Current Levels of Primary Polybrominated Diphenyl Ethers (PBDEs) in Norwegian Seafood

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Introduction

The brominated flame retardants (BFRs) represent major industrial chemicals whose use has increased dramatically over the past few decades. Polybrominated diphenyl ethers (PBDEs) are BFRs that are used in many types of consumer products. PBDEs have come to the attention of regulators and scientists due to their bioaccumulation, increasing exposure and persistence in the environment, and adverse health effects in animals and humans[1-4]. Although PBDE contamination in marine fish was first detected in Sweden in 1979, and certain PBDEs are now banned as of this year, the European Union has yet to establish regulations on PBDE levels in either feed or seafood[5]. The determination of these contaminants in consumer seafood is important for dietary exposure assessment and protection of human health. This study reports on the levels of 6 prominent PBDE congeners (28, 47, 99, 100, 153, and 154) in a selection of common Norwegian seafood.

Materials and Methods

Samples and sites: All samples were collected in 2003 from coastal areas in Norway and stored at -20°C until analysis. Five farmed Norwegian salmon (Salmo salar) were each collected from the regions of Hordaland, Trøndelag, Møre og Romsdal, and Sogn og Fjordane. Twenty-five wild mackerel (Scomber scombrus) were collected from the Møre og Romsdal region and fillets were pooled into five samples. Herring (Clupea harnegus) was collected from the Møre og Romsdal (n=25) and Sogn og Fjordane (n=25) and pooled into 5 samples from each location. Atlantic halibut (hippoglossus hippoglossus, n=18) were collected from the regions of Nordland, Lofoton, and eastern Finnmark. Fifty wild Cod (Gadus morhua L.) were collected from the north Finnmark region and fillets were pooled into 10 samples. Cod livers were from fish collected in the Troms region, and livers from 25 cod were pooled into 5 samples. Blue mussels (Mytilus edulis) were collected (n=50) and pooled (n=14) from Nordland, Finnmark, Trøndelag, Møre og Romsdal, Rogaland, Sogn og Fjordane, and Hordaland regions. Crabs (Cancer pagurus) were collected from Nordland, Sogn og Fjordane, Møre og Romsdal, Rogaland, and Hordaland regions. Eight crab samples were separated into muscle and shell meat portions. For all samples, total lipid was measured gravimetrically after ethyl acetate extraction.

Sample extraction and cleanup: Muscle and tissue samples were weighed, freeze-dried, homogenized, and 2-4 g was extracted with a Dionex ASE 300 (Dionex, Sunnyvale, CA, USA)

accelerated solvent extraction (ASE). Liver samples were not freeze-dried due to high lipid concentrations. Weighed samples were mixed with acidified and heat treated (600°C for 4 h) pelleted diatomaceous earth and added to 66-mL ASE cells. The internal standards PBDE 66 and 119 (Cambride Isotope Laboratories, Andover, MA, USA) were added at 10 ng to each cell prior to extraction. The following ASE parameters were used: solvent 80:20 dichloromethane:hexane (v/v), temperature 40°C; pressure 1500 psi; heating time 5 min; static time 1 min; flush volume 100%; purge time 60 s; static cycles 1. The extract was then concentrated to 1 mL in a Turbovap II (Zymark, Hopkinton, MA,USA). Samples were then washed twice with hexane and concentrated sulphuric acid (5:1, v/v) to remove lipids. Then 1 mL of toluene was added to the samples and they were concentrated under N_2 to approximately 1 mL and analyzed by GC/MS.

GC/MS analysis: Analysis of the PBDEs (28, 47, 99, 100, 153, and 154) was performed on a Thermo Finnigan Trace GC coupled to a Trace DSQ mass spectrometer with helium (1.2 mL/min) as the carrier gas. Splitless with surge injections of 1 µL were made by a Thermo Finnigan AS 3000 autosampler with a surge time of 1.5 min and the injector temperature set at 275°C. The GC column was a 30 m x 0.25 mm i.d. (0.25-µm film thickness) RTX-5MS capillary column (Restek, Bellefonte, PA, USA). The GC oven program was as follows: isothermal at 110 °C for 2 min, 8°C/min to 180°C, and 10°C/min to 325°C for 3 min. The GC to MS transfer line was held at 300°C. The mass spectrometer was operated in the negative chemical ionization mode (NCI) using methane (2 mL/min) as the reagent gas and the ion-source temperature was 250°C. Selective ion monitoring of the two bromide ions at *m/z* 79 and 81 was used to detect the PBDEs. The response factors for all compounds were determined using quantitation standards (Cambride Isotope Laboratories, Andover, MA, USA) with known amounts of target compounds and internal standards.

Results and Discussion

The PBDE results reported here (Tables 1 and 2) are in good agreement with respect to levels and congener pattern to the values for farmed salmon and wild herring reported in previous studies[4, 6]. The average concentrations for the sum of PBDE congeners found in 20 Atlantic farmed salmon was 19.5 ng/g lipid, with the higher range of concentrations found in the more populated regions of Hordaland and Trøndelag. The PBDE levels found in these salmon were an order of magnitude lower then those found recently for salmon from the Atlantic and more polluted waters of the Baltic[4, 7]. As with PCBs, it has been suggested that PBDE contamination for farm-raised salmon comes via marine oils used in aquaculture feed[8]. We found the range of PBDEs in 21 Norwegian fish feeds (3-19 ng/g lipid) from 2003 to be close to the levels observed in herring (11-24 ng/g lipid) slightly lower then levels found in Norwegian farmed salmon (8-34 pg/g lipid) reported here[9]. As observed with PCBs, it is likely that PBDEs are also able to accumulate in adult salmon[10].

Also, the average levels of summed PBDEs observed in wild herring (16.63 ng/g lipid) were nearly those found for the farmed salmon (19.52 ng/g lipid). The highest level and greatest variability in PBDE concentrations were observed in Atlantic halibut, with the sum of average concentrations at 51.06 in a range of 11 to 191 ng/g lipid. The high concentrations of PBDEs measured in halibut were independent of location, and may be more related to their habitat in surface sediments. As far as regional differences in PBDE concentrations, only farmed salmon showed a gradient of higher concentrations (2 to 3-fold) in more urban areas of Norway. Also, there was no apparent correlation between the sum of PBDE concentrations and the amount of lipid

in the species examined (Table 2). Relatively low average concentrations of PBDEs were found in the muscle tissue of mackerel (5.54 ng/g lipid) with average an average fat content of 27%, and cod (2.65 ng/g lipid) with average fat values of 1%.

Cod liver contained approximately 6-fold greater concentrations (12.55 ng/g lipid) of the PBDEs than found in the muscle. Average concentrations of PBDEs in crab muscle and blue mussels were comparable at 5.32 and 7.57 ng/g lipid, respectively. However, as with cod liver, the internal organ concentration of PBDEs in the crab shell meat (18.41 ng/g lipid) was nearly 4-fold greater then that of the crab muscle. The localization of PBDEs in cod liver over muscle has been reported previously, however, the mechanism behind such accumulation is unknown. It has been suggested that PBDE accumulation in cod liver over muscle is different to that of planar PCBs and PCDD/Fs which bind with high affinity to liver enzyme CYP 1A[11].

As found with previous studies on PBDEs in seafood, the congener profiles for the measured PBDEs here indicate that PBDE 47, 99, and 100 are the most abundant congeners in Norwegian seafood[6,11,12]. PBDE 47 accounts for approximately 60 to 70% of the total PBDEs found in the fish and mussel species examined here. Congener specific accumulation of PCBs in farmed Atlantic salmon has been studied previously, where preferential accumulation of non-ortho PCBs with tetra chlorine substitution was found over other other non-ortho and mono-ortho substituted PCBs[13]. Since PBDE 47 is also a tetra substituted congener that is structurally similar to tetra-substituted non-ortho PCBs, similar mechanisms for PBDE 47 accumulation in seafood may also be present.

For crab, substantial levels of PBDE 153 were observed in the shell meat (4.11 ng/g lipid). As reported earlier for fish, it is possible that the high levels of PBDE 153 we observe in crab shell meat may be due to biotransformation of PBDE 209[14]. Compared to the lower PBDE congeners, the fully brominated PBDE 209 is found at considerably higher concentrations in surface sediments[15]. While PBDE 209 was not measured in this study, it is likely that biotransformation to PBDE 153, and possibly other lower brominated congeners, is present in these crab and halibut samples. In mussels, we were not able to isolate PBDE 28 due to considerable baseline inference. The source of the interference found with mussels is unknown and was not observed in any of the other seafood species analyzed here.

In regards to the possible toxicity of PBDEs to these seafood species, the effect of BFR exposure on marine organisms is limited. In mussels, PBDE 47 has been shown to damage sperm cell DNA at relatively low concentrations [16]. Since PBDE toxicity appears to follow that of PCBs, it is likely that exposure to both these contaminants in combination, along with their products of degradation and metabolism, puts both seafood and people at significant risk to biological injury. This study has focused on popular seafood species used for consumption and should help in assessing some of the sources and levels of PBDE exposure in humans.

Table 1: Concentrations of PBDE congeners (ng/g wet wt) in Norwegian seafood collected in 2003.

Sample		PBDE 28	PBDE 47	PBDE 99	PBDE 100	PBDE 153	PBDE 154	Sum
Atlantic Salmon	Average	0.12	1.66	0.27	0.30	0.05	0.11	2.51
(Salmo Salar, farmed, muscle, n=20)	SD	0.06	0.78	0.09	0.15	0.02	0.05	1.13
	Range	0,05-0,24	0,67-3,09	0,15-0,47	0,12-0,52	<0,03-0,07	0,05-0,16	1,14-4,49
Mackerel	Average	0.09	0.86	0.26	0.16	0.04	0.06	1.46
(Scomber scombrus, muscle, n=5)	SD	0.01	0.13	0.05	0.03	0.01	0.02	0.23
	Range	0,08-0,10	0,76-1,07	0,20-0,33	0,14-0,20	<0,03-0,05	0,05-0,08	1,26-1,78
Herring	Average	0.07	1.23	0.18	0.36	0.03	0.05	1.90
(Cupea harengus, muscle, n=10)	SD	0.01	0.38	0.09	0.39	0.01	0.02	0.82
	Range	0,06-0,08	0,75-1,81	0,08-0,29	0,01-1,39	<0,02-0,04	0,03-0,09	1,02-3,53
Atlantic Halibut	Average	0.21	4.68	0.15	0.44	0.07	0.19	5.65
(Hippoglossus hippoglossus, muscle, n=18)	SD	0.21	4.97	0.14	0.45	0.05	0.18	5.93
	Range	0,01-0,57	0,15-14,54	0,02-0,48	0,01-1,54	<0,02-0,18	<0,02-0,68	0,32-17,59
Cod	Average	<0,01	0.02	<0,01	<0,01	<0,02	<0,02	0.03
(Gadus morhua L., muscle, n=10)	SD		0.01					0.01
	Range	<0,01	0,02-0,04	<0,01-0,01	<0,01	<0,02	<0,02	0,02-0,04
Cod Liver	Average	0.37	5.70	0.15	0.67	0.03	0.40	7.32
(Gadus morhua L., liver, n=5)	SD	0.07	1.31	0.03	0.17	0.00	0.12	1.68
	Range	0,30-0,47	4,00-7,37	0,11-0,19	0,46-0,90	0,02-0,03	0,26-0,58	5,17-9,50
Blue mussels	Average	nd	0.08	0.04	0.02	0.02	0.02	0.15
(Mytilus edulis, n=14)	SD	nd	0.03	0.02	0.01	0.01	0.01	0.06
	Range	nd	0,03-0,12	0,01-0,07	<0,01-0,04	<0,01-0,03	<0,01-0,04	0,06-0,25
Crab	Average	<0,01	0.04	0.02	<0,01	<0,02	<0,02	0.05
(Cancer pagurus, muscle, n=6)	SD		0.01	0.01				0.02
	Range	<0,01	0,03-0,05	0,01-0,02	<0,01	<0,02	<0,02	0,03-0,07
Crab shell meat	Average	0.04	0.90	0.67	0.23	0.53	0.12	2.39
(Cancer pagurus, shell meat, n=8)	SD	0.03	1.24	1.00	0.34	0.48	0.15	2.60
	Range	<0,01-0,1	0,09-3,05	<0,01-2,34	0,02-0,80	0,06-1,61	<0,02-0,37	0,58-6,96

nd=not determined

Table 2: Concentrations of PBDE congeners (ng/g lipid) in Norwegian seafood collected in 2003.

Sample		% lipid	PBDE 28	PBDE 47	PBDE 99	PBDE 100	PBDE 153	PBDE 154	Sum
Atlantic Salmon	Average	13.2	0.94	12.90	2.08	2.39	0.40	0.86	19.52
(Salmo Salar, farmed, muscle, n=20)	SD	2.0	0.47	6.40	0.74	1.29	0.17	0.41	9.37
	Range	9,0-18,6	0,40-1,76	5,11-22,68	1,16-3,58	0,88-3,85	<0,03-0,63	0,39-1,52	8,42-33,63
Mackerel	Average	26.6	0.36	3.28	0.99	0.62	0.16	0.24	5.54
(Scomber scombrus, muscle, n=5)	SD	2.6	0.07	0.65	0.26	0.16	0.07	0.09	1.27
	Range	22,1-28,6	0,26-0,47	2,67-4,07	0,73-1,34	0,44-0,81	<0,1-0,21	0,17-0,37	4,42-7,27
Herring	Average	11.4	0.68	11.14	1.48	2.78	0.21	0.45	16.63
(Cupea harengus, muscle, n=10)	SD	3.7	0.28	2.44	0.34	2.44	0.05	0.11	4.02
	Range	6,7-15,6	0,35-1,22	7,89-14,78	0,95-1,92	0,98-9,42	<0,25-0,29	0,33-0,64	10,80-24,02
Atlantic Halibut	Average	11.0	1.90	42.31	1.55	3.86	0.48	1.50	51.06
(Hippoglossus hippoglossus, muscle, n=18)	SD	10.0	2.01	46.97	1.31	3.93	0.35	1.24	55.34
	Range	1,4–31,1	0,56-7,42	8,60-161,89	0,52-6,20	0,65-13,72	<0,20-1,17	<0,4-4,65	10,92-190,58
Cod	Average	0.9	<1,00	2.54	<0,01	<1,00	<2,00	<2,00	2.65
(Gadus morhua L., muscle, n=10)	SD	0.0		0.59					0.79
	Range	0,9-1,0	<1,00	1,91-3,85	<0,01-1,13	<1,00	<2,00	<2,00	1,91-4,24
Cod Liver	Average	58.7	0.64	9.77	0.25	1.15	0.05	0.69	12.55
(Gadus morhua L., liver, n=5)	SD	2.4	0.13	2.51	0.06	0.32	0.01	0.23	3.22
	Range	55,9-61,6	0,49-0,79	6,50-10,95	0,18-0,34	0,74-1,60	0,04-0,06	0,43-1,04	8,39-16,99
Blue mussels	Average	2.1	nd	3.78	1.70	0.86	0.85	1.19	7.57
(Mytilus edulis, n=14)	SD	0.5	nd	1.38	0.63	0.36	0.29	0.44	2.85
	Range	0,9-2,9	nd	1,29-5,77	0,55-2,64	<0,25-1,63	<0,40-1,27	<0,40-2,01	2,41-11,31
Crab	Average	1.0	<1,00	3.62	1.48	<1,00	<2,00	<2,00	5.32
(Cancer pagurus, muscle, n=6)	SD	0.1		0.83	0.51				1.52
	Range	0,09-1,0	<1,00	2,81-4,79	0,92-2,19	<1,00	<2,00	<2,00	3,07-6,98
Crab shell meat	Average	13.0	0.29	6.90	5.16	1.79	4.11	0.96	18.41
(Cancer pagurus, shell meat, n=8)	SD	0.1	0.26	9.53	7.70	2.62	3.69	1.12	20.02
	Range	12,80-13,4	<0,08-0,76	0,70-23,49	<0,08-17,98	0,16-6,18	0,47-12,41	<0,16-2,81	4,45-53,55

nd=not determined

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