Dioxin concentration in the blood of patients collected during medical check-up for Yusho in 2006

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Abstract

We measured the concentrations of polychlorinated dizenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and non-*ortho* coplanar polychlorinated biphenyls (non-*ortho* PCBs) in blood collected from 300 Yusho patients and 125 Yusho-suspected persons during medical check-up performed in 2006. The sums of toxic equivalents (TEQ) concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood of Yusho patients and Yusho-suspected persons were 122.2 and 34.0 pg TEQ/g lipid, respectively, and the concentrations were 3.3 and 0.9 times higher than those of normal controls that had been previously reported, respectively. Of the PCDFs congeners for Yusho patients and Yusho-suspected persons, the average concentrations of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) were about 9.9 and 1.1 times higher than those in the normal controls, respectively. However, the maximum concentration of 2,3,4,7,8-PeCDF in the Yusho-suspected persons group was 276.3 pg/g lipid, which suggested that some of the Yusho-suspected persons were in fact exposed to PCDFs. By continuing this follow-up survey in the future, some of Yusho-suspected persons will finally be registered as Yusho patients based on the new diagnostic criteria that officially became the diagnostic criteria for Yusho exposure on September 29, 2004, which included a concentration of 2,3,4,7,8-PeCDF in the blood.

Introduction

In 1968, over 1800 persons in western Japan developed a strange skin disease, later named Yusho disease, which found to have been caused by the ingestion of rice bran oil contaminated with polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), polychlorinated quarterphenyls (PCQs), and polychlorinated terphenyls (PCTs)[1]. Over 38 years have passed since the Yusho outbreak, and although almost all of the typical symptoms affecting Yusho patients have improved, some patients are still afflicted with subjective symptoms. From the results of extensive research by the Yusho study group investigating this disease, PCDFs were concluded to be the primary cause of Yusho disease symptoms[1]. However, the concentrations of PCDFs in the blood were not included in the diagnostic criteria because the amount of blood that can be collected from Yusho patients is restricted. Recently, the measurement of PCDDs, PCDFs, and non-*ortho* PCBs in blood has become possible using small amounts of blood collected from participants in an annual medical check-up for Yusho patients[2]. We measured the concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in blood samples collected from 78 Yusho patients living in Fukuoka Prefecture in 2001 for a preliminary study[3]. Moreover, we measured the concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in blood collected from 279, 269, 242, and 237 Yusho patients during medical check-up performed in 2002 to 2005, respectively, and 92, 74, 74, and 114 Yusho-suspected persons during those same years, respectively, and we also measured the concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood of 127 normal controls unaffected by Yusho living in Fukuoka Prefecture whose ages were similar to those of the Yusho patients[4,5]. Based on the results of these follow-up survey study, the concentration of 2,3,4,7,8-PeCDF in the blood officially became part of the diagnostic criteria for Yusho exposure on September 29, 2004. As a result, of 74, 74, and 114 Yusho-suspected persons measured during 2003 to 2005, respectively, 18, 7, and 14 persons were officially registered as Yusho patients based on the "New Diagnostic Criteria," respectively. Because Yusho patients still have much higher concentrations of PCDFs in their blood than unaffected people, the patients remain at high risk for PCDFs toxicity. Therefore, it is necessary for this follow-up investigation to be continued so that further data that will support the health care of the patients and the general population can be gathered.

In this study, we measured the concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in blood collected from 300 Yusho patients and 125 Yusho-suspected persons in 2006, and compared with concentrations of these dioxin-like compounds among the groups of Yusho patients, Yusho-suspected persons, and normal controls that had been previously reported.

Materials and Methods

Medical check-up for Yusho patients have been conducted annually to determine their health status since the outbreak of Yusho incident[1]. The medical check-up is open not only to those persons officially registered as Yusho patients but also to Yusho-suspected persons who regard themselves as potential victims. Both officially registered Yusho patients and Yusho-suspected persons are examined based on the "Diagnostic Criteria for

Yusho" [1]. The blood samples examined in this study were collected from 425, of whom gave his or her informed consent to participate in this study. The normal controls were persons unaffected by Yusho, and selected to be in the same age range as the Yusho patients. These 127 normal controls from whom blood samples were collected also gave their informed consent. The 425 participants were 300 Yusho patients and 125 Yusho-suspected persons. Blood samples of 10 ml were collected using a vacuum blood-collecting tube containing heparin and were stored at 4°C until analyses for concentrations of PCDDs, PCDFs, and non-*ortho* PCBs. The extraction of PCDDs, PCDFs, and non-*ortho* PCBs from the blood was performed using a previously reported method[2]. Concentrations of the PCDDs, PCDFs, and non-*ortho* PCBs were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent cut large-volume injection system[2]. To estimate the sum of TEQ concentrations of PCDDs, PCDFs, and non-*ortho* PCBs, we introduced ND (less than the detection limit) values to half values of the detection limit and the estimates based on the toxic equivalency factors (TEF) values proposed by the World Health Organization (WHO) in 1998.

Results and discussion

The concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood of Yusho patients and Yusho-suspected persons in 2006, including the dates of the normal controls that had been previously reported, are presented in Table 1.

Table 1. Concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood of Yusho patients and Yusho-suspected persons in 2006

	Concentrations (pg/g lipid)											
	Yusho Patients				Yusho-suspected persons				Normal controls			
	n=300				n=125				n = 127			
Congeners	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.
2,3,7,8-TCDD	1.8	1.0	ND	6.7	1.6	1.2	ND	6.7	1.9	0.8	ND	4.3
1,2,3,7,8-PeCDD	11	6.0	2.0	43	7.6	4.5	ND	29	8.9	3.3	3.2	20
1,2,3,4,7,8-HxCDD	3.3	1.9	ND	12	3.3	2.0	ND	11	3.5	1.9	ND	13
1,2,3,6,7,8-HxCDD	50	42	3.9	314	25	17	4.0	106	28	11	7.3	70
1,2,3,7,8,9-HxCDD	5.2	3.3	ND	24	5.1	3.4	ND	19	4.5	2.8	ND	16
1,2,3,4,6,7,8-HeCDD	52	30	13	292	57	33	21	191	78	56	18	471
OCDD	840	468	233	3988	922	554	272	3212	1213	941	181	7614
2,3,7,8-TCDF	2.2	1.9	ND	13	1.0	0.9	ND	8.8	0.9	0.7	ND	4.5
1,2,3,7,8-PeCDF	1.2	1.2	ND	9.6	0.8	1.1	ND	12	ND			
2,3,4,7,8-PeCDF	169	227	3.3	1792	19	29	1.1	276	17	7.6	5.0	37
1,2,3,4,7,8-HxCDF	45	77	ND	580	5.5	13	ND	138	5.0	2.8	ND	20
1,2,3,6,7,8-HxCDF	18	23	ND	181	5.1	4.9	ND	39	5.7	2.6	ND	16
2,3,4,6,7,8-HxCDF	ND				ND				ND			
1,2,3,7,8,9-HxCDF	ND				ND				ND			
1,2,3,4,6,7,8-HeCDF	2.8	3.1	ND	29	3.0	3.7	ND	24	2.2	2.1	ND	15
1,2,3,4,7,8,9-HeCDF	ND				ND				ND			
OCDF	ND				ND				ND			
3,4,4',5-TCB(81)	ND				ND				ND			
3,3',4,4'-TCB(77)	ND				ND				ND			
3,3',4,4',5-PeCB(126)	99	73	13	516	91	93	ND	669	109	72	17	519
3,3',4,4',5,5'-HxCB(169)	175	132	15	1149	71	60	ND	305	63	25	16	192
Total PCDDs	963	506	271	4262	1022	602	307	3432	1338	1007	214	8171
Total PCDFs	243	326	12	2558	40	48	11	468	37	14	15	86
Total PCDDs/PCDFs	1206	631	294	4765	1062	615	320	3484	1375	1013	229	8257
Total Non-*ortho* PCBs	286	167	42	1236	174	146	20	939	186	94	59	744
Total	1493	730	347	5153	1235	707	368	4291	1561	1045	288	9000
PCDDs-TEQ	19	11	4.0	80	13	7.6	2.0	46	15	5.6	5.0	35
PCDFs-TEQ	91	123	2.1	971	11	16	1.1	156	10	4.3	3.5	22
PCDDs/PCDFs-TEQ	111	132	6.2	1049	24	21	3.1	173	25	9.1	8.5	54
Non-*ortho* PCBs-TEQ	12	7.7	1.6	53	9.8	9.7	0.6	69	12	7.4	2.0	54
Total of TEQ	122	134	8.0	1068	34	27	3.6	178	37	15	16	132

ND: less than the determination limit; S.D.: standarrd deviation; TEQ: toxic equivalent quality.

The sum of TEQ concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood of Yusho patients and Yusho-suspected persons in 2006 were 122.2 and 34.0 pg TEQ/g lipid, respectively, and the concentrations were 3.3 and 0.9 times higher than those in the normal controls, respectively. The average PCDFs concentrations of Yusho patients and Yusho-suspected persons were 91.3 and 11.0 pg TEQ/g lipid, respectively, and the concentrations were 9.1 and 1.1 times higher than those in the normal controls, respectively. The average

concentration of 2,3,4,7,8-PeCDF, which was the highest among PCDFs congeners for Yusho patients, was about 9.9 times higher than that of the normal controls. In the case of Yusho-suspected persons, the concentration was about 1.1 times higher than those of normal controls. However, the maximum concentration of 2,3,4,7,8-PeCDF in the Yusho-suspected persons group was 276.3 pg/g lipid, which suggested that some of the Yusho-suspected persons were in fact exposed to PCDFs.

Between 279 and 300 Yusho patients who received medical check-up in 2002 and 2006, the concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood of 199 patients were measured in both 2002 and 2006 (Table 2). The average PCDFs concentrations for the 199 Yusho patients in 2002 and 2006 were 105.8 and 95.8 pg TEQ/g lipid, respectively, and the concentration was slightly decrease during 2002 to 2006. The concentrations of 2,3,4,7,8-PeCDF among PCDFs congeners were 194.9 and 177.4 pg/g lipid in 2002 and 2006, respectively, and the concentration has slightly decreased during four years. These findings suggest that the PCDFs remained in the blood of Yusho patients for a very long time, 38 years having passed since the outbreak of Yusho, have a very high persistency.

Table 2. Concentrations of each congeners for 199 Yusho patients in whom the concentrations of PCDDs, PCDFs, and non-*ortho* PCBs in the blood were measured in both 2002 and 2006

	Concentrations (pg/g lipid)									
	2002 (n = 199)					2006 (n = 199)				
Congeners	Mean	Median	SD	Min.	Max.	Mean	Median	SD	Min.	Max.
2,3,7,8-TCDD	1.6	1.6	0.8	ND	4.4	1.7	1.6	0.9	ND	4.8
1,2,3,7,8-PeCDD	11	9.7	5.8	1.5	47	11	10	5.9	2.2	43
1,2,3,4,7,8-HxCDD	3.0	2.7	1.8	ND	11	3.3	3.1	1.9	ND	12
1,2,3,6,7,8-HxCDD	53	42	41	6.0	291	52	41	42	4.6	314
1,2,3,7,8,9-HxCDD	5.1	4.4	3.3	ND	18	5.2	4.4	3.4	ND	24
1,2,3,4,6,7,8-HpCDD	67	52	58	11	556	50	44	29	13	292
OCDD	891	706	770	172	9159	824	693	492	233	3988
2,3,7,8-TCDF	1.3	1.2	0.9	ND	5.2	2.3	1.7	1.9	ND	12
1,2,3,7,8-PeCDF	1.0	0.5	0.9	ND	6.3	1.2	0.5	1.2	ND	9.6
2,3,4,7,8-PeCDF	195	113	251	3.1	1890	177	90	229	3.3	1792
1,2,3,4,7,8-HxCDF	58	20	93	ND	670	47	17	77	ND	580
1,2,3,6,7,8-HxCDF	22	11	27	ND	167	18	10	22	ND	155
2,3,4,6,7,8-HxCDF	ND					ND				
1,2,3,7,8,9-HxCDF	ND					ND				
1,2,3,4,6,7,8-HpCDF	3.3	2.5	4.4	ND	40	2.9	2.1	3.5	ND	29
1,2,3,4,7,8,9-HpCDF	ND					ND				
OCDF	ND					ND				
3,4,4',5-TCB(81)	ND					ND				
3,3',4,4'-TCB(77)	11	11	6.4	ND	37	ND				
3,3',4,4',5-PeCB(126)	103	84	69	15	416	95	82	60	13	332
3,3',4,4',5,5'-HxCB(169)	202	170	151	13	1131	186	154	141	15	1149
Total PCDDs	1031	830	824	244	9802	948	822	527	271	4262
Total PCDFs	285	154	368	13	2744	254	129	328	12	2558
Total PCDDs/PCDFs	1317	1112	893	281	9886	1202	1112	630	294	4765
Total Non-*ortho* PCBs	321	297	179	59	1220	293	266	164	42	1236
Total	1638	1432	969	410	10294	1495	1370	716	347	5153
PCDDs-TEQ	19	17	10	3.3	78	20	18	10	4.0	77
PCDFs-TEQ	106	60	137	2.1	1029	96	49	124	2.1	971
PCDDs/PCDFs-TEQ	125	80	145	5.4	1108	115	63	133	6.2	1049
Non-*ortho* PCBs-TEQ	12	11	7.3	1.6	45	11	9.9	6.4	1.6	35
Total of TEQ	138	92	147	7.0	1126	127	74	134	8.0	1068

ND: less than the determination limit; S.D.: standarrd deviation; TEQ: toxic equivalent quality.

A similar poisoning caused by the ingestion of rice oil contaminated by PCBs and their thermal products occurred in Taiwan, and the symptoms of Yu-Cheng disease were very similar to those of Yusho disease[6]. The principal causal compounds of Yu-Cheng and Yusho disease were PCDF congeners: 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF) in Yu-Cheng disease and 2,3,4,7,8-PeCDF in Yusho disease. Accidental exposure to PCDDs, PCDFs, and dioxin-like PCBs also occurred at Seveso in northern Italy, and in Russia and Austria[7,8,9]. In the Seveso, Italy, and Austrian chloracne cohorts, the cause was exposure to 2,3,7,8-tetrachlorodizenzo-*p*-dioxin (TCDD). In the two cohorts in Russia, 2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD) were the primary causal compounds. In the Seveso, Austrian, and Russian chloracne cohorts, members of all three cohorts exhibit the production of an excess of female children associated with paternal exposure to 2,3,7,8-TCDD. Following the Yusho incidence, however, no such

phenomenon has been observed, and this is true for the Yu-Cheng incident in Taiwan as well, which may suggest different mechanisms of action between 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF on the sex ratio. Further study is necessary to elucidate the effect of dioxin-like compounds on human reproduction.

Because Yusho patients and Yusho-suspected persons have become older, it has become difficult to distinguish their age-related senile clinical symptoms from symptoms peculiar to Yusho disease. By continuing this follow-up survey in the future, some of Yusho-suspected persons will finally be registered as Yusho patients based on the new diagnostic criteria.

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