NEUROBEHAVIORAL TOXICITY OF BROMINATED FLAME RETARDANTS: DIFFERENTIAL EFFECTS OF PBDE-99, TBBPA AND HBCD AND ENDOCRINE RELATION

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Introduction

The reported increases in concentrations of brominated flame retardants (BFRs) in environmental matrices[1] have motivated studies of their possible toxic properties. In particular, the presence of polybrominated diphenyl ethers (PBDEs) in human milk[2,3] has guided investigations of developmental effects. Several studies indicated influences on thyroid hormones[4,5] and sexual development[6] in experimental animals. Since thyroid hormones and sex steroids are important regulators of neural development[7,8], effects on the nervous system are likely[9]. The present report summarizes three experiments with PBDE-99, one of the most abundant PBDE congeners, tetrabromobisphenol A (TBBPA), and hexabromocyclododecane (HBCD). Compared to PBDEs, information about environmental levels and toxicity of TBBPA and HBCD is scarce.

Materials and Methods

Time-pregnant Long Evans rats were SC injected with 0; 1; or 10 mg PBDE-99/kg bw, daily from gestational day 10-18. Also, a group exposed to the technical PCB mixture Aroclor 1254 (A1254; 30 mg/kg bw). At weaning, randomly selected dams and pups were sacrificed, taking blood and organ samples. Concentrations of estradiol and testosterone were determined in serum. Littermates were studied for puberty onset. Sweet preference, which is a sexually dimorphic behaviour in rats, was tested in adult offspring to examine behavioral feminization. In addition, effects on haloperidol-induced catalepsy were studied in naive male offspring. A full description of the methods is given elsewhere[10,11]. Dissections were repeated in adult offspring. Tissues for analyses of PBDE-99 concentrations were taken at different time-points.

Experiments with TBBPA and HBCD were part of the FIRE project and were conducted according to OECD guideline 415, using a benchmark design. Dietary exposure of Wistar rats started before conception and was continued throughout mating, gestation, lactation, and after weaning of the offspring. There were eight dose groups in the TBBPA study (vehicle; 3; 10; 30; 100; 300; 1000; or 3000 mg/kg bw) and nine in the HBCD experiment (0; vehicle; 0.1; 0.3; 1; 3; 10; 30; or 100 mg/kg bw). Behavioral testing was conducted in adult offspring. Since results of a subacute study with TBBPA indicated predominant effects on the thyroid hormone system, brain stem auditory evoked potentials (BAEPs) were selected to study neurotoxicity. It is well-known that insufficient supply with thyroid hormones in the early postnatal period affects hearing in the lower frequency range which is related to function of the apical part of the cochlea[12]. The methods used for BAEP recording are described elsewhere[11]. According to a subacute HBCD experiment, there were effects on circulating thyroid hormones. In addition, in vitro studies indicated impairment of dopamine uptake into synaptosome preparations[13]. Therefore, BAEPs and haloperidol-induced catalepsy were selected for investigation of HBCD effects on the nervous system in vivo. Benchmark analyses were conducted according to the procedures described by Slob[14].

Results and Discussion

Exposure to PBDE-99 did not cause marked effects on number of implantations, number of still-borne pups, and pup body weights. There were decreases in circulating estradiol and to a lesser extent in testosterone in male pups at weaning. These decreases became even more pronounced in adulthood. Also, there were reductions in anogenital distance at the higher dose of PBDE-99 in males at weaning which did not recover in adulthood.

Puberty onset was delayed by PBDE-99 in female offspring at the higher dose, while in males a slight acceleration was observed at the lower dose. Sweet preference showed a dose-related increase in PBDE-exposed adult males (figure 1). In females, some signs of supernormality were seen, but these were not significant. A1254 did not cause any effect on this sexually dimorphic behavior. Haloperidol-induced catalepsy was elevated by exposure to PBDE-99 and A1254 in the early phase of the measuring period, but no differences to controls were found 60 min after the application of haloperidol. The observed effects on sweet preference indicate feminization of this sexually dimorphic behavior in adult males and in line with similar effects of a PCB mixture which was reconstituted according to the congener pattern found in human breast milk[15,16]. Together with the effects on steroid hormone levels and sexual development, which are consistent with the outcome of exposure to a commercial mixture of PBDEs (DE-71)[6], the present results suggest that PBDE-99 affects effects related to sex steroids, in addition to reductions in circulating thyroid hormones[4,5]. Also, the outcome of the sweet preference test indicates that not all effects of PBDE-99 can be ascribed to dioxin-like impurities, since A1254 did not alter this behavior, thus, confirming results of a previous experiment[15]. This conclusion is supported by in vitro studies which also indicate differential effects of PBDE-99 and A1254 on astrocytoma cells[17]. Also, the PBDE-99 used here was shown not to induce CYP1A1 and EROD in mice (Darnerud, personal communication). The tissue concentrations of PBDE-99 in exposed rats were approximately 8-15-fold higher than the 95-percentile of levels found in North America[3]. These factors are about 10 to 100-fold higher in Europe[2].

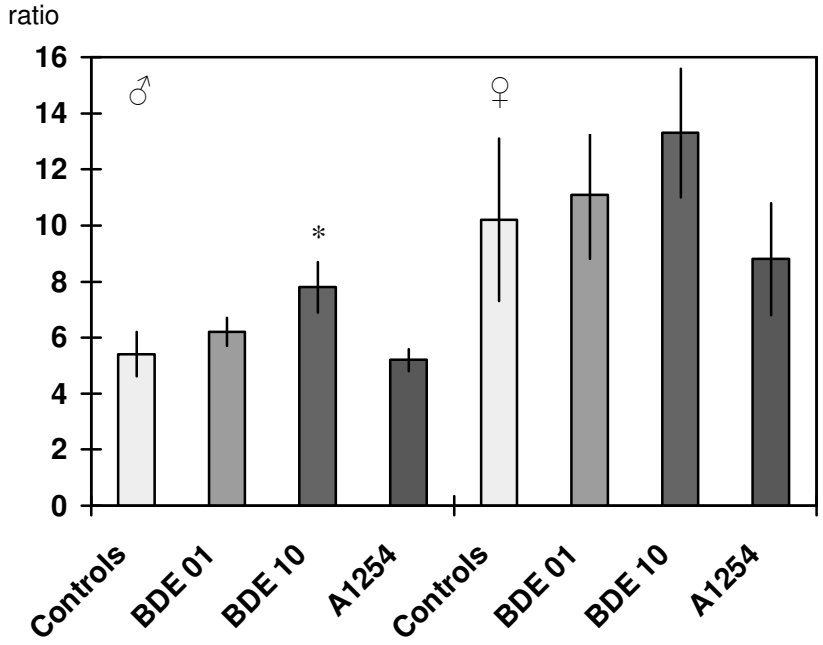

Figure 1: Sweet preference in male (left) and female (right) rat offspring (means ± SEM). Bars show the ratio of saccharin to water consumption. Exposure to PBDE-99, but not Aroclor 1254, induced elevated sweet preference in males ($p<0.05$). Females exhibited some non-significant signs of supernormality. The result indicates feminization of this sexually dimorphic behaviour. BDE 01 – 1 mg PBDE-99/kg bw, BDE 10 - 10 mg PBDE-99/kg bw, A1254 – 30 mg Aroclor 1254/kg bw.

Exposure to TBBPA elevated thresholds and prolonged latencies in the BAEP in adult rat offspring. Effects were found in the lower frequency range and the prolongation of latencies was more pronounced on later waves. This outcome is in general accordance with reported auditory effects of developmental PCB exposure in rats[18] which was related to damage in the apical part of the cochlea due to a lack of supply with thyroid hormones in the

neonatal period[12]. However, the more marked increase in latencies of later waves in the BAEP indicates an additional neural effect of TBBPA. According to benchmark calculations, the lowest BMD-L values were 0.9 mg/kg bw for threshold at 2 kHz in female offspring and 7.7 and 8.3 mg/kg bw for latencies of wave IV at 0.5 kHz in males and females, respectively.

In contrast to TBBPA, effects of HBCD on the BAEP were only seen in male offspring. Also, no progressive delays in peak latencies were detected in later waves of the BAEP, indicating a cochlear origin of the impairment. The relation to thyroid hormone system remains unclear, as in contrast to the subacute study, no effects on circulating thyroid hormones were detected in littermates in the present experiment. However, HBCD is reported to alter thyroid hormone receptor-mediated gene expression in vitro[19]. The lowest BMD-L values were 0.2 and 0.9 mg/kg bw for thresholds at 0.5 kHz and for clicks, respectively. BMD-L values for wave II latency were approximately 30 mg/kg bw for clicks and about 40 mg/kg bw at 1 kHz. In the catalepsy test, HBCD exposure resulted in decreased latencies to movement onset in all three situations used to measure cataleptic behaviour, namely, bar, grid, and box. This outcome may be due to HBCD-related induction of xenobiotic enzymes in the liver, resulting in enhanced metabolism of haloperidol, or to a lower dopaminergic activity in the brain. Further studies are needed to identify the mechanism underlying HBCD-induced alterations in cataleptic behavior. The lowest BMD-L values were approximately 3 mg/kg for foreleg retraction in male offspring on the box. In females, these values were 0.6 mg/kg bw for foreleg retraction on the box and about 4 mg/kg on the grid and the sum of latencies in all three situations.

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