CONGENER-SPECIFIC ANALYSIS AND TOXICOLOGICAL EVALUATION OF PCDDS, PCDFS AND CO-PCBS IN YUSHO RICE OIL

Yuan Yao[1,2], Takumi Takasuga[3], Shigeki Masunaga[1,2] and Junko Nakanishi[1,2]

[1]Institute of Environmental Science and Technology, Yokohama National University, 79-7 Tokiwadai, Hodogaya, Yokohama 240-8501, Japan
[2]CREST, Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[3]Shimadzu Techno-Research Inc., 2-4 Nishinokyo, Sanjo, Bocho, Nakagyo, Kyoto 604-8435, Japan

Introduction

In 1968, the Yusho poisoning incident occurred in Western Japan and involved more than 1,800 people. Although it was found that Yusho rice oil ingested by the victims was contaminated with polychlorinated biphenyls (PCBs), subsequent investigations revealed the presence of polychlorinated dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) in the causal rice oil[1-3]. The objective of this study is to investigate the levels of PCDD/Fs and PCBs including dioxin-like coplanar PCBs (Co-PCBs) in Yusho rice oil using the newest analytical techniques and to further evaluate their relative toxicological contribution.

Methods and Materials

One bottle of Yusho rice oil was obtained from a Yusho family in Fukuoka City in 1998. Since the obtained causal oil had spontaneously divided into two layers, namely, the liquid layer (701 g) and the sediment layer (15 g), we analyzed them separately and performed weighted average for concentration calculation. The concentrations of PCDD/Fs and PCBs in the causal oil were analyzed by Yokohama National University and Shimadzu Techno-Research Inc. with two different approaches shown below for cross-checking. The toxic equivalent (TEQ) levels were calculated based on the toxic equivalency factors (TEFs) for humans revised by the World Health Organization (WHO) in 1998.

Approach 1: The Yusho rice oil sample (0.20 g) of each layer was initially dissolved in *n*-hexane (10 mL). After the addition of ^{13}C-labeled internal standards, an aliquot (0.50 mL) of the *n*-hexane solution was treated with alkaline hydrolysis and concentrated sulfuric acid. Sample cleanup included chromatography on silica gel, aluminum and carbon columns. The final PCDD/F and Co-PCB fractions were further concentrated to 25μL and spiked with $^{13}C_{12}$-labeled recovery standards for high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analysis. The tetra- to octachlorinated PCDD/Fs and four non-ortho substituted Co-PCBs (PCB-77, PCB-81, PCB-126 and PCB-169) were analyzed by congener-specific analysis. The rice oil was analyzed twice (A and B) by this approach in the present study.

Approach 2: The oil sample of each layer was initially dissolved in *n*-hexane containing 10 % toluene. For the analysis of PCDD/Fs, an aliquot containing 1 g of the causal oil was extracted with *n*-hexane-saturated dimethyl sulfoxide (DMSO) after the addition of $^{13}C_{12}$-labeled internal standards. The DMSO phase was back-extracted with *n*-hexane and *n*-hexane-extracted water. The concentrated *n*-hexane phase was further cleaned up using multi-layer silica and carbon

column chromatography. In the case of PCB analysis, an aliquot containing 1 g of the causal oil was directly treated using multi-layer silica and carbon columns after the addition of $^{13}C_6$- and $^{13}C_{12}$-labeled internal standards. The obtained PCDD/F and PCB fractions were concentrated and congener-specifically analyzed by HRGC/HRMS.

Results and Discussion

Nearly all the tetra- to octachlorinated PCDD/Fs and all the Co-PCBs were detected from the rice oil sample. The results are presented in Tables 1 and 2. The individual concentrations of all the 2,3,7,8-substituted PCDD/F and Co-PCB congeners in Yusho rice oil were elucidated for the first time. Good reproducibility was obtained using approach 1. Furthermore, the results obtained from the two approaches agreed well, indicating the reliability of the data obtained in this study.

The concentrations of PCDDs and PCDFs were found to be 0.59 and 8.8 ppm, respectively. These results are comparable to those of Tanabe et al.[3], who congener-specifically investigated two Yusho oil samples and reported that the oil contained 0.83 (0.81 and 0.84) ppm of PCDDs and 12 (9.2 and 14) ppm of PCDFs[3]. For PCBs, more than 130 PCB peaks were observed and a total concentration of 850 ppm including 140 ppm of Co-PCBs was obtained in the present study. The mean concentration of PCBs in Yusho oil reported by Nagayama et al.[1] and Mimura et al.[4] was 920 (830-1030) and 830 (769 and 899) ppm, respectively. Additionally, Mimura et al. indicated that 130 –140 PCB congeners were present in Yusho rice oil[4]. On the other hand, Miyata et al. found relatively low levels of these compounds in Yusho causal oils[2]. The concentrations of PCDDs, PCDFs and PCBs were reported to be 0.14 (0.13 and 0.14), 1.5 (1.3 and 1.6) and 160 (150 and 160) ppm, respectively[2]. In addition, only 74 PCB components were detected from Yusho oil by Tanabe et al. and the mean PCB concentration was 380 (330 and 420) ppm[3]. The differences in dioxin and PCB concentrations between the Yusho oils mentioned above might be attributed to the difference in production date[5]. Based on the comparison of the observed PCDF and PCB levels and their ratio (PCDFs/PCBs) with those of various Yusho oils produced on different dates[5], the rice oil analyzed in this study is believed to be produced during the initial period of the rice oil contamination.

The TEQs of PCDDs, PCDFs, and Co-PCBs were calculated to be 17, 470 and 120 ppb, respectively. Thus, the relative contribution of these classes to the total TEQ in Yusho oil is 3, 77, 20 %, respectively, indicating that PCDFs played a major role in the toxicity of Yusho oil. These percentages of TEQ contribution are consistent with those found in Yusho blood[6]. Furthermore, it was confirmed that 2,3,4,7,8-PeCDF contributes 58 % to the total TEQ, supporting the view that this compound is the principal causal agent in Yusho poisoning[3]. 3,3',4,4',5-PeCB and 1,2,3,4,7,8-HxCDF were found to be the second and third causative agents, contributing 16 % and 12 % to the total TEQ, respectively. Previous studies indicated that 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF are present at high levels in blood[6, 7] and sebum[7] of Yusho patients compared to normal control. It is noteworthy that the most toxic 2,3,7,8-TCDD was newly discovered, although it contributes only 0.1 % to the total TEQ. This finding gives the explanation for the existence of 2,3,7,8-TCDD in sebum and blood of Yusho patients[7]. Based on the data of Tanabe et al.[3], Masuda calculated the TEQ contribution of PCDDs, PCDFs, and PCBs in Yusho oil to be 1, 91 and 8 %, respectively. Furthermore, the smallest TEQ intake during the latent period was estimated to be 0.11 mg[8]. The difference in the evaluation results of TEQ contribution in Yusho oil mentioned above is mainly attributable to the significant difference in the concentration of 2,3,4,7,8-PeCDF between our data and those reported by Tanabe et al.[3]. Consequently, the TEQ of 2,3,4,7,8-PeCDF obtained in the present study was only about 1/2 that of Tanabe et al.[3]. Based on our data, the smallest TEQ intake during the latent period was estimated to be 0.067 mg for

Yusho patients, according to the calculation method of Masuda[8]. This value is 61 % of that estimated by Masuda[8], and suggests that a lower minimum amount is necessary for developing the toxic symptoms of Yusho.

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Table 1. Concentrations of PCDD/Fs in Yusho rice oil (ppb)

Homolog	Isomer	Approach 1-A	Approach 1-B	Approach 1	Homolog	Isomer	Approach 1-A	Approach 1-B	Approach 1
TCDD	1368	2.2	1.9	2.1	PeCDF	13678	83	60	72
	1379	1.5	1.1	1.3		12368/12478/13467/13478/12467	1000	690	850
	1369	0.3	0.2	0.3		13479/14678	170	110	140
	1247/1248/1378/1469	1.6	1.2	1.4		12478	0.0	0.0	0.0
	1246/1249/1268/1478	0.7	0.5	0.6		13469	0.0	0.0	0.0
	1279	0.3	0.2	0.3		23468/12469/12347/12348	1000	700	850
	1234/1236/1269	0.2	0.1	0.1		23468/12469/12347/12348	0.0	0.0	0.0
	1237/1238	0.9	0.6	0.8		12348	400	280	340
	2378	0.7	0.4	0.6		12378	100	71	86
	1239	0.1	0.1	0.1		12367	41	30	36
	1278	0.4	0.3	0.4		12678/12379	210	140	180
	1267	0.0	0.0	0.0		23478/12489/12679/12369	780	530	660
	1289	0.1	0.1	0.1		23467	520	340	430
TCDF	1368	3.0	6.0	4.5		12349	6.9	5.8	6.4
	1468	29	28	29		12389	4.2	3.4	3.8
	2468	27	25	26	HxCDD	124679/124689	34	21	28
	1247/1347/1378/1348/1246	330	350	340		123468	81	50	66
	1247/1347/1378/1346/1246	120	0.0	60		123679/123689	100	63	82
	1367/1348/1379/1248	330	250	290		123469	3.8	1.2	2.4
	1268/1467/1478	45	87	66		123478	7.9	6.2	7.1
	1268/1467/1478	68	0.0	34		123678	39	32	36
	1369/1237/2368	280	210	250		123467/123789	31	23	27
	2467/1239/1238/1469/1678/1234	130	180	160	HxCDF	123468	160	110	140
	2467/1238/1238/1469/1678/1234	85	0.0	43		134678/124678	430	300	370
	1278	58	47	53		134679	10	4.9	7.4
	1267/1349	29	24	27		124679	11	11.0	11
	2348/2378/2347/2348/1249/1279	1400	990	1200		124689	7.7	5.1	6.4
	2367	110	73	92		123467/123478	1800	1200	1400
	3467/1269	19	14	17		123678	170	110	140
	1239	0.0	0.0	0.0		123479	39	23	31
	1289	3.1	2.5	2.8		123489/123679	31	28	30
PeCDD	12468/12479	35	27	31		123689	8.0	8.7	8.4
	12469	1.0	0.8	0.9		234678	200	160	180
	12368	30	23	27		123789	2.0	2.3	2.2
	12478	5.5	3.9	4.7		123489	33	36	35
	12379	17	14	16	HpCDD	1234678	88	78	87
	12389	1.8	1.3	1.6		1234678	130	100	120
	12467/12469	2.9	2.0	2.5	HpCDF	1234678	330	250	290
	12347	2.0	1.5	1.8		1234679	29	25	27
	12348	0.2	0.3	0.3		1234689	27	23	25
	12378	8.8	7.2	8.0		1234789	24	16	20
	12367	2.3	1.6	2.0	OCDD		66	53	60
	12389	2.5	1.7	2.1	OCDF		38	30	34
PeCDF	13468/12468	110	84	97	PCDD/Fs		11000	8100	9600

Table 2. Concentrations and TEQs of 2,3,7,8-PCDD/Fs and Co-PCBs in Yusho rice oil (ppb)

	Concentration (ppb)					TEQ (ppb)		
	Approach 1-A	Approach 1-B	Approach 1	Approach 2	Average	Approach 1	Approach 2	Average
TCDD	8.0	6.7	7.8	7.4	7.6			
PeCDD	110	85	98	82	90			
HxCDD	300	200	250	250	250			
HpCDD	230	180	210	160	180			
OCDD	66	53	60	54	57			
TCDF	3100	2300	2700	2300	2500			
PeCDF	4500	3000	3800	3700	3700			
HxCDF	2700	2000	2400	1900	2100			
HpCDF	410	320	370	380	370			
OCDF	37	30	34	31	32			
PCDDs	710	620	620	570	580			
PCDFs	11000	7600	9300	8300	8800			
PCDD/Fs	11000	8100	9800	8900	9200			
2,3,7,8-D	0.7	0.4	0.8	0.6	0.5	0.8	0.5	0.5
1,2,3,7,8-D	8.8	7.2	8.0	7.5	7.8	8.0	7.5	7.8
1,2,3,4,7,8-D	8.5	6.8	7.7	7.8	7.8	0.8	0.8	0.8
1,2,3,6,7,8-D	44	35	40	37	38	4.0	3.7	3.8
1,2,3,7,8,9-D	27	22	25	24	24	2.5	2.4	2.4
1,2,3,4,6,7,8-D	130	100	120	110	110	1.2	1.1	1.1
OCDD	66	53	60	54	57	0.0	0.0	0.0
2,3,7,8-F	150	100	130	110	120	13	11	12
1,2,3,7,8-F	100	71	86	200	140	4.3	10	7.2
2,3,4,7,8-F	730	570	650	710	680	330	360	350
1,2,3,4,7,8-F	890	640	760	720	740	76	72	74
1,2,3,6,7,8-F	170	110	140	110	130	14	11	13
2,3,4,6,7,8-F	200	160	180	140	160	18	14	16
1,2,3,7,8,9-F	3.2	3.0	3.1	2.7	2.9	0.3	0.3	0.3
1,2,3,4,6,7,8-F	330	250	280	280	280	2.8	2.8	2.8
1,2,3,4,7,8,9-F	24	16	20	20	20	0.2	0.2	0.2
OCDF	36	30	34	31	33	0.0	0.0	0.0
2,3,7,8-PCDDs	290	230	260	240	250	17	16	17
2,3,7,8-PCDFs	2600	2000	2300	2300	2300	460	480	470
2,3,7,8-PCDD/Fs	2900	2200	2600	2500	2500	470	490	480
PCB 81	680	550	620	510	560	0.1	0.1	0.1
PCB 77	13000	10000	12000	11000	11000	1.2	1.1	1.1
PCB 126	1100	860	980	980	980	98	98	98
PCB 169	50	39	45	31	38	0.4	0.3	0.4
Non-ortho PCBs	15000	11000	13000	13000	13000	100	99	100
PCB 123				3300			0.3	
PCB 118				58000			5.8	
PCB 105				49000			4.9	
PCB 114				4500			2.3	
PCB 156				9700			4.8	
PCB 157				2400			1.2	
PCB 167				2900			0.0	
PCB 189				800			0.1	
Mono-ortho PCBs				130000			19	

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