FOLLOW-UP INVESTIGATION OF POLYCHLORINATED BIPHENYL CONCENTRATIONS IN FISH FROM TSUNAMI-STRICKEN AREAS OF JAPAN

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Introduction

Since the Great East Japan Earthquake in 2011 there has been much concern about the contamination of foods produced in tsunami-stricken areas by chemical pollutants such as radioactive materials and hazardous organic compounds. Old electrical conductors and transformers containing polychlorinated biphenyls (PCBs) were washed away by the tsunami[1]. To manage the potential health risks associated with daily PCB intake, we have investigated PCB concentrations in marine fish consumed as the main source of PCB exposure in the Japanese diet. Our previous analysis of 101 fish samples revealed that fish purchased from markets in tsunami-stricken areas were unlikely to be contaminated with PCBs at high concentrations[2]. However, we considered that further investigation of tsunami-related PCB contamination of fish was needed.

We therefore quantified PCBs and the concentrations of 209 congeners as percentages of the total PCB concentration in 80 follow-up fish samples obtained not only from tsunami-stricken areas but also from an area unlikely to have been affected by the tsunami. For our analyses we used high-resolution gas chromatography–high-resolution mass spectroscopy (HRGC-HRMS)

Materials and methods

Samples

In 2014, a total of 80 fish samples, consisting of fat greenling and flounder, were purchased from general food markets located in tsunami-stricken areas (Aomori, Iwate, and Miyagi prefectures) and in Kanagawa prefecture as a negative control, as this latter prefecture was not likely to have been affected by the tsunami. Each sample was uniformly homogenized in a food processor and stored at –20°C until analysis.

Materials and equipment

Dioxin-analysis-grade *n*-hexane, acetone, toluene, dichloromethane, and alumina were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). A multilayer silica gel column filled, from bottom to top, with 2 g of anhydrous sodium sulfate, 0.9 g silica gel, 3 g of 44% (w/w) sulfuric acid–impregnated silica gel, 0.9 g silica gel, and 2 g of anhydrous sodium sulfate was purchased from GL Science Inc. (Tokyo, Japan). PCB standards for calibration (TPCB-CSL-A, CS1-A, CS2-A, CS3-A, CS4-A, and CS5-A) and $^{13}C_{12}$-labeled internal standards (TPCB-CL-A100 and TPCB-SY-A100) were obtained from Kanto Chemical Co., Inc. A PCB standard (209 congeners mixture) for checking retention times was prepared by mixing equal amounts of M-1668A-1-0.01x, 2-0.01x, 3-0.01x, 4-0.01x, and 5-0.01x standards (AccuStandard Inc., New Haven, CT, USA). HRGC-HRMS was performed with an HP-6890 Plus gas chromatograph (Hewlett Packard Co., Palo Alto, CA, USA) coupled with a JEOL JMS-700 MStation mass spectrometer (JEOL, Tokyo, Japan).

Extraction, cleanup, and measurement of PCBs by HRGC-HRMS

The homogenized sample (20.0 g) spiked with $^{13}C_{12}$-labeled internal standards was digested with alkaline 1M KOH–ethanol for 16 h at room temperature. The digested sample was then extracted with *n*-hexane. The extract was treated with concentrated sulfuric acid and purified on a multilayer silica gel column followed by an alumina column. The eluate was evaporated and spiked with $^{13}C_{12}$-labeled recovery standards.

All 209 PCBs were quantified by HRGC-HRMS with an HT8-PCB column (0.25 mm × 60 m, Kanto Chemical Co., Inc.), as described previously[3], with minor modifications. The limit of detection for PCBs was 0.034 to 0.93 pg/g and the limit of quantitation (LOQ) for PCBs was 0.11 to 3.1 pg/g. Each PCB congener concentration was calculated by assuming that PCB congener concentrations lower than the LOQ were equal to the LOQ/2.

Results and discussion

Total PCB concentrations

PCBs were detected in all 80 fish samples. Statistical data on each PCB congener concentration in the samples from tsunami-stricken areas (A to C) and from the negative control area (K) are shown in Table 1. Total PCB concentrations in the samples from the tsunami-stricken areas ranged from 0.32 to 223 ng/g, whereas those in samples from the negative control area ranged from 0.75 to 128 ng/g. The maximum PCB concentration found was lower than the provisional regulatory limit (oceans, 500 ng/g) in Japan. Furthermore, the maximum concentration of the sum of six indicator PCBs (#28, #52, #101, #138, #153, and #180) was 57 ng/g; this value was below the EU regulation limit (marine fish, 75 ng/g); moreover, the concentration of #52 might have been overestimated owing to incomplete separation of #52 and #69 under the HRGC-HRMS analysis.

Table 1. Statistical data on each PCB congener concentration (ng/g)

Area	Fish group		M1CBs	D2CBs	T3CBs	T4CBs	P5CBs	H6CBs	H7CBs	O8CBs	N9CBs	D10CB	Total
A to C (tsunami-stricken areas)	fat greenling (n = 30)	Ave.	0.0033	0.025	0.41	2.3	5.1	3.2	0.91	0.15	0.015	0.0067	12
		S.D.	0.00062	0.043	1.6	10	21	6.8	0.88	0.11	0.0083	0.0049	40
		Max.	0.0052	0.24	8.6	56	114	39	5.0	0.53	0.035	0.018	223
		Median	0.0031	0.014	0.080	0.45	1.2	1.9	0.69	0.10	0.013	0.0052	4.4
		Min.	0.0025	0.0052	0.031	0.15	0.40	0.68	0.22	0.034	0.0046	0.0012	1.6
	flounder (n = 30)	Ave.	0.0030	0.016	0.096	0.38	0.73	0.86	0.31	0.049	0.0063	0.0057	2.4
		S.D.	0.00087	0.014	0.10	0.38	0.60	0.61	0.20	0.029	0.0037	0.0052	1.9
		Max.	0.0058	0.060	0.50	1.9	2.4	2.3	0.91	0.14	0.016	0.023	7.3
		Median	0.0027	0.011	0.053	0.25	0.55	0.71	0.27	0.046	0.0056	0.0041	2.0
		Min.	0.0020	0.0051	0.015	0.047	0.079	0.10	0.046	0.012	0.0030	0.00042	0.32
K (negative control)	fat greenling (n = 10)	Ave.	0.0048	0.059	0.84	3.4	6.4	9.6	4.9	1.1	0.13	0.043	26
		S.D.	0.0017	0.050	0.64	2.6	4.9	7.1	3.8	0.96	0.14	0.047	20
		Max.	0.0074	0.15	2.1	9.6	18	27	15	3.5	0.45	0.15	76
		Median	0.0048	0.050	0.67	2.7	5.5	8.5	4.0	0.87	0.093	0.025	22
		Min.	0.0028	0.0062	0.076	0.32	1.1	2.6	1.0	0.15	0.015	0.0022	6.7
	flounder (n = 10)	Ave.	0.0030	0.035	1.3	6.2	5.8	3.7	1.3	0.24	0.032	0.013	19
		S.D.	0.00033	0.047	3.4	16	12	5.6	1.5	0.24	0.032	0.012	39
		Max.	0.0036	0.17	11	51	40	19	5.3	0.85	0.10	0.036	128
		Median	0.0029	0.023	0.32	1.3	2.1	2.3	0.95	0.18	0.026	0.0090	7.3
		Min.	0.0025	0.0057	0.025	0.082	0.23	0.28	0.10	0.019	0.0030	0.00042	0.75

We compiled histograms of the total PCB concentrations in samples of fat greenling (a) and flounder (b) obtained from areas A to C (black bars) and K (gray bar) (Figure 1). The histograms showed a pattern typical of the concentrations of hazardous chemicals in foods, and this pattern was consistent with that obtained in our previous investigation[2]. We also compiled box plots of total PCB concentrations in combination with area and fish group (Figure 2). In each fish group, the ranges of PCB concentrations between the 25th and 75th percentile values in areas A to C appeared to be lower than those in area K. In each type of fish we consider that there was no substantial difference in the concentrations of PCBs among tsunami-stricken areas, with the exception of one fat greenling sample in area B.

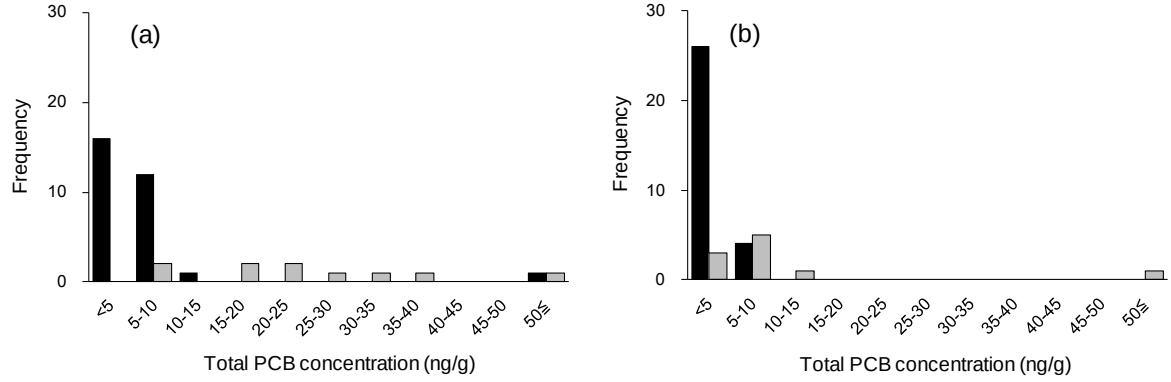

Figure 1. Histograms of total PCB concentrations (ng/g) in fat greenling (a) and flounder (b) obtained from areas A to C (black bars) and K (gray bars).

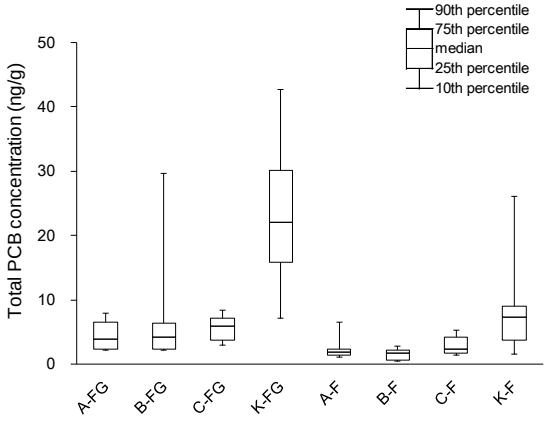

Figure 2. Box plots of total PCB concentrations classified by area and fish type.
FG, fat greenling; F, flounder

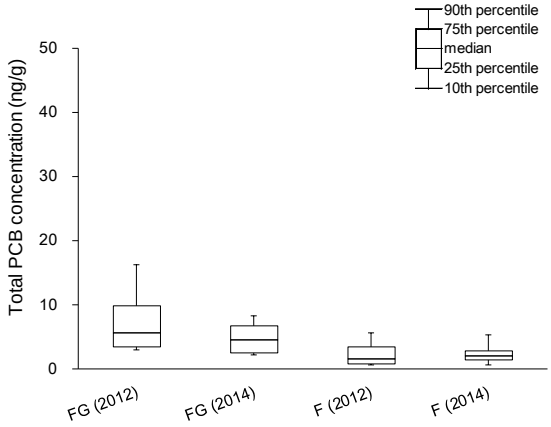

Figure 3. Box plots of total PCB concentrations in fat greenling (FG) and flounder (F) obtained from tsunami-stricken areas (A to C) in 2012 and 2014.

Next, we compared the concentrations of total PCBs in each fish group between 2014 (Figure 3) and 2012 (the time of our previous investigation[2]) in areas A to C. The median total PCB concentrations in each fish group in 2014 were similar to those in 2012. In addition, the ranges of the PCB concentrations between the 25th and 75th percentile values in each fish group in areas A to C in 2012 (Figure 3) were lower than those in area K in 2014 (Figure 2). The concentration of total PCBs in fat greenling appeared higher than that in flounder, likely because of a difference in fat content between the two types of fish.

Analysis of each PCB congener as a percentage of total PCBs

We calculated the chlorinated congener concentrations and their percentages of the total PCB concentration (Figure 4). The percentage of each chlorinated congener appeared similar among the samples; tetra- to hepta-chlorinated congeners dominated. Sample B-FG10 had the maximum total PCB contantration and was unique in that penta-chlorinated congeners accounted for 50% of the total PCBs. In addition, the percentages of tetra-chlorinated congeners in samples A-F4, A-F5, and K-F3, and of hepta-chlorinated congener in sample K-FG2 were higher than in the other samples. However, it would be difficult to further evaluate any association between these results and the effects of the tsunami.

Our results support our previous findings that fish samples from markets in tsunami-stricken areas were unlikely to have been contaminated with PCBs at high concentrations. However, to obtain more detailed information on the situation, we intend to continue our investigations by increasing the numbers of fish and the areas sampled and by performing a multivariate analysis of the data.

Acknowledgments

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References

1. Bird WA, Grossman E. (2011); *Environ Health Perspect* 119(7): A290-301
2. Uekusa Y, Takatsuki S, Watanabe T, Kataoka Y, Tsutsumi T, Matsuda R, Hachisuka A, Teshima R. (2014); *Organohalogen Compounds* 76 : 1074-1077
3. Matsumura C, Tsurukawa M, Nakano T, Ezaki T, Ohashi M. (2002); *J Environ Chem* 12(4):855-865

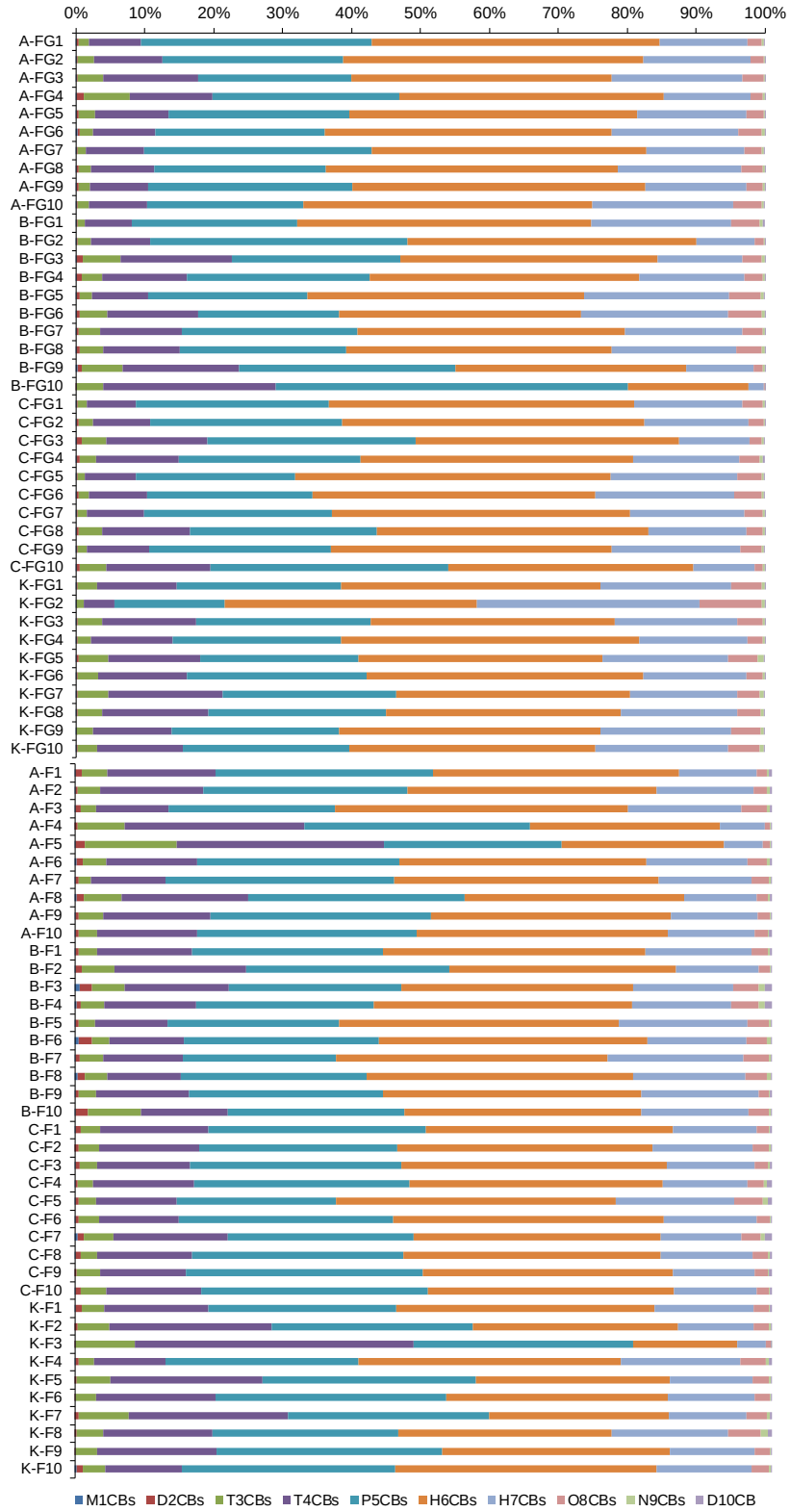

Figure 4. Concentration of each chlorinated congener as a percentage of the total PCB concentration.