INVESTIGATION OF SELECTED PCBs AND ORGANOCHLORINE PESTICIDES IN FARMED ATLANTIC SALMON (*Salmo salar*), SALMON AQUACULTURE FEED AND FISH OIL COMPONENTS OF THE FEED

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Introduction

Data are available on the concentrations of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-*p*-furans (PCDFs) in fish from wild stocks[1]. With farmed fish, potential hazards associated with the ingredients and additives used in aquaculture feed were not considered significant until very recently[2-3]. Existing data on the levels of PCDDs, PCDFs and PCBs consumed in the UK have been mainly derived from total diet surveys and surveillance data for specific food types[4]. These data indicate the presence of elevated organochlorine contamination of farmed fish. Of 161 salmon samples tested in 1997, all were found to contain residues of PCBs (ICES 7 set of PCBs) at concentrations of 23-620 ng/g fat. All 80 trout samples contained residues of PCBs at concentrations of 61-550 ng/g fat, mean 220 ng/g fat[5]. The determination of these contaminants in fish and identifying the original sources of the contamination is, therefore, important for dietary exposure assessment and the protection of public health, particularly in view of the increasing availability to the consumer of farmed salmon. The farmed salmon industry has trebled in production from 225,462 metric tons (mt) to 687,906 mt during the years from 1990 to 1998, Norway and the UK are the major world producers[6]. Use of fish meal in aquaculture feeds for farmed fish and shrimp was 2,016,361 mt in 1996[7], and continues to increase from 10% in 1988 to 17% in 1994 and 33% in 1997[8,9]. In contrast world wide fish oil production has fallen from 768,952 mt in 1995 to 492,364 mt in 1998[6], but use in salmon feeds has increased significantly since 1995 from less than 30% to 33% and even 36.3% of the total diet for farmed salmon[9,10]. This paper presents the results of the analyses of mainly farmed European Salmon, feed and fish oil samples for a wide range of PCBs and organochlorine pesticides. It confirms and extends previous reports of relatively high concentrations of PCBs in farmed Scottish Salmon feed and fish oils. Although preliminary, this investigation strongly indicates the need for further study of farmed salmon and salmon feed, including feed fortified with fish oil and feed fortified with selected vegetable oils, for PCBs and organochlorine pesticides. Although banned in Europe and most of the world, these pollutants are still being delivered to the consumer via foods and feeds of fish origin. In some cases there are indications of recent usage of DDT.

Materials and methods

Sites and sampling: Seven British salmon (*Salmo salar)* samples and one Norwegian sample that enter the European fish market were analyzed. Data on the levels of PCDD/Fs and selected coplanar PCBs for these samples is available elsewhere[11,12]. The samples were of variable age, both farm raised and wild, and were obtained from seven different Scottish sites, 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 were obtained from the same source, on the same date, but originally from varying sources). Data on other POPs, principally polybrominated diphenyl ethers (PBDEs) for the same sample set are presented elsewhere[13]. 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 raw 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^{0}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 stored at -20^{0}C. The extraction, clean up and analysis methods used are described elsewhere[14,15]. Approximately 10g fish tissue (or 3g feed) were extracted with hexane:methylene chloride:acetone=3:1:1 into a hot Soxhlet manifold. The samples obtained from Scotland were analyzed for 74 PCB congeners of which four PCBs were the dioxin like coplanar PCBs 105, 118, 156, and 157 with a designated toxic equivalency to 2,3,7,8-TCDD4[16]. The samples obtained in Belgium were analyzed for 23 PCBs. All samples were also analyzed for HCHs (3 isomers), HCB and DDT and metabolites (6 *op*- and *pp*-isomers). All analyses were performed on a Hewlett Packard (Palo Alto, CA, USA) 6890 GC equipped with a 50m x 0.22mm x 0.25μm, HT-8 capillary column (SGE), connected via direct interface with a HP 5973 mass spectrometer. Method limits of detection (LOD) for individual PCB congeners ranged between 0.1–0.5 ng/g fat. For HCHs and DDTs, the detection limit was 0.2 ng/g fat for each isomer. Recoveries of target compounds ranged between 72 and 80%. The procedure was validated through regular analysis of blanks, spiked samples, certified material CRM 349, 350 (PCBs and organochlorine pesticides in cod liver and mackerel oil) and through successful participation in a Belgian Ministry of Health interlaboratory study.

Results and discussion

All samples examined contained detectable residues of organochlorine contaminants. With most of the samples the predominant pesticide isomers were *pp*-DDE and γ-HCH. Results are presented in Table 1, with total sum (Σ) values given using the LOD equal to zero where there were no detections. The sample size was insufficient to make comparisons between farmed and wild salmon.

Fish samples: PCB concentrations in the farmed samples were between 145.1 and 482.1 ng/g lipid. These concentrations are rather high and indicate possible local sources of pollution with PCBs. Profiles of PCBs were similar and of the same order of magnitude for all the farmed samples, but lower in a Norwegian sample (M13) and a magnitude lower in a wild Irish sample (M21). The 7 ICES marker PCBs constituted 39.2 % of the total ΣPCB congeners for Scottish salmon. In the Norwegian sample (M13), the 7 markers accounted for 48% of the total PCB concentration. The mean concentration for HCB was 16.7 ± 12.2 ng/g fat, but was under the detection limit of 0.2 ng/g fat in sample M13. The mean concentration for HCHs (Σα,β,γ-HCH) was 11.2 ± 10.1 ng/g fat. The mean concentration of DDTs (Σ*pp* isomers) was 79.2 ± 74.4 ng/g fat. Concentration of DDTs in M13 was lower (5.1 ng/g fat). The PCB fraction provides a greater contribution to the total TEQ value[12,13]. The ratio PCB-TEQ/ dioxins-TEQ was 2.14 ± 0.54, while the total TEQ was 29.95 ± 12.98 pg TEQ/g fat. PCB 126 was the principal contributor (70%) to the PCB-TEQ, followed by PCB 118 (12.5%), PCB 156 (4.7%) and PCB 105 (3.6%).

Aquaculture feeds: Concentrations of PCBs ranged between 75.6 and 1153.2 ng/g fat. Six feed samples presented the same profile of PCBs, with the hexa- and penta-PCB congeners representing up approximately 80% of the total PCB content. PCBs concentrations were high (147.4 ± 41.3

ng/g fat), probably due to the high fish oil content of some samples (up to 35%). Two samples with the highest concentrations came from the same manufacturer (M02 and M03) and presented a total different profile of PCB congeners when compared with the other six feed samples. Tri and tetra congeners (approximately 55 and 28%, respectively) dominated, and the profile was close to that of Aroclor 1242. This profile could point to the existence of local PCB sources or illegal dumping of used Aroclor mixtures in the animal food chain (see the Belgian PCB poisoning episode-summer 1999[14]). The HCB, HCHs and DDTs concentrations were in a comparable range to that reported recently in Belgium[15]. The mean for HCB was 5.2 ± 2.9 ng/g fat, for HCHs the mean was 24.9 ± 13.7 ng/g fat. The mean ΣDDTs was 41.5 ± 7.5 ng/g fat with a mean ratio of pp-DDT/ΣDDTs at 0.13 ± 0.04. The 7 marker PCBs constituted approximately 40% of the total ΣPCB congeners.

Fish oils: The fish oil samples showed varying PCB contents of 3.1, 14.0, 72.0 and 250.3 ng/g fat (ND=0 LOD). Penta- and hexa-congeners dominated the PCB profile for all samples. Mean HCB, ΣHCHs and ΣDDT concentrations were 1.4 ± 1.7, 10.1 ± 7.9 and 27.5 ± 19.4 ng/g fat, respectively. The mean ratio pp-DDT/ΣDDTs was 0.15 ± 0.07 ng/g fat. The levels and ratios of the HCB and HCHs are reflected in the aquaculture feed, while the DDT ratios are reflected (with *pp*-DDE dominating) but the levels in the feed were an order of magnitude higher. The ICES7 marker PCBs constituted approximately 40% of the total sum of PCB congeners (for the samples with high content of PCBs-M06 and M08). In samples with a high number of non detections (M09 and M10), the contribution of the ICES7 PCBs was greater, at 70%.

The results from this study are in good agreement with the values for Salmon reported in recent studies[4,5] with surprisingly high detections of PCB congeners. Previous reports have detected significant levels of PCBs and organochlorine pesticides in fatty fish such as herring and salmon[1, 17,18], in fish meal[14] and fish oils[19].

Conclusions

Fish are significant sources of organochlorine contaminants in the diet and so consequently the use of fish meal and fish oil of European origin are considered to be of critical concern when used in diets for farmed fish and other food producing animals. In terms of risk assessment the potential contribution to the human diet of PCBs and organochlorine pesticides from farmed European Salmon will vary according to the diet and age of the fish, the frequency of consumption, portion size, cooking practices and the age of the consumer. The possible contribution to dietary intakes of organochlorines from farmed salmon could clearly be significant for high consumers, 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: PCBs and organochlorine pesticides in European, Scottish, Irish and one Norwegian Atlantic Salmon: Lipid Adjusted (ng/g, ppb)

Sample codes		Further information	% lipid	Sample Wt (g)*	Lipid Wt (g)	PCBs Σ tri	Σ tetra	Σ penta	Σ hexa	Σ hepta	Σ octa	Σ PCBS[a] (ND=0)	Σ7 PCB[b]	HCB	α-HCH	β-HCH	γ HCH	Σ HCHs	*pp*-DDE	*pp*-DDT	*pp*-DDD	Σ DDTs	*pp*DDT/ ΣDDT
M11	Salmon: Scotland Jan 1999	(f) 3+ yrs fresh	13.5	8.19	1.10	13.5	50.3	113.4	151.8	42.5	3.8	367.1	162.8	19.5	6.8	ND	16.1	22.9	71.4	10.5	22.7	104.6	0.10
M12		(f) 2+ yrs fresh	14	8.13	1.14	33.2	46.2	79.6	110.2	29.4	2.0	284.5	119.5	13.1	4.7	ND	10.3	15.0	50.1	8.9	18.4	77.4	0.11
M13		Norway frozen	13.7	8.39	1.15	1.5	1.5	25.2	75.1	38.3	3.8	143.2	69.9	ND	1.1	ND	9.7	10.8	2.8	1.3	1.0	5.1	0.25
M14		(w) 2+yrs frozen	4.9	9.73	0.47	20.6	76.1	145	167.2	47.3	3.6	448.6	183.0	43.5	4.0	2.0	12.4	18.4	123.0	40.1	46.0	249.4	0.16
M24		(f) 3+ yrs fresh	19.9	9.69	1.93	10.2	34.2	80.4	112.6	37.1	4.6	272.5	118.4	15.6	ND	ND	ND	ND	29.0	9.1	8.7	46.8	0.21
M25		(f) 3+ yrs fresh	19.1	9.04	1.73	10.2	34.5	79.5	111.3	37.0	4.6	270.7	116.6	15.7	ND	ND	ND	ND	29.6	10.3	8.5	48.4	0.26
M28		(w)	12.3	9.5	1.17	9.2	29.8	77.6	131.5	47.3	4.8	294.7	125.9	13.8	ND	ND	ND	ND	30.0	12.0	3.3	45.3	0.29
M31		(f) N=2, fresh, smolt	9.3	9.15	0.85	19.5	33.1	71.4	105.2	29.7	2.5	251.1	103.2	12.6	ND	8.7	14.1	22.8	33.10	14.0	9.3	56.4	0.25
M18	Salmon: B.Market March 2001	(f) Sm Bio.	3.9	4.60	0.18							226.6[c]	140.4	ND	ND	ND	ND	ND	37.1	9.2	ND	46.3	0.20
M19		(f) Fresh Bio.	16.5	5.15	0.84							265.1[c]	167.9	ND	ND	ND	ND	ND	42.2	19.1	ND	61.3	0.31
M20		(f) Fresh	11.0	5.48	0.60							219.8[c]	141	ND	ND	ND	ND	ND	46.1	18.5	ND	64.6	0.29
M21		(w) Ireland, sm.	11.6	4.51	0.52							94.0[c]	60.6	4.1	ND	ND	ND	4.1	38.8	11.5	ND	50.4	0.23
M22		(f) Ireland, sm.	14.8	7.25	1.07							284.4[c]	176.6	12.0	ND	ND	ND	12.0	39.2	9.2	ND	48.4	0.19
M01	S. Feed Jan 1999	Fry 1-5g (A)	18.1	3.00	0.54	14.6	15.8	29.2	53.7	9.8	0.6	123.8	49.3	2.3	4.2	ND	11.2	15.4	22.1	7.6	5.9	35.6	0.21
M02		1000-2200g (B)	30.1	3.00	0.90	688.4	306.3	84.4	43.4	10.1	0.2	1153.2	258.7	4.2	31.0	ND	15.7	46.7	25.6	4.6	7.8	38.0	0.12
M03		350-1000g (B)	31.0	3.03	0.94	412.6	210.4	65.4	52.8	8.0	0.1	749.4	168.6	4.9	15.0	ND	12.5	27.5	23.0	4.3	7.7	35.0	0.12
M04		1000-2200g (C)	35.9	3.07	1.10	3.3	18.1	63.5	81.0	14.4	0.9	181.3	68.5	5.9	4.6	1.8	36.6	43.0	24.4	4.7	9.6	38.7	0.12
M05		Fry (C)	19.8	2.26	0.45	2.0	5.9	23.0	40.9	3.7	ND	75.6	31.7	0.6	2.3	ND	ND	8.3	21.4	5.4	6.9	33.7	0.16
M15		500- 1300g (D)	28.1	2.00	0.56	1.6	26.8	50.6	72.6	11.0	0.6	163.1	65.5	7.6	5.6	ND	15.7	21.3	33.5	6.0	12.2	51.6	0.12
M16		1300-2200g (D)	34.3	2.11	0.72	3.8	27.8	57.4	79.4	14.7	0.8	181.0	74.7	9.3	5.2	ND	9.5	14.7	35.6	2.8	12.8	51.2	0.05
M17		500-1300g (D)	32.7	1.97	0.64	3.9	22.9	49.8	68.8	10.6	0.4	157.3	63.7	7.1	5.9	ND	12.7	18.6	30.4	6.0	11.4	47.8	0.13
M06	Fish oils Jan 1999	Fish oil (C)		0.50		1.4	11.5	24.3	30.9	3.7	ND	71.8	27.7	3.7	6.7	ND	5.1	11.8	19.3	3.4	7.2	29.9	0.11
M07		Fish oil (C)		0.50		0.3	ND	1.5	1.3	ND	ND	3.1	2.2	1.5	1.3	ND	1.4	2.7	7.8	3.8	3.7	15.3	0.25
M08		Fish oil (C)		0.50		0.2	15.2	51.5	132.6	46.3	4.4	250.2	101.8	0.2	4.7	ND	15.7	20.4	36.1	5.5	12.3	53.9	0.10
M09		Fish oil (C)		0.50		0.5	ND	2.9	9.2	1.6	0.0	14.2	7.9	0.2	2.0	2.0	1.5	5.5	7.4	1.6	1.8	10.8	0.15
M10		Vegetable Oil (C)		0.50		ND	ND	ND	ND	ND	ND	ND	ND	ND	1.1	ND	14.5	15.6	ND	ND	ND	<5	ND
MX		Fish oil (E)		0.50		0.6	31.5	130.4	225.6	57.5	4.7	450.3	220.6	2.3	4.2	0.9	4.4	9.5	152.4	21.4	44.4	218.2	0.10

Sample Wt taken for analyses; ND= Non Detects (treated as 0 for Σcalculations); L= loch, R=river; LB=loch B; (w)=wild; (f)=farmed; smolt = very immature fish, not market size; Sources A, B, C, D, E denoted in brackets; S. Feed=Scotland aquaculture salmon feed; B. Market=Belgium market; Sm=smoked; Bio=bioculture; [a] 74 PCB congeners - PCBs IUPAC no's: 16, 17, 18, 20, 22, 28, 31, 33, 42, 44, 47, 48, 49, 52, 56, 60, 64, 66, 70, 74, 84, 85, 87, 91, 95, 97, 99, 101, 105, 110, 114, 118, 123, 128, 130, 132, 135, 137, 138, 141, 144, 146, 149, 151, 153, 156, 157, 158, 163, 167, 170, 171, 174, 176, 177, 178, 179, 180, 183, 187, 189, 191, 194, 195, 196, 199, 200, 201, 202, 203, 205, 206, 208, 209; [b] ICES 7 marker PCBs: IUPAC no's; 28, 52, 101, 118, 138, 153, 180; [c] 23 PCBs: 28+31, 52, 74, 101, 99, 110, 118, 105, 149, 153, 138+163, 128+167, 156, 187, 183, 180, 199, 170+190, 194