INVESTIGATION OF POLYBROMINATED DIPHENYL ETHERS IN SCOTTISH AND EUROPEAN FARMED ATLANTIC SALMON (*Salmo salar*), SALMON AQAUCULTURE FEED AND FISH OILS

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Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used flame retardants. As persistent and ubiquitous organic pollutants (POPs), they may have endocrine disrupting effects[1-4]. Due to low solubility in water they bioaccumulate in the lipid compartment of the aquatic food chain in a similar and possibly more efficient way than other lipophilic contaminants such that fatty fish may be contaminated with appreciable amounts of PBDEs due to biomagnification[5, 6]. Although available data, including our report, suggests that current levels of PBDEs are an order of magnitude lower than PCBs, the increase of PBDE levels in human breast milk and blood[2, 7-10] raises concern about risks to human health, particularly infants. A major source of human exposure to the brominated congeners is through food, but there are other exposure routes. Although these compounds are in extensive production and use, data on the amounts of PBDEs consumed in the UK is very limited. Data on PBDE's in wild fish from the Northern Hemisphere are available[1], and surveys to determine the distribution and fate of PBDEs in marine life in waters around the UK are underway[11]. However, there is little or no data in the public domain for aquaculture products and farmed fish. In this preliminary study, salmon (*Salmo salar*), salmon aquaculture feed and fish oil samples were extracted and initially analyzed for polychlorinated biphenyl (PCB) congeners and organochlorine pesticides[11]. It was demonstrated for these samples that the same extract fraction contained PBDEs. The results are in good agreement with recent reports on wild fatty fish (herring and salmon)[5], and markedly lower than Lake Michigan Coho and Chinook salmon (*Oncorhynchus kisutch* and *Oncorhynchus tshawytscha*)[12]. Further larger scale investigations of a comprehensive range of PBDEs and other POPs should be conducted in farmed salmon and salmon feed, including feed fortified with fish oil and feed fortified with selected vegetable oils, to protect the consumer from increasing exposure to newly identified POPs.

Materials and Methods

Sites and sampling: Seven British salmon (*Salmo salar)* samples and one Norwegian salmon sample that enter the European fish market were analyzed. Data on the levels of PCDD/Fs, selected coplanar PCBs[13, 14], PCB and organochlorine pesticides[15] for these samples is available elsewhere. The samples were of variable age, both farm raised and wild, and were obtained from seven different Scottish sites, as documented previously and a Norwegian sample for which no information is available. In addition, five salmon samples, two originating from Ireland (one wild, one farmed) and three farmed samples for which no further information was available, purchased from the Belgian market, were analyzed. Eight salmon feeds were analyzed (from four different Scottish sources) and five fish oils, one vegetable oil were analysed, (all but one of which were

obtained from the same source, on the same date, but originally from widely varying sources). The fresh and frozen samples were wrapped in polyethylene bags and frozen immediately at –20°C. Whole body weights of the fish ranged from 400g to 5.2kg, the fish ranged in age from 1 year to 3+ years. The fish oil and feed were not samples fed directly to the salmon collected, as these were not available. Table 1 gives sample details.

Sample preparation: The samples were thawed, filleted, skinned and the epaxial muscle homogenized before being subdivided into smaller replicate portions of approximately 100 grams. The portions were weighed, stored in tightly sealed polythene bags and frozen at -20°C. One sample consisted of homogenized samples of two fish (from the same source and of the same age and size) to ensure similar sample quantities of tissue. The samples were sent packed in dry ice to the Toxicological Center, Antwerp, where they were logged-in and stored at -60°C prior to analysis.

Sample analysis: The samples were analyzed for the following PBDEs: (IUPAC no's) 28, 47, 66, 71, 75, 99, 100, 153 and 154. After addition of internal standards (a mixture of ^{13}C-BDE 47, 99 and 153), approximately 10g fish tissue (or 3g feed) were grounded with anhydrous sodium sulphate and extracted for 2h with 75 ml hexane:methylene chloride:acetone=3:1:1 (v/v) into a hot Soxhlet manifold. After concentration and determination of lipid content, the extract was subjected to clean-up on 2 successive solid phase cartridges containing acid silica and acid silica : neutral silica : deactivated basic alumina (from top to bottom), respectively. PBDEs (and PCBs) were eluted with 50 ml hexane. All analyses were performed on a Hewlett Packard 6890 GC equipped with a 10m x 0.10mm x 0.10µm AT-5 (5% phenyl polydimethyl siloxane) capillary column (Alltech), connected via direct interface with a HP 5973 mass spectrometer. Recoveries of internal standards, ^{13}C-labeled BDEs (calculated based on PBB 80 added prior to injection) ranged between 81 and 103% with a standard deviation of less than 21%. Method limits of detection (LOD) for individual PBDE congeners ranged between 0.1 and 0.4 ng/g fat. Analysis of two samples of biota (eel and porpoise liver) used for the first interlaboratory test on PBDE[16], showed a variation of less than 10-15% from mean values.

Results

PBDEs were detected in all fish samples, all feed samples but only one of the fish oil samples. The summed concentration of selected PBDEs is the sum of concentrations of the congeners measured and is an underestimate of the total BDE concentration as other present isomers were not measured. The sum of the BDEs (ΣBDEs) ranged from 1.2 ng/g fat to 85.2 ng/g fat for the thirteen salmon samples, from 8.1 ng/g fat to 23.8 ng/g fat for the eight feed samples and from not detected to 12.7 ng/g fat for the four fish oil and one vegetable oil samples. The fry feed samples, with a far lower fish oil content had lower residue levels than the feeds designed for smolts and adult salmon with a lipid content over 20%. As expected, BDE 47 dominated the profiles, ranging between 50 to 100% of the ΣBDEs included in the analyses.

All values were adjusted to the lipid content of the sample by dividing the whole weight concentration by the percent lipid in each sample and are reported in Table 1 together with details of weight, age and lipid content for each sample analyzed. Quality control (QC) samples were included in batches of samples analysed. The concentration of lipid found in the fish samples ranged from 3.9% to 19.9%, and in the feed samples 18.1%-35.9%. The immature (smolt) fish are fed lower levels of oils, and tend to store lower levels of lipid in the flesh compared to mature fish and this is reflected in the lipid content of the samples, with two unusual exceptions (Sample M14 and the sample sourced in Belgium M18). With the former, the supplier gave the provenance as wild. Whether these fish had a similar dietary lipid intake to the other fish sampled is not known,

but it has been observed that there can be marked variation in flesh lipid content within fish fed the same dietary oil level such that certain individual fish utilize high-energy diets but deposit little lipid in their flesh while others tend toward greater adiposity. All fish contained detectable levels of certain PBDEs, the highest levels of 85.2 ng/g fat in a wild (or possible farm escapee) salmon sample and over 50 ng/g fat were observed in the oldest fish, 3 years + as expected. The ratio of BDE 47 to the ΣBDEs ranged from 0.37 to 1 with the majority of the samples being around 0.55 for all fish and feed samples and one of the fish oil samples (originally from the Northern Hemisphere).

Discussion

The average concentration across all salmon samples of the ΣBDE congeners was 33.7 ng/g fat, and 16.6 ng/g fat for the feed samples. This is a magnitude lower than the average respective ΣPCB concentrations[15]. Moreover, the results from this study are an order of magnitude lower than the respective PBDE values for salmon reported in recent studies from polluted open waters. Previous reports have detected significant levels of PBDEs in fatty fish such as sprats, herring and salmon from the Atlantic and more polluted waters such as the Baltic[1, 2, 5] showing a similar age related accumulation and biomagnification to that shown in this study. Salmon from Lake Michigan tributaries in the US appear to have the highest PBDE concentrations so far reported worldwide, several orders of magnitude greater than the concentrations reported here, but with a similar congener distribution[12]. The potential contribution to the human diet of PBDEs and other contaminants from farmed salmon will vary according to environmental levels, the age of the fish, whether the individual fish had a predisposition to adiposity, or not, the frequency of consumption, portion size, cooking practices and the age of the consumer. The possible contribution of PBDEs to the total dietary intakes of organohalogen compounds from farmed salmon could be significant for high consumers, particularly breast feeding mothers, but national extrapolations cannot be made on the basis of this study due to the relatively small sample size.

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Table 1: Selected PBDEs in European and Scottish Atlantic Salmon, aquaculture feed and fish oils: Lipid Adjusted (ng/g, ppb)

Sample codes		Further information	% lipid	Sample Wt (g)*	Lipid Wt (g)	BDE 28	BDE 71	BDE 47	BDE 75	BDE 66	BDE 100	BDE 99	BDE 154	BDE 153	sum BDEs	BDE47 /sum
M11	Salmon Scotland	(f) 3+ yrs fresh Jan 1999	13.5	8.19	1.10	1.3	7.1	29.3	0.3	2.0	6.5	4.9	1.3	1.0	53.7	0.55
M12		(f) 2+ yrs fresh Jan 1999	14	8.13	1.14	0.9	4.4	14.7	ND	ND	2.9	2.8	0.7	0.8	27.2	0.55
M13		Norway frozen Jan 1999	13.7	8.39	1.15	ND	ND	0.4	ND	ND	ND	0.7	ND	ND	1.1	0.37
M14		(w) 2+yrs frozen Jan 1999	4.9	9.73	0.47	0.8	1.7	12.9	ND	0.9	2.4	5.9	ND	ND	24.6	0.52
M24		(f) 3+ yrs fresh Jan 1999	19.9	9.69	1.93	1.5	7.1	28.9	0.2	2.2	6.4	5.0	1.6	ND	52.9	0.55
M25		(f) 3+ yrs fresh Jan 1999	19.1	9.04	1.73	1.5	7.1	28.0	0.4	1.8	6.1	4.4	1.0	ND	50.3	0.56
M28		(w) Jan 1999	12.3	9.5	1.17	2.0	7.9	43.0	0.3	3.2	9.9	14.0	3.6	1.3	85.2	050
M31		(f) N=2, Smolt, fresh , Jan 1999	9.3	9.15	0.85	1.3	6.7	25.0	0.5	1.6	5.6	4.0	0.9	ND	45.6	0.55
M18	Salmon B. Market	(f) Smoked bioculture, March 2001	3.9	4.60	0.18	ND	ND	3.1	ND	ND	ND	ND	ND	ND	3.1	-
M19		(f) Fresh bioculture, March 2001	16.5	5.15	0.84	0.5	2.8	10.2	ND	0.8	2.8	1.6	0.2	ND	18.9	0.54
M20		(f) Fresh March 2001	11.0	5.48	0.60	0.5	2.4	9.4	ND	0.4	1.5	1.5	ND	ND	15.7	0.60
M21		(w) Ireland, smoked March 2001	11.6	4.51	0.52	0.2	0.7	5.0	ND	0.1	1.1	1.3	ND	ND	8.4	0.59
M22		(f) Ireland, smoked March 2001	14.8	7.25	1.07	1.0	8.1	25.8	0.7	1.7	7.9	5.6	1.3	ND	52.1	0.49
M01	Salmon Feed	Fry 1-5g (A)	18.1	3.00	0.54	0.3	1.4	7.0	ND	ND	ND	ND	ND	ND	8.7	0.81
M02		1000-2200g (B)	30.1	3.00	0.90	0.5	3.1	8.2	0.2	0.5	1.5	1.7	ND	ND	15.6	0.53
M03		350-1000g (B)	31.0	3.03	0.94	0.5	3.1	8.2	ND	0.7	1.5	1.4	ND	ND	15.4	0.54
M04		1000-2200g (C)	35.9	3.07	1.10	0.7	4.3	12.3	0.3	0.7	1.8	2.7	ND	ND	22.8	0.54
M05		Fry (C)	19.8	2.26	0.45	ND	1.4	5.1	ND	ND	0.9	0.7	ND	ND	8.1	0.63
M15		500- 1300g (D)	28.1	2.00	0.56	0.6	4.6	13.2	ND	ND	2.3	2.3	ND	ND	23.0	0.57
M16		1300-2200g (D)	34.3	2.11	0.72	0.6	4.4	13.0	0.3	0.9	2.4	2.2	ND	ND	23.9	0.54
M17		500-1300g (D)	32.7	1.97	0.64	0.5	3.0	9.5	ND	ND	1.8	1.4	ND	ND	16.2	0.59
M06	Fish oil	Details withheld (C)		0.50		0.3	2.7	7.1	ND	ND	1.4	1.2	ND	ND	12.7	0.56
M07, 08, 09, 10				0.50		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-

Non Detects=ND; (w)=wild; (f)= farmed; Salmon Feed=Scotland aquaculture salmon feed; B. Market=Belgium market; Sources A, B, C, D, E denoted in brackets