Exposure Alters Sensitivity to Scopolamine's Effects on Attention

Although controls demonstrated the expected attentional deficits in response to cholinergic blockade, performance by the DE-71 animals was relatively resistant to this manipulation. More specifically, when compared to controls, the DE-71 animals demonstrated subsensitivity to the effects of scopolamine on omission errors at the middle and high doses. There was also a significant interaction between treatment, dose, and delay for omission errors: the subsensitivity to scopolamine in the DE-71–exposed rats was greatest for trials in which the cue was preceded by the longest delays (3 s and 6 s). The specificity

of the treatment differences in sensitivity to scopolamine's effects suggests that developmental DE-71 exposure produced alterations specifically in cholinergic networks that are important for sustaining attention, such as the basal forebrain neurons that project to the frontoparietal cortex (Robbins *et al.*, 1989). In contrast, the treatment groups did not differ in their response to scopolamine's effects on motivation or motor function, as was evidenced by nonsignificant treatment by dose interactions for alcove, response, or reward latencies.

These findings suggest that early postnatal DE-71 exposure produced lasting alterations in cholinergic functioning, specifically in systems that normally mediate sustained attention. This evidence for altered cholinergic activity is consistent with research in which rats orally administered 8.0 mg BDE 99/kg on PND 10 demonstrated altered responsivity to nicotine, a nicotinic cholinergic agonist (Viberg *et al.*, 2002), and in which animals orally administered 9.0 mg BDE 153/kg on PND 10 showed a significant decrease in the density of nicotinic receptors in the hippocampus (Viberg *et al.*, 2003). These findings, together with the subsensitivity to scopolamine noted in the present study, may indicate that exposure to DE-71 produces lasting changes in some presynaptic aspect of cholinergic functioning, such as neuron number, acetylcholine production, or acetylcholine release.

One possible mechanism by which DE-71 could alter cholinergic system development is through its disruption of thyroid hormone homeostasis (Hallgren *et al.*, 2001; Zhou *et al.*, 2001, 2002). During the early postnatal period in rodents, experimentally induced hypothyroidism leads to reduced expression of ChAT, the enzyme responsible for catalyzing acetylcholine production (Patel *et al.*, 1987). Therefore, it is plausible that reduced ChAT activity would be found in animals exposed to PBDEs, particularly when the exposure corresponds with the brain growth spurt, as was the case with the animals in the current study. However, because thyroid hormone levels were not assessed in this study, the possibility cannot be ruled out that the altered sensitivity to scopolamine in the DE-71–exposed rats is due to other known effects of this compound, such as androgen receptor antagonism (Stoker *et al.*, 2005), aryl hydrocarbon receptor (AhR)-mediated induction of hepatic enzymes (Kuiper *et al.*, 2004; Zhou *et al.*, 2001), or alterations in protein kinase C activation or intracellular calcium uptake (Kodavanti and Ward, 2005). Furthermore, if thyroid hormones were altered in the DE-71–exposed rats, the possibility cannot be ruled out that the observed effects in this study were due at least in part to hypothyroid-induced alterations in other neurotransmitter systems, such as the dopaminergic system, that are sensitive to thyroid hormone variations in development (Vaccari *et al.*, 1990).

If exposure to DE-71 disrupted the development of cortical cholinergic functioning, then why did the DE-71–exposed animals fail to demonstrate impairments in the sustained attention task in the absence of scopolamine? A recent lesion study suggests that neonatal cholinergic forebrain lesions, unlike adult lesions, do not produce attentional deficits (Pappas *et al.*, 2005). It is possible that other structures or systems take over the function of the missing cholinergic projections if the insult is incurred early in life. Therefore, while the DE-71–exposed animals demonstrated an altered response to scopolamine, neural or behavioral compensation for impaired cholinergic development may have prevented deficits in the attention task. Further characterization of the state of cholinergic neurons in DE-71–exposed animals will be required to test this hypothesis.

CONCLUSIONS AND FUTURE DIRECTIONS

The findings in this study provide evidence for lasting behavioral alterations in rats exposed to DE-71 for a brief period in neonatal development. The exposed rats demonstrated impaired visual discrimination learning, in addition to a blunted response to the effects of cholinergic blockade on sustained attention. The DE-71 rats' subsensitivity to the effects of scopolamine in the attention task provides evidence that even this brief period of exposure produced lasting alterations in the cholinergic system. Polybrominated diphenyl ethers may cause their neurotoxic effects by inducing developmental hypothyroidism, which in turn leads to reduced ChAT levels and low levels of cortical acetylcholine in adulthood. Future studies should investigate whether brief postnatal DE-71 exposure is accompanied by both reduced thyroid hormone levels and reduced ChAT levels or cholinergic neuron numbers in the adult forebrain.

ACKNOWLEDGMENTS

This research was funded by Colorado College Research and Development and Venture Grants to L.L.D. and G.P. and a Howard Hughes Undergraduate Research Program Grant to C.D.

REFERENCES

Ali-Melkkila, T., Kanto, J., and Iisalo, E. (1993). Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. *Acta Anaesthesiol. Scand.* **37,** 633–642.

Alvarez-Dolado, M., Iglesias, T., Rodriguez-Pena, A., Bernal, J., and Munoz, A. (1994). Expression of neurotrophins and the Trk family of neurotrophin receptors in normal and hypothyroid rat brain. *Mol. Brain. Res.* **27,** 249–257.

Auso, E., Lavado-Autric, R., Cuevas, E., Del Rey, F. E., Morreale, D. E., and Berbel, P. (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. *Endocrinology* **145,** 4037–4047.

Behrmann, M., and Haimson, C. (1999). The cognitive neuroscience of visual attention. *Curr. Opin. Neurobiol.* **9,** 158–163.

Berger, A., and Posner, M. I. (2000). Pathologies of brain attentional networks. *Neurosci. Biobehav. Rev.* **24,** 3–5.

Branchi, I., Alleva, E., and Costa, L. G. (2002). Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. *Neurotoxicology* **23,** 375–384.

Bucci, D. J., Holland, P. C., and Gallagher, M. (1998). Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. *J. Neurosci.* **18,** 8038–8046.

Bushnell, P. J., Levin, E. D., Marrocco, R. T., Sarter, M. F., Strupp, B. J., and Warburton, D. M. (2000). Attention as a target of intoxication: Insights and methods from studies of drug abuse. *Neurotoxicol. Teratol.* **22,** 487–502.

Carli, M., Robbins, T. W., Evenden, J. L., and Everitt, B. J. (1983). 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.* **9,** 361–380.

Darnerud, P. O., Eriksen, G. S., Johannesson, T., Larsen, P. B., and Viluksela, M. (2001). Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* **109(Suppl 1),** 49–68.

Domingo, J. L. (2004). Human exposure to polybrominated diphenyl ethers through the diet. *J. Chromatogr. A* **1054,** 321–326.

Eriksson, P., Jakobsson, E., and Fredriksson, A. (2001). Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* **109,** 903–908.

Eriksson, P., Viberg, H., Jakobsson, E., Orn, U., and Fredriksson, A. (2002). A brominated flame retardant, 2,2′,4,4′,5-pentabromodiphenyl ether: Uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. *Toxicol. Sci.* **67,** 98–103.

Figueiredo, B. C., Otten, U., Strauss, S., Volk, B., and Maysinger, D. (1993). Effects of perinatal hypo- and hyperthyroidism on the levels of nerve growth factor and its low-affinity receptor in cerebellum. *Brain Res. Dev. Brain Res.* **72,** 237–244.

Fowles, J. R., Fairbrother, A., Baecher-Steppan, L., and Kerkvliet, N. I. (1994). Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology* **86,** 49–61.

Gould, E., and Butcher, L. L. (1989). Developing cholinergic basal forebrain neurons are sensitive to thyroid hormone. *J. Neurosci.* **9,** 3347–3358.

Gould, E., Woolf, N. J., and Butcher, L. L. (1991). Postnatal development of cholinergic neurons in the rat: I. Forebrain. *Brain Res. Bull.* **27,** 767–789.

Hale, R. C., Alaee, M., Manchester-Neesvig, J. B., Stapleton, H. M., and Ikonomou, M. G. (2003). Polybrominated diphenyl ether flame retardants in the North American environment. *Environ. Int.* **29,** 771–779.

Hallgren, S., Sinjari, T., Hakansson, H., and Darnerud, P. O. (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.* **75,** 200–208.

Hayashi, M., and Patel, A. J. (1987). An interaction between thyroid hormone and nerve growth factor in the regulation of choline acetyltransferase activity in neuronal cultures, derived from the septal-diagonal band region of the embryonic rat brain. *Brain Res.* **433,** 109–120.

Hites, R. A. (2004). Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environ. Sci. Technol.* **38,** 945–956.

Jones, D. N., and Higgins, G. A. (1995). Effect of scopolamine on visual attention in rats. *Psychopharmacology (Berl.)* **120,** 142–149.

Kodavanti, P. R., and Ward, T. R. (2005). Differential effects of commercial polybrominated diphenyl ether and polychlorinated biphenyl mixtures on intracellular signaling in rat brain in vitro. *Toxicol. Sci.* **85,** 952–962.

Kuiper, R. V., Bergman, A., Vos, J. G., and van den Berg, M. (2004). Some polybrominated diphenyl ether (PBDE) flame retardants with wide environmental distribution inhibit TCDD-induced EROD activity in primary cultured carp (*Cyprinus carpio*) hepatocytes. *Aquat. Toxicol.* **68,** 129–139.

Mazdai, A., Dodder, N. G., Abernathy, M. P., Hites, R. A., and Bigsby, R. M. (2003). Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environ. Health Perspect.* **111,** 1249–1252.

McGaughy, J., Turchi, J., and Sarter, M. (1994). Crossmodal divided attention in rats: Effects of chlordiazepoxide and scopolamine. *Psychopharmacology (Berl.)* **115,** 213–220.

Meerts, I. A., Letcher, R. J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J. G., van der Burg, B., and Brouwer, A. (2001). *In vitro* estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. *Environ. Health Perspect.* **109,** 399–407.

Muir, J. L., Dunnett, S. B., Robbins, T. W., and Everitt, B. J. (1992). Attentional functions of the forebrain cholinergic systems: Effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task. *Exp. Brain Res.* **89,** 611–622.

Oh, J. D., Butcher, L. L., and Woolf, N. J. (1991). Thyroid hormone modulates the development of cholinergic terminal fields in the rat forebrain: Relation to nerve growth factor receptor. *Brain Res. Dev. Brain Res.* **59,** 133–142.

Orn, U., and Klasson-Wehler, E. (1998). Metabolism of 2,2′,4,4′-tetrabromodiphenyl ether in rat and mouse. *Xenobiotica* **28,** 199–211.

Oros, D. R., Hoover, D., Rodigari, F., Crane, D., and Sericano, J. (2005). Levels and distribution of polybrominated diphenyl ethers in water, surface sediments, and bivalves from the San Francisco Estuary. *Environ. Sci. Technol.* **39,** 33–41.

Pappas, B. A., Payne, K. B., Fortin, T., and Sherren, N. (2005). Neonatal lesion of forebrain cholinergic neurons: Further characterization of behavioral effects and permanency. *Neuroscience* **133,** 485–492.

Patel, A. J., Hayashi, M., and Hunt, A. (1987). Selective persistent reduction in choline acetyltransferase activity in basal forebrain of the rat after thyroid deficiency during early life. *Brain Res.* **422,** 182–185.

Ridley, R. M., Barefoot, H. C., Maclean, C. J., Pugh, P., and Baker, H. F. (1999). Different effects on learning ability after injection of the cholinergic immunotoxin ME20.4IgG-saporin into the diagonal band of Broca, basal nucleus of Meynert, or both in monkeys. *Behav. Neurosci.* **113,** 303–315.

Risbrough, V., Bontempi, B., and Menzaghi, F. (2002). Selective immunolesioning of the basal forebrain cholinergic neurons in rats: Effect on attention using the 5-choice serial reaction time task. *Psychopharmacology* **164,** 71–81.

Robbins, T. W., Everitt, B. J., Marston, H. M., Wilkinson, J., Jones, G. H., and Page, K. J. (1989). Comparative effects of ibotenic acid induced lesions of the substantia innominata on attentional functions in the rat: Further implications for the role of the cholinergic system of the nucleus basalis in cognitive processes. *Behav. Brain Res.* **35,** 221–240.

Robbins, T. W. (2002). The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl.)* **163,** 362–380.

Ruotsalainen, S., Miettinen, R., MacDonald, E., Koivisto, E., and Sirvio, J. (2000). Blockade of muscarinic, rather than nicotinic, receptors impairs attention, but does not interact with serotonin depletion. *Psychopharmacology (Berl.)* **148,** 111–123.

Sawin, S., Brodish, P., Carter, C. S., Stanton, M. E., and Lau, C. (1998). Development of cholinergic neurons in rat brain regions: Dose-dependent effects of propylthiouracil-induced hypothyroidism. *Neurotoxicol. Teratol.* **20,** 627–635.

Schecter, A., Papke, O., Joseph, J. E., and Tung, K. C. (2005). Polybrominated diphenyl ethers (PBDEs) in U.S. computers and domestic carpet vacuuming: Possible sources of human exposure. *J. Toxicol. Environ. Health A* **68,** 501–513.

Schecter, A., Pavuk, M., Papke, O., Ryan, J. J., Birnbaum, L., and Rosen, R. (2003). Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environ. Health Perspect.* **111,** 1723–1729.

She, J., Petreas, M., Winkler, J., Visita, P., McKinney, M., and Kopec, D. (2002). PBDEs in the San Francisco Bay Area: Measurements in harbor seal blubber and human breast adipose tissue. *Chemosphere* **46,** 697–707.

Sjodin, A. (2000). Occupational and dietary exposure to organohalogen substances, with special emphasis on polybrominated diphenyl ethers. Doctoral dissertation, Stockholm University.

Stoker, T. E., Cooper, R. L., Lambright, C. S., Wilson, V. S., Furr, J., and Gray, L. E. (2005). *In vivo* and *in vitro* anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. *Toxicol. Appl. Pharmacol.* **207,** 78–88.

Stoker, T. E., Laws, S. C., Crofton, K. M., Hedge, J. M., Ferrell, J. M., and Cooper, R. L. (2004). Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols. *Toxicol. Sci.* **78,** 144–155.

Sutin, E. L., Shiromani, P. J., Kelsoe, J. R., Jr., Storch, F. I., and Gillin, J. C. (1986). Rapid-eye movement sleep and muscarinic receptor binding in rats are augmented during withdrawal from chronic scopolamine treatment. *Life Sci.* **39,** 2419–2427.

Vaccari, A., Rossetti, Z. L., de Montis, G., Stefanini, E., Martino, E., and Gessa, G. L. (1990). Neonatal hypothyroidism induces striatal dopaminergic dysfunction. *Neuroscience* **35,** 699–706.

Viberg, H., Fredriksson, A., and Eriksson, P. (2002). Neonatal exposure to the brominated flame retardant 2,2′,4,4′,5-pentabromodiphenyl ether causes altered susceptibility in the cholinergic transmitter system in the adult mouse. *Toxicol. Sci.,* **67,** 104–107.

Viberg, H., Fredriksson, A., and Eriksson, P. (2003). 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.* **192,** 95–106.

Viberg, H., Fredriksson, A., and Eriksson, P. (2004). Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl ethers in mice. *Toxicol.Sci.* **81,** 344–353.

Zhou, T., Ross, D. G., DeVito, M. J., and Crofton, K. M. (2001). Effects of short-term *in vivo* exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.* **61,** 76–82.

Zhou, T., Taylor, M. M., DeVito, M. J., and Crofton, K. M. (2002). Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol.Sci.* **66,** 105–116.