A MASS BALANCE FEEDING STUDY OF A COMMERCIAL OCTABROMODIPHENYL ETHER MIXTURE IN 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. Recent production figures indicate that 40,000 tons/year are manufactured worldwide.[1] They are structurally similar to other environmentally persistent contaminants, i.e. dioxins, PCBs, and furans. Therefore, there is a growing belief that PBDEs 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] Concentrations in human milk appear to be increasing,[4] as do concentrations in organisms that inhabit the deep oceans.[5] The most abundant PBDEs found in biota are tetra- to hexa-brominated congeners (BDE-47, 99, 100, 153, and 154); the most abundant congener in environmental samples is the deca-brominated compound (BDE-209).

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 penta-brominated diphenyl ethers only as minor contaminants and are mainly composed of hexa- to nona-BDEs. Although we have found little data reporting hepta-, octa-, or nona-BDEs in the environment, production figures would suggest that these PBDEs are present. Hepta-BDEs have been reported in fish species,[6] but octa- and nona-BDEs are seldom, if ever, reported. The low occurrence of these higher brominated congeners may be due to limited bioavailability and bioaccumulation, to debromination reactions that give rise to lower brominated congeners, to instability in the environment, or merely to inadequate detection limits in the analytical methods.

In this study we have examined the bioavailability, bioaccumulation, and possible biotransformation of the more highly substituted PBDEs at low exposure levels. The initial results are presented for a mass balance study in male rats fed a low dose of a commercial octa-BDE mixture for 21 days.

Materials and Methods

A commercial octa-BDE mixture (DE-79; Great Lakes Chemical) was added to peanut oil and administered in the feed for 21 days at the rate of 33 ng/day/rat or approximately 3 ppb in the feed. Male Sprague-Dawley rats (n=8; 250–300 g; Taconic Labs) were trained to consume the treated feed within a 1 h feeding period. Control rats (n=8) consumed peanut oil vehicle in the feed within the same time period. 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 24 h after the final feeding; 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. For the controls, the respective tissues were pooled before processing. All samples (~10 g) were fortified with seven ^{13}C-labeled recovery

standards (1 ng each except for ^{13}C-BDE-209 at 20 ng) and extracted in an Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) with 50:50 hexane:$MeCl_2$ (1500 psi, 100 °C). The extracts were purified by a modification of EPA Method 1613[7] 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, a basic alumina column, and a charcoal column. Samples were analyzed by GC-MS in the electron impact selective ion monitoring mode using a 30 meter DB-5MS column, on-column injection, and pressure programming. 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. For unknown hepta- and octa-BDEs, quantitation was estimated based on the average relative response factors for the known hepta-BDEs. For the nona-BDEs, quantitation was estimated using the relative response factor of ^{13}C-deca-BDE.

The PBDE congener composition of the DE-79 formulation was determined by the isotope dilution GC-MS method using the estimates described above. PBDEs have been analyzed in four dosed rats and four control rats. Background PBDE levels measured 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

To date, only four of the dosed animals and the control composites have been fully analyzed by GC-MS. A fecal sample from one of the dosed rats was excluded from this data due to low recoveries of the ^{13}C-standards and an apparent contamination with lower brominated congeners. 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[8,9] indicated PBDEs were not excreted in urine. 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 yet been analyzed. Several of the dosed congeners were below the limits of detection in certain matrices. Nona-BDEs were not found in any of the dosed animals, although analysis of the octa-formulation showed a major nona-BDE component. The insensitivity of nona-BDEs in the GC-MS method may be the reason for this lack of detection.

Tissue retention and fecal excretion in the dosed rats for eight of the major BDE congeners are shown in Table 2. Of the congeners detected 0.9–1.7 % of the dose remained in the liver, and 16.2–62.9 % remained in the carcass. Bioaccumulation of the PBDEs decreased with increasing bromination. Total retentions in the liver and carcass were 64.6 % for BDE-153, 27–37 % for the three hepta homologs, and 16–23 % for two of the octa homologs. Excretion in the feces accounted for 20–40 % of most of the dosed congeners. Except for BDE-154 and an octa-BDE that were near the background levels, total recoveries of the dosed BDEs ranged from 48 to 80 %. These recoveries indicate that the higher brominated congeners were metabolized; however, we saw no evidence that lower brominated diphenyl ethers were among the metabolites produced.

Compared to a previous dosing experiment with a commercial penta-BDE mixture,[10] the recovery and tissue distribution of BDE-153, a congener common to both doses, were similar. In the present study, liver contained 1.7 % of the dosed BDE-153, carcass contained 63 %, and feces contained 16 %; in the previous study, the respective amounts were 1.2 %, 44 %, and 13 %. In the previous study, no differences were seen in the bioavailability or accumulation of tetra- to hexa-brominated congeners; however, for the hexa- to nona-brominated compounds in this study, decreasing bioavailability with increasing bromination was apparent. Although PBDEs above the hepta-substituted level have not been reported in biota, this study demonstrated that they are absorbed by rats. Factors such as transport and

Table 1. Concentration (pg/g ± s.d.) of the major PBDEs in control and dosed rats from a feeding study with an octa-BDE formulation. Dosed rat values are averages with n = 4 for liver and carcass; n = 3 for feces; zero = not detected.

BDE #	Control liver	Average dosed liver	Control carcass	Average dosed carcass	Control feces	Average dosed feces
154	5	8 ± 2	8	11 ± 3	20	26 ± 4
153	19	87 ± 39	38	177 ± 20	41	255 ± 45
183	0	190 ± 112	19	357 ± 41	104	1716 ± 206
hepta	0	0	0	18 ± 5	9	103 ± 20
190	0	0	0	10 ± 4	0	47 ± 7
octa	0	52 ± 33	0	53 ± 11	49	622 ± 56
octa	0	0	0	15 ± 14	35	83 ± 52
octa	0	0	0	0	99	353 ± 68

Table 2. Average control-subtracted PBDE amounts (ng ± s.d.) in liver, carcass, and feces of male rats administered a commercial octa-BDE formulation for 21 days. n = 4 for liver and carcass; n = 3 for feces; zero = not detected.

BDE #	Total Dosed	Amount in Liver	% of Dose	Amount in Carcass	% of Dose	Amount in Feces	% of Dose	Total % Recovered
154	5	0.04 ± 0.03	0.9	0.82 ± 0.65	18.1	0.22 ± 0.19	4.9	23.9
153	49	0.81 ± 0.10	1.7	30.89 ± 5.6	62.9	7.77 ± 4.84	15.9	80.4
183	216	2.22 ± 0.29	1.0	75.1 ± 11.8	34.6	58.56 ± 35.1	27.1	62.8
hepta	11	0	0	4.05 ± 1.3	37.1	3.43 ± 1.74	31.5	68.6
190	8	0	0	2.19 ± 1.0	27.0	1.69 ± 0.99	20.9	47.9
octa	55	0.61 ± 0.17	1.1	11.87 ± 3.0	21.7	20.81 ± 11.8	38.2	61.0
octa	10	0	0	0	0	1.71 ± 2.1	16.7	16.7
octa	21	0	0	3.41 ± 3.6	16.2	9.21 ± 5.9	44.3	60.5

stability in the environment or mode of exposure (sediment-bound or water soluble versus dissolved in oil) will affect the uptake in wildlife. Detection limits for the octa- and nona-BDEs may be another reason that they are seldom found. We did not detect nona-BDEs in any matrix, although we estimated that the dosed nona-BDE may have been present in as high as 7 ppb in the feces.

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