

# The changes in the concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls in the blood of Yusho patients from 2004 to 2014

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## Introduction

The 1968 Yusho poisoning accident affected over 1800 people in western Japan, and was caused by the accidental ingestion of rice bran oil containing polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated quarterphenyls (PCQs), and polychlorinated terphenyls (PCTs). Since the Yusho outbreak, the National Study Group for the Therapy of Yusho has carried out medical care and health examinations of the affected population. In 2001, technological advances made it possible to measure PCDDs, PCDFs, and non-*ortho* PCBs in small amounts of blood<sup>1,2</sup>. We have measured the concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood collected from Yusho patients in medical health examinations since 2002<sup>3</sup>. Moreover, we have conducted a congener-specific analysis of non-dioxin-like PCBs in the blood of these patients since 2004<sup>4</sup>. Based on these results, 2,3,4,7,8-PentaCDF has been recognized as the most important causative agent for the subjective symptoms of Yusho<sup>5</sup>. We also reported that Yusho patients continue to have higher concentrations of PCDFs in their blood than unaffected people, and that the concentration of PCDFs in the blood is significantly correlated with the intensity of Yusho symptoms. To provide useful information related to the health risks of PCDDs, PCDFs, and dioxin-like PCBs in Yusho patients, we previously reported on changes in the individual congener concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood of Yusho patients<sup>6</sup>. The objective of this study was to extend our previous studies.

Out of 243 and 246 Yusho patients who received medical health examinations in 2004 and 2014, respectively, there were 118 patients in whom the blood concentrations of PCDDs, PCDFs, and PCBs were measured both years. In these 118 Yusho patients, we compared the individual congener concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood measured in 2004 with those measured in 2014.

## Materials and Methods

Medical health examinations have been performed annually on Yusho patients to determine their health status since the Yusho incident. The medical health examination 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". The blood samples examined in this study were collected from 243 and 246 participants who received medical health examinations in 2004 and 2014, respectively, each of whom gave informed consent to participate in this study. Blood samples of 10 ml were collected using a vacuum blood-collecting tube containing heparin and were stored at 4°C until analyses for the concentrations of PCDDs, PCDFs, and PCBs.

The extraction and purification of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs from blood samples were performed using a previously reported method<sup>2,4</sup>. Concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs were determined by a previously reported method<sup>2,4</sup>.

To evaluate the accuracy and reliability of the analysis of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs, our laboratory participated quality control studies of the analysis of PCDDs, PCDFs, and dioxin-like PCBs in 2013, 2015, 2017, and 2019 and non-dioxin-like PCBs in 2014, 2016, and 2018. Each quality control study involved the participation of various laboratories that perform measurements for these compounds in human blood in Japan. In each quality control study, our results were compared with those of participating laboratories, and tests confirmed that the average variation among values obtained by each organization performing the analysis was within 10%. These results indicated that our laboratory's analytical methods regarding PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like PCBs in human blood provided accurate results.

We introduced ND (less than the detection limit) values to half values of the detection limit to estimate the toxicity equivalence (TEQ) concentrations and calculated based on the toxic equivalency factor (TEF) values proposed by the WHO. Statistical analysis was conducted using the Wilcoxon signed-rank test in the software programs from IBM SPSS Statistics 24 (Advanced Analytics, Inc). Significant probabilities (*p* values) were calculated for the respective number of samples analyzed.

## Results and Discussion

The objective of the present study was to extend our previous studies by reporting the changes in the individual congener concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood of Yusho patients from 2004 to 2014<sup>6</sup>. Out of 243 and 246 Yusho patients who received medical health examinations in 2004 and 2014, respectively, there were 118 patients in whom the blood concentrations of PCDDs, PCDFs, and dioxin-like PCBs were measured both years. In these 118 Yusho patients, the individual congener concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood samples measured in 2004 were compared with those measured in 2014 (Tables 1). The total TEQ concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in the blood of 118 Yusho patients in 2004 and 2014 were 5.2–533 (mean: 80, median: 52) and 11–545 (mean: 78, median: 73) pg TEQ g<sup>-1</sup> lipid, respectively. The TEQ concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in the blood of Yusho patients were 16, 51, 12, and 1.7 pg TEQ g<sup>-1</sup> lipid in 2004, and 16, 45, 14, and 2.0 pg TEQ g<sup>-1</sup> lipid in 2014, respectively, indicating that the concentrations of PCDFs in the blood of Yusho patients significantly decreased from 2004 to 2014.

**Table 1** Concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood of 118 Yusho patients

Congeners	Concentration (pg/g lipid)										Ratio 2014/2004	p Values
	2004					2014						
	Mean	Median	SD	Maximum	Minimum	Mean	Median	SD	Maximum	Minimum		
2,3,7,8-TetraCDD	1.3	1.3	0.7	4.4	0.5	1.6	0.9	1.5	4.2	0.5	1.2	<0.001
1,2,3,7,8-PentaCDD	8.7	8.1	4.3	33	1.0	9.6	5.4	8.9	41	1.9	1.1	0.001
1,2,3,4,7,8-HexaCDD	2.5	2.4	1.5	8.3	1.0	2.2	1.6	2.1	12	1.0	0.9	0.011
1,2,3,6,7,8-HexaCDD	46	37	35	247	4.8	41	35	29	248	3.0	0.9	<0.001
1,2,3,7,8,9-HexaCDD	4.3	3.3	3.4	23	1.0	3.2	3.0	2.6	18	1.0	0.7	<0.001
1,2,3,4,6,7,8-HeptaCDD	49	44	25	196	16	37	24	33	208	11	0.8	<0.001
OctaCDD	714	624	359	2305	181	511	292	424	1779	95	0.7	<0.001
Total PCDDs	825	741	393	2459	205	605	318	517	1930	138	0.7	<0.001
2,3,7,8-TetraCDF	1.6	1.3	1.3	7.2	0.5	2.6	5.5	1.4	43	0.5	1.6	0.329
1,2,3,7,8-PentaCDF	0.8	0.5	0.7	4.1	0.5	1.1	1.1	0.5	5.6	0.5	1.4	0.002
2,3,4,7,8-PentaCDF	151	79	192	1240	4.1	137	180	67	1261	5.8	0.9	<0.001
1,2,3,4,7,8-HexaCDF	37	14	63	514	1.0	25	48	8.6	402	1.0	0.7	<0.001
1,2,3,6,7,8-HexaCDF	15	8.2	20	176	1.0	12	19	6.9	174	1.0	0.8	<0.001
2,3,4,6,7,8-HexaCDF	ND					ND						
1,2,3,7,8,9-HexaCDF	ND					ND						
1,2,3,4,6,7,8-HeptaCDF	2.5	1.5	2.6	24	1.0	1.8	2.3	1.0	21	1.0	0.7	<0.001
1,2,3,4,7,8,9-HeptaCDF	ND					ND						
OctaCDF	ND					ND						
Total PCDFs	213	109	273	1946	13	186	244	101	1875	16	0.9	<0.001
TriCB-77	ND					ND						<0.001
TriCB-81	ND					ND						0.500
PentaCB-126	82	66	62	441	5.0	91	65	77	428	17	1.1	<0.001
PentaCB-169	121	105	79	361	11	180	124	149	677	23	1.5	<0.001
Total Non- <i>ortho</i> PCBs	219	193	120	696	26	283	161	254	856	50	1.3	<0.001
PentaCB-105	3111	2239	2425	15888	563	3197	2503	2654	19926	606	1.0	0.134
PentaCB-114	1721	1339	1363	8660	208	2033	1712	1622	10177	234	1.2	<0.001
PentaCB-118	14872	11534	11468	80220	2355	16238	14513	12544	133844	2853	1.1	0.005
PentaCB-123	265	195	220	1385	5.0	249	223	196	1502	16	0.9	0.150
HexaCB-156	23217	17570	19537	90316	5.0	28518	27967	19752	175909	1961	1.2	<0.001
HexaCB-157	6639	4980	5510	25277	351	7332	7635	5311	47932	318	1.1	0.050
HexaCB-167	3057	2360	2108	13497	483	3957	2838	3318	20467	541	1.3	<0.001
HeptaCB-189	3402	2629	2720	11402	5.0	4276	3843	3261	23833	137	1.3	<0.001
Total Mono- <i>ortho</i> PCBs	56283	45958	34608	159176	5971	65802	47855	56430	268679	6767	1.2	<0.001
TEQ from PCDDs	16	14	8.3	63	2.4	16	9.2	15	72	3.7	1.0	0.288
TEQ from PCDFs	51	26	66	442	1.7	45	60	23	437	2.7	0.9	<0.001
TEQ from PCDDs/PCDFs	67	40	72	505	4.1	62	68	38	509	6.4	0.9	<0.001
TEQ from non- <i>ortho</i> PCBs	12	10	7.4	50	0.8	14	8.6	13	52	2.4	1.2	<0.001
TEQ from mono- <i>ortho</i> PCBs	1.7	1.4	1.0	4.8	0.2	2.0	1.4	1.7	8.1	0.2	1.2	<0.001
TEQ from dioxin-like PCBs	14	12	8.2	54	1.0	16	9.7	15	57	2.6	1.2	<0.001
Total TEQ	80	52	75	533	5.2	78	73	54	545	11	1.0	0.288

ND (less than the determination limit) values introduced to half values of the detection limit.

SD : standard deviation.

CDD : chlorinated dibenzo-*p*-dioxin.

CDF : chlorinated dibenzofuran.

CB : chlorinated biphenyl.

We previously reported that the concentrations of 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, and 1,2,3,6,7,8-hexaCDF in the blood of Yusho patients were higher than those of normal controls<sup>3</sup>. These can be considered the characteristic congeners in the blood of Yusho patients. Of these four congeners, the concentrations of 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, and 1,2,3,6,7,8-hexaCDF were 46, 151, 37, and 15 pg g<sup>-1</sup> lipid in 2004, respectively, and 41, 137, 25, and 12 pg g<sup>-1</sup> lipid in 2014, respectively,

indicating that these congeners decreased significantly from 2004 to 2014 ( $p < 0.001$ ). In addition, the concentrations of 1,2,3,7,8,9-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, octaCDD, and 1,2,3,4,6,7,8-heptaCDF showed a trend of decrease from 2004 to 2014 ( $p < 0.001$ ). However, among individual congeners of dioxin-like PCBs, most congeners did not significantly decrease from 2004 to 2014.

Among the 118 patients, the blood concentrations of 2,3,4,7,8-pentaCDF were under 100 pg g<sup>-1</sup> lipid in 67 patients and over 100 pg g<sup>-1</sup> lipid in 51 patients. In the 51 over 100 pg g<sup>-1</sup> lipid group, the arithmetic mean TEQ concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in the blood were 21, 101, 13, and 2.2 pg TEQ g<sup>-1</sup> lipid in 2004, respectively, and 22, 90, 17, and 2.7 pg TEQ g<sup>-1</sup> lipid in 2014, respectively, with the total TEQ concentrations of these dioxin-like compounds ranging from 50 to 533 (mean: 137, median: 84) and 47 to 545 (mean: 132, median: 83) pg TEQ g<sup>-1</sup> lipid in 2004 and 2014, respectively (Table 2). With respect to the characteristic congeners in the blood of Yusho patients, the concentrations of 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, and 1,2,3,6,7,8-hexaCDF were found to decrease slightly from year to year. These results in the high-2,3,4,7,8-pentaCDF patients were almost the same as those in the total 118 Yusho patients.

**Table 2** Concentrations of PCDDs, PCDFs, and dioxin-like PCBs in the blood of 51 Yusho patients

Congeners	Concentration (pg/g lipid)										Ratio 2014/2004	p Values
	2004					2014						
	Mean	Median	SD	Maximum	Minimum	Mean	Median	SD	Maximum	Minimum		
2,3,7,8-TetraCDD	1.4	0.8	1.3	4.4	0.5	1.8	0.8	1.7	4.0	0.5	1.2	0.003
1,2,3,7,8-PentaCDD	11	5	11	33	4.1	13	6.1	11	41	5.0	1.1	0.010
1,2,3,4,7,8-HexaCDD	2.67	1.33	2.61	6.74	1.00	2.4	1.4	2.3	6.3	1.0	0.9	0.042
1,2,3,6,7,8-HexaCDD	69	39	61	247	23	64	40	49	248	17	0.9	0.001
1,2,3,7,8,9-HexaCDD	5.0	4.1	3.6	23	1.0	3.9	3.5	3.0	18	1.0	0.8	<0.001
1,2,3,4,6,7,8-HeptaCDD	48	19	44	98	16	35	14	33	84	13	0.7	<0.001
OctaCDD	687	297	612	1760	265	542	254	493	1317	107	0.8	<0.001
Total PCDDs	825	330	738	1973	324	662	269	653	1463	161	0.8	<0.001
2,3,7,8-TetraCDF	2.22	1.64	1.92	7.20	0.50	2.3	2.5	1.8	16	0.5	1.1	0.687
1,2,3,7,8-PentaCDF	0.95	0.69	0.50	3.10	0.50	1.1	1.0	0.5	4.8	0.5	1.2	0.256
2,3,4,7,8-PentaCDF	301	211	231	1240	106	273	203	211	1261	87	0.9	<0.001
1,2,3,4,7,8-HexaCDF	76	81	49	514	14	52	64	29	402	3.7	0.7	<0.001
1,2,3,6,7,8-HexaCDF	27	26	20	176	6.6	23	26	15	174	2.8	0.8	<0.001
2,3,4,6,7,8-HexaCDF	ND					ND						0.893
1,2,3,7,8,9-HexaCDF	ND					ND						0.180
1,2,3,4,6,7,8-HeptaCDF	2.5	1.8	2.0	8.4	1.0	2.0	2.9	1.0	21	1.0	0.8	0.002
1,2,3,4,7,8,9-HeptaCDF	ND					ND						0.317
OctaCDF	ND					ND						0.317
Total PCDFs	415	314	333	1946	135	359	290	262	1875	112	0.9	<0.001
TriCB-77	ND					ND						0.180
TriCB-81	ND					ND						<0.001
PentaCB-126	78	55	64	354	27	92	56	79	323	24	1.2	<0.001
PentaCB-169	174	78	156	361	49	264	128	241	677	79	1.5	<0.001
Total Non- <i>ortho</i> PCBs	268	119	256	696	91	367	161	333	856	132	1.4	<0.001
PentaCB-105	2761	1988	2180	12894	941	2977	1665	2607	9721	909	1.1	0.694
PentaCB-114	2516	1493	1964	8660	848	3065	1769	2474	10177	837	1.2	0.001
PentaCB-118	13441	9333	10335	59893	4613	15409	8394	12698	47970	4863	1.1	<0.001
PentaCB-123	223	198	180	1268	5.0	219	149	168	827	64	1.0	0.023
HexaCB-156	36739	20764	31628	90316	7320	46431	32682	38547	175909	9250	1.3	<0.001
HexaCB-157	10130	5947	8477	25277	1996	12335	8943	10501	47932	2437	1.2	<0.001
HexaCB-167	3444	2031	2626	10040	1002	4467	2038	3956	10483	1655	1.3	0.001
HeptaCB-189	5035	2718	4422	11402	960	6539	4243	5543	23833	1348	1.3	<0.001
Total Mono- <i>ortho</i> PCBs	74289	35228	68154	159176	22680	91442	49916	79465	268679	28912	1.2	<0.001
TEQ from PCDDs	21	9.1	20	63	7.5	22	10	20	72	8.0	1.0	0.238
TEQ from PCDFs	101	73	79	442	34	90	69	67	437	28	0.9	<0.001
TEQ from PCDDs/PCDFs	122	81	107	505	44	112	78	87	509	38	0.9	<0.001
TEQ from non- <i>ortho</i> PCBs	13	6.8	12	43	4.7	17	8.0	16	41	6.2	1.3	<0.001
TEQ from mono- <i>ortho</i> PCBs	2.2	1.1	2.0	4.8	0.7	2.7	1.5	2.4	8.1	0.9	1.2	<0.001
TEQ from dioxin-like PCBs	15	7.7	14	48	5.5	20	9.2	18	45	7.5	1.3	<0.001
Total TEQ	137	84	119	533	50	132	83	113	545	47	1.0	0.023

ND (less than the determination limit) values introduced to half values of the detection limit.

SD : standard deviation.

CDD : chlorinated dibenzo-*p*-dioxin.

CDF : chlorinated dibenzofuran.

CB : chlorinated biphenyl.

Of the 118 Yusho patients, 61 were men and 57 were women. The arithmetic mean TEQ concentrations of PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in the blood of the 61 men were 5.2–252 (mean: 60, median: 45) in 2004 and 11–249 (mean: 58, median: 43) pg TEQ g<sup>-1</sup> lipid in 2014. The concentrations in the 57 women were

12–533 (mean: 102, median: 93) in 2004 and 15–545 (mean: 100, median: 91) pg TEQ g<sup>-1</sup> lipid in 2014, indicating that the total TEQ concentrations in women in 2004 and 2014 were significantly higher than those in men. Regarding the characteristic congeners in the blood of Yusho patients, the concentrations of 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, and 1,2,3,6,7,8-hexaCDF showed a trend of decrease from 2004 to 2014 in both men and women. The decreasing ratios of individual congener concentrations of PCDDs, PCDFs, and dioxin-like PCBs from 2004 to 2014 in women were almost the same as those in men, suggesting that there is probably no sex difference regarding the enzyme that catalyzes the metabolism of dioxin-like compound in humans.

According to the results of the present study, among the individual congeners of PCDDs, PCDFs, and PCBs, most congeners of these compounds did not significantly decrease from 2004 to 2014. However, the concentrations of 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, and 1,2,3,6,7,8-hexaCDF in the blood of Yusho patients significantly decreased from 2004 to 2014. In addition, the concentrations of 1,2,3,7,8,9-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, octaCDD, and 1,2,3,4,6,7,8-heptaCDF were also showed a trend of decrease from 2004 to 2014.

Although over 50 years have passed since the outbreak of Yusho, many patients still suffer various symptoms such as chloracne, general fatigue and neuropathy. There are patients who continue to have much higher concentrations of PCDDs, PCDFs, and dioxin-like PCBs in their blood than unaffected persons. The investigations conducted in the present study suggest that the PCDDs, PCDFs, and dioxin-like PCBs that have remained in the bodies of Yusho patients are very difficult to excrete from the body, and that the half-lives of individual congener concentrations of these dioxin like-compounds in the blood are proving to be long to near infinity in the majority of Yusho patients.

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