Polybrominated Diphenyl Ether Analysis in Fish Tissue and Other Matrices by GC–ECD

This article describes the development of a method for the extraction, clean-up, and successful analysis of polybrominated diphenyl ethers (PBDEs) in fish tissue, soil, and water. A summary of the initial demonstration of capabilities and low-level recoveries is presented for each matrix. The method also was used to evaluate native PBDE concentrations found in each matrix. Some concern existed regarding the mode of PBDE exposure for fish species taken from the Yakima River and for sturgeon taken from the Columbia River below Priest Rapids Dam and above McNary Dam. Sewage treatment plant effluents and biosolids were suspected as a major source of PBDEs. Therefore, sewage treatment plant biosolid and effluent samples, along with a hops field where biosolids were applied as a fertilizer, were collected and analyzed. In addition, sediments, water, and fresh water clam tissue from the river systems in question were analyzed and found to contain PBDEs. Furthermore, sediment samples from many other fresh and marine areas show that PBDEs are ubiquitous. EPA Method 8082A, using gas chromatography–electron capture detection in conjunction with ancillary clean-up procedures, was validated for this analysis.

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While analyzing approximately 300 fish tissue samples for chlorinated pesticides and Aroclors by gas chromatography–electron capture detection (GC–ECD) using EPA Method 8082A (1), several significant peaks were observed to be eluted after Aroclor 1260. These peaks also had been observed in other tissue samples taken previously from other studies and had been identified as brominated compounds using atomic emission detection (AED) for bromine (478 nm). These late-eluting peaks were suspected to be polybrominated diphenyl ethers (PBDEs) from the fire retardant Bromkal 70-5DE or Bromkal 71 (pentabrominated formulations). The pentabrominated formulations are used as fire retardants for polyurethane foam, imitation wood, carpet padding, packaging, sound insulation panels, cushioning, rubber cables, paints, plastic electrical parts, fabric backings, and coatings and are a major source of PBDEs in the environment. Because a standard for Bromkal 70-5DE and standards for major individual components present in Bromkal 70-5DE were not available at the time, a "Bromkal" standard was generated by bromination of diphenyl ether in the presence of iron using a procedure similar to the perchlorination procedure in EPA Method 508A. The resulting mixture was analyzed by GC–mass spectrometry (MS) to establish the identities of the major peaks (that is, tetra-, penta-, and hexasubstituted diphenyls) and their corresponding retention times. The mixture was assayed using the compound independent calibration procedure described in EPA Method 8085 (2) for each of the PBDE congeners at 478 nm, which is linear over a wide range for bromine. Retention times were also determined by AED. This work allowed quantification of the PBDEs in the tissue extracts using this synthesized standard.

Approximately 70 sample tissue extracts from five fish species were analyzed for PBDEs. The result was positive analytical determination of bromine containing compounds in virtually every sample. Using the laboratory-generated standard, concentrations of these compounds were estimated. Because PBDEs were not target analytes in the original project plan, the analyses were performed without optimizing the extraction and clean-up procedure, nor were PBDE recoveries determined because the samples were not fortified with PBDEs. The results were reported as a total of the individual homologues — that is, tetrabromodiphenyl, pentabromodiphenyl, and hexabromodiphenyl congeners. Note: There

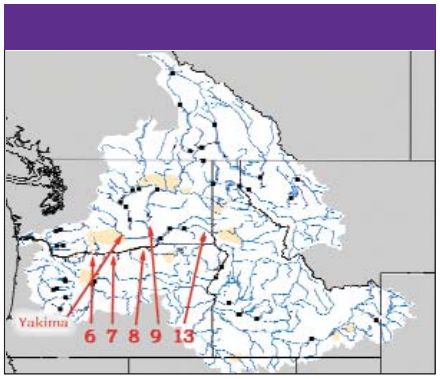

Figure 1: Map of Columbia River basin station locations.

was no official validated method for PBDE analysis at the time and one still does not exist. There is, however, a funded study proposal through the Office of Water to validate both EPA Methods 1668 (HR/MS) and 8082A (GC–ECD) over the next two years.

The results of this current study demonstrate that commonly used methods are appropriate for the analysis of this important class of compounds in environmental matrices. Although PBDEs have not been regulated as yet, it is important that a validated method is in place before regulation, especially considering the ubiquitous nature of PBDEs. Based on the findings of the subsequent study, the advisability of using biosolids containing elevated concentrations of PBDEs as soil conditioner or crop fertilizer is questionable. Available information suggests that PBDEs, which have chemistry similar to polychlorinated biphenyls, are persistent. Although the I am unaware of any harmful effects due to PBDEs, the practice of applying them to crops remains questionable. Potential leaching of the material into receiving waters and airborne dispersion by wind are a concern. I would also question the advisability of using biosolids containing triclosan, which is a broad-spectrum antibacterial–antimicrobial agent, as a soil-conditioner or crop fertilizer. The question should be asked: "Will use of this compound affect bacterial activity in soil, or are we creating more antibacterial-resistant bacteria?"

Subsequent to the finalization of this PBDE study, Varian (Palo Alto, California) personnel at a training session in Portland, Oregon, presented material regarding the bioaccumulation of PBDEs, their toxicity with regard to neurodevelopment, neurobehavior, endrocrine disruptor and liver, their persistency, and the movement in certain countries to ban the production and importation of goods treated with PBDEs.

Supplies and Equipment

Agilent 6890 gas chromatograph with dual micro electron capture detectors and a Gerstel CIS 4 high-volume injector using 30 µL injections.

Individual PBDE congener standards at 50 µg/mL included BDE numbers 17, 28, 47, 66, 77, 85, 99, 100, 138, 153, and 154 from Cambridge Isotopes (Andover, Massachusetts). The final working standards were in iso-octane.

"Predominant" PBDE congener mix: Included BDE numbers 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190, and 209 at 50 µg/mL with BDE-209 at 250 µg/mL (Cambridge Isotopes). The final working standards were in isooctane.

Chromatography columns: Rtx–CLPEST, 30 m × 0.25 mm, 0.25-µm film thickness and Rtx-CLPEST II, 30 m × 0.25 mm, 0.20-µm film thickness (3). The columns were plumbed in parallel. 30-µL injections in solvent vent mode were made.

Chromatography Column-Column, plain, 250 mm × 11 mm with a PTFE stopcock plug, Kontes 420280-0213, 200-mL reservoir capacity.

Granular anhydrous sodium sulfate was heated overnight at 430 °C in a muffle furnace in a stainless steel pan to remove interferences and stored in an air-tight jar with a PTFE-lined lid.

Florisil (60–100 mesh), J.T. Baker (Phillipsburg, New Jersey) 3372-08, Baker Analyzed Reagent: "Suitable for use in chromatographic cleanup of pesticide residues" or equivalent. It was necessary to heat the Florisil at 430 °C to obtain consistent separation performance. The Florisil was cooled, transferred to a large Erlenmeyer flask, capped loosely with aluminum foil, and stored at least overnight in a drying oven at 130 °C before use.

Solvents: Hexane, diethyl ether (preserved with 2% ethanol), acetone, and isooctane (all solvents distilled in glass grade; available from Burdick and Jackson [Muskegon, Michigan] or equivalent).

Procedures for sample extraction and cleanup of extracts: Tissue was extracted according to EPA Method 3540C (4) using 10 g of tissue mixed with 50 g of anhydrous sodium sulfate. The extraction was performed overnight with 1:1 acetone and hexane. The extracts were concentrated

Table I: Retention times for PBDEs and miscellaneous compounds

Compound	Number of Bromines	CLPEST Rt (min)	CLPEST RRt	CLPEST II Rt (min)	CLPESTII RRt
BDE-17	3	18.113	0.636	22.100	0.708
BDE-28	3	18.992	0.667	22.623	0.724
BDE-47	4	23.776	0.835	26.608	0.852
BDE-66	4	24.389	0.856	27.081	0.867
BDE-77	4	25.291	0.888	27.791	0.890
BDE-85	5	28.171	0.989	30.847	0.988
BDE-99	5	26.944	0.946	29.506	0.945
BDE-100	5	26.204	0.920	28.907	0.926
BDE-138	6	30.511	1.071	34.104	1.092
BDE-153	6	29.423	1.033	32.192	1.031
BDE-154	6	28.491	1.003	31.243	1.0005
DCB (S.S.)	----	28.080	0.986	29.423	0.942
HBB (S.S.)	6	28.479	1.000	31.226	1.000
Cl8-Dibenzodioxin	-----	30.914	1.086	33.100	1.060
Cl8-Dibenzofuran	----	30.824	1.082	33.315	1.067
Cl8-Napthalene	---	27.608	0.969	29.825	0.955
Perchlorinated DDE	----	34.943	1.227	40.905	1.310

(S.S. = Surrogate Spike)

Table II: Florisil® elution pattern for PBDEs by semi-macro column (8,9)

Congener	0% Fraction	6% Fraction	50% Fraction	Total % Recovery
BDE-47	0%	99.6%	0%	99.6%
BDE-99	4.7%	94.6%	0%	99.3%
BDE-100	7.2%	95%	0%	102%
BDE-153	55.9%	43.8%	0%	99.7%
BDE-154	60.3%	40.8%	0%	101%

using Kuderna-Danish concentrators on a steambath and then an N-Evap. The extracts were transferred to precalibrated disposable centrifuge tubes, and the final volume of 10 mL was adjusted with isooctane. A 2-mL volume of concentrated sulfuric acid was added to each test tube and mixed well. The phases were allowed to settle overnight. Centrifugation was utilized when needed. A portion of the sample was transferred to an autosampler vial and analyzed using both a single-point serial dilution of the spiking solution (where the same volume of spiking solution was diluted to volume with isooctane), and against eight-point calibration curves. Either a linear or a quadratic multipoint curve was generated with a correlation coefficient of 0.995 or better. Smaller samples of whale blubber were extracted as noted.

Extraction of soil, sediment, and biosolids by EPA Method 3540C (5) was accomplished using the equivalent of 10 g of dry soil. The soil was extracted overnight with acetone. The extract was solvent-exchanged to 10 mL of isooctane as shown earlier.

Extraction of water also was accomplished by a modification of EPA Method 3510C using a stir-bar for mixing directly in the sample jar with methylene chloride. In this case, 3–3.5 L of sample was extracted. The extract was solvent-exchanged to 1 mL of isooctane as described earlier. Laboratory reagent water in the amount of 1 L was used to develop initial demonstration of capability data.

Cleanup of Extracts with Sulfuric Acid, EPA Method 3665A (6) Florisil cleanup of extracts by EPA Method 3620B (7,8); semi-macro modification: A small glass wool plug was placed in the bottom of a 250 mm × 11 mm chromatography column. Approximately 0.25 in. of anhydrous sodium sulfate was placed on top of the glass wool. A 10-g amount of prepared Florisil was added on top of the sodium sulfate and packed tightly by vertically tapping the column. A 0.5-in. layer of anhydrous sulfate was placed on top. The column was rinsed with 40 mL of hexane, which was discarded. The column must not go dry otherwise separation characteristics might be compromised, and the column will need to be prepared again. The column was now ready for extract introduction, and the elution proceeded as described in Method 3620B.

Sulfur Removal from Extracts by EPA Method 3660 (9) with Tetrabutyl Ammonium Sulfite

Experimental: Before reanalysis of any fish tissue samples, several parts of the analytical procedure were explored. The first step was to make a composite standard cocktail of all 11 PBDE congeners and determine their retention times on the two analytical columns. The GC oven conditions were as follows: Detectors were maintained at 350 °C; helium flow was 3 mL/min; injection volume was 30 µL; the oven program comprised a hold at 80 °C for 1.8 min, then 80–180 °C at 50 °C/min, then 180–210 °C at 2 °C/min, then 210–320 °C at 8 °C/min, and hold at 320 °C for 5 min. The elapsed time was 37.5 min.

Using these GC conditions, the retention times for the 11 PBDE congeners as well as some other late-eluted compounds of interest on both capillary columns were determined and are listed with relative retention times compared with 2,2′,4,4′,5,5′-hexabromobiphenyl. See Table I. This particular GC program is also useful for the analysis of polychlorinated biphenyl residues as the Aroclors or as the polychlorinated biphenyl congeners. Later, the final temperature of the GC oven was increased to 330 °C, and the run was extended to 45 min so that the PBDE BDE-209 could be determined. The analytical runs can be shortened substantially by increasing the helium flow to 5 mL/min. Then BDE-209 had a retention time of 20 min on the primary column and 31 min on the secondary column. The Florisil elution pattern was determined for the five major congeners associated with Bromkal 70-5DE. The Florisil column was spiked with 1000 µL of a 500-pg PBDE/µL solution and the eluant was diluted to 10 mL. The resulting concentration analyzed was 50 pg/µL. The chemistry of PBDEs is similar to polychlorinated biphenyls. They are stable to acid treatment and slightly more polar as demonstrated by their elution by 6% diethyl ether in hexane. The coplanar polychlorinated biphenyl congeners also are eluted in the 6% fraction (8). See Table II.

Low-level recoveries were determined next using seven 5-g tissue samples spiked at 200 pg/g wet weight basis, which is between five

Table III: Low-level recoveries for PBDEs in fish tissue by EPA Method 8082A, EPA reg. 10 laboratory*

BDE	Native Conc. (pg/g)	Rep 1 (pg/g)	Rep 2 (pg/g)	Rep 3 (pg/g)	Rep 4 (pg/g)	Rep 5 (pg/g)	Rep 6 (pg/g)	Rep 7 (pg/g)	Avg. Result (pg/g)	% Recovery	Std Dev	%RSD
17	2800	204	184	188	204	184	164	196	189	95	14.0	7.4
28	3200	166	176	180	192	188	170	186	180	90	9.6	5.4
47	61,000	208	188	188	206	184	156	200	190	95	17.7	9.3
66	2300	180	184	200	176	146	192	183	180	90	17.0	9.5
77	380	174	160	174	182	166	156	170	169	85	8.9	5.3
85	240	170	156	176	172	152	156	144	161	81	11.9	7.4
99	3400	218	194	198	224	194	174	212	202	101	17.2	8.5
100	10,000	218	198	196	212	196	168	208	199	100	16.3	8.2
138	34	190	208	238	180	248	158	172	199	100	33.8	17.0
153	2900	206	176	178	200	182	184	204	190	95	12.9	6.8
154	2500	218	194	192	216	188	172	216	199	100	17.6	8.8

*5 g of tissue fortified with 200 pg/g, final extract volume = 5mL, 30 µL injected.

Table IV: IDCs for PBDEs in fish tissue by EPA method 8082A, EPA reg. 10 laboratory*

BDE	Native 1 (pg/g)	Rep 1a (pg/g)	Rep 1b (pg/g)	Native 2 (pg/g)	Rep 2a (pg/g)	Rep 2b (pg/g)	Native 3 (pg/g)	Rep 3a (pg/g)	Rep 3b (pg/g)	Avg. Recovery (pg/g)	% Recovery	% RSD
17	4100	1200	970	5200	1030	870	4000	830	980	980	98	13
28	1000	1140	960	2100	960	880	3000	740	850	920	92	15
47	14,000	1090	990	26,000	1010	920	31,000	850	1010	980	98	8.5
66	940	920	740	1900	800	680	1300	730	740	770	77	11
77	190	800	730	290	740	700	400	550	690	700	70	12
85	120	820	730	400	540	560	300	720	830	720	72	19
99	540	1030	940	15,000	900	810	390	830	800	890	89	10
100	90	1140	950	5100	970	840	170	800	910	940	94	13
138	39	830	850	72	660	680	70	740	680	740	74	11
153	490	1120	900	2400	710	710	1500	760	810	840	84	19
154	290	1020	850	1300	700	700	990	740	860	820	82	15

*10 g of tissue, three different fish samples fortified in duplicate with 1000 pg/g, final extract volume = 10 mL, 30 µL injected.

and ten times the expected detection limit for each of the 11 PBDE congeners and corrected for the native concentrations of one of the sturgeon samples used for the determination of the low-level recoveries. Because of elevated BDE-47, BDE-99, and BDE-100 concentrations in the native sample, the samples were diluted tenfold and reanalyzed. The results were corrected for the background native concentration and are summarized in Table III. Lower reporting limits for the minor constituents can be determined by increasing the sample size and then decreasing the final extract volume if necessary.

The initial demonstration of capability was determined using duplicate 10-g subsamples of three different fish samples: two sturgeon and a catfish. Each sample was fortified with all 11 PBDE congeners at 1000 pg/g (ppt) wet weight basis and surrogates and treated as described earlier. A portion of the treated sample extract was transferred to an autosampler vial and analyzed utilizing both a single-point serial dilution of the spiking solution and eight-point calibration curves. The initial demonstration of capabilities were conducted concurrently with the low-level recovery determinations. Because of elevated BDE-47, BDE-99, and BDE-100 concentrations in some of the native samples, the samples were diluted tenfold and reanalyzed. The results, corrected for the background native concentration, are summarized in Table IV. The recovery and precision data were considered acceptable for the duplicates, and the precision data of the fortified samples were judged acceptable also.

Extraction and analysis procedure for initial demonstration of capabilities and minimum detectable levels in soil screened through eight mesh (< 2.4 mm) with 97% solids: four fortified samples used to determine initial demonstration of capabilities were spiked with 1.00 mL at 25 pg/µL added to 10 g of soil. This was equivalent to 2.5 ng/g (ppb). Seven fortified samples used to determine low-level recoveries were spiked with 40 µL at 25 pg/µL added to 10 g of soil. This was equivalent to 0.10 ng/g (ppb). The spiking solution contained 13 PBDE congeners at 25 pg/µL in acetone. The BDE-209 congener was five times higher at 125 pg/µL. A serial dilution was made for both fortification levels — that is, 1.00 mL diluted to 10 mL isooctane and 40 µL diluted to 1 mL isooctane — at the time of setting up the samples. These solutions were analyzed against eight-point calibration curves along with the initial demonstration of capabilities and low-level recovery extracts so that % recoveries could be determined. Method blanks were treated in the same manner.

Soil from a marine environment contained substantial elemental sulfur, which is an analytical interference with electron capture detectors. The sulfur was removed by addition of tetrabutyl ammonium sulfite reagent (9) to the centrifuge tubes and mixed with the sample by shaking. The extracts were transferred to a second centrifuge tube and mixed with 2 mL of concentrated sulfuric acid and allowed to settle or centrifuge as needed. Approximately 1 mL of extract used for initial demonstration of capabilities determination was transferred to an autosampler vial. Approximately 250 µL of extract used for low-level recovery determination was transferred to an autosampler vial with a micro insert. The extracts along with the serial dilutions were analyzed against eight-point calibration curves for the 14 PBDE congeners. Both linear and quadratic curves, correlation coefficients of 0.995 or better, were used as appropriate. The initial demonstration of capability results are summarized in Table V. The low-level recoveries are summarized in Table VI. There were no detectable PBDEs in the soil.

Table V: PBDE congener IDCs in soil as ng/g (ppb)-dry weight basis

BDE *	IDC 1	IDC 2	IDC 3	IDC 4	Average	% Rec.	% RPD
17	2.40	2.35	2.58	2.63	2.49	99.6	5.5
28	2.68	2.60	2.23	2.80	2.58	103	9.5
47	2.35	2.48	1.93	2.30	2.28	91.2	10.3
66	2.35	2.35	1.98	2.28	2.25	90.0	7.8
71	2.23	2.33	2.10	2.38	2.25	90.0	5.5
85	2.48	1.63	2.05	1.85	2.00	80.0	18.1
99	2.08	2.10	1.75	2.05	2.00	80.0	8.2
100	1.90	2.00	1.80	1.93	1.90	76.0	4.4
138	2.05	1.88	1.50	2.10	1.88	75.2	14.5
153	2.18	2.08	1.70	2.15	2.03	81.2	11.0
154	2.08	2.08	1.63	2.10	1.98	84.0	11.5
183	1.90	1.75	2.03	2.03	1.93	77.2	6.9
190	2.25	1.88	2.28	2.48	2.23	89.2	11.2
209	16.65	15.40	13.65	17.75	15.86	127	11.1

* All BDE congeners were spiked at 2.5 ng/g except for BDE-209, which was spiked at 12.5 ng/g. The final extract volume was 10 mL with a 30-µL injection.

Table VI: PBDE congener low-level recoveries in soil as ng/g (ppb) dry weight basis

BDE *	Rep 1 (ng/g)	Rep 2 (ng/g)	Rep 3 (ng/g)	Rep 4 (ng/g)	Rep 5 (ng/g)	Rep 6 (ng/g)	Rep 7 (ng/g)	Average (ng/g)	Std. Dev.	%Recovery
17	0.127	0.135	0.108	0.092	0.072	0.094	0.096	0.103	0.022	103
28	0.099	0.108	0.098	0.087	0.068	0.081	0.074	0.088	0.015	88
47	0.116	0.134	0.120	0.117	0.098	0.103	0.122	0.116	0.012	116
66	0.083	0.070	0.084	0.071	0.064	0.068	0.057	0.071	0.010	71
71	0.106	0.124	0.116	0.103	0.084	0.112	0.116	0.109	0.013	109
85	0.102	0.099	0.092	0.090	0.085	0.091	0.098	0.094	0.010	94
99	0.110	0.120	0.109	0.111	0.099	0.102	0.124	0.110	0.010	110
100	0.110	0.119	0.123	0.149	0.064	0.099	0.096	0.109	0.026	109
138	0.082	0.082	0.086	0.067	0.065	0.073	0.060	0.074	0.010	74
153	0.082	0.086	0.082	0.073	0.072	0.078	0.111	0.083	0.013	83
154	0.065	0.064	0.053	0.052	0.050	0.052	0.065	0.057	0.010	57
183	0.096	0.094	0.102	0.079	0.089	0.103	0.052	0.088	0.018	88
190	0.094	0.110	0.121	0.123	0.108	0.103	0.126	0.112	0.012	112
209	0.595	0.625	0.555	0.535	0.495	0.595	0.555	0.565	0.044	113

* All BDE congeners were spiked at 0.10 ng/g except for BDE-209, which was spiked at 0.50 ng/g. The final extract volume was 1 mL with a 30-µL injection.

Four 1-L samples of reagent water were extracted after being fortified with 5 ppt of "predominant" PBDE mix (25 ppt for BDE-209). These extracts were used to determine the low-level recoveries for the 14 PBDE congeners. (There were no detectable PBDE congeners in reagent water; see Table VII.

Results

Fish tissue results: Based on the data obtained earlier from the GC–AED system, another 10-g aliquot was extracted and analyzed for each of 41 samples (four species from the Yakima River and 20 sturgeon from the Columbia River and Snake River) along with fortified samples and method blanks using EPA Methods 3540C and 3620B, Modified (8). Method 3620B was modified by decreasing the weight of Florisil from 20 g to 10 g, using a narrower 250 mm × 11 mm column, and reducing the volume of eluent for each fraction from 200 mL to 100 mL. The extracts and PBDE standards were analyzed as described earlier. There was good agreement between the original results obtained using the lab-generated standard and AED and the results obtained with certified standards and GC–ECD.

Also, use of the GC–ECD produced much lower detection limits, and results for the six minor constituents also were determined. They are not included in Table VIII, but are available upon request. Recent data indicate that the less brominated congeners appear to be the most abundant of the PBDEs accumulated. PBDEs can be characterized as persistent and highly lipophilic substances with low vapor pressure, similar to well-known environmental polychlorinated biphenyl contaminants. While the tetra-congener of BDE-47 is more prevalent in literature reports, it appears that certain species of fish (for example, suckers) accumulate certain penta-congeners preferentially (10). Other studies have shown BDE-47 to be the most common and have the highest concentrations, followed by BDE-99 and BDE-100. BDE-153 and BDE-154 are found in the environment also (11–16).

Note that the percentage of the three major congeners for Bromkal 70-5DE is given at the bottom of the table to be used to facilitate as a point of reference. Bromkal 71, another pentabromo formulation, is similar in composition. The results for mountain whitefish, listed in Table VIII, show that BDE-47 and BDE-99 are about the same concentration, which is the case with the Bromkals 70 and 71 formulations, and the other three congeners are much lower. This is the only species where this correlation was observed. Although potentially of interest, this difference cannot be explained, but it must have some significance. It is not believed that this is a case of recent exposure because the other three species were collected in the same area of the river at the same time. Apparently, whitefish do not seem to be able to metabolize BDE-99 congener. This table demonstrates that it is not feasible to use Bromkal 70-5DE as a calibration standard and that the individual congeners should be used instead.

The average PBDE data for the three major congeners in five smallmouth bass from the Yakima River are listed in Table VIII. BDE-47 is substantially higher than the rest of the congeners. Although not presented here, the whole body concentrations are higher than the fillet-plus-skin concentrations. By and large, the BDE-99 and BDE-100 are approximately the same with the BDE-153 and BDE-154 much lower. The latter two congener results are not included in Table VIII. The percentage of lipid data was not available.

Table VIII summarizes the PBDE data for the three major congeners in six channel catfish from the Yakima River. Although not presented here, the fillet-plus-skin BDE-47 concentrations seem to be slightly higher than the whole body concentration but about twice the BDE-99 concentration. The BDE-100 concentration appears to be about half the BDE-99 concentration. The percentage of lipid data was not available.

Table VIII summarizes the PBDE data for the three major congeners in four largescale suckers from the Yakima River. The BDE-47 concentrations are greatly elevated compared to the rest of the congeners. The BDE-99 is virtually lost, while the BDE-100 concentration is greater. Although potentially of interest, this difference cannot be fully explained (10).

Table IX summarizes the PBDE data for BDE-47 and the total PBDE concentrations in 20 white sturgeon taken from the Columbia and Snake Rivers. The station locations are noted on the map (Figure 1).

The results for the total PBDEs and BDE-47 are presented as the percentage of lipid basis. The lowest downstream designation at each station is "A," and the uppermost is "I" (that is, 9I is just below Priest Rapids Dam, and 9B is just above McNary Dam). The highest concentrations observed for the Columbia are at Station 9I. The concentration decreases generally as samples are taken farther downstream, especially as when con-

Table VII: PBDE congener low-level spike recoveries in water as ng/L (ppt)

BDE *	Rep 1 (ng/L)	Rep 2 (ng/L)	Rep 3 (ng/L)	Rep 4 (ng/L)	Avg. (ng/L)	% Rec.	Std. Dev.	% RSD
17	2.5	3.0	2.6	3.6	2.9	58	0.50	17
28	2.6	3.1	2.6	3.5	3.0	60	0.44	15
47	3.5	3.9	3.8	4.2	3.9	78	0.29	7.4
66	3.6	3.9	3.7	4.2	3.9	78	0.26	6.7
71	3.3	3.6	3.5	4.0	3.6	72	0.29	8.1
85	3.9	4.1	4.1	4.3	4.1	82	0.16	3.9
99	3.7	4.0	4.0	4.1	4.0	80	0.17	4.3
100	3.7	4.0	3.8	4.2	3.9	78	0.22	5.6
138	4.2	4.5	4.0	4.0	4.2	84	0.24	5.7
153	4.1	4.4	3.9	3.9	4.1	82	0.24	5.9
154	4.1	4.4	3.9	4.1	4.1	82	0.21	5.1
183	4.1	4.4	3.7	3.8	4.0	80	0.32	8.0
190	4.1	4.3	3.5	3.3	3.8	76	0.48	13
209	19	21	16	14	17.5	70	3.1	18

* All BDE congeners were spiked at 0.005 μg/L (5 ng/L) except for BDE-209, which was spiked at 0.025 μg/L (25 μg/L). The final extract volume was 1 mL with a 30-μL injection.

Table VIII: Yakima River-average PBDE results as μg/kg, wet-weight basis*

Species (# of composites)	BDE-47	BDE-99	BDE-100	Total PBDEs
Mountain Whitefish (6)	54	50	12	130
Small Mouth Bass (5)	53	8.3	8.2	91
Channel Catfish (6)	37	13	6.8	73
Largescale Sucker (4)	39	0.8	5.3	53
Bromkal 70-5DE as % Composition	27.1 %	39.6 %	7.2 %	100%

*If one considers using % lipid basis, the results are over ten times higher.

Table IX: White sturgeon-Columbia/Snake Rivers, proceding downstream below Priest Rapids Dam to: PBDE results as μg/kg (ppb), lipid-weight basis

Station	Number	Fish Station Description	BDE-47	Total PBDEs
9-I	2	Hanford Reach	840	1220–1410
9-B &A	2	Just Above McNary Dam	160–410	300–800
8-F	3	John Day Dam Pool	82–110	130–190
8-E	2	John Day Pool	210–400	370–560
8-B	1	Just Above John Day Dam	650	850
7-A	3	The Dalles Dam	120–170	230–320
6-C	4	Bonneville Dam	320–570	550–880
13-E	2	Lower Granite Dam, Snake River	140	260–280

sidering the percentage of lipids to normalize the results. Station 13 is on the Snake River just below the confluence of the Clearwater River. The results are lower here as one might expect due to the relatively pristine nature of these rivers.

Generally, there is limited passage for the sturgeon from one station to another due to the intervening dams. PBDE residues are diminished at the station located between McNary Dam and John Day Dam and between John Day Dam and The Dalles Dam. There seems to be a substantial increase in concentration at the station located between Bonneville Dam and The Dalles Dam. The source of the elevated PBDE values at either Stations 9 and 6 is not known and should be investigated.

To further test the methodology, 11 sediment samples were collected from both the Columbia and Willamette Rivers near Portland, Oregon, and four marine and fresh water sediment samples were collected near Tacoma, Washington. The two predominant congeners, BDE-47 and BDE-99, were found in every sediment sample. Sediment samples consisting of mainly sand had the lowest residue level. Those sediments consisting of silt had more elevated concentrations. PBDEs appear to be ubiquitous. Three of the sediment samples were spiked in duplicate, and two of the sediment samples were extracted and analyzed in duplicate. There is good precision for both the fortified samples, corrected for the native concentration, and the duplicate samples. The results are summarized in Table X and

Table X: QA data for sediments (% recovery)

BDE Congener	A, MS	A, MSD	%RPD	K, MS	K, MSD	%RPD	3, MS	3, MSD	%RPD
28	115%	95%	19%	90%	85%	5.7%	130%	102%	24%
47	97%	87%	11%	89%	106%	17%	108%	85%	24%
99	101%	90%	12%	91%	97%	6.4%	115%	83%	32%
100	91%	80%	13%	79%	82%	3.7%	93%	73%	24%
153	98%	88%	11%	90%	90%	0%	101%	78%	25%
154	90%	81%	11%	81%	82%	1.2%	90%	78%	14%
183	106%	98%	7.8%	102%	112%	9.3%	98%	82%	18%
190	121%	114%	6.0%	93%	90%	3.3%	158%	150%	5.2%
209	130%	113%	14%	107%	99%	7.8%	*	*	N/A
Avg. rec.	105%	94%	11%	91%	94%	3.2%	112%	91%	21%

Station A = Columbia River near Portland International Airport, Portland, Oregon; Station K = Willamette River at Milwaukie, Oregon; 3 = Puyallup River at Tacoma, Washington.
* Unable to correct for native concentration for BDE-209 due to native concentration being very high at 28 μg/kg.

Table XI: Duplicate results for sediments, μg/kg (ppb); dry weight basis

BDE Congener	Sample F	Sample F, DUP.	%RPD	Sample H	Sample H, Dup.	%RPD
#28	0.55	0.32	51%	0.18	0.15	18%
#47	2.5	2.4	4.1%	0.59	0.57	3.4%
#99	2.9	2.8	3.5%	0.59	0.58	1.7%
#100	0.66	0.65	1.5%	0.14	0.14	0%
#153				0.13	0.13	0%
#154					0.15	N/A
#183	1.2	0.66	58%	0.059	0.042	34%
#190	1.4	0.62	77%			N/A
#209	47	36	27%	1.5	1.6	6.5%
Total	56.2	43.5	25%	3.19	3.36	5.2%1

Station F = Willamette River, Portland Harbor, Portland, Oregon; Station H = Willamette River at entrance to Multnomah Channel, Portland, Oregon.
The blank cells indicate that the congener was not detected at or above the reporting level, which may vary tremendously considering the wide variability of the % solids encountered in sediments and the non-homogenous nature of sediments.

Table XII: PBDE congener concentrations in STP biosolids as μg/kg (ppb); dry weight basis

BDE Congener	STP 1	STP 1, Dup.	STP 2	STP 2, Dup.
28	58	58	130	150
47	2000	2100	1700	1700
99	2400	2400	2000	2000
100	400	410	350	360
153	240	230	240	240
154	150	160	190	200
183	40	34	54	64
190	< 6.4	< 7.4	20	26
209	4600	3900	4000	3900
Total	10,064	9457	8852	8823

Table XIII: PBDE congener concentrations in STP effluents as μg/L (ppb)

BDE	STP 1	STP 2	STP 3	STP 3, Dup.
47	6.10	1.20	7.0	10.0
99	4.90	0.93	7.8	12.0
100	0.84	0.16	1.2	1.8
153	0.56	0.075	0.67	1.0
154	0.34	0.054	0.45	0.72
Total PBDEs	15.3	3.0	20.1	29.0
Triclosan	Not detected	Not detected	0.26 J	1.2 J

Table XIV: PBDE congener concentrations in sediments as μg/kg (ppb)-dry-weight basis

BDE	Columbia River	Marina	Yakima River
47	0.027–0.22	0.60	0.38–0.60
99	0.053–0.23	0.52	0.36–0.72
Total PBDEs	0.40–2.00	5.75	2.03–4.10

Table XI. The concentrations for total PBDEs ranged from 0.072 to 50 μg/kg-dry weight basis (ppb).

Station F is the Willamette River, Portland Harbor, Portland, Oregon, and Station H is the Willamette River at the entrance to Multnomah Channel, Portland.

The blank cells indicate that the congener was not detected at or above the reporting level, which can vary tremendously considering the wide variability of the percentage of solids encountered in sediments and the nonhomogenous nature of sediments.

Concurrently, two sewage treatment plant biosolid samples were collected near Tacoma, Washington. All major and some minor PBDE congeners were present in every sample. According to the literature, one can expect a total of 1–2 ppm PBDEs in sewage treatment plant biosolids. However, the results of biosolids from both plants were as much as 5–10 times higher. See Table XII. Also, duplicate results are excellent. The data suggest additional sampling of both biosolids and sewage treatment plant effluents should be done to confirm that sewage treatment plant effluent is one of the major pathways for exposure to fish. Although the laboratory did not have a certified standard at the time, elevated concentrations of triclosan were present in both biosolid samples. The identity was confirmed by GC–MS. A certified triclosan standard was obtained and used for the Columbia/Yakima River Sediment Survey.

Columbia/Yakima Sediment Survey

In an attempt to determine PBDE "hot spots" in the Columbia/Yakima River area, sediment and other types of samples were taken from the Columbia/Yakima River area where fish tissue samples with high PBDE burden had been captured. The object was to determine the routes of exposure. Sediment, water, and clam samples were collected above Wanapum Dam, which is upstream from Priest Rapids Dam on the main stem of the Columbia River, and above the collection site for sturgeon with the highest observed PBDE burden. Sediment and water samples were collected throughout the affected area both in the main stem of the Columbia River and the Yakima River. Samples of biosolids and sewage treatment plant effluent were collected from three plants situated along the Yakima River. Three soil samples, one of which was a control, were collected from a hops field where biosolids had been applied as a soil conditioner or fertilizer.

Results for water samples: Eight water grab samples from the rivers were collected and extracted directly in the sample container. The observed results in the actual samples ranged from 0.030 to 0.13 ng/L (ppt) for BDE-47, 0.048 to 0.30 ng/L (ppt) for BDE-99, and 0.35 to 0.88 ng/L (ppt) for total PBDEs. One sample contained some suspended solids that had been stirred up during sample collection, and the correspon-

Table XV: PBDE congener concentrations in STP biosolids as μg/kg (ppb)-dry-weight basis

BDE	Plant 1 Activated	Plant 1 Dewatered	Plant 2 Activated	Plant 2 Dewatered	Plant 3 Activated	Plant 3 Dewatered
47	1200	760	200	240	950	1000
99	1200	860	260	320	1100	1200
100	200	130	43	48	180	190
153	120	77	29	33	110	120
154	87	67	25	26	100	92
Total PBDEs	2850	5410	850	960	3600	4100
Triclosan	620 J	23,000 J	4200 J	3500 J	4100 J	16,000 J

ding concentrations were 0.40, 0.49, and 2.30 μg/L, (ppb) respectively. This site just happened to be where the very highest PBDEs were observed in all of the fish tissues taken.

The effluents were collected as grab samples from three sewage treatment plants located along the Yakima River. (Note: the third effluent was collected in duplicate.) There is fair comparison for the duplicates considering the nonhomogeneous nature of the effluent. The results expressed μg/L (ppb), including triclosan, are summarized in Table XIII.

There are substantial differences for the results from the three plants. The results for triclosan are denoted by a "J" qualifier because the method was not validated for triclosan.

Results for sediment samples: Seven sediment grab samples were collected along the Yakima River, and 12 were collected along the Columbia River from above Wanapum Dam down to the Hanford Reach. Sturgeon collected from the Hanford Reach area had the highest PBDE burden. See Table IX. The samples were extracted, cleaned up, and analyzed using the same procedure used for initial demonstration of capabilities and minimum detectable level soil samples. Generally, the PBDE congener concentration ranges, except for a marina on the Columbia, were higher for BDE-47, BDE-99, and total PBDEs for Yakima River sediments as compared to Columbia sediments. The results are expressed as μg/kg (ppb)-dry weight basis and summarized in Table XIV.

Biosolids were collected as activated sludge (0.3% to < 1% solids) and as dewatered sludge (15–20% solids) from three sewage treatment plants located along the Yakima River. There is fair comparison for the duplicates considering the nonhomogeneous nature of the biosolids and the time elapse from the activated sludge stage and the final dewatered step. The results are expressed in μg/kg (ppb) dry-weight basis, including triclosan, and are summarized in Table XV.

It is interesting to note that there are some substantial differences within and between each sewage treatment plant.

The dewatered biosolids from Plant 2 supposedly had been applied to a hops field as a soil conditioner and fertilizer. Three samples were taken from the hops field. The control sample was taken from a section of the field that had not had biosolids applied. Another sample was taken between the hops plants where the biosolids had been disked in. Also, one sample, which obviously had not been disked in, was taken adjacent to one of the supporting poles. The results are summarized in Table XVI.

Because there was no correlation between the PBDE congener and triclosan results between the support pole and Plant 2, the farmer was questioned further about the perceived discrepancy of a factor of six to eight. Upon his further reflection, he remembered that biosolids from sewage treatment plants in the Seattle area had been applied to the field previously, where one might expect higher PBDE and triclosan concentrations.

Results for fresh water clams: Four composites of fresh water clams were collected and analyzed for PBDEs. The PBDE congener concentrations were much lower than for fish taken from the same area. This likely is due to their much lower percentage of

Table XVI: PBDE congener concentrations in HOPS field samples as μg/Kg (ppb)-dry-weight basis

BDE	Control	Between Hops	Support Pole
47	Not Detected	33	1300
99	0.14	39	1500
100	0.15	6.7	260
153	0.12	3.8	140
154	N. D.	3.1	120
Total PBDEs	0.49	140	7600
Triclosan	Not detected	150 J	29,000 J

Table XVII: Analytical results for Killer Whale blubber, μg/Kg, wet-weight basis

Compound	Resident (μg/kg)	Resident Dup. (μg/kg)	Transient, (μg/kg)	Transient, Dup. (μg/kg)
AROCLOR®1254	2700 J	2500 J	230,000 J	260,000 J
AROCLOR®1260	5000 J	5700 J	620,000 J	680,000 J
TOTAL PCBs	7700 J	8200 J	850,000 J	940,000 J
PCB-77	23	28	9800	11,000
PCB-81			450	160
BDE-47	530	630	5900	6100
BDE-99	79	99	900	970
BDE-100	99	120	1200	1300
BDE-153	4.3	4.4	61	55
BDE-154	8.5	9.7	100	90
TOTAL PBDEs	721	863	8300	8560

lipids, feeding habits, and shorter life span. The BDE-47 concentrations ranged from 0.23 to 1.30 μg/kg; the BDE-99 concentrations ranged from 0.13 to 0.52 μg/kg, and the total PBDEs ranged from 0.62 to 2.36 μg/kg.

Results for killer whale blubber: As a further test of the procedure, two killer whale blubber samples were obtained from the National Oceanic and Atmospheric Administration (NOAA, Washington, DC). The resident whale was from the Puget Sound area and fed predominantly on salmon. The transient whale was from the open ocean and fed primarily on pinnipeds such as seals, sea lions, and other large mammals with high lipid content. The lipid content of the samples was high and smaller sample aliquots (that is, 1.5–3 g) were used. The blubber was cut into small pieces with a scalpel and extracted as described previously for fish. Each sample was analyzed in duplicate. After extraction, the residue in the extraction thimble was ground with a mortar and pestle and re-extracted to test the completeness of the original extraction. The second extraction confirmed that the first extraction was complete. The extract was cleaned up with the semimacro Florisil procedure using first 80 mL of hexane for the "0% fraction," followed by 100 mL of 6% diethyl ether for the "6% fraction," followed by sulfuric acid clean-up and subsequent analysis by GC–ECD using dual dissimilar capillary column confirmation.

The results are summarized in Table XVII. There is good precision for the duplicate samples. Due to the different feeding habits of the transient whale, the Aroclor results and as well as the coplanar polychlorinated biphenyl congeners are about a factor of 100 times higher than for the resident whale. Because the transient whales spend most of their time in the open ocean, it is believed that pinnipeds make up the majority of their diet. Pinnipeds as a group are at the top of the food chain, biomagnification of polychlorinated biphenyls and other lipophilic, nonpolar organic compounds, is pronounced. But resident whales feed mainly on fish, particularly salmon, which have generally low concentrations of these types of compounds. Polychlorinated biphenyl-81 congener was not detected in the resident whale at the detection limit. Due to the convoluted polychlorinated biphenyl pattern for the two Aroclors, the results were reported with a "J" qualifier to designate that the results are estimated. The PBDE results were about 10 times higher for the transient compared with the resident whale and exhibited good precision as summarized in Table XVII. Also, the total polychlorinated biphenyl concentrations as Aroclors in the resident were 10 times higher compared with the total PBDE concentrations. On the other hand, the total polychlorinated biphenyl concentrations as Aroclors in the transient were 100 times higher compared with the total PBDE concentrations. Perhaps the difference might be explained simply by the fact that PBDEs have been used for only about 20 years compared with a much longer period of time and perhaps higher volume/weight for polychlorinated biphenyls. Therefore, one would expect the total PBDE burden to be less than the total polychlorinated biphenyls.

Conclusion

In summary, PBDEs are extracted readily from fish and clam tissue, soil and sediment, whale blubber, and sewage treatment plant biosolids using EPA Method 3540C and from water using EPA Method 3510C (Modified). They are cleaned up with concentrated sulfuric acid treatment by Method 3665A and with Florisil by Method 3620B (Modified) where needed, sulfur is removed by Method 3660 where needed, and the PBDE extracts are subsequently analyzed by GC–ECD using Method 8082A. There is sufficient selectivity and sensitivity for this analysis. Furthermore, the data demonstrate that EPA Method 8082A is validated for PBDE analyses in the presence of elevated concentrations of Aroclor residues in a variety of environmental matrices. It also has been demonstrated that the effluent from sewage treatment plants, where PBDEs are absorbed onto particulates, is one potential source of PBDEs in the sediment and ultimately biological uptake into bottom-feeding fish species. Data suggest that PBDEs are biomagnified in the tissue samples.

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References

(1) SW-846, Method 8082A: Polychlorinated Biphenyls (PCBs) by Gas Chromatography (U.S. Environmental Protection Agency, Washington, DC, 1996).

(2) SW-846, Method 8085: Pesticide Screening and Compound Independent Elemental Quantitation by Gas Chromatography with Atomic Emission Detection (AED), (U.S. Environmental Protection Agency, Washington, DC, 1996).

(3) Restek Catalog (Restek Corporation, Bellefonte, Pennsylvania).

(4) SW-846, Method 3540C: Soxhlet Extraction (U.S. Environmental Protection Agency, Washington, DC, 1996).

(5) SW-846, Method 3510C-Modified: Separatory Funnel Liquid-Liquid Extraction (U.S. Environmental Protection Agency, Washington, DC, 1996).

(6) SW-846, Method 3665A: Sulfuric Acid/Permanganate Cleanup (U.S. Environmental Protection Agency, Washington, DC, 1996).

(7) SW-846, Method 3620B: Florisil® Cleanup (U.S. Environmental Protection Agency, Washington, DC, 1996).

(8) R.H. Rieck, *LCGC* **21**(10), 992–1004 (2003).

(9) SW-846, Method 3660: Sulfur Removal (U.S. Environmental Protection Agency, Washington, DC, 1996).

(10) A. Johnson and N. Olson, *Archives Environ. Contamination Toxicol.*, **41**:339–344, October 2001.

(11) M. Alaee, J.M. Luross, D.M. Whittle, and D.B. Sergeant, 4th Annual Workshop on Brominated Flame Retardants in the Environment, Burlington, Ontario, Canada, June 17–18, 2002.

(12) B.G. Loganathan, K. Kannan, I. Watanabe, M. Kawano, K. Irvine, K. Subodh, and H.C. Sikka, *Environ. Sci. Technol.* **29**, 1832–1838 (1995).

(13) H.M. Stapleton, R.J. Letcher, and J.E. Baker, 4th Annual Workshop on Brominated Flame Retardants in the Environment, Burlington, Ontario, Canada, June 17–18, 2002.

(14) P.S. Haglund, D.R. Zook, H.-R. Buser, and J. Hu, *Environ. Sci. Technol.* **31**(11), 3281–3287 (1997).

(15) J.J. De Kok,A. De Kok, and U.A.Th. Brinkman, *J. Chromatogr.* **171**, 269–278 (1979).

(16) U. Sellstrom, A. Kierkegaard, C. De Wit, and B. Jansson, *Environ. Sci. Technol.* **17**(6), 1065–1072 (1998). ■