

## TRANSITION OF PCB AND PCDF CONCENTRATIONS IN YUSHO PATIENTS FOR 38 YEARS

Yoshito Masuda<sup>1</sup>, Takesumi Yoshimura<sup>2</sup>, Jumboku Kajiwara<sup>2</sup> and John Jake Ryan<sup>3</sup><sup>1</sup>Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan<sup>2</sup>Fukuoka Institute of Health and Environmental Sciences, Dazaifu, Fukuoka, Japan<sup>3</sup>Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada**Abstract**

Concentrations of total PCBs in the blood of Yusho patients in Fukuoka Prefecture have been gradually decreased from 7 ppb in 1974 to 3 ppb in 2006. The concentrations of 2,2',4,4',5,5'-hexaCB (PCB153), 2,3,3',4,4',5-hexaCB (PCB156) and PCBs in the blood of Yusho A (Typical Yusho) were higher than those of Yusho B and Yusho C in order throughout the 32 years. The concentrations of 2,3',4,4',5-pentaCB (PCB118) in the blood of Yusho A were, however, lower than those of Yusho B and Yusho C. Simple correlation coefficients between the concentrations of PCB118, PCB153, PCB156, PCBs and their ages were examined from 1974 to 2006. The significant correlations are rather rare among them from 1974 to 1991, increasing number to 2000, and finally almost all to 2006. However, the correlation coefficients between the concentrations of PCB118 and PCB156 have been insignificant throughout the time with a few exceptions. By the use of the data of 3 Taiwan Yucheng patients and 5 Fukuoka Yusho patients, chronological concentrations of PCB118, PCB153, PCB156, 2,3,4,7,8-pentaCDF (23478F) and 1,2,3,4,7,8-hexaCDF in the blood of Yusho patients are figured out from outbreak of the poisoning to the present for 38 years. Very high concentrations of PCBs (97 ppb) and PCDFs (600 ppt) just after the onset were gradually decreased to 1.3 ppb of PCBs and 3.6 ppt of PCDFs in 2005 with the biological half-lives of 4.1, 6.7, 7.5, 5.2 and 4.4 years, respectively. The Yusho particular PCB156 and 23478F have accelerated metabolizing PCB118 to hydroxy-PCBs, which have been disturbing the hormonal effects.

**Introduction**

A mass food poisoning occurred in northern Kyushu, Japan in 1968. The poisoning is called Yusho, oil disease, because it was caused by ingestion of rice bran oil that had been contaminated with polychlorinated biphenyls (PCBs). It was later found that the rice bran oil had been contaminated not only with PCBs but also with polychlorinated dibenzofurans (PCDFs) and polychlorinated quaterphenyls (PCQs). Consequently, Yusho is a poisoning by a mixture of PCBs, PCDFs, PCQs and related compounds<sup>1</sup>. Blood samples of Yusho patients have been surveyed for PCB concentrations by annual medical examinations from 1974. Very small amounts of PCDFs in the blood were quantitatively analyzed from 1980 for Taiwan Yucheng patients and from 1997 for Fukuoka Yusho patients. Using the data of PCBs and PCDFs in the blood of Yusho and Yucheng patients, the transition of PCB and PCDF congeners in Yusho patients for 38 years is elucidated.

**Materials and Methods**

**Blood samples** Blood of Yusho patients in Fukuoka Prefecture have been surveyed at the annual medical examination and analyzed for PCB concentrations by Fukuoka Institute of Health and Environmental Sciences, Kitakyushu City Institute of Environmental Sciences, Fukuoka City Institute for Hygiene and the Environment and Daiichi College of Pharmaceutical Sciences. The data for 30 years from 1974 to 2006 were used for this study. Blood samples of 3 Taiwan Yucheng patients and 5 Yusho patients were supplied and stored in a freezer and analyzed for PCBs and PCDFs by Daiichi College of Pharmaceutical Sciences and Health and Welfare Canada.

**PCB analysis** After 1974, total PCBs were determined by comparing the total gas chromatographic peak heights of blood samples to that of Kanechlor, a commercial PCB mixture in Japan. Relative peak heights of

Peak1/Peak2 and Peak5/Peak2 have been used for characterization of Yusho<sup>2</sup>. After 1988, concentrations of individual PCB peaks were determined using the calculation method for PCB congeners<sup>3</sup>. Recently, concentrations of individual PCB peaks have been determined by using individual PCB congeners as standard substances<sup>4</sup>. Therefore, the validation for PCB congeners have much developed during the 32 years from 1974.

**PCDF analysis** Blood samples of patients with Yucheng or Yusho were collected and stored in a freezer and later analyzed by the developed method<sup>5</sup>, using <sup>13</sup>C-PCDD and <sup>37</sup>Cl-PCDF as internal standards and silica gel and activated carbon column chromatography for clean-up and high-resolution mass spectrometry with high-resolution capillary column for quantification of individual PCB and PCDF congeners. Blood samples, 10 ml, collected at the annual medical examination in Fukuoka Prefecture were analyzed for very minute amounts of PCDF congeners by newly developed methods<sup>6</sup> using <sup>13</sup>C-PCDFs as internal standards, double layer silica gel and activated carbon column chromatography, and special high-resolution mass spectrometry with high-resolution capillary column.

**Calculation of biological half-lives of PCBs and PCDFs.** Blood samples of Yucheng and Yusho patients collected at about 15 years after the incidents were determined for PCB and PCDF congeners. By the use of these data, concentrations of PCB and PCDF congeners in the blood of Yusho patients just after the incident were figured out. Biological half-lives from the incidents are calculated by the following equation:  $-0.693 (T_2 - T_1) / \ln(C_2 / C_1)$ , where  $T_2 - T_1$ : Time difference from the first blood sampling after the incident to the next blood sampling,  $C_1$ : Concentrations in the blood sampled just after the incident, and  $C_2$ : Concentrations in the blood of next sampling.

## Results and Discussion

Concentrations of PCB118, PCB153, PCB156 and total PCBs in the blood of Yusho patients were determined at the annual medical examination in Fukuoka Prefecture from 1974 to 2006. When the concentrations of PCB congeners were not determined, they were calculated from the peak ratios and total PCB concentrations. Yusho patients are classified into 3 types of Yusho, namely, Yusho A: having typical gas chromatogram (GC) pattern, Yusho B: between Yusho A and Yusho C, and Yusho C: having GC pattern similar to those of Controls. Average concentrations of PCBs in 1976, 7.39 ppb for Yusho A, 4.00 ppb for Yusho B, and 3.56 ppb for Yusho C, had decreased to about half values of the original concentrations, 3.39 ppb for Yusho A, 2.06 for Yusho B and 1.53 ppb for Yusho C, in 2005 for 30 years. Average concentration of PCBs, 3.84 ppb, in the blood of Controls in 1976 had decreased to one fourth, 0.83 ppb in 2005. The concentrations of PCB153, PCB156 and PCBs in the blood of Yusho A were higher than those of Yusho B and Yusho C in order. However, the concentrations of PCB118 in the blood of Yusho A were lower than those of Yusho B and Yusho C in order.

Simple correlation coefficients between the concentrations of PCB118, PCB153, PCB156 and PCBs in the blood of Yusho patients and their ages at the blood sampling were examined from 1974 to 2006. The correlations in 1976, 1988, 1997, 2003 and 2006 are shown in Table 1. Correlations between them in the blood of Controls in Fukuoka are also shown in Table 1 as reference data. The significant correlations were rather rare among them from 1974 to 1991, increasing number to 2000, finally almost all to 2006, as observed in the blood of Fukuoka Controls in 1999, in which all the correlations were significant. However, the concentration coefficients between the concentrations of PCB118 and PCB156 in the blood of Yusho patients have been insignificant throughout from 1974 to 2006 with a few exceptions.

By the use of the data of 3 Taiwan Yucheng patients and 5 Fukuoka Yusho patients, chronological concentrations of PCB118, PCB153, PCB156, 2,3,4,7,8-pentaCDF (23478F) and 1,2,3,4,7,8-hexaCDF (123478F) in the blood of Yusho patients were figured out from the outbreak of the poisoning to the present for 38 years. Table 2 shows the concentrations and half-lives of PCB118, PCB153, PCB156, 23478F and 123478F in the blood of Yucheng patients (n=3) and Yusho patients (n=5), 233 days, 14-15 years and 37-38 years after the incidents.

**Table 1 Simple correlation coefficients between concentrations of PCB118, PCB153, PCB156 and PCBs in the blood of Yusho patients and Age of the patients at blood sampling**

1976						2003					
n=43	Age	PCB118	PCB153	PCB156	PCBs	n=114	Age	PCB118	PCB153	PCB156	PCBs
Age	1	0.3631	0.4722	0.1891	0.4722	Age	1	0.4330	0.5620	0.3200	0.5500
PCB118	0.3631	1	-0.0843	-0.3241	-0.0843	PCB118	0.4330	1	0.5880	0.1610	0.4670
PCB153	0.4722	-0.0843	1	0.7380	1	PCB153	0.5620	0.5880	1	0.7570	0.8990
PCB156	0.1891	-0.3241	0.7380	1	0.7380	PCB156	0.3200	0.1610	0.7570	1	0.8320
PCBs	0.4722	-0.0843	1	0.7380	1	PCBs	0.5500	0.4670	0.8990	0.8320	1
1988						2006					
n=100	Age	PCB118	PCB153	PCB156	PCBs	n=120	Age	PCB118	PCB153	PCB156	PCBs
Age	1	0.2380	0.0900	0.0530	0.0940	Age	1	0.4550	0.3790	0.3530	0.5400
PCB118	0.2380	1	0.3580	0.0990	0.3590	PCB118	0.4550	1	0.5640	0.1430	0.5280
PCB153	0.0900	0.3580	1	0.8280	0.9960	PCB153	0.3790	0.5640	1	0.7220	0.7720
PCB156	0.0530	0.0990	0.8280	1	0.8390	PCB156	0.3530	0.1430	0.7220	1	0.8300
PCBs	0.0940	0.3590	0.9960	0.8390	1	PCBs	0.5400	0.5280	0.7720	0.8300	1
1997						Controls in Fukuoka, 1999					
n=88	Age	PCB118	PCB153	PCB156	PCBs	n=151	Age	PCB118	PCB153	PCB156	PCBs
Age	1	0.2525	0.1704	0.0565	0.1928	Age	1	0.6405	0.6929	0.7437	0.7439
PCB118	0.2525	1	0.4053	0.0616	0.2635	PCB118	0.6405	1	0.8436	0.8087	0.8918
PCB153	0.1704	0.4053	1	0.7506	0.8368	PCB153	0.6929	0.8436	1	0.8980	0.9746
PCB156	0.0565	0.0616	0.7506	1	0.7749	PCB156	0.7437	0.8087	0.8980	1	0.9370
PCBs	0.1928	0.2635	0.8368	0.7749	1	PCBs	0.7439	0.8918	0.9746	0.9370	1

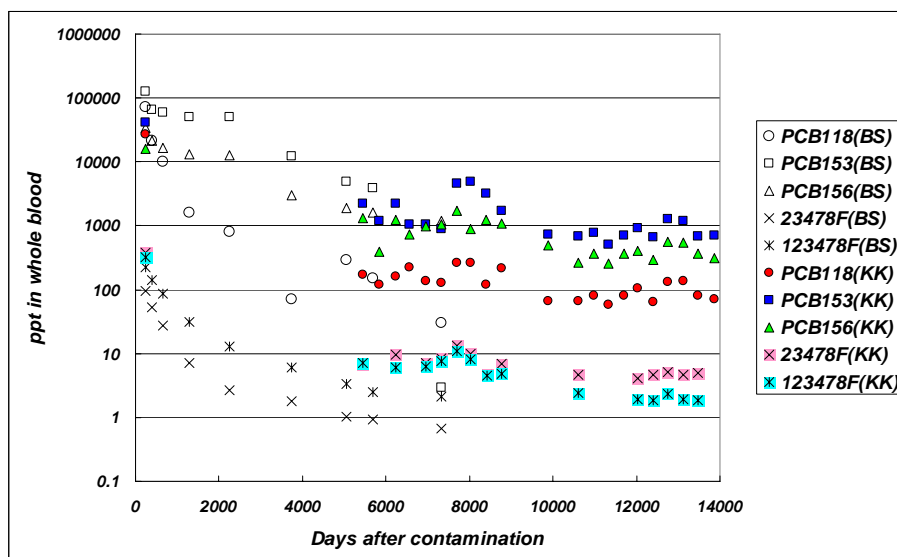
Significance, blue:  $p < 0.01$ , yellow:  $0.01 < p < 0.05$ , red:  $p > 0.05$ **Table 2 Average concentrations and half-lives of PCB and PCDF congeners in the blood of Yucheng patients (n=3) and Yusho patients (n=5) after the incidents**

	233 days after		14-15 years after				37-38 years after	
	Yucheng	Yusho	Yucheng		Yusho		Yusho (n=4)	
	Conc ppb	Conc * ppb	Conc ppb	Half-life year	Conc ppb	Half-life year	Conc ppb	Half-life year
PCB118	39	39	0.2	1.9	0.24	1.9	0.05	4.1
PCB153	80	46	5.1	3.7	2.7	3.4	0.81	6.7
PCB156	22	12	2.3	4.6	1.1	4.1	0.43	7.5
23478F	0.058	0.35	0.0013	2.7	0.0068	2.4	0.0027	5.2
123478F	0.14	0.25	0.0047	3.0	0.0067	2.7	0.0009	4.4

\* Calculated value from the concentrations 14-15 years after the incidents

In Yusho patients, very high concentrations of PCB118, PCB153, PCB 156, 23478F and 123478F just after the incident (data in Table 2) were gradually decreased to two orders of magnitude lower concentrations 37-38 years after the incident with the biological half-lives of 4.1, 6.7, 7.5, 5.2 and 4.4 years, respectively. Concentrations of PCB118 in the Yucheng patients were once reduced to 0.07 ppb in 1980, 11 years after the incident. As 0.07 ppb was lower than the values of Controls, most of the PCB118 in the patients after that time were not from the rice oil of Yusho incident but from the daily foods polluted with PCBs. As the concentrations of other PCB and PCDF congeners in Yusho patients were always much higher than those values of Controls, transition of these compounds in the patients can be elucidated.

Transition of blood concentrations of PCB118, PCB153, PCB156, 23478F and 123478F in the Yusho patient from the outbreak of the poisoning to present for 38 years is shown in Figure 1, using Yucheng BS and Yusho KK as an example. The high concentrations of PCB and PCDF congeners in 1969 were reduced to 0.25-3.4 % of the original concentrations in 2005, 37 years after the incident. The PCB concentrations were two orders of magnitude higher than those of PCDFs and the difference have been continued to the present for 23 years.



**Figure 1** Concentration trend of PCB118, PCB153, PCB156, 23478F and 123478F in patients with Yucheng (BS) and Yusho (KK) for 38 years

The original high concentrations of PCBs and PCDFs were estimated to be produced by ingestion of the polluted rice oil and the high levels were maintained for 2 or 3 years after the incident. Then the levels were gradually decreased for 15 years with about 4 years of half-lives except for PCB118, of which half-life was about 2 years. The concentration decreases were further slowed down after the 15 years as shown in Table 2 and Figure 1.

The particular declining mode of PCB118 during 38 years and the correlation coefficients between the concentrations of PCB118, PCB153, PCB156 and 23478F in the blood of Yusho patients indicated that PCB118 concentrations have been decreased by the coexisting PCB156, 23478F and others which have very strong enzyme inducing activities and the PCB118 has been metabolized by the enzyme inducers to hydroxyl-PCBs, which firmly bind to transthyretin<sup>7</sup> (thyroxine transport protein) in the blood and disturb the hormonal effects.

#### Acknowledgement

This work was supported in part by Grant-in-Aid for scientific research from the Ministry of Health, Labor and Welfare, Japan

#### References

1. Masuda Y. In: YUSHO, Kuratsune M (ed.), Kyushu University Press, Fukuoka, 1996: 49.
2. Masuda Y, Kagawa R, Shimamura K, Takada M, Kuratsune M. Fukuoka Igaku Zasshi 1974; 65: 25.
3. Ugawa M, Nakamura A, Kashimoto T. Shokuhin Eiseigaku Zasshi 1973; 14: 415.
4. Masuda Y, Yamaguchi S, Kuroki H, Haraguchi K, Fukuoka Igaku Zasshi 1985, 76: 150.
5. Ryan JJ, Levesque D, Panopio LG, Masuda Y, Kuroki H. Arch Environ Contam Toxicol 1993; 24: 504
6. Todaka T, Hirakawa H, Tobiishi K, Iida T. Fukuoka Igaku Zasshi 2003; 94: 148.
7. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. Toxicol Ind Health 1998; 14: 59