CONCENTRATION OF POLYCHLORINATED BIPHENYLS (PCBs) AND HYDROXYLATED PCBs IN SEAFOOD SAMPLES COLLECTED IN KYUSHU DISTRICT, JAPAN

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Introduction

The hydroxylated polychlorinated biphenyls (OH-PCBs) are well known as metabolites of polychlorinated biphenyls (PCBs). The major route of OH-PCB formation in biota is oxidation via the cytochrome P450 enzyme system.[1] OH-PCBs of para- and meta-substituted OH with an adjacent chlorine atom have relatively high affinity for the thyroid hormone transport proteins.[2] The concentrations of OH-PCBs and their distribution in human serum and other biological and environmental media have been reported recently.[3–5]

The thorough elucidation of OH-PCBs in foods is very important to our understanding of both the metabolism of OH-PCBs in biota and the persistence of OH-PCBs in human tissues, neither of which has been determined.

Materials and Methods

Chemicals and reagents

Non-labeled OH-PCBs and PCBs, $^{13}C_{12}$-labeled OH-PCBs and PCBs were purchased from Wellington Laboratories (Guelph, ON, Canada) and AccuStandard (New Haven, CT, USA). Solvents such as acetonitrile, n-hexane and methanol of dioxin-analysis grade were purchased from Kanto Chemical (Tokyo).

Samples

The seafood samples for the OH-PCBs and PCBs analysis were purchased from fish markets in the Kyushu district, Japan during the period 2013–2014 (Table 1). Each edible parts were collected, cut into small pieces and blended. The samples were maintained at temperatures below −20°C until analysis.

Table 1. The seafood samples used in this study

No	Sample	Production regions	Fat content (%)
1	Sardine	Chugoku-Shikoku	1.3
2	Mackerel-1	Kyushu	3.8
3	Mackerel-2	Kyushu	4.1
4	Yellowtail	Kyushu	3.5
5	Japanese seabass-1	Kyushu	0.54
6	Japanese seabass-2	Kyushu	0.41
7	Sea bream-1	Chugoku-Shikoku	5.0
8	Sea bream-2	Kyushu	0.96
9	Tuna-1	Kyushu	18
10	Tuna-2	Kyushu	2.8
11	Horse mackerel-1	Kyushu	0.39
12	Horse mackerel-2	Kyushu	0.11
13	Horse mackerel-3	Kyushu	0.32
14	Horse mackerel-4	Kyushu	1.4
15	Cod	Tohoku	0.078
16	Largehead hairtail	Kyushu	2.9

Sample preparation for OH-PCBs

The protocol for the OH-PCBs analysis is illustrated in Figure 1.[4] First, $^{13}C_{12}$-labeled OH-PCBs (mono- to heptachlorinated 11 congeners) were spiked as surrogate standards in the samples. The samples were homogenized twice in acetonitrile, added to 10% NaCl(aq.) and extracted two times with n-hexane. The extracts were fractionated using a Florisil cartridge column (Sep-Pak Florisil Plus, Waters, Milford, MA). Non-polarity compounds were removed with 0.5% diethyl ether/n-hexane, and then the OH-PCBs were eluted with 50% acetone/methanol. OH-PCBs were

derivatized to methoxylated PCBs (OMe-PCBs) by reaction with dimethyl sulfate and 3N KOH/ethanol (70°C for 1 h). OMe-PCBs were extracted twice with n-hexane and then purified using a Florisil cartridge column. The fractions were concentrated to 0.05mL and spiked with $^{13}C_{12}$-labeled PCB111 as an injection standard.

Sample preparation for PCBs

The protocol for the PCB analysis is illustrated in Figure 2.[6] The sample was loaded into the extraction cell filled with Isolute. $^{13}C_{12}$-labeled PCBs (tri- to decachlorinated 20 congeners) were spiked as surrogate standards in the extraction cells. N-hexane was used as the extraction solvent of an accelerated solvent extractor. The extract was cleaned up using 1N KOH/ethanol, concentrated sulfuric acid, and a multilayer silica gel column. The clean-up solution was concentrated to 0.05 mL and spiked with $^{13}C_{12}$-labeled PCB111 as an injection standard.

Analytical methods and instrumentation

We identified and quantified the OH-PCBs (OMe-PCBs) and PCBs by gas chromatograph (GC; 6890GC, Agilent, Santa Clara, CA)/high-resolution mass spectrometer (Autospec Premier, Waters) at the resolution of R > 10,000 (10% valley). The injector temperature was 280°C, and 2 μL samples were injected in the splitless mode. The HT8-PCB capillary column (60 m × 0.25 mm i.d., Kanto Chemical) was used for the GC. Tri- to decachlorinated congeners of PCBs and mono- to heptachlorinated congeners of OH-PCB were determined.

Results and Discussion:

The concentrations of OH-PCBs in the seafood samples

Table 2 shows the concentrations of OH-PCBs in the seafood samples. OH-PCBs were detected in all of the seafood samples collected in this study. The concentrations of ΣOH-PCBs were 0.014–0.98 ng/g wet weight (ww); the mean concentration was 0.18 ng/g ww. The ΣOH-PCBs in the seafood samples were unrelated to the fish species or the fat content of the fish. The dominant congeners were classified as those in the fish species that exhibited OH-MonoCBs and OH-DiCBs and those that exhibited OH-PentaCBs and OH-HexaCBs.

The concentration of PCBs in the seafood samples

Table 3 shows the concentrations of PCBs in the seafood samples. PCBs were detected in all of the seafood samples. The concentrations of ΣPCBs were 0.20–50 ng/g ww, and the mean concentration was 9.2 ng/g ww. The ΣPCBs in the seafood samples were related to the fat content of the fish samples. The dominant congeners were pentaCBs and hexaCBs.

Comparison of the persistence of OH-PCBs and PCBs

Table 4 summarizes the presence of ΣOH-PCBs/ΣPCBs in the seafood samples. The concentrations of ΣOH-PCBs/ΣPCBs were 0.00094–0.25, and the mean was 0.051. The ΣOH-PCBs/ΣPCBs in the fish samples were not related to the fish species or the fat content. The levels of ΣOH-PCBs were low compared with those of ΣPCBs in all of the seafood samples. Our study findings demonstrate that OH-PCBs are accumulated in the human body by the intake of fish.

Acknowledgements:

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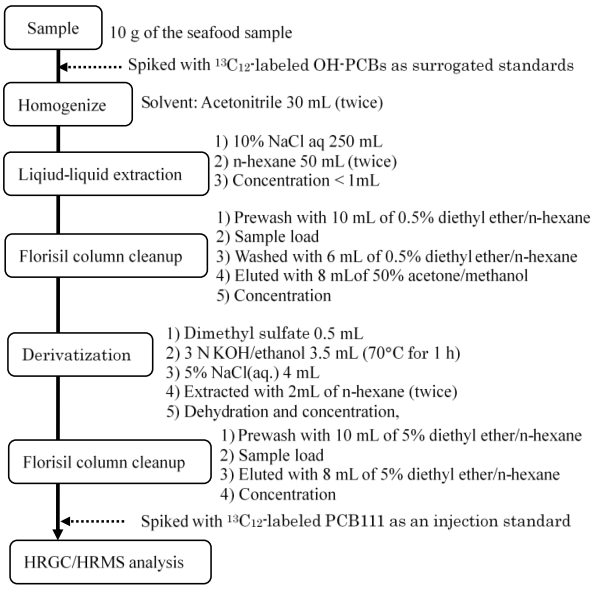

Figure 1. The protcol for OH-PCBs analysis.

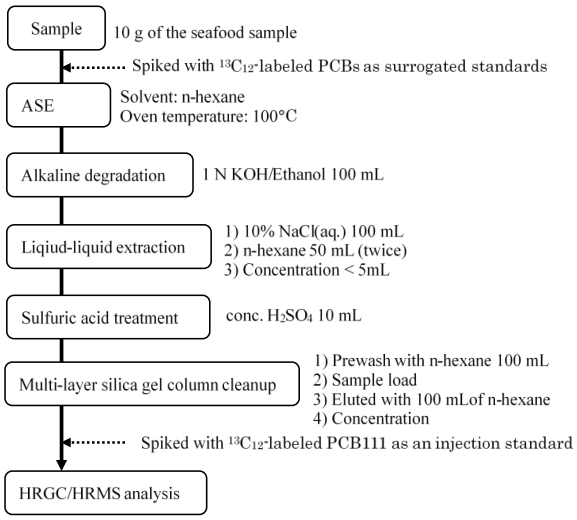

Figure 2. The protcol for PCBs analysis.

Table 2. The concentrations of OH-PCBs in the seafood samples.

(ng/g ww)

	OH-MoCBs	OH-DiCBs	OH-TriCBs	OH-TeCBs	OH-PeCBs	OH-HxCBs	OH-HpCBs	ΣOH-PCBs
Sardine	0.14	0.12	0.010	0.023	0.0058	0.0032	0.0034	0.31
Mackerel-1	0.016	0.084	0.0035	0.010	0.0063	0.0029	0.0029	0.12
Mackerel-2	0.038	0.19	ND	0.032	0.010	0.0040	0.0031	0.28
Yellowtail	0.016	0.074	0.00071	0.0097	0.0080	0.0084	0.0040	0.12
Japanese seabass-1	0.037	ND	0.0031	0.013	0.0015	0.0064	0.0032	0.064
Japanese seabass-2	0.0038	0.0026	0.0010	0.0019	0.0021	0.0033	0.0037	0.018
Sea bream-1	0.011	0.020	0.0016	0.0077	0.0042	0.0031	0.0028	0.050
Sea bream-2	0.0010	0.0020	0.0010	0.0023	0.0020	0.0035	0.0031	0.015
Tuna-1	0.27	0.44	0.0018	0.039	0.21	0.0082	0.0083	0.98
Tuna-2	0.066	0.097	0.00023	0.011	0.058	0.0079	0.0051	0.24
Horse mackerel-1	0.17	0.16	0.014	0.026	0.0049	0.0042	0.0039	0.38
Horse mackerel-2	0.011	0.0088	0.0013	0.0040	0.014	0.0035	0.0058	0.049
Horse mackerel-3	0.013	0.016	0.0090	0.026	0.024	0.012	0.0062	0.11
Horse mackerel-4	0.0058	0.013	ND	0.0015	0.0044	0.0043	0.00012	0.029
Cod	ND	0.00098	0.00059	0.0018	0.0016	0.0044	0.0051	0.014
Largehead hairtail	0.013	0.036	0.0013	0.0020	0.0053	0.0037	0.0046	0.066
Mean	0.051	0.079	0.0031	0.013	0.023	0.0052	0.0041	0.18
Min.	ND	ND	ND	0.0015	0.0015	0.0029	0.00012	0.014
Max.	0.27	0.44	0.014	0.039	0.21	0.012	0.0083	0.98

Table 3. The concentration of PCBs in the seafood samples.

(ng/g ww)

	TrCBs	TeCBs	PeCBs	HxCBs	HpCBs	OcCBs	NoCBs	DeCB	ΣPCBs
Sardine	0.053	0.22	0.41	0.75	0.38	0.066	0.0094	0.0074	1.9
Mackerel-1	0.062	0.18	0.43	0.76	0.41	0.049	0.014	0.012	1.9
Mackerel-2	0.19	0.96	2.4	3.3	1.4	0.19	0.038	0.028	8.5
Yellowtail	0.26	1.6	5.4	10	4.9	0.66	0.093	0.086	23
Japanese seabass-1	0.14	0.65	1.2	1.5	0.47	0.072	0.0053	0.0019	4.1
Japanese seabass-2	0.34	1.3	2.5	2.7	0.90	0.14	0.012	0.0046	7.8
Sea bream-1	0.35	1.1	1.9	2.4	0.99	0.12	0.015	0.011	6.9
Sea bream-2	0.94	2.6	4.5	5.6	2.4	0.31	0.038	0.023	16
Tuna-1	0.79	5.1	15	20	8.0	1.1	0.20	0.12	50
Tuna-2	0.16	0.82	2.5	3.3	1.4	0.22	0.043	0.027	8.5
Horse mackerel-1	0.030	0.12	0.35	0.62	0.34	0.046	0.0028	0.0011	1.5
Horse mackerel-2	0.047	0.23	0.58	1.1	0.77	0.12	0.0083	0.0047	2.8
Horse mackerel-3	0.039	0.11	0.20	0.35	0.22	0.038	0.0049	0.0042	0.96
Horse mackerel-4	0.065	0.20	0.44	0.71	0.43	0.071	0.012	0.014	1.9
Cod	0.029	0.061	0.064	0.040	0.0093	0.0010	ND	0.00037	0.20
Largehead hairtail	0.18	0.80	1.9	4.4	2.5	0.32	0.018	0.0047	10
Mean	0.23	1.0	2.5	3.6	1.6	0.22	0.034	0.022	9.2
Min.	0.029	0.061	0.064	0.040	0.0093	0.0010	ND	0.00037	0.20
Max.	0.94	5.1	15	20	8.0	1.1	0.20	0.12	50

Table 4. The presence of ΣOH-PCBs/ΣPCBs in the seafood samples.

	Fat content (%)	ΣPCBs (ng/g ww)	ΣOH-PCBs (ng/g ww)	ΣOH-PCBs/ΣPCBs
Sardine	1.3	1.9	0.31	0.16
Mackerel-1	3.8	1.9	0.12	0.063
Mackerel-2	4.1	8.5	0.28	0.033
Yellowtail	3.5	23	0.12	0.052
Japanese seabass-1	0.54	4.1	0.064	0.016
Japanese seabass-2	0.41	7.8	0.018	0.0023
Sea bream-1	5.0	6.9	0.050	0.0072
Sea bream-2	0.96	16	0.015	0.00094
Tuna-1	18	50	0.98	0.020
Tuna-2	2.8	8.5	0.24	0.028
Horse mackerel-1	0.39	1.5	0.38	0.25
Horse mackerel-2	0.11	2.8	0.049	0.018
Horse mackerel-3	0.32	0.96	0.11	0.11
Horse mackerel-4	1.4	1.9	0.029	0.015
Cod	0.078	0.20	0.014	0.070
Largehead hairtail	2.9	10	0.066	0.0066
Mean	2.9	9.2	0.18	0.051
Min.	0.078	0.20	0.014	0.00094
Max.	18	50	0.98	0.25