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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

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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).

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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,