

ANA - Bioanalytical Approaches for POPs Detection

dioxin-like compounds were converted into Ah-I-based, 2,3,7,8-TCDD equivalents (DEQs), and were corrected by subtraction of the blank concentration for the sample preparation procedure. The minimum concentration measurable in samples was 1.0 pg/g.

HRGC/HRMS: Dioxins were extracted, prepared and analyzed as described previously⁴.

Results and Discussion

Two-fold serial dilutions of the prepared extracts of the fish samples were tested in the PCB-EIA and Ah-I, to check for any interference by contaminants from the natural environment. Two or three different fish extracts were diluted with DMSO and assayed. In the PCB-EIA, the concentrations measured were within 83.5 – 107.9% of those expected from the starting concentrations (Figure 2a), suggesting that the matrix did not significantly interfere with this assay, using samples prepared in this way. In contrast, in the Ah-I, dioxin concentrations in some samples, in particular sea bass and yellowtail, appeared to increase with dilution (Figure 2b). This suggested interference, in some cases, with the Ah-I by the sample matrix or AhR antagonists in the samples. Therefore, serial dilutions of the prepared samples were measured in the Ah-I, and the maximum concentration obtained was taken as the concentration of non-*ortho* PCBs and PCDD/Fs fraction.

Dioxin concentrations were measured in twenty samples of retail fish by PCB-EIA and Ah-I, and compared to TEQ concentrations obtained by HRGC/HRMS. Both the concentrations of mono-*ortho* PCBs fraction obtained in the EIA (Figure 3a) and the concentrations of non-*ortho* PCBs and PCDD/Fs fraction obtained in Ah-I (Figure 3b) showed good correlations with TEQ concentrations measured by HRGC/HRMS ($r > 0.98$ and $r = 0.97$, respectively). These results showed that using a combination of the PCB-EIA and the Ah-I would offer a practical method for estimating the TEQ levels of dioxins in retail fish.

Further, the concentrations by the PCB-EIA and the concentrations of PCB 118 by HRGC/HRMS showed a good correlation (Figure 4), with the slope of the linear regression equation being roughly 1. This suggested that a positive reading in the EIA was mainly attributable to PCB 118 in the samples. Results obtained for concentrations in the Ah-I were compared to the expected results, calculated by multiplying the concentrations of 4 non-*ortho* PCBs and 17 PCDD/Fs determined by HRGC/HRMS and their relative potency values in the Ah-I⁵. As shown in Figure 5, a good correlation was observed between obtained and expected values, with the slope of the linear regression equation being roughly 1. This showed that a positive reading in the Ah-I was largely attributable to the target compounds in the samples, and that the difference in the toxic equivalent measurements in the Ah-I and HRGC/HRMS was mainly due to the differences between the relative potency values in the Ah-I and the WHO-TEFs in HRGC/HRMS.

Overall our results indicate that using the PCB-EIA and Ah-I in combination is a useful approach to measuring TEQs of dioxins in retail fish.

Acknowledgements

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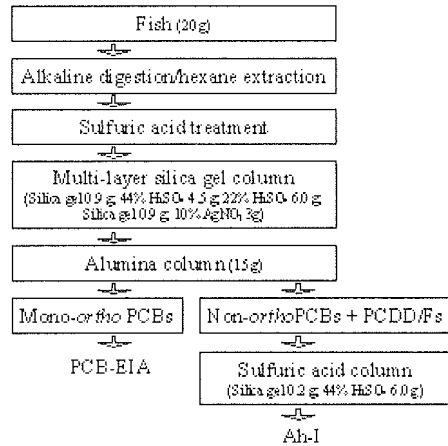


Figure 1. Sample preparation for retail fish

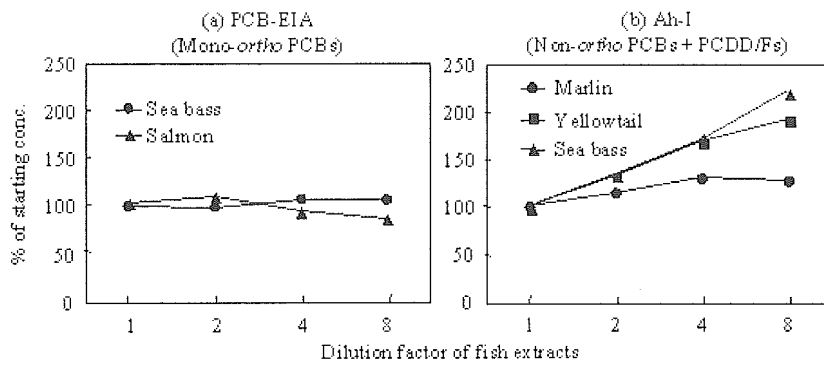


Figure 2. Effect of dilution factor on the determination of dioxins in fish

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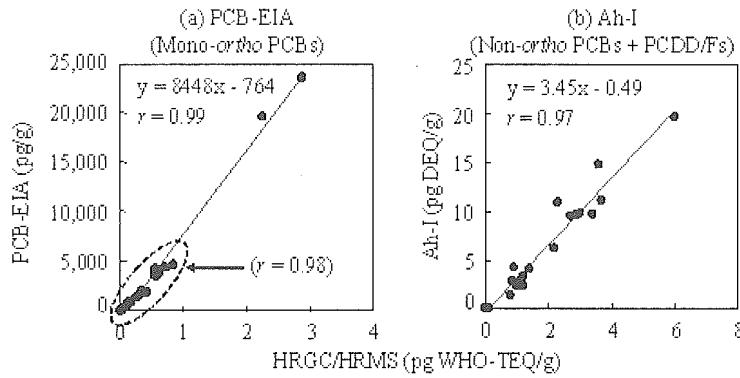


Figure 3. Comparison of EIA and Ah-I with HRGC/HRMS measurements of 20 fish samples

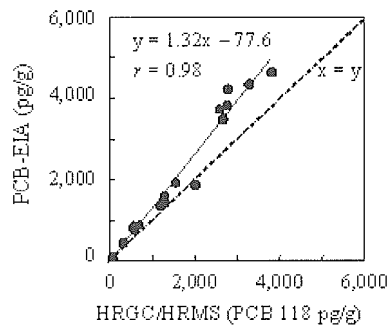


Figure 4. Comparison of concentrations measured in EIA with PCB 118 concentrations measured by HRGC/HRMS in fish samples. Two highly contaminated samples ($> 5,000$ pg/g of PCB 118) were excluded from the regression calculation.

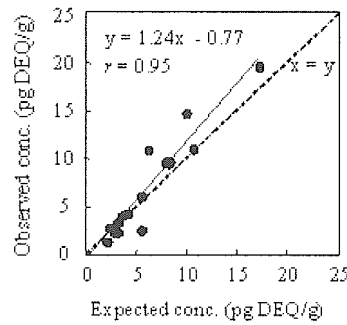


Figure 5. Comparison of observed and expected concentrations in fish, in the Ah-I. Two samples with undetectable levels were excluded from the regression calculation.

Determination of Brominated Flame Retardants in Fish and Market Basket Food Samples of Japan

Reiko Nakagawa¹, Yuki Ashizuka¹, Tsuguhide Hori¹, Kazuhiro Tobiishi¹, Daisuke Yasutake¹, Kumiko Sasaki²

¹Fukuoka Institute of Health and Environmental Sciences

²National Institute of Health Sciences

Introduction

Brominated flame retardants (BFRs) have been used all over the world and detected in effluents such as those from sewage treatment plants and textile plants¹. In most cases, such effluents drain into rivers or estuaries. BFRs are suspected as sources of poly-brominated dioxins. Consequently, social concern is increasing regarding the pollution of the environment and marine products by BFRs. Polybrominated diphenyl ethers (PBDE), a well-known group of BFRs, are lipophilic and easily bio-accumulated in organisms through the food web; however, the toxicities have not yet been fully clarified. Tetra-brominated bisphenol A (TBBPA) is another representative group of BFRs whose demand by Japan industries in 2001 has increased to nearly 32,000 tons², which is more than ten times the demand for DecaBDE (2,200 tons), the only PBDE used in Japan. Since TBBPA has a more polar structure than PBDEs and has been reported to be readily metabolized, there has been a limited number of studies on the pollution in food such as marine products, compared with studies on the pollution caused by PBDEs. In this paper, we report the pollution levels of these BFRs in marine products collected at food market stores in Japan. Additionally, we discuss the estimated daily intakes of the BFRs by analyzing food group samples prepared following the Market Basket Method.

Materials and Methods

Marine products: Eighteen fish of 15 species.

Market basket food samples: Thirteen mixed food samples were prepared in 2002, following the method of the Market Basket Study, alternatively termed the Total Diet Study³. One hundred and sixty-six food items were chosen from 85 categories of foods that the Japanese populace commonly consumes, and the respective amounts of food items composing each of 13 food groups were decided by referring to the data of the latest national and prefectural surveys.

Preparation of samples: A homogenized sample (5~10 g) was spiked with ¹³C-labeled TBBPA as a clean-up standard and then packed in a stainless steel tube and extracted with methanol. The extraction conditions are listed in Table 1. The methanol extract (ca. 30~50 mL) was defatted by liquid-liquid partition with 20 mL of hexane. Then, 120 mL of 5% sodium chloride solution was added to the methanol layer fraction and re-extracted twice with 30~50 mL of dichloromethane. The extract was concentrated to dryness and then 1 mL of 1N potassium hydroxide and 0.2 mL of diethyl sulfate (Cica-Reagent) were added to it and the mixture was kept at 25~30 °C for 30 min. After the mixture was treated with 4 mL of 1N potassium hydroxide at 70°C for 1 hour, 3 mL of water was added to it and the solution was re-extracted with hexane. The hexane extract was cleaned by chromatography, the first, a florisil mini-column using an elution solvent of 8 mL of 2% diethyl ether/hexane, and when necessary, the second, a sulfuric-acid-impregnated silica-gel mini-column using an elution solvent of 15 mL of dichloromethane. The final eluate was concentrated, re-dissolved in 25 mL of nonane with 5 ng of chrysene-d₁₂ as a syringe spike and subjected to measurement by GC/MS. The analytical conditions are listed in Table 2.

Analysis of PBDEs was described in our paper, presented at the Dioxin 2004 in Berlin⁴.

Results and Discussion

The levels of TBBPA and ΣPBDEs in 16 mixed-food samples prepared based on the nutritional classification are

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shown in Table 3. In this study, the LODs were 0.1 ng/g w.w. for TBBPA and 0.0001 ng/g w.w. for each PBDE congener. TBBPA was only detected at 0.46 ng/g w.w. in one of the two X samples, constituted from fish, shellfish and processed fish products. On the other hand, PBDEs were detected ranging from 0.00012 to 1.7 ng/g w.w. in all of the mixed-food samples except VI. The total consumption of TBBPA was estimated to be 18.8 ng/day at ND=0 and 98.4 ng/day at ND=1/2LOD. The 5-fold differences in the values between the two conditions suggested dependence upon the high-level LOD, in comparison with the non-significant difference in the values of Σ PBDEs between 114 ng/day at ND=0 and 115 ng/day at ND=1/2LOD.

The levels of TBBPA and Σ PBDEs in marine products are shown in Table 4. TBBPA ranged from ND (<0.1 ng/g) ~3.0 ng/g (saury), while Σ PBDEs ranged from ND (<0.0001 ng/g)~1.2 ng/g (yellowtail) in the raw fish. Although Σ PBDEs appeared to be correlated with the fat contents, there is no correlation for TBBPA. Considering the likelihood of it being easily metabolized by organisms to the possible metabolites such as methoxy- or glucuronized-TBBPA, the exposure to TBBPA may not be considered to continue long, even if there is temporarily highly pollution with TBBPA by sewage or sludge from plants. The exposure to Σ PBDEs is thought to occur when eating food highly contaminated by food web accumulation as same as PCBs. In this study, the medians of TBBPA and Σ PBDEs in raw fish were 0.05 ng/g and 0.22 ng/g, respectively, on the condition that ND is assumed as 1/2 LOD. Regarding all the statistical values of average, median and frequency, the latest pollution by BFRs is considered more considerable by Σ PBDEs than by TBBPA, though the demand for PBDEs (only DecaBDE is now used in Japan) was far below that of TBBPA. For the toxicities in vivo, PBDEs have been reported to pose various toxicities, such as liver toxicity, disruption of thyroid hormones, developmental neuro-toxicity and carcinogenicity on animal tests⁵. In contrast, TBBPA have shown no adverse effects in vivo⁶. However, unexpected nephro-toxicity has been recently found in newborn rats treated with TBBPA⁷. In addition, the detection of TBBPA was reported in human serum by highly sensitive analysis using LC/MS⁸. Therefore, the monitoring of those BFRs in food should be continued to prevent the further pollution that may cause and/or increase risk for the health of humans and wildlife.

Acknowledgements

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Table 1 The extraction conditions

Apparatus: ASE-300 (Dionex)
Extraction Temp.: 50°C, Extraction Pressure: 1500psi
Cell Capacity: 33mL, Extraction Time: 10 min
Extraction Cycle: 3, Flash Capacity: 90%, Purge Time: 120 sec

Table 2 The GC/MS conditions

GC/MS: HP6890/5973MSD
Column: DB-5(J&W) 0.25mm.i.d.x30m, film thickness: 0.25mm
Injection Temp: 280°C
Column Temp: 120°C(1min)~20°C/min~300°C

Table 3 Average composition of total diet of average person in Japan

Group	Foods in group	Av. Weight g/day	TBBPA (ng/g,wet weight)	ΣPBDEs (ng/g,wet weight)
I	Rice and rice products	409	<0.1	0.00026
II	Grains, seeds and potatoes	192.8	<0.1	0.0019
III	Sugar and confectionaries	32.6	<0.1	0.0053
IV	Oils	15.2	<0.1	0.12
V	Legume and legume products	73.2	<0.1	0.0041
VI	Fruits	113.9	<0.1	ND
VII	Carrots and green leafy vegetables	86.9	<0.1	0.00056
VIII	White leafy vegetables, mushrooms, and seaweeds	184.6	<0.1	0.00016
IX	Seasonings and beverages	172.2	<0.1	0.00012
Xa	Fish and fish products	82.3	0.46	0.83
Xb		81.8	<0.1	1.7
X I a	Meat and eggs	110.5	<0.1	0.1
X I b		105.3	<0.1	0.07
X II a	Milk and milk products	122.5	<0.1	0.0062
X II b		122.5	<0.1	0.011
XIII	Other processed foods	38.1	<0.1	0.0016
XIV	Water	—	—	—
Total estimated consumption(ng/day) at ND=0			18.8 ^a	114 ^a
Total estimated consumption(ng/day) at ND=1/2LOD			98.4	115

^a calculated using the average TBBPA(or ΣPBDEs) values of Xa and Xb
LODs of 14 PBDE congeners are all below 0.0001ng/g.

Table 4 TBBPA and ΣPBDEs Concentrations in Various Marine Products

Raw fish and shellfish	Fat Contents (%)	TBBPA (ng/g,wet weight)	ΣPBDEs (ng/g,wet weight)
yellowtail	15	<0.1	1.2
horse mackerel	13	3.0	0.087
saury	11	1.8	0.47
sea bream A	10	0.14	0.50
salmon trout	10	<0.1	0.52
sardine	9.8	0.19	0.24
mackerel	9.1	0.81	0.55
grunt	4.8	1.6	0.11
sea bream B	3.5	<0.1	0.28
oyster-l	3.2	<0.1	0.01
amberjack	2.5	1.4	0.29
oyster-k	1.7	<0.1	0.04
striped beakperch	1.3	<0.1	0.22
cuttlefish	1.3	<0.1	0.26
razor -shell	0.98	<0.1	0.12
tuna	0.89	<0.1	0.0086
sea bass	0.54	<0.1	0.045
leatherfish	0.04	0.67	<0.0001