Bioaccumulation of Decabromodiphenyl Ether (BDE-209) from the diet into Sprague-Dawley Rats

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Introduction

Decabromodiphenyl ether is the major polybrominated diphenyl ether (PBDE) flame retardant in production today with a market demand of 56,100 metric tons in 2001.[1] Studies have shown that decaBDE is absorbed by rats and fish from the diet.[2-5] The extent to which decaBDE is bioavailable, however, remains questionable. In three studies with rats, the amount of decaBDE bioavailable from the diet has ranged from not detectable after one year of feeding to 10% of a single dose.[2,3,6] Using radiolabeled decaBDE to quantitate bioavailability, El Dareer and coworkers found <1% of a daily dose taken up into rats;[2] however, Morck et al. found 10% of a single oral dose was absorbed.[3] This difference may have been due to a number of factors including matrix effects. A dose dissolved in a lipophilic, oily matrix may provide better bioavilability than a dry dose mixed in the feed. In studies with radiolabeled decaBDE, the amount absorbed by rats from the diet was found to be dose dependent but ranged as high as 1.4 mg.[2,7] In this study, we have dosed rats for 21 days with low levels of decaBDE (3 ug/day) to determine its bioavailability from repeated, low exposures. This dose level was below the amounts absorbed in previous studies and was administered in corn oil to optimize absorption.

Materials and Methods

Six male Sprague-Dawley rats (80 days old) were trained to eat ground rat chow (12 g) topped with corn oil (200 uL) over a 1 hr period each morning. Water was allowed ad libitum, but only one feeding period was allowed. The rats were maintained on 12 g of feed and 200 uL of corn oil for the entire experiment. A dose was prepared using a decaBDE formulation that was 99% pure (DE-83R, Great Lakes Chemical) dissolved in corn oil and a small amount of toluene. The solution was stirred overnight under a nitrogen stream to remove most of the toluene. The final concentration was 18.9 ug decaBDE/mL oil to give a daily dose of 3.78 ug/rat (0.3 ppm in the total diet). Rats were randomly divided into dosed and control groups and housed in individual cages. Control rats (n=3; 217.0 ± 6.2 g) received feed and oil for 21 days; dosed rats (n=3; 203.3 ± 11.6 g) received feed and the oil dose for 21 days.

The rats were sacrificed 24 hrs after the last feeding. Blood, liver, gastrointestinal tract, and the remaining carcass were collected and frozen until analyzed. The livers and carcasses (sans GI) were individually homogenized, and subsamples were purified by a previously described procedure.[8] Briefly, the subsample (2-10 g) was spiked with seven ^{13}C-labelled PBDE recovery standards, extracted in an Accelerated Solvent Extractor (Dionex, Sunnyvale, CA), sequentially washed with 20% aqueous potassium hydroxide, water, concentrated sulfuric acid, and water, and then chromatographed on a triphasic silica column, an alumina column, and a charcoal column. The oil dose was purified through the triphasic silica and alumina column only. PBDEs were analyzed by isotope dilution GC/MS on a 30 m DB5-MS column at a mass resolution of 5000. The method was validated with spiked liver and carcass samples for quantitation of 20 PBDE congeners with an accuracy and precision better than 35%. Recoveries of ^{13}C-PBDE standards were 31-128%. The amount of the dose that was bioaccumulated was calculated by subtracting the average control levels from the dosed levels.

Results and Discussion

The decaBDE dose administered in this study was 300 ppb in the diet, a dose that we estimated would provide a measurable increase in the amount of decaBDE in the dosed rats relative to the controls. Table 1 shows that tissues from the control rats had low amounts of deca- and nona-BDEs either due to background exposures, low level laboratory contamination, or, in the case of nonaBDEs, degradation of the decaBDE. However, the tissues of dosed rats were elevated 10-20 times over those of control rats. The daily dose was roughly 100-1000 times lower than the

daily doses used in previous studies.[2,3] The rats showed no adverse health effects. All the rats gained weight during the course of the experiment even though they were held on a restricted diet (average gain = 19 g). Liver weights were not noticeably different between the control and dosed groups.

The concentration of decaBDE in the liver of dosed rats was 3-times higher than that of the carcass both on a whole weight or lipid weight basis (Table 1). Other studies of decaBDE in rats and rainbow trout have also shown concentrations 3-20 times higher in the liver than in other major tissue compartments after oral dosing.[2-4] These results are not surprising given that the liver is an organ highly prefused by the blood supply. In 21 day feeding studies in rats with the penta- and octa-BDE formulations, the lower brominated congeners (tetra to octa) appeared to concentrate to an equal or higher extent in the carcass compared to the liver.[8,9] The disposition of decaBDE appears different than that of the other PBDEs.

In addition to decaBDE, Table 1 shows that other congeners were present in the dose and in the tissue samples. These congeners were identified as nona-, octa-, and hepta-BDEs by GC retention times and/or the presence of diagnostic molecular ions. Several of these congeners were recovered in higher amounts than could be accounted for by the dose (>100%). The formation of lower brominated congeners from decaBDE has been reported in fish. Carp exposed to decaBDE in the diet for 60 days showed penta- through octa-BDEs accumulating in their tissues but not decaBDE.[5] The lower brominated congeners were attributed to metabolic debromination of the decaBDE and gave an estimated bioavailability for decaBDE of 0.4%. Although debromination of decaBDE has not previously been observed in rats, it is possible that metabolic debromination occurs at a low rate, and these congeners magnify to a larger extent than the decaBDE due to differences in uptake and half life. Another explanation is degradation of decaBDE during cleanup and handling. The excess nonaBDEs may be accounted for by degradation during the cleanup process, because ^{13}C-nonaBDEs are observed in the GC/MS analysis as a result of degradation of the ^{13}C-decaBDE recovery standard; and we have observed degradation of spiked samples during cleanup. However, we have never observed degradation of nona- or deca-BDEs to octa-BDEs during the course of the cleanup at greater than our detection limits (0.6% of decaBDE, 4% of nonaBDE). Based on this observation, only octa-2, which represents 2-3% of the decaBDE in the tissues, may be forming in the rats. Stapleton and coworkers also observed one octaBDE in the tissues of carp fed decaBDE but were unable to identify the specific congener.[5] Further experiments are planned to investigate this possible metabolic debromination in rats and positively identify end products.

The amount of decaBDE in the liver and carcass of the dosed rats was roughly equivalent to one daily dose (4.3% of the total or 3.36 ug). Although daily fluxes of decaBDE into and out of the rats were not measured in this study, bioconcentration factors (BCFs), another estimate of bioavailability, were measured in both the liver and carcass and are given in Table 2. These values are compared with relative BCFs for various other PBDEs that have been studied in our laboratory in 21 day dosing experiments.[8,9] For decaBDE, the BCFs calculated after 21 days of dosing may represent true steady state values, because the half life in rats is thought to be as short as 2 days.[2-4] Half lives of other PBDEs are estimated to be over 20 days,[10] so that BCFs calculated after 21 days of dosing represent only pseudo-steady state values and may be much higher at true steady state. The BCFs show that decaBDE is bioconcentrated to a similar extent as the other PBDEs in the liver after 21 days (BCF = 0.11-0.33). However, decaBDE is concentrated to a much lower extent in the carcass, only one-tenth that of other PBDEs. This is in agreement with previous studies that found low accumulation of decaBDE in rats during long term feeding studies.[2,6] Reasons for the different accumulation patterns of decaBDE in liver and the rest of the carcass may include selective transport processes, rapid metabolism, or numerous other possibilities.

The methods used in this study selectively analyzed PBDEs. Previous studies in rats have reported extensive metabolism of absorbed decaBDE to either bound residues or phenolic compounds.[2, 3] For example, only 26% of the radioactivity in the liver of rats dosed with ^{14}C-decaBDE was identified as parent compound.[3] In the current study, we have quantified the bioaccumulation of the parent compound, decaBDE, along with several other PBDEs. DecaBDE was minimally bioconcentrated (BCF=0.05 for the carcass) and evidence for debromination to other PBDEs was inconclusive.

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Table 1. Average PBDE amounts (ng) in the dose, average concentrations in control and dosed rat tissues (ng/g wet weight), and total amounts (ng) found in the dosed rat tissues from a feeding study with a deca-BDE formulation; n = 3. For non-detected congeners (nd), the maximum amount possible based on the detection limit is given in parentheses.

BDE #	Total Dose (ng)	Average Liver Concentration		Total in Dosed Liver (ng)[a] (corrected for control)	Average Carcass Concentration		Total in Dosed Carcass (ng)[a] (corrected for control)
		Control (ng/g)	Dosed (ng/g)		Control (ng/g)	Dosed (ng/g)	
209	78,194	2.11 ± 1.06	50.7 ± 12.2	321 ± 59.7	1.25 ± 0.50	15.2 ± 4.0	3028 ± 811
Nona-1[b]	41	0.08 ± 0.03	1.08 ± 0.86	6.4 ± 5.1	Nd (0.04)	0.36 ± 0.10	68.9 ± 19.5
Nona-2[b]	130	0.13 ± 0.07	7.81 ± 4.32	50.3 ± 26.2	Nd (0.05)	2.92 ± 0.12	621 ± 14.5
206	395	0.13 ± 0.07	1.05 ± 0.90	5.9 ± 5.4	0.06 ± 0.02	0.43 ± 0.12	81.2 ± 26.4
Octa-1[b]	Nd (0.2)	Nd (0.003)	0.19 ± 0.08	1.2 ± 0.5	Nd (0.002)	0.09 ± 0.008	18.1 ± 1.7
Octa-2[b]	Nd (0.2)	Nd (0.003)	0.80 ± 0.25	5.3 ± 1.4	Nd (0.002)	0.36 ± 0.01	78.7 ± 4.9
203	9	Nd (0.004)	0.04 ± 0.008	0.2 ± 0.05	Nd (0.002)	0.02 ± 0.003	3.8 ± 0.8
Octa-3[b]	19	Nd (0.003)	0.22 ± 0.09	1.4 ± 0.5	Nd (0.002)	0.11 ± 0.02	23.1 ± 3.1
183	5	Nd (0.006)	0.03 ± 0.01	0.2 ±0.06	Nd (0.006)	0.03 ± 0.006	6.1 ± 1.4
Weight (g)	256	6.43 ± 0.32	6.67 ± 0.38		205.3 ± 8.1	216.7 ± 6.4	
% lipid		3.46 ± 0.58	3.43 ± 1.34		4.13 ± 0.11	4.32 ± 1.30	

[a]Formation of hepta- to nona-BDEs during the analytical clean up can not be distinguished from formation in vivo. [b] These values are estimates because exact standards were not available to validate the analytical method.

Table 2. Bioconcentration factors (BCFs) calculated for select PBDEs from 21 day feeding experiments in male rats with penta-, octa-, and deca-BDE formulations at the specified dose levels. BCF= concentration in tissue (ng/g)/concentration in feed (ng/g).

	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209
Liver BCF	0.17	0.24	0.31	0.33	0.11	0.28	0.16
Carcass BCF	0.41	0.59	0.49	0.46	0.34	0.33	0.05
Dose ng/g	0.83	1.33	0.22	0.24	0.11	0.92	305.2