Environ. Sci. Technol. **1999**, *33,* 1612−1617

Dietary Uptake and Biological Effects of Decabromodiphenyl Ether in Rainbow Trout (*Oncorhynchus mykiss*)

AMELIE KIERKEGAARD,* LENNART BALK, ULLA TJÄRNLUND, CYNTHIA A. DE WIT, AND BO JANSSON

Institute of Applied Environmental Research, Stockholm University, SE-106 91 Stockholm, Sweden

Dietary uptake and effects of decabromodiphenyl ether (DeBDE), a widely used flame retardant, were studied in rainbow trout. Fish were fed for 16, 49, or 120 days with control or DeBDE-treated food (7.5−10 mg of DeBDE/kg of body weight/day). One group was fed DeBDE for 49 days and then control diet for 71 days to study depuration. Chemical analyses were performed using GC/MS(ECNI). Several physiological and biochemical variables were also measured. DeBDE concentrations in muscle increased from <0.6 ng/g of fresh weight to 38 (±14) ng/g after 120 days. Corresponding liver concentrations were <5 and 870 (±219) ng/g of fresh weight. Several hexa- to nonabromodiphenyl ethers, present in both liver and muscle, increased in concentration with exposure length. These congeners originate from metabolism of DeBDE and/or selective uptake of minor components in the DeBDE product. After depuration, DeBDE concentrations declined significantly, but concentrations of some lower brominated congeners were unaffected. Liver body index and plasma lactate concentrations were higher in fish exposed for 120 days and in the depuration group, indicating delayed chronic effects, possibly from lower brominated congeners. DeBDE uptake (0.02−0.13%) and possible metabolism seem not to be major sources of tetra- and pentabromodiphenyl ethers found in wild fish.

Introduction

Polybrominated diphenyl ethers (PBDE) are flame retardants used in plastics and textiles (*1*). Tetra- (TeBDE), penta- (PeBDE), and hexabromodiphenyl ethers (HxBDE) are widespread in biota in the northern hemisphere including the Arctic (*2*−*6*). Time trend studies of guillemot eggs and pike from background areas in Sweden show increasing concentrations of one TeBDE and two PeBDEs during the 1970s and 1980s (*3*, *7*, *8*). Currently, the most frequently used PBDE is decabromodiphenyl ether (DeBDE), with an annual worldwide production (1992) of 30 000 tons (*1*).

There are few studies on DeBDE concentrations in the environment. In 1979, DeBDE was identified in soil, air particulate, and sediment samples and in human hair collected near plants manufacturing brominated flame retardants (*9*−*11*). BDE congeners containing 6−10 bromine atoms were found in human adipose tissue samples collected in a U.S. survey in 1987 (*12*). In Japan, DeBDE was found in airborne dust, sediment, and a mussel sample (*13*−*15*). Recently, high DeBDE concentrations were reported in sediment from the Swedish River Viskan, which is polluted by several textile industries that have used DeBDE (*16*). A few pike muscle samples showed traces of DeBDE; however, the concentrations were below the quantification limit (100 ng/g of lipid weight).

An effective molecular cross section of 9.5 Å is suggested to limit uptake via the gills and possibly the gut (*17*). However, dietary uptake efficiencies of 2,2′,4,4′-TeBDE (BDE47), 2,2′,4,4′,5-PeBDE (BDE99), and 2,2′,4,4′,5,5′-HxBDE (BDE153) in pike were 92, 62, and 40%, respectively, despite effective cross sections larger than 9.5 Å for BDE99 and BDE153 (*18*). BDE congeners are numbered in analogy to IUPAC PCB nomenclature.

Radiolabeled BDE47 was highly absorbed in rats and mice dosed orally (*19*). However, ^{14}C-DeBDE uptake was low following oral administration to rats, and between 90 and 99% of the dose was recovered in the feces and gut (*20*, *21*). A 2-year feeding study of DeBDE in rats confirmed low accumulation; however, the small portion that reached the adipose tissue, measured as the total bromine content, remained unaffected for 90 days of recovery (*20*, *22*). This agrees with the half-lives determined for TeBDE to HxBDE in rats; the longest half-life was for one of the higher brominated congeners examined (*23*).

The objective of this study was to investigate if DeBDE is absorbed from the gastrointestinal tract of fish despite its extreme lipophilicity and large molecular size. Another aim was to investigate if lower brominated PBDEs found in wildlife could be linked to the use of the DeBDE product. Several biological variables were also studied.

Experimental Section

Chemicals. Technical decabromodiphenyl ether (DOW FR-300BA) was purified on a charcoal column (Norit A, United States Biochemical Corp., Cleveland, OH) from planar compounds, such as polybrominated dibenzo-*p*-dioxins and dibenzofurans. The solution contained detectable amounts of nona- (NoBDE), octa- (OcBDE), and heptabromodiphenyl ethers (HpBDE). Organic solvents were either glass distilled or of analytical grade. The purified DeBDE was used as reference standard for the quantitative determination of DeBDE (BDE209). The commercial flame retardant Bromkal 70-5DE (Chemische Fabrik Kalk GmbH, Cologne, Germany) was used as reference standard for 2,2′,4,4′-tetrabromodiphenyl ether (BDE47, 37%), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE99, 35%), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE100, 6.8%), and 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE154, 2.5%) (*24*). 2,2′,4,4′,5,5′-Hexabromodiphenyl ether (BDE153) was a gift from Eva Jakobsson, Stockholm University. Dechlorane 603 (Occidental Chemicals, Dallas, TX) was used as internal standard.

Fish. Juvenile rainbow trout (*Oncorhynchus mykiss*) were purchased from Delta Lax AB, Timrå, Sweden. Fish were kept in standard square fish tanks (2.1 × 2.1 m), supplied with a continuous flow of charcoal-filtered brackish water (Baltic Sea, salinity = 7.0−7.5‰); natural light conditions and outdoor water temperatures (Figure A1, Supporting Information) for the period June−September were followed.

Food Preparation. Food was prepared from Barents Sea cod (*Gadus morhua*). Soft and semisoft tissues (gonads, gall bladder, and liver excluded) were homogenized in a meat mincer and mixed with an equal volume of 3% gelatin solution. DeBDE was dissolved/suspended in 10 mL of corn

* Corresponding author phone: +46 8 674 71 82; fax: +46 8 674 76 37; e-mail: amelie.kierkegaard@itm.su.se.

10.1021/es9807082 CCC: $18.00
Published on Web 04/01/1999

TABLE 1. Selected Morphological and Physiological Effects of DeBDE Exposure on Rainbow Trout[a]

		condition factor			liver body index (%)			lactate (mg/100 mL of blood)			hemoglobin (mg/mL blood)			leucocytes (% of blood cells)			lymphocytes (% of leucocytes)		
	days	mean	CI	*n*	mean	CI	*n*	mean	CI	*n*	mean	CI	*n*	mean	CI	*n*	mean	CI	*n*
control	0	0.894	±0.018	44	1.39	±0.083	44	na[b]			na			na			na		
control	16	0.945	±0.025	32	1.24	±0.090	32	7.19	±2.22	12	75.5	±10.2	12	8.58	±1.31	12	57.1	±7.35	12
expt	16	0.994	±0.033	32	1.23	±0.068	32	5.81	±1.54	12	58.5*	±6.23	12	9.17	±1.38	11	67.2	±6.00	11
control	49	0.871	±0.019	41	0.904	±0.048	41	na			na			7.22	±1.23	12	70.6	±5.82	12
expt	49	0.907	±0.019	41	0.950	±0.050	41	na			na			6.69	±1.09	12	61.4	±6.05	12
control	120	0.889	±0.020	34	0.819	±0.055	34	6.65	±1.17	12	60.6	±6.56	12	3.40	±0.405	12	64.2	±5.63	12
expt	120	0.926	±0.028	30	0.945*	±0.062	30	17.0*	±6.59	11	69.7	±7.52	11	4.21	±0.721	12	53.4*	±4.00	12
depur	71	0.876	±0.030	30	1.01*	±0.124	30	10.6*	±2.72	11	63.6	±6.60	12	5.16*	±1.02	12	62.1	±5.77	12

[a] Results are given as mean values ± 95% confidence intervals (CI) derived from *n* fish analyzed. An asterisk indicates a significant difference at the $p < 0.05$ level, between treated and control group from the same exposure time (Student's *t* test). [b] na, not analyzed.

oil and vigorously shaken to a slurry and then mixed with the cod/gelatin solution. Another batch of cod/gelatin solution was mixed with corn oil only. The mixtures were divided into 24 g portions in Petri dishes, air-dried in darkness for 24−36 h, and stored frozen. Before feeding, the cod chips were thawed, divided into small pieces, and allowed to soak in water. Both DeBDE-treated food and control food were consumed within 20−40 s by the fish.

Exposure. Rainbow trout were randomly divided into control and treated groups. Trout were fed either control cod chips or cod chips containing DeBDE in doses of 7.5−10 mg of DeBDE/kg of body weight/day.

Sampling. Fish were sampled for biological and chemical studies after 16, 49, and 120 days of exposure and after 49 days exposure plus 71 days of depuration. Some biological variables were studied on day 0. To avoid contamination from the gastrointestinal tract, the fish were starved for 24−48 h before sampling and dissected via the heart sac downward. Fish were killed by a blow to the head, and blood samples were taken directly from the dorsal aorta using a heparinized syringe. The sex, total length, and weight of each fish were recorded. Condition factor, liver body index, hematocrit, lactate, hemoglobin, glucose, protein, and differential blood cell counts as well as ethoxyresorufin-*O*-deethylase (EROD) and ethoxycoumarin-*O*-deethylase (ECOD) were analyzed according to methods described in Balk et al. (*25*) and references cited therein. Transketolase was analyzed according to the procedure of Tate and Nixon (*26*).

Chemical Methods. Liver and muscle (skin removed) samples were homogenized, solvent extracted, and treated with sulfuric acid as previously described (*27*). After being soaked in water, cod chips were homogenized and centrifuged, and aliquots of 1 g each were extracted and treated as above. A few sample extracts (1 mL each) were treated with 1 mL of potassium hydroxide (0.5 M in 50% ethanol). The alkaline phase was acidified (pH 2) with hydrochloric acid and reextracted with 2 mL of hexane/methyl tributyl ether (1:1). Pentabromophenol was used as a positive control.

The extracts were analyzed by GC/MS (Trio-1000, Fisons Instrument) measuring the negative ions formed at chemical ionization (ECNI), using the following conditions: ion source temperature, 200 °C; interface temperature, 300 °C; emission current, 150 μA; and electron energy, 70 eV. The reagent gas was ammonia (99.998%). The gas pressure was set to maximize sensitivity for the ions of interest. The ions monitored were m/z −79 and −81 for PBDE and m/z −237 and −239 for Dechlorane.

The gas chromatograph (Carlo Erba Mega MFC 500) used helium (99.995%) as carrier gas. The split/splitless injector temperature was 280 °C. The column was a 15 m DB-5 (J&W, 0.25 mm i.d., 0.25 μm film thickness). The temperature program was 80 °C, 1 min with the split valve closed, 25 °C/min to 240 °C, 5 °C/min up to 315 °C, and held there for 17 min.

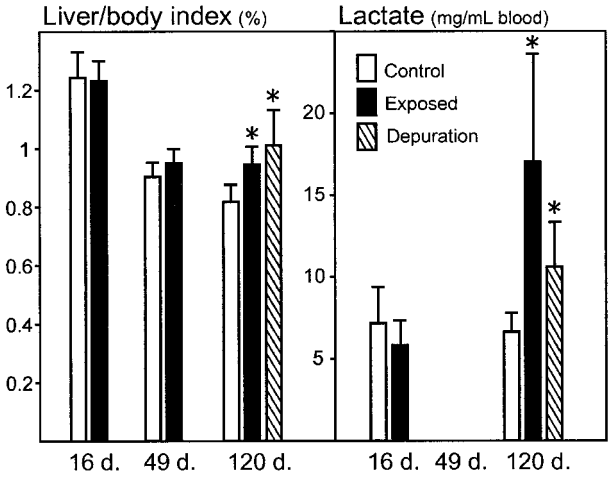

FIGURE 1. Plasma lactate concentration (mg/mL of blood) and liver body index (%) in rainbow trout (±95% confidence intervals). A significant difference at the $p < 0.05$ (Student's *t* test) level is indicated (*).

Results and Discussion

Biological Results. Results from the biological studies are shown in Table 1 and as Supporting Information (SI). Despite a record warm summer with peak water temperatures of 23.5 °C, 50−57 days into the experiment (Figure A1, SI), the mean weight and length of the control fish increased during the experiment (Table A1, SI). From 16 to 49 days, however, the control fish showed significantly decreased liver weight and condition factor (especially male fish), probably as a result of water temperature (Table 1, statistical results not shown). Also, blood glucose concentrations (Table A2, SI) and number of leucocytes decreased after 120 days (Table 1).

Condition factor (pooled sexes) and mortality (not shown) were not affected by DeBDE exposure, indicating that exposure levels were not acutely toxic (Table 1). However, DeBDE caused an increased liver weight after 120 days compared to controls (Table 1; Figure 1). This effect was not observed after 16 or 49 days, but was seen in the recovery group, indicating a delayed effect of DeBDE possibly due to metabolites. Liver enlargement was reported in rats fed DeBDE (*21*, *28*), while another study found no such effect in rats fed DeBDE for 2 years (*22*). Compared to controls, blood lactate concentrations were significantly higher after 120 days of exposure and after depuration, also implying delayed effects (Table 1; Figure 1). The number of lymphocytes was significantly lower after 120 days of exposure compared to controls; hemoglobin decreased significantly at 16 days of exposure, but the differences disappeared at 120 days, indicating reversible effects; and the number of leucocytes

TABLE 2. Concentrations of BDE Congeners in Muscle and Liver (Fresh Weight Basis) from Fish Fed DeBDE-Treated or Control Food[a]

			% lipid		BDE47 (ng/g of fresh wt)		BDE99 (ng/g of fresh wt)		BDE100 (ng/g of fresh wt)		BDE153 (ng/g of fresh wt)		BDE154 (ng/g of fresh wt)		DeBDE (ng/g of fresh wt)	
	days	*n*	mean	SD[b]	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Muscle																
control	16	10	3.9	1.1	2.0	0.26	0.62	0.075	0.34	0.044	0.064	0.0094	0.1	0.02	nd[d]	
expt	16	10	3.3	0.65	2.1	0.31	0.70	0.094	0.35	0.046	0.071	0.011	0.4	0.1	10	3.2
control	49	10	1.6	0.59	2.1	0.54	0.73	0.19	0.36	0.087	0.080	0.022	0.1	0.03	nd[e]	
expt	49	10	2.4	0.92	1.7	0.20	0.64	0.082	0.32	0.036	0.13	0.016	2	0.5	23	4.3
control	120	14	0.97	0.39	1.4	0.70	0.42	0.21	0.25	0.11	0.056	0.019	0.1	0.03	nd[e]	
expt	120	14	1.3	0.45	1.3	0.40	0.38	0.14	0.23	0.066	0.25	0.045	7	2	38	14
depur	71	10	0.88	0.40	1.2	0.52	0.41	0.18	0.25	0.10	0.16	0.077	3	3	9.5	2.8
Liver																
control	16	6	2.7	0.24	0.87	0.32	0.36	0.082	0.13	0.031	0.063	0.079	0.05	0.01	nd	
expt	16	6	2.7	0.21	0.80	0.40	0.44	0.09	0.11	0.047	0.050	0.022	0.7	0.2	560	210
control	49	8	3.0	0.81	2.7	2.8	1.0	0.87	0.39	0.46	0.12	0.11	0.2	0.1	nd	
expt	49	8	3.1	1.3	4.6	3.1	1.7	1.3	0.53	0.59	0.29	0.23	6	3	240	180
control	120	8	2.9	0.58	4.7	3.3	1.5	0.94	0.84	0.54	0.18	0.12	0.2	0.1	nd	
expt	120	8	2.5	0.36	2.9	1.1	0.74	0.23	0.40	0.16	0.42	0.23	10	9	870	220
depur	71	8	2.9	1.4	4.7	2.3	1.5	0.9	0.91	0.55	0.48	0.31	10	7	30	18

[a] Levels of BDE154 are semiquantitative. The quantification limits for DeBDE in muscle and liver were 0.6 and 5 ng/g of fresh weight, respectively. [b] SD, standard deviation. [c] nd, not detected. [d] Two samples contained DeBDE. [e] One sample contained DeBDE.

in the depuration group was significantly higher than in controls and the 120 day exposure group (Table 1). However, because the leucocyte number was also possibly affected by water temperature, it is difficult to interpret this result. The results did not differ between the sexes except for male fish, which as compared to the controls had significantly higher condition factor after 49 days of DeBDE exposure (not shown). This could possibly be related to the water temperature dependent decrease seen in the male control fish after 49 days.

No significant effects were seen on EROD, ECOD, or transketolase activities (Table A3, SI). A previously presented study showed significant inhibition of the EROD activity in rainbow trout fed BDE47 or BDE99 (*29*).

Chemical Results. In the DeBDE analysis, some decomposition in the GC column was observed, expressed as a raised baseline immediately before the peak. However, from the linearity of the calibration curves, the decomposition did not seem to be limiting within the concentration interval used. Recoveries of DeBDE, BDE47, BDE99, and BDE100 in fish muscle in relation to dechlorane have previously been determined to be 114, 113, 111, and 111%, respectively (*16*). The recoveries of BDE154 and BDE153 have not been investigated. The concentrations presented are not corrected for recovery.

Muscle tissues and livers from each individual were analyzed separately for PBDE. No significant sex differences in PBDE concentrations were found, and therefore results within each group were pooled (Table 2). During the 120 day exposure period, the muscle lipid content decreased from 3.3 to 1.3% in exposed fish and from 3.9 to 0.97% in control fish. The lipid content of the livers remained more constant. The decrease in muscle lipids may reflect lipid loss or redistribution within the fish. So as to not confuse changes in PBDE concentrations in muscle with changes in lipid content, the concentrations will be compared on a fresh weight rather than a lipid weight basis.

The DeBDE-fortified chips had a higher proportion of the latest eluting NoBDE (nona-3) than the DeBDE standard (Figure 2) and traces of peaks eluting in the region of OcBDEs and HpBDEs. DeBDE is known to photolytically degrade to lower brominated BDEs and dibenzofurans in UV light and sunlight (*30*). Although the chips were kept in darkness until used and precautions were taken to avoid ultraviolet light, possible degradation may have occurred during preparation

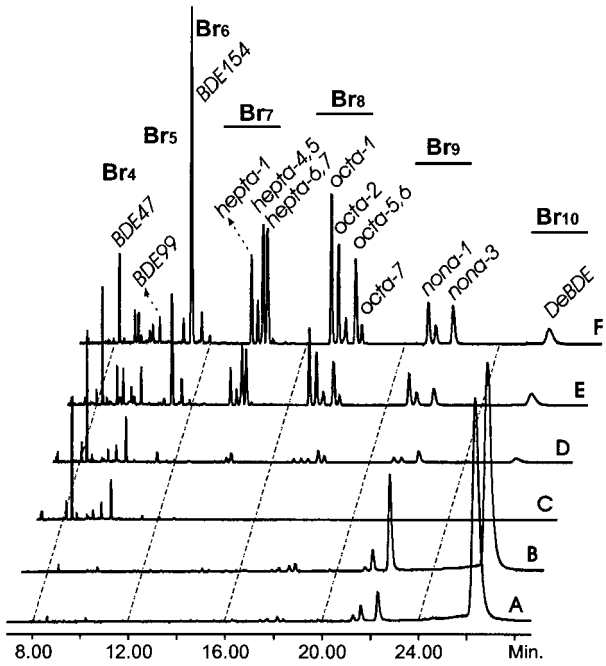

FIGURE 2. Mass chromatograms (sum of *m/z* −79 + −81) of the DeBDE technical product (A), DeBDE prepared cod chips (B), muscle tissue from control (16 days) rainbow trout (C), and after 16 (D), 49 (E), and 120 days of exposure (F). Traces of BDE47 and BDE99 in chromatograms A and B correspond to concentrations in blanks.

of the chips. The control chips did not contain any late eluting brominated substances.

At 16 days of exposure, the mean muscle concentration of DeBDE in exposed fish was 10 ± 3.2 ng/g of fresh weight. The concentration in treated fish increased with exposure length, and highest concentrations of 38 ± 14 ng/g of fresh weight were seen after 120 days of exposure (Figure 3; Table 2). Four (of 34) muscle samples from the control groups contained traces of DeBDE (<8% of the corresponding exposed groups).

Muscle concentrations of DeBDE in fish exposed for 49 days decreased significantly after 71 days of depuration to about half their value on a fresh weight basis, while no decrease was seen on a lipid weight basis. DeBDE concen-

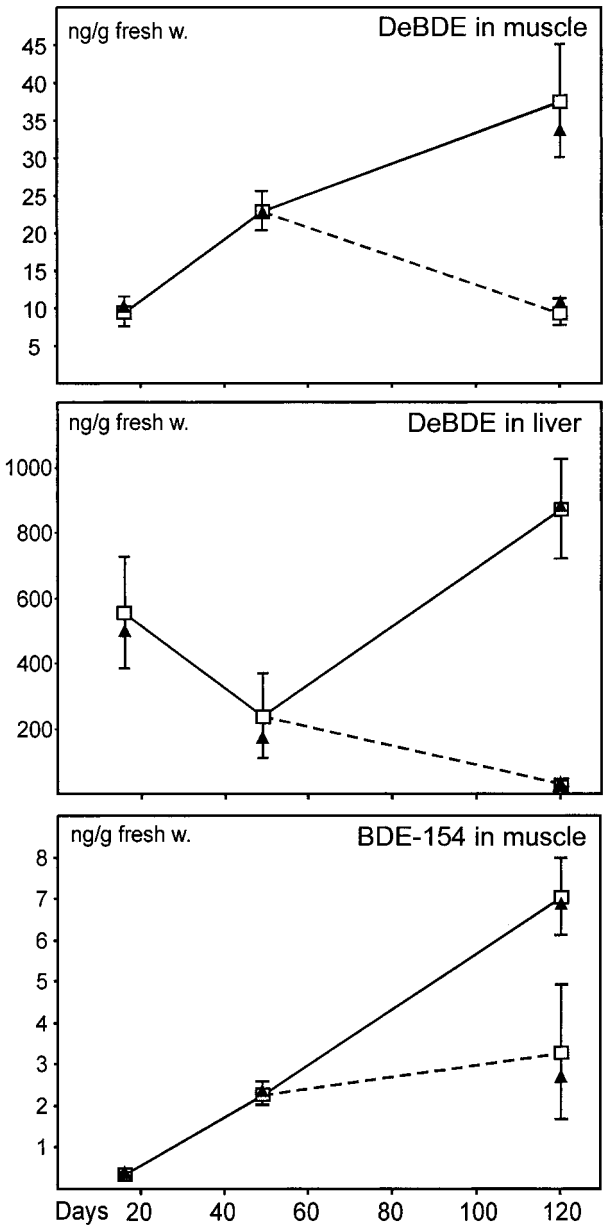

FIGURE 3. Concentrations (fresh weight basis) of DeBDE and BDE154 in muscle and liver from rainbow trout fed DeBDE-treated food for 16, 49, and 120 days (solid line) or 49 days with 71 days of depuration (broken line). The concentrations are presented as arithmetic mean values (□) and as median values (▲), ±95% confidence intervals.

trations in the livers of treated fish exceeded those in the muscle in all individuals analyzed on both fresh weight and lipid weight bases (Table 2). Mean liver concentrations were 560 ng/g of fresh weight after 16 days of exposure and 870 ng/g of fresh weight after 120 days.

In both liver and muscle samples from treated fish a number of bromine-containing compounds chromatographically eluting before DeBDE were detected (Figure 2). Peaks with retention times corresponding to BDE47, BDE99, BDE100, BDE153, and BDE154 were quantified (Table 2). The initial concentrations of BDE47, BDE99, and BDE100 in all fish were of the same order of magnitude as in, for example, Baltic herring (*3*). The fresh weight muscle concentrations of these congeners decreased significantly in all fish from 120 days compared to both exposed and control fish from 16 days, which may be the result of growth dilution. Liver concentrations were significantly higher after 120 days, probably because of redistribution due to the reduced amount of lipids in the muscle tissues. Concentrations given for BDE154 are semiquantitative due to interfering coeluting compounds. The concentrations of BDE153 and BDE154 increased with exposure length in both muscle and liver from the treated fish (Table 2; Figure 3). The BDE154 concentration in muscle increased from 0.4 ng/g on day 16 to 7 ng/g on day 120, a 20-fold increase on a fresh weight basis. For liver, the corresponding increase was 22-fold. BDE153 concentrations also increased, but the magnitude of the increase was less. Furthermore, unlike DeBDE, the concentrations of both of these HxBDEs did not decrease during the depuration period.

The retention times of the remaining unknown peaks present in the chromatograms from liver and muscle extracts were identical. The pattern successively shifted to lower brominated species, and peak areas increased with length of exposure (Figure 2D−F). The potassium hydroxide treatment did not indicate the presence of hydroxylated PBDEs. The mass spectra from scanning concentrated extracts of muscle and liver samples in ECNI mode matched spectra of single congeners of PBDE (up to HxBDE) and of congeners present in technical octaBDE products containing HpBDE to DeBDE. Single BDE congeners fragmented in ECNI mainly by the successive loss of Br (M − 79), HBr (M − 80), Br_2 (M − 158), HBr_2 (M − 159), Br_3 (M − 237), or HBr_3 (M − 238). From HxBDE to DeBDE, an increasing proportion of tri- to pentabrominated phenoxide anions was observed. The presence of phenoxide ions in the ECNI spectra of HpBDE and OcBDEs has been reported earlier (*31*). The major peaks in the extracts, corresponding to GC retention times of HxBDEs to NoBDEs, all fragmented to phenoxide ions with different degrees of bromination. The phenoxide ions formed from expected HxBDEs contained three bromine atoms, from HpBDEs three and four, and from OcBDEs four bromines, indicating an even distribution of the bromine substituents between the two rings. ECNI fragmentation of tetra- to hexabrominated dibenzofurans, which have been reported as possible degradation products of PBDE, is characterized by the successive loss of bromine, and not the formation of phenoxides (*31*). Furthermore, no traces of the typical ions from methoxylated PBDEs, recently reported in Baltic fish, were detected (*32*). From the presence of the phenoxide anions together with the retention times, the major components in sample extracts were characterized as congeners of lower brominated diphenyl ethers.

For the NoBDEs and OcBDEs in the liver/muscle extracts, the pattern showed increasing peak heights for the first eluting peak (nona-1, octa-1) as compared to the DeBDE standard and the DeBDE-treated chips (Figure 2). None of these congeners were available as pure reference standards. A qualitative analysis was made for different BDE congeners in which peak areas were normalized to the internal standard and fresh weight for each sampling time. Because the response of HxBDE and DeBDE differed by up to 1 order of magnitude in ECNI, ratios of the normalized peak areas against their respective values at 16 days of exposure were calculated to compare the relative increase of the BDE congeners (Figure 4). The results indicate that the first eluting congener for each bromination degree is formed/taken up preferentially.

Concentrations of HxBDE to NoBDE congeners increased with exposure length in both muscle and liver. A selective uptake could possibly explain the presence of the nona-, and to some extent the octa- and heptaBDEs, because they were also present as impurities in the DeBDE used in this study (Figure 2). The HxBDEs and the first eluting OcBDE were not detected in the original DeBDE mixture, and thus their presence in the fish is the result of a metabolic process and/or an efficient absorption compared to that of the other congeners. For instance, Octa-1 concentrations seem to

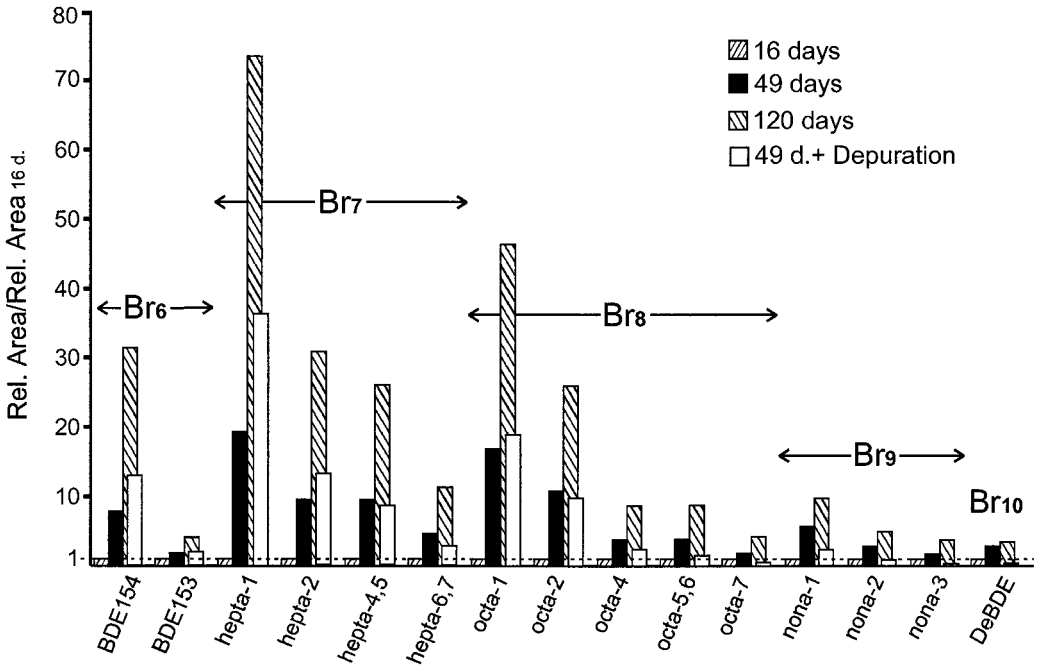

FIGURE 4. Relative increase of brominated substances (normalized to 16 days of exposure) in muscle tissue from rainbow trout. The bars represent mean values of the ratio of the normalized peak areas (fresh weight basis).

exceed those of Octa-7, the dominating OcBDE in the food, by a factor of ~400 (assuming identical response factors), indicating that absorption is comparatively extremely efficient or else that metabolism plays a role. During depuration, DeBDE concentrations decline significantly, whereas no decline was seen for the HxBDEs, two HpBDEs, and one OcBDE (Figure 4). BDE154 concentrations were 4% of DeBDE concentrations in muscle at 16 days, whereas at 120 days the ratio increased to 18% and 30% after depuration (Table 2). Enzymatic debromination has been reported as a metabolic pathway for hexabromobenzene (HBB) administered to rats (*33*). Debromination was also observed in Atlantic salmon that had been fed commercial hexa- and octabromobiphenyl preparations (*34*).

BDE47, BDE99, and BDE100 dominate in environmental samples and bioaccumulate. A possible explanation for this could be that they are metabolites formed after DeBDE exposure. However, no evidence of debromination to these congeners was found in this study.

BDE congener distribution between liver and muscle was examined by calculating liver/muscle ratios of normalized peak areas from individual fish samples on a lipid weight basis. For DeBDE and NoBDE congeners the ratios at 16 days are very high, due to higher concentrations in the liver. The ratios decrease at 49 and 120 days of exposure and after depuration. The ratios at 16 days decrease with decreasing bromine substitution on the diphenyl ether molecule with the HxBDE's ratio close to 1 after 16 days of exposure. This agrees with the high log K_{ow} values for the higher brominated PBDEs of 8.9 and 10 for octa- and DeBDE, respectively (*35*), and their large molecular size, which hinders their ability to cross membranes.

The uptake, calculated from DeBDE concentrations in muscle and the mean dietary dose of DeBDE, was low, ~0.005%. This value is underestimated if the lower brominated congeners are metabolites of DeBDE, as the true uptake should include the sum of all metabolites produced. Due to the lack of reference compounds and the large differences in response from the BDE congeners in ECNI, only a rough estimation of the amount of unknown congeners could be made. By making the assumption that the ECNI responses of the unknown BDE congeners are between the responses of BDE153 and DeBDE, the sum of the congeners was calculated to be within the range of 85−1000 ng/g of fresh weight in muscle after 120 days. Taking into account that each congener derives from one molecule of DeBDE, the uptake based on the sum of all congeners in muscle was estimated to be 0.02−0.13% after 120 days of exposure.

Acknowledgments

Barents Sea cod were obtained with the kind support of Dr. Odd Smestad, Bergen University, Norway. Purified DeBDE was a kind gift from Ulrika Örn, Stockholm University. We thank Per-Åke Hägerroth and Linda Bergander for their assistance. The study was financed by the Swedish Environmental Protection Agency.

Supporting Information Available

Figure A1, time versus water temperature curve, and Tables A1−A3, complete results from morphological, physiological and biochemical measurements (4 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) *Environmental Health Criteria 162: Brominated Diphenyl Ethers*; World Health Organization: Geneva, Switzerland, 1994.
(2) Jansson, B.; Asplund, L.; Olsson, M. *Chemosphere* **1987**, *16*, 2343−2349.
(3) Sellström, U.; Jansson, B.; Kierkegaard, A.; de Wit, C.; Odsjö, T.; Olsson, M. *Chemosphere* **1993**, *26*, 1703−1718.
(4) de Boer, J. *Chemosphere* **1989**, *18*, 2131−2140.
(5) Stafford, C. J. *Chemosphere* **1983**, *12*, 1487−1495.
(6) Kuehl, D. W.; Haebler, R.; Potter, C. *Chemosphere* **1991**, *22*, 1071−1084.
(7) Sellström, U. *Polybrominated Diphenyl Ethers in the Swedish Environment*; ITM Report 45; Institute of Applied Environmental Research, Stockholm University: Stockholm, Sweden, 1996.
(8) Sellström, U.; Kierkegaard, A.; de Wit, C.; Jansson, B.; Olsson, M. *Organohalogen Compd.* **1993**, *14*, 211−213.
(9) DeCarlo, V. J. *Ann. N. Y. Acad. Sci.* **1979**, *320*, 678−681.
(10) Zweidinger, R. A.; Cooper, S. D.; Pellizzari, E. D. In *Measurement of Organic Pollutants in Water and Wastewater*; Van Hall, C. E., Ed.; ASTM STP 686; ASTM: Philadelphia, PA, 1979; pp 234−250.
(11) Zweidinger, R. A.; Cooper, S. D.; Erickson, M. D.; Michael, L. C.; Pellizzari, E. D. In *Monitoring Toxic Substances*; Schuetzle, D.,

Ed.; ACS Symposium Series 94; American Chemical Society: Washington, DC, 1979; pp 217−231.
(12) Stanley, J. S.; Cramer, P. H.; Thornburg, K. R.; Remmers, J. C.; Breen, J. J.; Schwemberger, J. *Chemosphere* **1991**, *23*, 1185−1195.
(13) Watanabe, I.; Kawano, M.; Tatsukawa, R. *Organohalogen Compd.* **1995**, *24*, 337−340.
(14) Watanabe, I.; Kashimoto, T.; Tatsukawa, R. *Bull. Environ. Contam. Toxicol.* **1986**, *36*, 839−842.
(15) Watanabe, I.; Kashimoto, T.; Tatsukawa, R. *Chemosphere* **1987**, *16*, 2389−2396.
(16) Sellström, U.; Kierkegaard, A.; de Wit, C.; Jansson, B. *Environ. Toxicol. Chem.* **1998**, *17*, 1065−1072.
(17) Opperhuizen, A.; Sijm, D. T. H. M. *Environ. Toxicol. Chem.* **1990**, *9*, 175−186.
(18) Burreau, S.; Axelman, J.; Broman, D.; Jakobsson, E. *Environ. Toxicol. Chem.* **1997**, *16*, 2508−2513.
(19) Örn, U.; Klasson-Wehler, E. *Xenobiotica* **1998**, *28*, 199−211.
(20) Norris, J. M.; Kociba, R. J.; Schwetz, B. A.; Rose, J. Q.; Humiston, C. G.; Jewett, G. L.; Gehring, P. J.; Mailhes, J. B. *Environ. Health Perspect.* **1975**, *11*, 153−161.
(21) El Dareer, S. M.; Kalin, J. R.; Tillery, K. F.; Hill, D. L. *J. Toxicol. Environ. Health* **1987**, *22*, 405−415.
(22) Kociba, R. J.; Frauson, L. O.; Humiston, C. G.; Norris, J. M.; Wade, C. E.; Lisowe, R. W.; Quast, J. F.; Jersey, G. C.; Jewett, G. L. *Combust. Toxicol.* **1975**, *2*, 267−285.
(23) Von Meyerinck, L.; Hufnagel, B.; Schmoldt, A.; Benthe, H. F. *Toxicology* **1990**, *61*, 59−274.
(24) Sjödin, A.; Jakobsson, E.; Kierkegaard, A.; Marsh, G.; Sellström, U. *J. Chromatogr.* **1998**, *A822*, 83−89.
(25) Balk, L.; Ericson, G.; Lindesjöö, E.; Petterson, I.; Tjärnlund, U.; Åkerman, G. *Effects of Exhaust from Two-Stroke Outboard Engines on Fish. Studies of Genotoxic, Enzymatic, Physiological and Histological Disorders at the Individual Level*; TemaNord 1994:528; Nordic Council of Ministers: Copenhagen, Denmark, 1994; ISBN 92 9120 439 0.
(26) Tate, J. R.; Nixon, P. F. *Anal. Biochem.* **1987**, *160*, 78−87.
(27) Jansson, B.; Andersson, R.; Asplund, L.; Bergman, Å.; Litzén, K.; Nylund, K.; Reutergårdh, L.; Sellström, U.; Uvemo, U.-B.; Wahlberg, C.; Wideqvist, U. *Fresenius' J. Anal. Chem.* **1991**, *340*, 439−445.
(28) Carlson, G. P. *Toxicol. Lett.* **1980**, *5*, 19−25.
(29) Tjärnlund, U.; Ericson, G.; Örn, U.; de Wit, C.; Balk, L. *Mar. Environ. Res.* **1998**, *46*, 107−112.
(30) Watanabe, I.; Tatsukawa, R. *Bull. Environ. Contam. Toxicol.* **1987**, *39*, 953−959.
(31) Buser, H.-R. *Anal. Chem.* **1986**, *58*, 2913−2919.
(32) Haglund, P.; Zook, D. R.; Buser, H.-R.; Hu, J. *Environ. Sci. Technol.* **1997,** *31*, 3281−3287.
(33) Yamaguchi, Y.; Kawano, M.; Tatsukawa, R. *Arch. Environ. Contam. Toxicol.* **1988**, *17*, 807−812.
(34) Zitko, V. *Uptake and Excretion of Chlorinated and Brominated Hydrocarbons by Fish*; Fisheries and Marine Service Technical Report 737; Fisheries and Environment Canada: St. Andrews, NB, 1977.
(35) Watanabe, I.; Tatsukawa, R. *Proceedings of the Workshop on Brominated Aromatic Flame Retardants*; Swedish National Chemicals Inspectorate: Solna, Sweden, 1989; pp 63−71.

Received for review July 14, 1998. Revised manuscript received February 8, 1999. Accepted February 24, 1999.

ES9807082