A MASS BALANCE STUDY OF A COMMERCIAL PENTABROMODIPHENYL ETHER MIXTURE IN MALE SPRAGUE-DAWLEY RATS

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Introduction

Polybrominated diphenyl ethers (PBDEs) are common additive flame-retardants used in high impact polystyrene, polyurethane foam, and textile coatings. The most recent production figures indicate that 40,000 tons/year are manufactured worldwide.[1] They are structurally similar to other environmentally persistent aromatics, i.e. dioxins, PCBs, and furans. Therefore, there is a growing belief that this family may be the next environmental contaminant of concern. Environmental sampling for the past 20 years has shown that PBDEs are persistent in sediment and bioaccumulate in tissues.[2,3] Levels in human milk are increasing,[4] as are levels in organisms that inhabit the deep oceans.[5] BDE-47 is the most abundant PBDE in environmental tissues, followed by BDE-99, 100, 153, and 154. Production of commercial penta-BDE formulations, which contain predominantly BDE-47 and 99, accounts for 10% of the PBDE market, while production of octa- and deca-BDE mixtures accounts for 15% and 75%, respectively. Commercial octa-BDE mixtures contain tetra- and pentabrominated diphenyl ethers only as minor contaminants. The predominance of lower brominated congeners in biota, therefore, may be due to preferential bioavailability and bioaccumulation of these PBDEs, debromination of the higher brominated congeners, or factors such as transport and stability in the environment.

In order to examine the bioavailability and bioaccumulation of PBDEs at low exposure levels, we have performed a mass balance study in male rats fed a low dose of a commercial penta-BDE mixture for 21 days. The initial results of this study are presented here.

Materials and Methods

A commercial penta-BDE mixture (DE-71; Great Lakes Chemical) was added to peanut oil and administered in the feed for 21 days at the rate of 32 ng/day/rat (672 ng total). The male Sprague-Dawley rats (n=8; 258-288 g; Taconic Labs) were trained to consume the treated feed in 1h. Control rats (n=8) consumed peanut oil vehicle in the feed. The rats were housed individually in stainless steel metabolism cages, which allowed for separation of urine and feces, and the room was kept at 25°C with a 12h light:12 h dark cycle. The rats were killed; feces, livers and carcasses were frozen at -70° C until analyzed.

Carcasses from dosed rats were individually homogenized in a Hobart grinder. Livers were diced to homogeneity with a razor blade, and feces were lyophilized. Control carcasses, livers, and feces were pooled for processing. All samples (~10 g) were spiked with ^{13}C-labelled recovery standards and extracted in an Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) with 50:50 hexane:$MeCl_2$. The extracts were purified by a modification of EPA Method 1613[6] including sequential washing of the extracts with 20% aqueous potassium hydroxide, water, concentrated sulfuric acid, and water, followed by chromatography on a triphasic silica column, an

alumina column, and a charcoal column. Feces were further purified on Florisil columns from which most PBDEs eluted with 10% ether:hexane. GC-MS analyses were performed on a VG Autospec instrument operating in the electron impact selective ion monitoring mode at a resolution of >2500. Gas chromatography was performed on a 30 meter DB-5MS column (J&W Scientific, Folsom CA) using on-column injection and pressure programming to elute BDE-209 in under 45 min. Two ions were monitored for each homologue group (mono- to deca-BDEs); ion ratios were within 15% of the theoretical value. PBDEs were quantitated by comparison to an internal standard (^{13}C-BDE-77 for mono- to tetra-BDEs, ^{13}C-BDE-139 for penta- to deca-BDEs). Recoveries were adjusted based on the ^{13}C-recovery standards.

The PBDE congener composition of the DE-71 formulation was also determined by the isotope dilution GC-MS method. PBDEs were analyzed in four each of the dosed rats and the control rats. The background PBDE levels found in the controls were subtracted from the levels in the dosed rats to provide the mass balance data. Urine was not analyzed.

Results and Discussion

Each dosed rat received PBDEs in their feed at a total concentration of 2.9 ppb, a rate designed to mimic environmental levels. Thus far, only four of the dosed animals and the control composites have been fully analyzed by GC-MS. The feces sample from one of the dosed rats was excluded from our data due to low recoveries of the ^{13}C-standards (<10%). Other samples had recoveries over 30% for most labeled standards. The urine was not analyzed because the lipophilic nature of these compounds and previous dosing studies[7,8] indicated PBDEs would not be found there. PBDEs were detected in the control rats but at levels much lower than in the dosed rats (Table 1). These background levels were probably due to air-born or method contamination or may have been present in the feed or peanut oil which has not been analyzed yet.

Tissue retention and fecal excretion in the dosed rats for the six most abundant BDE congeners in the penta formulation dose are shown in Table 2. On average 0.3 - 1.2% of the dosed congeners remained in the liver, and 17.9 – 42.7% remained in the carcass. The BDE congeners with the highest bioaccumulation in the liver and carcass were BDE-99 and BDE-153 with totals of 46.2% and 45.3%, respectively. Excretion in the feces was similarly low for all congeners, except BDE-85, and showed that at least 80% of the dose was bioavailable to rats. High amounts of BDE-85 in the feces (56% of dose) may indicate that this congener was poorly absorbed. Overall, only 40 - 88% of the dosed congeners were accounted for, suggesting that significant metabolism occurred for some of the congeners.

These chronic feeding study results paralleled some of the observations made in a limited number of metabolism studies. Bioavailability and tissue retention were high for the lower brominated congeners. Rat liver and carcass retention of ^{14}C-BDE-99 after a single oral dose of 8.8 mg/kg was 0.9 and 49.1%, respectively, after 3 days.[7] Somewhat higher levels of BDE-47 (86%) than seen in this study remained in the bodies of rats 5 days after receiving a single 14.5 mg/kg dose[8]. While most studies, both *in vivo* and *in vitro*, have concluded that metabolic activity towards PBDEs is low,[5,7,8] this study indicated significant metabolism of most of the tetra to hexa-BDEs. The induction of metabolizing enzyme systems during the course of long-term exposure may explain the increased amounts of metabolism found in the chronic study. Non-extractable residues or lipid-bound metabolites were identified in both previous short-term studies and appeared to increase as a percent of the excreted fraction with time.[7,8] Cytochrome P450 enzyme activity has also been shown to be induced in mice and rats given subchronic doses of penta-BDE formulations.[9,10]

Results from this long-term dosing experiment in rats do not show any obvious differences between the bioavailability or accumulation of lower brominated congeners, especially BDE-47 and 99, which could be used to explain the elevated amounts of BDE-47 in biota. The congener distribution pattern in the rat carcasses and livers was the same as in the dosing formulation. A similar congener pattern resembling the penta-BDE formulation has been reported in chickens[11]. While species differences, length of exposure, or dose vehicle may explain the differences between the congener patterns in rats and wildlife, it may also involve the environmental transport and stability of the different PBDE congeners.

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Table 1. Concentration (pg/g) of PBDEs in control rats and dosed rats from a feeding study with a penta-BDE formulation. Dosed rat values are averages with n = 4 for liver and carcass; n = 3 for feces.

BDE #	Control liver	Average dosed liver	Control carcass	Average dosed carcass	Control feces	Average dosed feces
47	214	400 ± 95	210	497 ± 74	357	765 ± 52
85	5	19 ± 4	5	30 ± 6	20	287 ± 166
99	135	565 ± 135	126	753 ± 176	319	1471 ± 83
100	27	79 ± 20	52	130 ± 22	67	190 ± 27
153	15	112 ± 24	34	148 ± 55	42	240 ± 17
154	9	23 ± 8	9	39 ± 14	37	144 ± 4

Table 2. Average control-subtracted PBDE amounts (ng) in liver, carcass, and feces from male rats administered a commercial penta-BDE formulation for 21 days. n = 4 for liver and carcass; n = 3 for feces.

BDE #	Total Dosed	Amount in Liver	% of Dose	Amount in Carcass	% of Dose	Amount in Feces	% of Dose	Total % Recovered
47	190	1.30 ± 0.66	0.7	61.97 ± 15.1	32.6	14.36 ± 1.88	7.6	40.9
85	20	0.10 ± 0.03	0.6	5.44 ± 1.19	32.0	9.48 ± 5.99	55.8	88.3
99	300	3.04 ± 1.00	1.0	135.6 ± 36.4	45.2	40.60 ± 3.14	13.5	59.8
100	50	0.37 ± 0.13	0.7	16.93 ± 4.48	33.9	4.34 ± 0.91	8.7	43.3
153	56	0.69 ± 0.17	1.2	24.68 ± 12.1	44.1	6.98 ± 0.67	12.5	57.8
154	24	0.10 ± 0.06	0.4	6.57 ± 3.12	27.4	3.78 ± 0.13	15.8	43.5