DIOXINS AND OTHER ORGANOHALOGEN COMPOUNDS IN FISH OIL SUPPLEMENTS ON THE JAPANESE MARKET

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Abstract
Dioxin (PCDD/Fs and DL-PCBs) concentrations of 30 fish oil supplements on the Japanese market were determined to estimate the dioxin intakes resulting from their consumption. The dioxin intakes from most products were under 10% of the tolerable daily intake (TDI) of dioxins (4 pg-TEQ/kg bw/day) set in Japan. However, only a product, no. 1, had extremely high dioxin concentrations and dioxin intake of the product greatly exceeded the TDI. Four products with relatively high dioxin concentrations, including product no. 1, were further analyzed in terms of PBDD/Fs, PXDD/Fs, PCBs and PBDEs. PCBs and PBDEs were found in all samples, especially product no. 1 had much higher concentrations of PCBs and PBDEs than the other products did. By contrast, PBDD/Fs and PXDD/Fs were detected much less often in all samples.

Introduction
Fish oil supplements are a source of long-chain *n*-3 polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acid, which are thought to have health benefits. Recently, the popularity of these supplements has increased in Japan. They are produced from various types of fish, especially from fatty tissues, and can be a major source of persistent organic pollutants such as dioxins (PCDD/Fs and DL-PCBs) and PCBs. Recently, there has been increasing concern over brominated compounds such as PBDEs, which are used as flame retardants, as well as brominated and mixed chlorinated-brominated dioxins (PBDD/Fs and PXDD/Fs, respectively). Although it is important to determine the levels of these compounds in fish oil supplements, only a few previous studies have considered them[1–4]. Here, we examined the dioxin levels in fish oil supplements on sale in Japan and estimated the dioxin intakes resulting from their consumption. Products with relatively high dioxin concentrations were further analyzed in terms of PBDD/Fs, PXDD/Fs, PCBs and PBDEs.

Materials and Methods
Fish oil supplements: In total, 30 products (29 capsule formulations and 1 bottled formulation) were purchased between 2002 and 2005 from retail outlets, or by post, in Tokyo, Japan. The analysis of encapsulated products included the capsules. All samples were stored at 4°C until they were analyzed.

PCDD/F and DL-PCB analyses: Dioxins were extracted, prepared and analyzed as described previously[5]. The TEQ concentrations were calculated using WHO-TEFs (1998).

PBDD/F and PXDD/F analyses: Samples (5–20 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with aqueous potassium hydroxide (KOH) and then kept for 16 h at room temperature (RT). The alkaline hydrolysates were extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silica gel column followed by a Florisil column (deactivated with 1% water). After washing with *n*-hexane, the elute obtained with 60% dichloromethane/*n*-hexane was loaded onto an activated carbon column. This was washed by *n*-hexane followed by 25% dichloromethane/*n*-hexane, and the fraction containing PBDD/Fs and PXDD/Fs was eluted with toluene. The fraction was spiked with $^{13}C_{12}$-labelled recovery standards, and subjected to HRGC/HRMS. The determinations of 12 2,3,7,8-substituted PBDD/Fs (2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8/1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF and OBDF) and seven PXDD/Fs (2-Br-3,7,8-TrCDD, 1-Br-2,3,7,8-TeCDD, 2-Br-3,6,7,8,9-PeCDD, 1-Br-2,3,6,7,8,9-HxCDD, 1-Br-2,3,4,6,7,8,9-HpCDD, 3-Br-2,7,8-TrCDF and 1-Br-2,3,7,8-TeCDF) were performed using a DB-5HT and BP1 column. The WHO-TEFs for the chlorinated isomers were provisionally used to evaluate the toxicities of the corresponding PBDD/F and PXDD/F isomers.

PCB analyses: Samples (5–10 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with ethanolic KOH and then kept for 16 h at RT. The alkaline hydrolysates were added to water and extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silica gel column. The elute obtained with *n*-hexane was subjected to gel-permeation chromatography (GPC) using 5% cyclohexane/acetone. The fraction containing PCBs was concentrated and spiked with $^{13}C_{12}$-labelled recovery standards. The PCBs were quantified by HRGC/HRMS, and their determination was performed on an HT8-PCB column.

PBDE analyses: Samples (5–10 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with ethanolic KOH and then kept for 16 h at RT. The alkaline hydrolysates were added to water and extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silver nitrate/silica gel column. The elute obtained with *n*-hexane was subjected to GPC using acetone. The fraction containing PBDEs was concentrated and spiked with $^{13}C_{12}$-labelled recovery standards. The PBDEs were quantified by HRGC/HRMS, and their determination was performed using a DB-5HT and BP1 column.

Results and Discussion

The dioxin concentrations in the 30 products and the associated intakes are presented in Table 1. The dioxin concentrations varied significantly. Product no. 1, which was made from tiger shark liver (crude extract), had extremely high dioxin concentrations; however, most samples had levels below 10 pg-TEQ/g. These values were low compared with the dioxin concentrations in the source species. The fish oil purification processes could thus have effectively removed dioxins from the samples. Indeed, Hilbert and colleagues[6] reported that steam distillation, which is a refining process for fish oil, reduced the amounts of organochlorine contaminants, including PCBs.

The total dioxin intake from the most contaminated product reached 1,500 pg-TEQ/person/day, corresponding to 30 pg-TEQ/kg bw/day for an adult weighing 50 kg. This was about eight times higher than the tolerable daily intake (TDI) of dioxins (4 pg-TEQ/kg bw/day) set by the Japanese government in 1999[7]. The intakes from most products were under 10% of the TDI, although those of samples no. 2 and no. 3 corresponded to about 30 and 14%, respectively. The major contributors to the total TEQ were DL-PCBs, which accounted for more than 90% in the most contaminated sample. This was in agreement with previous reports on fish oil supplements[1,2,4].

We determined the dioxin concentrations in different batches of the same products and found no significant differences (Figure 1). Product no. 1 had the highest variation in dioxin concentrations between batches, although the ratio of the maximal/minimum dioxin concentrations was only about 2. The dioxin intakes from the four batches of product no. 1 ranged from 16 to 30 pg-TEQ/kg bw/day. Thus, if an individual consumed the product regularly over a long period, their daily dioxin intake would continuously exceed the TDI.

The four products with relatively high dioxin concentrations were also analyzed for PBDD/F, PXDD/F, PCB and PBDE (Table 2). PCBs and PBDEs were found in all samples, although their concentrations varied significantly between products. Two batches of product no. 1 had much higher concentrations of PCBs and PBDEs than the other products, with respective intakes of 32,000 to 57,000 ng/person/day and 480,000 to 670,000 pg/person/day. By contrast, PBDD/Fs and PXDD/Fs were detected much less often in all samples. Only one isomer, 2,3,7,8-TeBDF, was quantified in two products (nos. 3 and 6). The intakes of PBDD/Fs and PXCDD/Fs were calculated assuming that the levels of non-detected isomers were equal to half of their limits of detection (LODs). Overall, the intakes were much lower that those of dioxins. The dioxin-like toxicity of the PBDD/Fs and PXDD/Fs in fish oil supplements appeared to be negligible.

Thus, although rare, fish oil supplements may contain significantly high concentrations of dioxins, PCBs and PBDEs. Continuous monitoring of the levels of these compounds in fish oil supplements is therefore recommended.

Acknowledgements

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Table 1 Dioxin concentrations in individual fish oil supplements and associated intakes

Product no. [a]	Fish source [b]	Daily intake (g) [c]	Dioxin conc. (pg-TEQ/g) [d]			Dioxin intake [e] (pg-TEQ/person/day)
			PCDD/Fs	DL-PCBs	Total	
1	Tiger shark	3.17	37	450	480	1,500 (30)
2	Sardine	4.80	4.2	7.7	12	58 (1.2)
3	Lampern	2.84	2.6	7.8	10	28 (0.57)
4	Cod	2.00	< 0.10	8.4	8.4	17 (0.34)
5	Gulper shark	2.91	0.51	2.8	3.3	10 (0.19)
6	Tuna	1.25	< 0.10	6.5	6.6	8.3 (0.17)
7	Gulper shark	3.15	1.3	0.65	1.9	6.0 (0.12)
8	Lampern etc.	2.10	0.58	1.6	2.2	4.6 (0.092)
9	Sardine	1.53	0.17	2.4	2.5	3.8 (0.077)
10	Gulper shark	1.28	1.3	0.64	2.0	2.6 (0.051)
11	Herring, sardine	2.84	< 0.10	0.5	0.56	1.6 (0.032)
12	NS	3.60	0.11	0.22	0.32	1.2 (0.023)
13	Tuna	1.76	< 0.10	0.45	0.46	0.81 (0.016)
14	Lampern etc.	1.32	0.13	0.43	0.56	0.74 (0.015)
15	Sardine	2.70	< 0.10	0.17	0.25	0.68 (0.014)
16	NS	2.82	< 0.10	0.17	0.18	0.51 (0.010)
17	NS	2.23	< 0.10	0.12	0.20	0.45 (0.0089)
18	Sardine etc.	2.67	< 0.10	0.11	0.14	0.37 (0.0075)
19	Gulper shark	1.52	< 0.10	0.16	0.16	0.24 (0.0049)
20	Tuna, sardine	1.80	< 0.10	< 0.10	0.10	0.18 (0.0036)
21	NS	1.66	< 0.10	< 0.10	< 0.10	0.11 (0.0023)
22	NS	1.16	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
23	NS	1.76	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
24	NS	1.40	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
25	NS	1.28	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
26	Tuna etc.	3.87	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
27	Tuna etc.	2.10	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
28	Tuna etc.	2.19	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
29	NS	1.92	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)
30	Tuna etc.	0.93	< 0.10	< 0.10	< 0.10	< 0.10 (< 0.0020)

[a] All samples except sample 4 (bottled) were capsule formulations.

[b] Unspecified fish oil content is expressed as "NS". Nine products (nos. 3, 8, 13, 14, 26, 27, 28, 29 and 30) were a mixture of fish oils and vegetable oils.

[c] Daily intakes of each product were calculated from the maximal recommended dosages on the product labels.

[d] Dioxin concentrations are presented on a whole weight basis. For encapsulated fish oil products, the entire samples, including capsules, were analyzed. The concentrations were calculated assuming that the non-detected isomers were equal to zero.

[e] Dioxin intakes in parentheses (pg-TEQ/bw kg/day) were based on a person weighing 50 kg.

Table 2 Summary of dioxins and other organohalogen compounds in the selected fish oil supplements

Product no. [a]	Dioxins [b]		PBDD/Fs+PXDD/Fs [b]		PCBs		PBDEs	
	Conc. (pg-TEQ/g)	Intake (pg-TEQ/ person/day)	Conc. (pg-TEQ/g)	Intake (pg-TEQ/ person/day)	Conc. (ng/g)	Intake (ng/ person/day)	Conc. (pg/g)	Intake (pg/ person/day)
1 (B)	510	1,600	0	0	18,000	57,000	210,000	670,000
	(510)	(1600)	(1.3)	(4.1)				
(D)	250	800	0	0	10,000	32,000	150,000	480,000
	(250)	(800)	(1.3)	(4.1)				
3 (D)	9.9	28	0.13	0.37	140	400	1,800	5,100
	(9.9)	(28)	(0.48)	(1.4)				
5	3.3	9.6	0	0	52	150	550	1,600
	(3.3)	(9.6)	(1.3)	(3.8)				
6 (D)	7.0	8.8	0.12	0.15	110	140	5,700	7,100
	(7.0)	(8.8)	(0.47)	(0.59)				

[a] Letters in parentheses indicate batch no. in Figure 1.

[b] Dioxin concentrations are presented on a whole weight basis. For encapsulated fish oil products, the entire samples, including capsules, were analyzed. The concentrations as well as intakes were calculated assuming that the non-detected isomers were equal to zero, and also calculated in parentheses assuming that non-detected isomers were equal to half of their LODs.

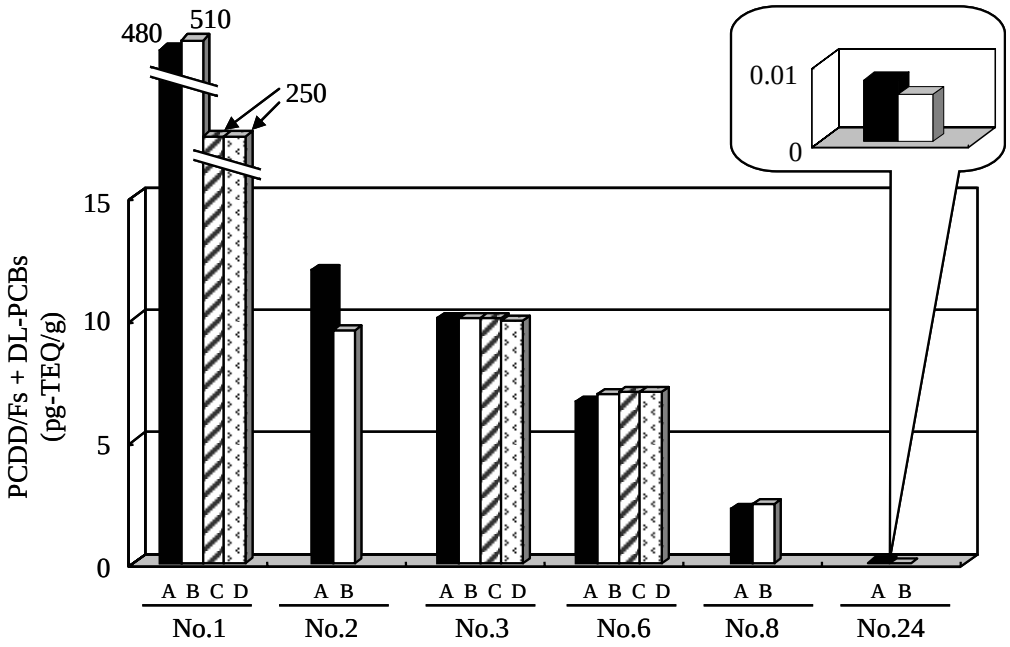

Figure 1 Batch differences in dioxin concentrations for several fish oil supplements
The same products, two to four batches, were purchased during 2002-2005. The samples designated "A" in each product are the same samples as in Table 1.