Chapter 4

Causal Agents of Yusho

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4.1. Toxic Agents in Rice Oil

When the Yusho incident first occurred in 1968, the rice oil collected from the home of the affected patients was analyzed for organochlorines using gas chromatography with electron capture detection, infrared absorption spectroscopy, the Volhard titrimetry and a radioactivation analysis (Tsukamoto et al., 1969). The PCB concentrations in the rice oil were thus estimated to be 2,000–3,000 ppm based on the chlorine contents of 1,000–1,500 ppm determined by the above methods, since KC-400 contains 48% chlorine. X-ray fluorometry also identified chlorine measuring up to 500 ppm in the rice oil produced or shipped from Kanemi at the beginning of February and none of the oils shipped in the other months were found to contain more than a trace amount of chlorine. Several years later, the rice oil produced on February 5 or 6 in 1968 was reanalyzed for PCBs by gas chromatography with electron capture detection using improved analytical techniques by means of total peak heights and by the perchlorination methods (Nagayama et al., 1975). The concentrations of PCBs in the rice oil were estimated to be 800–1,000 ppm, one-half to one-third of the first estimation. As shown in Fig. 4.1, PCBs in the rice oil showed a somewhat different gas chromatographic pattern from that of unused KC-400, indicating that the KC-400 which contaminated the rice oil had been heated under reduced pressure, causing an elimination of some amounts of lower chlorinated PCBs which have shorter retention times on the gas chromatogram (Masuda et al., 1974).

Vos et al., (1970) found by the chick embryo assay that the toxicity of commercial PCB preparations was greatly affected by their contaminants, PCDFs, which thus suggested that the contaminants were important to understand the total toxicity of the PCB mixtures. Column chromatography on the activated alumina was found to be effective in separating the PCDFs from a large amount of PCBs. After separation on alumina column chromatography, the PCDFs in the rice oil were first quantified by gas chromatography with electron capture detection (Nagayama et al., 1975). The oil was found to contain 5 ppm of PCDFs, or about 250 times the concentration (0.02 ppm) expected from the concentration of PCDFs in the unused KC-400 (Nagayama et al., 1976). This finding was later confirmed by Miyata et al., (1977). This marked increase in the amount of PCDFs in the oil could have occurred in the following manner. The KC-400 used as a heat transfer medium for deodorizing rice oil at the Kanemi plant was heated at higher than 200°C for a long time and the PCBs were thus gradually converted to PCDFs. It is said that the PCBs with increased PCDF concentrations then leaked into the rice oil through a hole formed in the heating pipe by a welding mistake (see Section 3.4.6). The

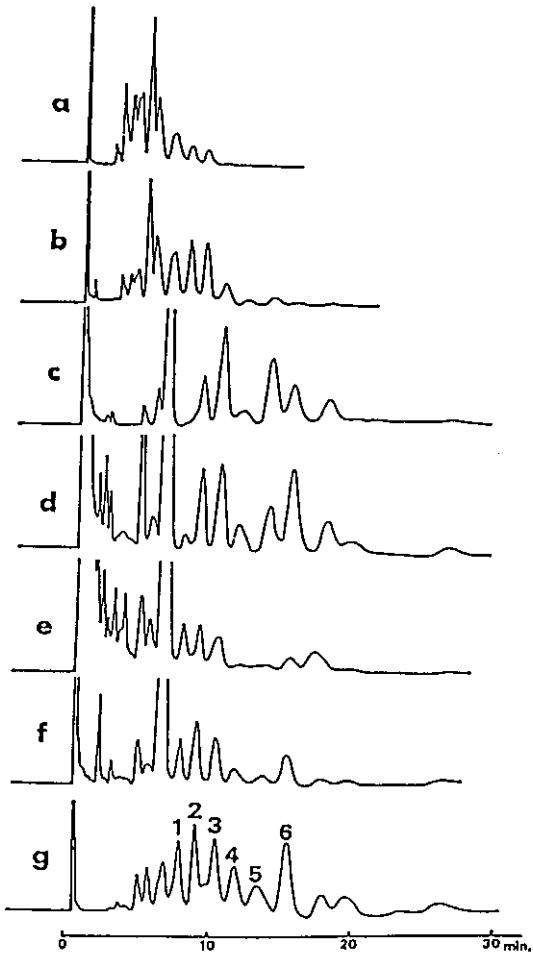


Fig. 4.1. Gas Chromatograms of PCBs on the 5% SE-30 Column
a: KC-400, b: Kanemi rice oil, c and d: blood of typical Yusho patients, e: blood from a Type C Yusho patient, f: blood from a normal control, g: KC-500 + KC-600 (1 : 1) (Masuda et al., 1974).

conversion of PCBs to PCDFs by heating at higher temperatures was confirmed by Miyata and Kashimoto (1978) and Nagayama et al. (1981). Concentrations of penta and hexa chlorinated PCDFs in the rice oil were then confirmed to have further increased during the deodorizing process under vacuum at high temperatures (Miyata and Kashimoto, 1979). Concentrations of PCBs and PCDFs in the rice oil, as well as KC-400 and their ratios were reported to be as shown in Table 4.1 by Kashimoto and Miyata (1987).

Two Yucheng rice oil samples were determined to contain PCBs, PCDFs and PCQs in 80 and 43.9, 0.10 and 0.141, and 48 and 17.3 ppm, respectively. These

Table 4.1. The Concentrations of PCBs, PCDFs and PCQs in Yusho Oil and KC-400

	Concentration (ppm)			Ratio (%)	
	PCBs	PCDFs	PCQs	PCDFs/PCBs	PCQs/PCBs
Yusho oil produced					
Feb. 5, 1968	968	7.4	866	0.76	89
Feb. 9, 1968	151	1.9	490	1.3	320
Feb. 10, 1968	155	2.3	536	1.4	350
Feb. 11, 1968	43.7	0.48	—	1.1	—
Feb. 15, 1968	12.3	0.085	—	0.69	—
Feb. 18, 1968	1.8	0.012	—	0.67	—
Unused KC-400	999,800	33	209	0.003	0.021
Used KC-400	968,400	510	31,000	0.052	3.2
Used KC-400	999,000	277	690	0.028	0.069
Used KC-400	971,900	20	28,000	0.002	2.9

(Kashimoto and Miyata, 1987)

concentrations were about 10–20 times lower than the corresponding concentrations in Yusho rice oil (Masuda et al., 1986). The gas chromatographic patterns of the PCBs from Yucheng rice oil were similar to that of KC-500, while the PCB patterns from Yusho rice oil also resembled the gas chromatogram of a mixture of KC-400 and KC-500 as shown in Fig. 4.2. The Yucheng PCBs consisted of more abundant higher chlorinated congeners than did Yusho PCBs.

PCDFs in the rice oil were composed of more than 40 congeners including toxic congeners of 2,3,7,8-tetra-, 1,2,3,7,8-penta-, 2,3,4,7,8-penta-, 1,2,3,4,7,8-hexa- and 1,2,3,6,7,8-hexa-CDFs (Buser et al., 1978; Rappe et al., 1979). A total of 74 PCB components and 47 congeners of tetra- through octa-PCDFs were quantitatively determined in the rice oil (Tanabe et al., 1989). The concentrations of major PCDF congeners are listed in Table 4.2. It was later found that highly chlorinated dibenzofurans decompose during the alkaline treatment of samples, that is, a common cleanup process for PCB and PCDF analyses, and by an improved analytical process, hepta-CDFs were found to be major components in the rice oil which contained 160 ppm of PCBs (Kashimoto et al., 1987).

Shortly after the Yusho incident, 2,000–3,000 ppm of PCBs were thought to be contained in the rice oil based on the finding of both a radioactivation analysis and X-ray fluorometry. The actual levels of PCBs, however, turned out to be around 1,000 ppm by gas chromatography using improved analytical techniques, and thus were equivalent to one-half or one-third of the originally suspected PCB levels. The rice oil was thus expected to contain a large amount of chlorinated compounds other than PCBs and PCDFs. PCQs, dimers of PCBs, consist of more than 100,000 congeners and show very wide peaks at very long retention times in the gas

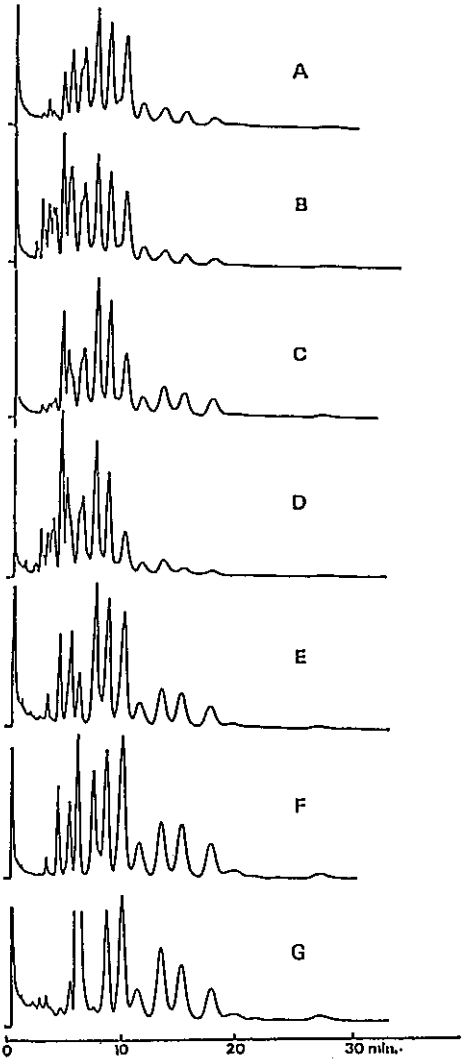


Fig. 4.2. Gas Chromatograms of PCBs on the 5% SE-30 Column
A: KC-500, B: KC-400 + KC-500 (1 : 1), C: from Yucheng rice oil, D: from Yusho rice oil, E and F: from the blood of Yucheng patients, G: from the blood of Yusho patient (Masuda et al., 1982).

Table 4.2. The Concentrations of PCDD, PCDF and PCB Congeners and 2,3,7,8-TCDD TEQ in the Rice Oil

	Concentration (ppb)	2,3,7,8-TCDD TEQ (ppb)
2,3,7,8-Tetra-CDD	nd	0
Other Tetra-CDDs	3	0
1,2,3,7,8-Penta-CDD	7	3.5
Other Penta-CDDs	77	0
1,2,3,4,7,8-Hexa-CDD	8	0.8
1,2,3,6,7,8-Hexa-CDD	40	4
1,2,3,7,8,9-Hexa-CDD	23	2.3
Other Hexa-CDDs	203	0
1,2,3,4,6,7,8-Hepta-CDD	185	1.9
Other Hepta-CDD	160	0
Octa-CDD	120	0.1
Total PCDDs	826	12.6
2,3,7,8-Tetra-CDF	660	66
Other Tetra-CDFs	2,570	0
1,2,3,7,8-Penta-CDF	525	26
2,3,4,7,8-Penta-CDF	1,350	675
Other Penta-CDFs	3,580	0
1,2,3,4,7,8-Hexa-CDF	890	89
1,2,3,6,7,8-Hexa-CDF	170	17
2,3,4,6,7,8-Hexa-CDF	165	16.5
Other Hexa-CDFs	1,259	0
1,2,3,4,6,7,8-Hepta-CDF	255	2.6
1,2,3,4,7,8,9-Hepta-CDF	11.5	0.1
Other Hepta-CDFs	42	0
Octa-CDF	76	0
Total PCDFs	11,600	892
3,3′,4,4′-Tetra-CB	11,500	5.75
3,3′,4,4′,5-Penta-CB	630	63
3,3′,4,4′,5,5′-Hexa-CB	27	0.27
2,3′,4,4′,5-Penta-CB	32,000	3.2
2,3,3′,4,4′-Penta-CB	28,000	2.8
2,3,3′,4,4′,5-Hexa-CB	2,950	1.5
Other Mono-ortho PCBs	91,800	0
Di-ortho PCBs	135,100	0
Tri-ortho PCBs	7,580	0
Other PCBs	71,000	0
Total PCBs	380,000	76.5
TOTAL	392,400	981

All values are the average of two samples.
(Data from Tanabe et al., 1989)

chromatography for PCB analysis. PCQs were, therefore, not determined by gas chromatography but were instead identified by chlorine analyses such as a radioactivation analysis and X-ray fluorometry. The quantification of PCQs was finally made by the perchlorination of PCQs to 6 simple skeletal isomers of octadecachloroquaterphenyls (Maeda and Kashimoto, 1978). Miyata et al. (1978a; 1978b) identified 866 ppm of PCQs consisting of penta- through deca-chlorinated congeners in the rice oil as shown in Table 4.1. The presence of PCQs in the rice oil was confirmed by Kamps et al. (1978) and Yamaguchi and Masuda (1985).

Polychlorinated quaterphenyl ethers (PCQEs), namely dipolychlorobiphenyl ethers, and polychlorinated terphenyls (PCTs) were also identified as minor components in the fraction of PCQs from the rice oil (Miyata et al., 1978a). The formation of PCQEs from PCBs was accompanied by the formation of PCQs in the heating process of KC-400 at high temperatures (Miyata et al., 1978b; Yamaryo et al., 1979). The causal rice oil was found to contain PCBs, PCQs and PCTs at the concentrations of 110, 380 and 7.2 ppm, respectively (Iida et al., 1985a). Polychlorinated sexiphenyl, namely trimers of PCBs, 70 ppm were separated from the PCQ fraction from the rice oil by gel permeation chromatography (Yamaguchi and Masuda, 1985). In the PCDF fraction from the rice oil, polychlorinated naphthalene (Buser et al., 1978) and polychlorinated phenyldibenzofurans (Kuroki et al., 1989) were identified in small quantities. Kashimoto et al. (1987) found 0.13 ppm of PCDDs and 1.41 ppm of 3 coplanar PCBs in a sample of the rice oil which contained 169 ppm of PCBs. Tanabe et al. (1989) also quantified the congeners of PCDDs and coplanar PCBs, and these concentrations are shown in Table 4.2.

The toxicities of the individual congeners of PCDDs, PCDFs and PCBs were evaluated in comparison with 2,3,7,8-TCDD by many organizations (Barnes et al., 1986; Safe and Phil, 1990; Safe, 1994). 2,3,7,8-TCDD toxic equivalents (TEQ) are calculated for the individual congeners using the TEQ factors of PCDDs/PCDFs and PCBs established by international organizations (Kutz et al., 1990) and WHO (Ahlborg et al., 1994), respectively. The TEQs calculated are also listed in Table 4.2 in order to better understand the toxicities converted to 2,3,7,8-TCDD. The total TEQ of the rice oil was calculated to be 0.98 ppm which was consisted of 91% PCDFs, 8% PCBs and 1% PCDDs. 2,3,4,7,8-Penta-CDF was also found to contribute to 69% of the total TEQ of the toxic rice oil.

4.2. The Intake of Toxic Agents by Yusho Patients

A survey of 141 Yusho patients who consumed the rice oil containing 920, 5 and 866 ppm of PCBs, PCDFs and PCQs, respectively, indicated that the average consumption of the rice oil was 688 ml in total and 506 ml during the latent period

Table 4.3. The Estimated Intakes of Rice Oil and 2,3,7,8-TCDD TEQ by Yusho Patients

Intake	Rice oil	TEQ
Average total intake per capita	688 ml[a] (195–3,375)[b]	0.62 mg[a] (0.18–3.04)[b]
Average intake during latent period	506 ml[a] (121–1,934)[b]	0.456 mg[a] (0.11–1.74)[b]
Average daily intake	0.171 ml/kg/day[a] (0.031–0.923)[b]	154 ng/kg/day[a] (28–832)[b]
Smallest intake during the latent period	121 ml	0.11 mg
Smallest daily intake during the latent period	0.031 ml/kg/day	28 ng/kg/day

The TEQs are calculated by 0.98 ppm in Yusho oil and 0.92 of oil density. [a]: Mean, [b]: range.
(Data from Hayabuchi et al., 1979.)

before illness became apparent. Therefore, the total amounts of PCBs, PCDFs and PCQs taken by a patient were calculated to be 633, 3.4 and 596 mg, respectively, on the average, while the amounts taken during the latent period were 466, 2.5 and 439 mg, respectively (Hayabuchi et al., 1979). The smallest amounts taken by a patient during the latent period were estimated to be 111, 0.6 and 105 mg, respectively. As the concentration of TEQ in the rice oil was determined to be 0.98 ppm (Table 4.2), the amounts of TEQ ingested by one patient were calculated to be 0.62 mg in total and 0.456 mg during the latent period. Table 4.3 lists the intake of rice oil and TEQ by Yusho patients. The smallest amount taken by a patient was also estimated and is shown in Table 4.3. The smallest daily intake during the latent period (28 ng/kg/day) was calculated from the oil intake (235 ml) of a particular patient (56 kg) during 135 days of the latent period. The clinical severity of illness and their blood PCB levels showed a close positive correlation with the total amount of oil consumed but not with the amount of oil consumed per kg body weight per day (Hayabuchi et al., 1981). This may indicate that after exposure to highly persistent toxic agents, the level of toxic agents in the body increased up to the level which developed the toxic symptoms of Yusho. The total amounts of ingested toxic agents may be important to estimate the severity of illness, as they are very accumulative in the body.

The intakes of PCBs, PCDFs and PCQs by Yucheng patients were estimated to be 673, 3.84 and 490 mg, respectively, for the total intakes per capita and 302, 1.26 and 192 mg, respectively, for the intakes during the latent period (Masuda et al., 1986). These values were about the same amounts as the corresponding intakes by Yusho patients, since the Yucheng intakes of the toxic rice oil were about 20 times greater than the Yusho intakes (Lan et al., 1981) and the concentrations of PCBs, PCDFs and PCQs in the Yucheng oil were respectively 10–20 times lower than

Table 4.4. The Concentrations of PCBs in the Tissue of Yusho Patients and Control Persons

Case	Sex	Age	Time of death or surgical operation	PCB concentration (ppm. wet basis)	
				Adipose tissue	Liver
1[a]	F	Stillborn	Oct., 1968	0.02	0.07
2[b]	M	About 17	Nov., 1968	76 (Face)	—
				13 (Abdomen)	—
3[b]	?	Adult	Nov., 1968	46	—
4[a]	M	17	July, 1969	1.3	0.14
5[a]	M	25	July, 1969	2.8	0.2
6[a]	M	73	Nov., 1969	3.8	0.07
7[a]	F	48	Dec., 1970	0.7	0.07
8[a]	M	46	May, 1972	4.3	0.08
9[c]	M	72	April, 1975	0.2	0.07
10[a]	M	59	March, 1977	1.2	0.006
11–21[d]	M, F	40–75	Nov., 1984	0.7–2.7	—
22–28[e]	M, F	43–55	Feb., 1986	1.0–5.7	—
Controls					
A (N = 31)[e]	M, F	0–61	1981	1.39[f] (0.09–13)	0.05[f] (0.01–0.2)
B (N = 10)[d]	M, F	23–83	Nov., 1984	0.09–1.1	—
C (N = 11)[e]	M, F	29–61	Feb., 1986	0.44–1.3	—

[a]: Masuda et al. (1974), [b]: Goto and Higuchi (1969), [c]: Masuda and Kuratsune (1979), [d]: Ohgami et al. (1987a), [e]: Iida et al. (1989), [f]: Mean and range in parenthesis.

those of the Yusho rice oil (Masuda et al., 1986).

4.3. Toxic Agents in the Tissue and Blood of Yusho Patients

4.3.1. PCBs

Just after the incident occurred in 1968, PCBs were identified in the subcutaneous fat, adipose tissue, sputum and other regions of patients with Yusho by gas chromatography (Goto and Higuchi, 1969; Tsukamoto et al., 1969; Kojima, 1971; Kikuchi et al., 1971). However, the quantification of the PCBs was inaccurate because of the complexity of the PCB compositions. The adipose and liver tissue of Yusho patients were later analyzed for PCBs with improved analytical methods (Tanabe, 1976). Table 4.4 summarizes the concentrations of PCBs in the adipose and liver tissue (Goto and Higuchi, 1969; Masuda et al., 1974; Masuda and Kuratsune, 1979; Iida et al., 1989). The PCB concentrations in the adipose tissue seemed to be very high, measuring as much as several tens ppm soon after the

Table 4.5. Concentrations of PCBs in the Blood of Yusho Patients

Grouping in Yusho or control	Sampling time	Place	ppb in whole blood			Reference
			Number	Mean	S. D.	
Yusho	1972	Fukuoka	25	4.8	2.9	Takamatsu et al. (1974)
Control			11	2.8	1.5	
Yusho A	1973	Fukuoka	24	8.9	5.8	Masuda et al. (1974)
Yusho B			15	4.0	2.1	
Yusho C			2	2.0		
Control			37	2.8	1.6	
Yusho A	1973	Fukuoka	43	7.2	4.9	Koda and Matsuda (1975)
Yusho B			26	4.3	3.1	
Yusho C			3	1.7	0.2	
Control			9	2.1	0.8	
Yusho	1973–74	Nagasaki	23	4.6	3.1	Baba et al. (1978)
Control			26	2.3	1.0	
Yusho		Goto	29	7.2	4.7	
Control			28	4.1	1.5	
Yusho	1973–78	Tamanoura	186	7.8	6.0	Baba et al. (1979)
Non approval[a]			254	4.4	3.0	
Yusho		Naru	86	5.7	4.5	
Non approval[a]			89	3.3	2.1	
Yusho		Nagasaki	29	3.6	2.3	
Non approval[a]			41	1.5	1.1	
Yusho Mother	1974	Goto	18	11.2[b]	7.3	Abe et al. (1975)
Yusho Infant			30	6.7[b]	4.3	
Control Infant			14	3.7[b]	2.0	
Yusho Male	1975–78	Kurume	10	16.5[b]	15.5	Takamatsu et al. (1975)
Control Male			13	6.1[b]	1.7	
Yusho Female			13	6.9[b]	4.8	
Control Female			15	4.6[b]	1.7	
Yusho A	1979	Fukuoka	31	9.6	6.4	Iida et al. (1981)
Yusho B			4	4.7	5.2	
Yusho C			29	2.6	1.1	
Control			23	2.9	1.0	
Yusho A	1979	Chikugo	11	6.2	4.9	Takamatsu et al. (1981)
Yusho B/C			20	2.7	1.4	
Control			18	3.3	1.2	
Yusho	1983	Fukuoka	18	3.1[c]	1.4	Masuda et al. (1985b)
Yusho	1984		26	3.5[c]	2.6	
Control	1983		27	0.8[c]	0.9	
Yusho	1983	Nagasaki	25	7.2	0.8	Okumura et al. (1987)
Control			7	1.9	0.1	
Yusho	1984	Nagasaki	11	6.5	2.4	Ohgami et al. (1987b)
Control			10	1.2	0.6	
Yusho	1988–89	Nagasaki	27	6.4	3.2	Ohgami et al. (1991)
Control			22	2.3	0.9	
Yusho	1990	Nagasaki	22	5.4	2.5	Ohgami et al. (1993)
Yusho	1991		16	6.6	2.8	
Yusho	1992		23	4.8	2.5	
Control			20	2.1	0.7	

[a]: Those who claim to be affected with Yusho but are not diagnosed as such, [b]: Concentration in serum,
[c]: Concentration of 7 PCBs.

Table 4.6. The concentrations of PCB Congeners in the Blood of Yusho Patients Sampled in 1983

	Concentration (ppt)		Ratio
	Yusho patient (N = 18)	Control (N = 27)	Yusho Control
2,3',4,4',5-Penta-CB	55 ± 44	71 ± 95	0.77
2,2',4,4',5,5'-Hexa-CB	1,086 ± 678	348 ± 430	3.12
2,3,3',4,4'-Penta-CB	38 ± 26	27 ± 35	1.41
2,2',3,4,4',5'-Hexa-CB	522 ± 294	139 ± 160	4.02
2,3,3',4,4',5-Hexa-CB	836 ± 343	50 ± 33	16.7
2,2',3,4,4',5,5'-Hepta-CB	311 ± 239	91 ± 124	3.42
2,2',3,3',4,4',5-Hepta-CB	198 ± 142	45 ± 55	4.40
Total	3,080 ± 1,440	763 ± 922	4.04

Masuda et al. (1985b). Values are the mean ± S. D.

onset of Yusho. The level of PCBs thereafter rapidly decreased to several ppm in the next year. However, these levels, which were only two to three times higher than the controls, were maintained until recent years. The level of PCBs in the liver was considerably lower than that in the adipose tissue.

The quantification of PCBs in the blood was developed after 1973, five years after the onset of Yusho. Since that time, many blood samples of Yusho patients have been analyzed for PCB levels, and the concentrations are shown in Table 4.5. The blood PCB levels of Yusho patients were always only two to three times higher than the control levels. However, the gas chromatographic patterns of PCBs in Yusho patients were different from those of the control persons (Masuda et al., 1974). The characteristic type was classified into the following three types: Type A: peculiar to Yusho, Type B: an intermediate pattern between Types A and C, and Type C: commonly observed in the blood PCBs of the general population (Masuda, 1985). Yusho patients showing Type A PCBs usually had significantly higher PCB concentrations than the control persons showing Type C. Fig. 4.1 shows gas chromatograms of PCBs from the blood of Yusho patients and control persons. Typical Yusho patients showed Type A which is a characteristic gas chromatogram with a relatively lower peak at 1 and a higher peak at 5 (Masuda et al., 1974). Table 4.6 shows that the composition of PCB congeners present in the blood obtained in 1983 from Yusho patients is quite different from those of the non-exposed persons, and were characterized by lower concentrations of 2,3',4,4',5- penta-CB and much higher concentrations of 2,3,3',4,4',5-hexa-CB (Masuda et al., 1985b). This characteristic difference has thus since been adopted as one of the criteria for diagnosis of Yusho (Kuratsune et al., 1987). In Nagasaki prefecture, the ratio of CB% was often used to evaluate Yusho patients (Ohgami et

al., 1989) instead of the characteristic types of A, B and C. The ratio of CB%, a concentration ratio of gas chromatographic peaks corresponding to 2,3,3′,4,4′,5-hexa-CB/2,3′,4,4′,5-penta-CB, was obtained by calculations from the CB%, or the percentage of individual PCB concentrations to the total PCB concentration (Ugawa et al., 1973). Since the concentrations of 2,3′,4,4′,5-penta-CB and 2,3,3′,4,4′,5-hexa-CB are relatively low and high respectively in typical Yusho patients, the higher the CB% ratio, the more typical of Yusho patients. As Iida et al. (1985b) observed that the concentrations of PCBs, especially 2,3,3′,4,4′,5-penta-CB, in the blood of normal persons gradually decreased over time from 1974 to 1980, the concentration differences of this congener have been minimized between Yusho patients and control persons in recent years. However, the typical Yusho type gas chromatogram of PCBs still persisted in Yusho patients even in 1994, more than 25 years after onset. Blood samples of patients with Yucheng, a disease similar to Yusho which occurred in Taiwan in 1979, have been analyzed for PCBs from 1980 just after the accident. Gas chromatograms of PCBs obtained from Yucheng patients one year after the incident resembled those of the PCBs in Yucheng rice oil, as shown in Fig. 4.2. It is expected, however, that their gas chromatographic pattern would gradually change to the PCB pattern similar to that of Yusho patients within ten years (Masuda et al., 1986). The biological half-lives of PCB congeners were determined in three Yucheng patients who had very high blood PCB levels of 156–397 ppb (Masuda et al., 1991). The half-lives of 2,3′,4,4′,5-penta-CB, 2,2′,4,4′,5,5′-hexa-CB and 2,3,3′,4,4′,5-hexa-CB were determined to be 1.16, 4.28 and 4.21 years on average, respectively, from 1980 to 1989. The medians of half-lives of the same congeners in 13 Yusho patients were 16, –34 and 84 years, respectively, from 1982 (14 years after the onset) to 1991, which thus suggested the overall elimination to be longer than the 10-year half-life (Masuda et al., 1993). When the blood PCB concentration is as high as 100 ppb, as observed in the Yucheng patients, the elimination of PCBs is relatively fast since the half-lives are 1–4 years, while, when the PCB concnetration is as low as 1 ppb, as seen in the Yusho patients 14 years after the onset, elimination is very slow since the half-lives are longer than 10 years. The shorter half-life of 2,3′,4,4′,5-penta-CB in the first 10 years after the exposure may thus partly cause the peculiar pattern in Yusho patients. Fig. 4.3 shows the elimination profiles of PCBs in a Yucheng patient after onset and a Yusho patient 14 years after onset. As the elimination lines of Yucheng and Yusho almost perfectly match with one another at 5,100 days (14 years) after the onset, the expected elimination of PCBs from the human body after 25 years is presented in Fig. 4.3.

Among the PCB congeners identified in Yusho patients, 2,3,3′,4,4′,5-hexa-CB showed a strong enzyme-inducing activity in the liver and marked atrophy of the

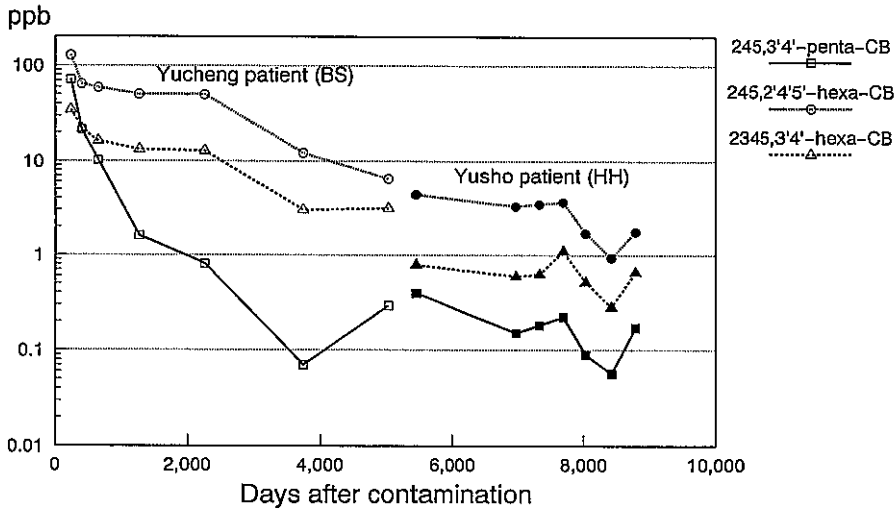


Fig. 4.3. The Elimination of PCB Congeners in the Blood of Yusho and Yucheng Patients (Whole blood basis)

The half-lives of 2,3′,4,4′,5-penta-CB, 2,2′,4,4′,5,5′-hexa-CB and 2,3,3′,4,4′,5-hexa-CB were 1.6, 3.5 and 3.9 years in Yucheng and 4.8, 4.9 and 12.6 years in Ysuho, respectively.

thymus in rats (Yoshihara et al., 1979; Masuda and Yoshimura, 1984). Therefore, 2,3,3′,4,4′,5-hexa-CB was considered to be one of the PCB congeners most causally related to the symptoms of Yusho. Recently, Kashimoto et al. (1987) and Tanabe et al. (1989) reported the presence of highly toxic coplanar PCBs in the tissue of Yusho patients, 3,3′,4,4′,5-penta-CB, a coplanar PCB, which measured 330 and 410 ppt in the intestines and 720 ppt in the adipose tissue, respectively. The levels of three coplanar PCBs were very low in the tissue relative to other PCBs, which were 0.06–0.6% in Yusho patients and 0.03–0.08% in the controls (Kashimoto et al., 1987). However, dioxinlike toxicity of 3,3′,4,4′,5-penta-CB in the adipose tissue was the highest among PCBs as shown in Table 4.7, since its TEQ factor is much higher than those of other PCBs. During the 13 years after 1977, the level of 3,3′,4,4′,5-penta-CB seemed to be lower relative to that of 2,3,3′,4,4′,5-hexa-CB, and the TEQ level of the latter was the highest among the PCBs in the blood of Yusho patients in 1990. Hirakawa et al. (1991) found coplanar PCBs in the subcutaneous adipose tissue resected from Yusho patients in 1986. The concentration of 3,3′,4,4′,5-penta-CB in Yusho patients (N = 7, 70 ppt) was lower than that of the controls (N = 8, 135 ppt). This relatively low level of the toxic PCB congener was also observed in the blood sampled from Yusho patients in 1990 (Masuda et al., 1994) as shown in Table 4.7.

Only several selected congeners of PCBs in the rice oil were retained in the body

Table 4.7. Concentrations of TEQ in the Adipose Tissue and Blood of Yusho Patients and Controls

	TEQ factor	TEQ concentration (ppt)		
		Yusho patient		Control
		Adipose[a] 1977 (Wet basis)	Blood[b] 1990/91 (Fat basis)	Serum[b] 1991/92 (Fat basis)
2,3,7,8-Tetra-CDD	1	0.9	2.25	3.10
1,2,3,7,8-Penta-CDD	0.5	9.0	3.60	4.58
1,2,3,4,7,8-Hexa-CDD	0.1	0.08	0.29	0.43
1,2,3,6,7,8-Hexa-CDD	0.1	16.0	3.57	3.88
1,2,3,7,8,9-Hexa-CDD	0.1	0.08	0.54	0.83
1,2,3,4,6,7,8-Hepta-CDD	0.01	0.06	0.17	0.46
Octa-CDD	0.001	0.23	0.53	1.14
Total PCDDs		26.35	10.94	14.41
2,3,7,8-Tetra-CDF	0.1	4.4	0.0	0.47
2,3,4,7,8-Penta-CDF	0.5	850.0	120.75	8.70
1,2,3,7,8-Penta-CDF	0.05	1.45	0.08	0.04
1,2,3,4,7,8-Hexa-CDF	0.1	130.0	15.25	1.19
1,2,3,6,7,8-Hexa-CDF	0.1	14.0	3.44	0.83
2,3,4,6,7,8-Hexa-CDF	0.1	0.08	0.0	0.0
1,2,3,7,8,9-Hexa-CDF	0.1	—	0.42	0.34
1,2,3,4,6,7,8-Hepta-CDF	0.01	0.95	0.17	0.09
1,2,3,4,7,8,9-Hepta-CDF	0.01	—	0.03	0.0
Octa-CDF	0.001	—	0.0	0.0
Total PCDFs		1,000.9	140.14	11.64
3,3′,4,4′-Tetra-CB	0.0005	0.35	0.01	0.01
3,3′,4,4′,5-Penta-CB	0.1	72.0	4.50	14.15
3,3′,4,4′,5,5′-Hexa-CB	0.01	3.8	1.26	0.92
Total Coplanar PCBs		76.20	5.77	15.07
2,3,3′,4,4′-Penta-CB	0.0001	0.25	0.35	1.00
2,3,4,4′,5-Penta-CB	0.0005	—	1.56	1.38
2,3′,4,4′,5-Penta-CB	0.0001	0.37	1.41	4.23
2′,3,4,4′,5-Penta-CB	0.0001	—	0.0	0.07
2,3,3′,4,4′,5-Hexa-CB	0.0005	16.9	16.69	8.04
2,3,3′,4,4′,5′-Hexa-CB	0.0005	—	4.38	1.81
2,3′,4,4′,5,5′-Hexa-CB	0.00001	—	0.05	0.08
2,3,3′,4,4′,5,5′-Hepta-CB	0.0001	—	0.24	0.09
Total Mono-ortho PCBs		17.52	24.68	16.70
2,2′,3,3′,4,4′,5-Hepta-CB	0.0001	3.5	2.49	1.92
2,2′,3,4,4′,5,5′-Hepta-CB	0.00001	0.7	0.57	0.82
Total Di-ortho PCBs		4.2	3.05	2.74
Total PCBs		97.92	33.50	34.51
Total TEQ		1,125.2	184.6	60.6

Data from [a]: Tanabe et al. (1989), [b]: Masuda et al. (1994)

of the patients as described above and most of the PCB congeners had disappeared from the body within a year or so either due to excretion or by being metabolized into hydroxy and methylsulfone PCBs. The methylsulfone PCBs, which were probably derived from the PCBs ingested with the rice oil, were identified in the tissue of Yusho patients (Haraguchi et al., 1986a; 1986b). The fat basis concentration of methylsulfone PCBs was higher in the lung (0.67 ppm) than in the adipose tissue (0.07 ppm), which contrasted with the concentration of PCBs in the tissue, and measured 0.8 and 1.3 ppm, respectively (Haraguchi et al., 1991). Some congeners of methylsulfone PCBs either induced or changed the enzyme condition in the human body. One of the metabolites, 3-methylsulfone-3′,4,4′,5-tetra-CB, was found to demonstrate a strong inhibition on the aromatic hydrocarbon hydroxylase (AHH) activity which was either previously or simultaneously induced by TCDD in a human lymphoblastoid cell culture (Kiyohara et al., 1990b; Nagayama et al., 1989). The same methylsulfone PCB inhibited methylcholanthrene-induced AHH activity in mouse liver microsomes in arylhydrocarbon (Ah) responsive strains of mouse, whereas it greatly enhanced the same enzymes in Ah non-responsive strains (Kiyohara et al., 1990a). Some 3-methylsulfone PCBs had stronger inductive effects on aminopyrin N-demethylase, 7-ethoxycoumarin O-deethylase and benzo(a)pyrene hydroxylase than the corresponding parent PCBs did, while 4-methylsulfone PCBs had little effect (Kato et al., 1993). Therefore, the health conditions of the patients are possibly altered by the accumulation of methylsulfone PCBs in the tissues. Human serum was found to contain 4-hydroxy-3,5-chlorinated PCBs at a concentration of 0.6 ppb which corresponded to one forth of the PCB level (Kuroki et al., 1993). According to animal experiments in mice and rats, the hydroxy PCBs bound to transthyretin and interfered the thyroxine transport in plasma (Brouwer, 1991), thus the human thyroxin levels are presumed to be disturbed in both the plasma and tissue. In fact, significantly elevated thyroxin levels were actually observed in the plasma of Yusho patients in 1984, 16 years after onset (Murai et al., 1985; 1987).

4.3.2. PCDFs

PCDFs were first identified in the tissue of Yusho patients by Nagayama et al. (1977), after the rice oil was proved to be contaminated with PCDFs in 1975 (Nagayama et al., 1975). Among the PCDF mixtures of tri to hexa chlorinated compounds in the rice oil, penta and hexa chlorinated PCDFs were mainly found to be retained in the tissue. The concentration of PCDFs, determined by comparing the peak heights of gas chromatographic peaks with those of synthesized PCDF mixture, are shown in Table 4.8. In contrast to the PCBs, which were found to be far more abundant in the adipose tissue than in the liver, PCDFs were retained at

Table 4.8. Concentrations of PCBs and PCDFs in the Tissue of Male Patients with Yusho

Case	Age (Y)	Time of death	Tissue	Concentration (ppb)				Ratio (%) PCDF/PCB
				PCB		PCDF		
				Whole basis	Fat basis	Whole basis	Fat basis	
1	17	July 1969	Adipose	1,400	3,400	13	30	0.9
			Liver	50	4,700	25	2,300	50
2	25	July 1969	Adipose	1,300	8,500	6	40	0.5
			Liver	60	5,600	10	1,100	17
3	46	May 1972	Adipose	1,200	2,100	7	1	0.6
			Liver	30	3,500	3	300	10

Data from Nagayama et al. (1977).

very similar levels in these two types of tissue. The property of toxic PCDF congeners to accumulate in the liver was also observed in the liver of monkeys and rats (Kuroki et al., 1980).

As individual PCDF congeners were separately synthesized from chlorophenols and chloronitrobenzenes (Kuroki et al., 1984), PCDF congeners in the tissue were quantitatively determined by the use of the individual congeners as standard compounds. Although Yusho patients ingested more than 40 types of various PCDF congeners with rice oil (Buser et al., 1978), only several particular PCDF congeners were retained in the patients' tissue (Kuroki and Masuda, 1978; Rappe et al., 1979). Most of the retained PCDF congeners had lateral (2, 3, 7 and 8) positions that were chlorinated and all of the congeners apparently excreted had two vicinal hydrogenated C-atoms in at least one of the two rings. The PCDFs chlorinated at lateral positions were identified in the tissue of not only Yusho patients but also Yucheng patients, as shown by the gas chromatograms in Fig. 4.4. However, the most abundant PCDF congener in the liver was 2,3,4,7,8-penta-CDF in Yusho patients and 1,2,3,4,7,8-hexa-CDF in Yucheng patients. Concentrations of the major PCDF congeners identified in the tissue, blood and breast milk of Yusho patients are shown in Table 4.9. Kashimoto et al. (1987) quantified the PCDF congeners with an improved pretreatment method which avoided the destruction of highly chlorinated PCDFs. Therefore, the concentrations of hexa and hepta-CDFs were greater than the other analyses as shown in case 4 in Table 4.9. Since high resolution gas chromatography and high resolution mass spectrometry were used in most of the analyses reported after 1987, the sensitivity increased in the quantification of PCDF congeners and very low levels as ppt are thus noted in Table 4.9. High concentrations of 2,3,4,7,8-penta-CDF and 1,2,3,4,7,8-hexa-CDF up to 25 and 72 ppb were observed in the liver and adipose tissues in 1969, one year after the out-

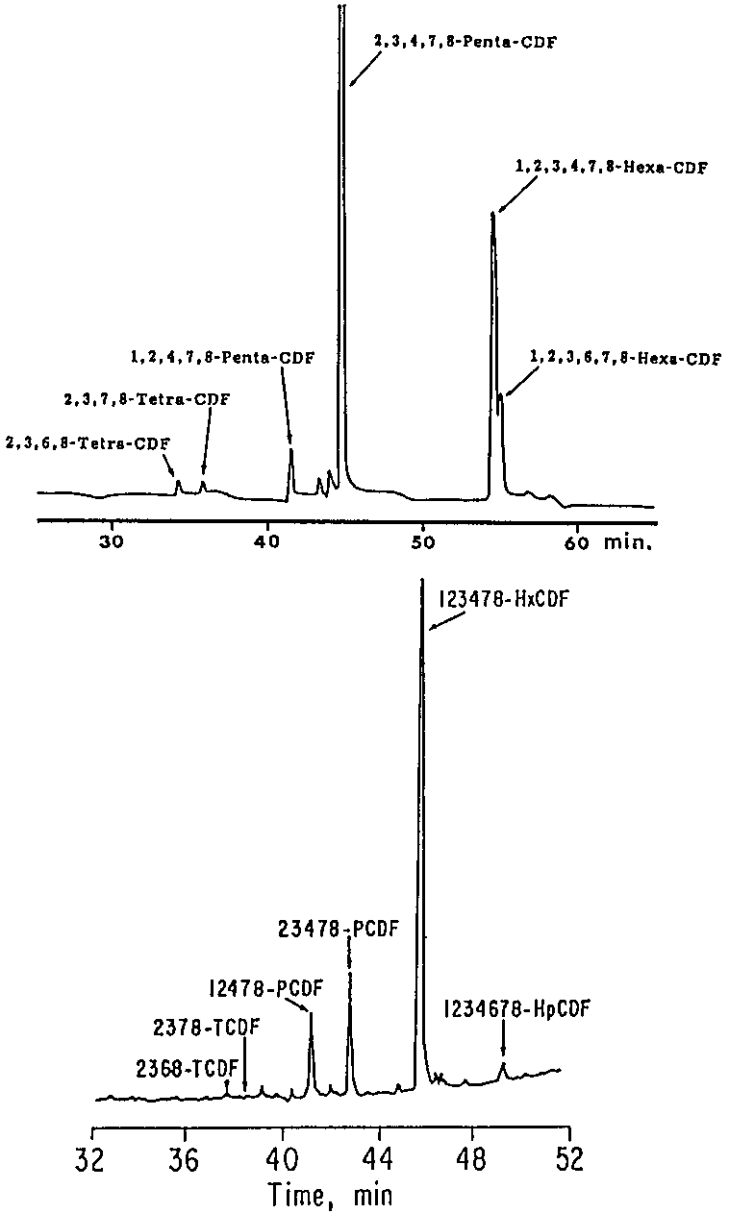


Fig. 4.4. The Gas Chromatograms of PCDFs from the Livers of Yusho (upper) and Yucheng (lower) Patients (Data from Masuda et al., 1985a; Chen and Hites, 1983)

Table 4.9. The Concentrations of PCDF Congeners in the Tissue of Yusho Patients

	Tissue	Year of sampling	PCBs (ppm)	PCDF concentration (ppb, wet basis)			
				2,3,7,8-	2,3,4,7,8-	1,2,3,4,7,8-/ 1,2,3,6,7,8-	1,2,3,4,6,7,8-
1	Liver	1969	1.4	0.3	6.9	2.6	
2	Liver	1969	0.2	0.02	1.2	0.3	
	Adipose		2.8	0.3	5.7	1.7	
3	Liver	1969		1.6	16.4	21.8	9.8
	Adipose			0.5	8.7	7.4	0.5
4	Liver	1969	0.22	0.11	25	72	140
	Intestine		3.6	0.13	5.2	7.2	15
5	Liver	1972	0.03	< 0.01	0.3	0.03	
	Adipose		4.3	nd	0.8	0.2	
6	Adipose	1975	0.2	nd	0.1	0.5	
7	Liver	1977	0.06	nd	1.49	5.31	1.39
	Adipose		3	0.002	1.45	1.99	0.22
	Lung		0.016	0.002	0.365	0.41	0.05
8	Liver	1977	0.036	0.047	2.3	8.4	1.5
	Adipose		1.8	0.044	1.7	1.3	0.095
9	Abscess	1977	2.44	nd	2.76	1.86	0.17
10	Comedo	1982	0.2	nd	0.36	0.39	0.1
11	Uterus	1985	0.005	nd	0.026	0.031	nd
12	Adipose	1986	1.0	nd	0.16	0.066	
	(N = 7)		–5.7	–0.018	–3.0	–1.22	
13	Adipose	1986	1.2	0.018	0.1	0.11	nd
	(N = 6)		–5.7	–0.034	–1.74	–1.44	–0.11
	Blood		0.003		0.0002	0.0002	0.0002
	(N = 6)		–0.022		–0.0066	–0.0061	–0.0006
14	Breast milk	1988		0.0047	0.67	0.347	0.0179
	(N = 7)			–0.024[a]	–0.793[a]	–0.598[a]	–0.063[a]
15	Breast milk	1990		0.0023	0.212	0.1076	0.0023
	(N = 6)			–0.0182[a]	–0.429[a]	–0.177[a]	–0.020[a]
Control							
16	Adipose	1981		0.0027	0.00423	0.0216	0.0043
17	Adipose	1986	0.44	nd	nd	nd	
	(N = 11)		–1.3	–0.019	–0.039		
18	Adipose	1986	0.071	0.004	0.013	0.009	nd
	(N = 3)		–1.3	–0.012	–0.027	–0.035	–0.019
	Blood		0.002		0.00005	0.00004	0.00008
	(N = 3)		–0.004		–0.00009	–0.0001	–0.0001
19	Breast milk	1991		0.0008	0.0037	0.0034	0.001
	(N = 9)			–0.0029[a]	–0.0123[a]	–0.0085[a]	–0.0025[a]

[a]: Fat basis

1, 2, 5, 6: Kuroki and Masuda (1978), 3, 16: Kuroki et al. (1987a), 4: Kashimoto et al. (1987), 7, 11: Ryan et al. (1987), 8: Tanabe et al. (1989), 9, 10: Kuroki et al. (1987b), 12, 17: Iida et al. (1989), 13, 18: Iida et al. (1992), 14, 15, 19: Matsueda et al. (1993).

break of this incident. Thereafter, no such high concentrations of PCDF congeners were observed in the tissue of Yusho patients, except for one case demonstrating 8.4 ppb of hexa-CDF in the liver in 1977. Higher levels than the control levels of PCDF congeners continued up to 1986, when the levels of PCDF congeners were 5 to 65 times higher than the control levels, while the PCB levels in the patients were only two to three times higher than the controls (Iida et al., 1992). It is noteworthy to mention that the PCDF concentrations in the liver were close to those in the adipose tissue, while the PCB concentrations were much lower in the former than in the latter. This relative abundance of PCDF congeners in the liver was also noted in normal persons (Miyata et al., 1977). The pharmacokinetics of PCDFs in humans was studied by monitoring the blood concentrations of the three Yucheng patients from 1980 to 1989 (Ryan et al., 1993). The half-lives of 2,3,4,7,8-penta-CDF, 1,2,3,4,7,8-hexa-CDF and 1,2,3,4,6,7,8-hepta-CDF were 2.1, 2.6 and 2.3 years, respectively, and these concentrations had decreased from 15, 43 and 5 ppb, fat basis, respectively, at the first sampling in 1980. These half-lives of PCDFs were shorter than those of very retainable PCB congeners, such as 2,2′,4,4′,5,5′-hexa-CB (4.28 years) and 2,3,3′,4,4′,5-hexa-CB (4.21 years), in the same Yucheng patients. From ten Yusho individuals with PCDF analyses at 3 to 6 time points from 1982 to 1990, the half-lives for the elimination of 2,3,4,7,8-penta-CDF and 1,2,3,4,7,8-hexa-CDF were estimated to be 9.6 and 7.8 years as medians with the ranges of 5.7–36 years and 4.3–54 years, respectively. Fig. 4.5 illustrates the elimination profiles of PCDF congeners in a Yucheng patient after the onset and a Yusho patient from 14 years after the onset. The figure demonstrates the elimination of PCDFs from the human body from high as 50 ppb to about 1 ppb during 25 years.

A toxicological assessment of individual PCDF congeners was made in rats (Yoshihara et al., 1981). All the PCDF congeners retained in the tissue of Yusho patients exhibited a strong enzyme induction in AHH and DT-diaphorase and caused a marked atrophy of the thymus and a hypertrophy of the liver in rats. PCDF congeners, with at least three chlorine atoms in the ring position at 2, 3, 7 and 8, exhibited a marked increase in the enzyme induction. 2,3,7,8-tetra-CDF and 2,3,4,7,8-penta-CDF significantly induced AHH and DT-diaphorase even at a single dose of 1 μg/kg. Safe and Phil (1990) summarized the structure of PCDFs and their toxicity in animals as follows: the AHH inducing activities of structural variation in PCDF congeners were linearly correlated with the thymic atrophy, body weight loss and immunotoxicity induced by the PCDF congeners. 2,3,4,7,8-penta-CDF was the most active compound among the PCDF congeners regarding both enzyme induction and toxicity in animals. Therefore, 2,3,4,7,8-penta-CDF is considered to be the most important etiologic agent for the Yusho symptoms. A

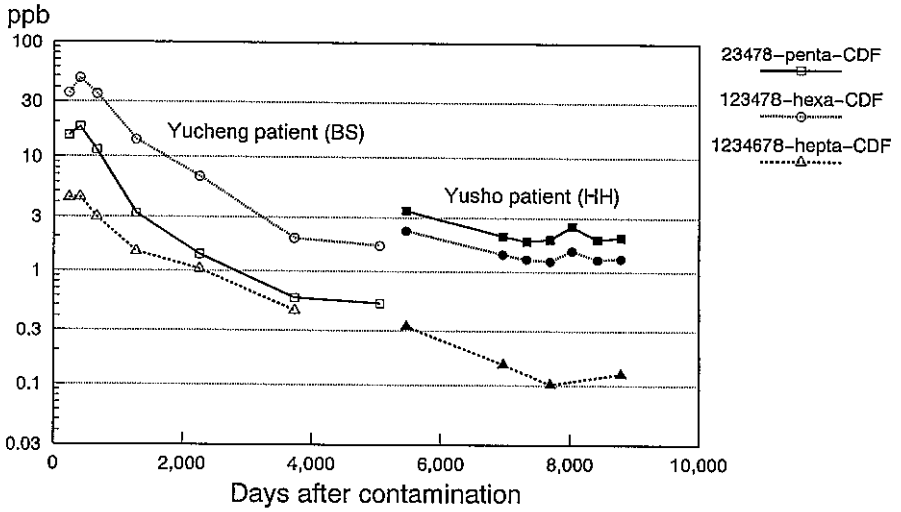


Fig. 4.5. The Elimination of PCDF Congeners in the Blood of Yusho and Yucheng Patients (Fat basis)

The half-lives of 2,3,4,7,8-penta-CDF, 1,2,3,4,7,8-hexa-CDF and 1,2,3,4,6,7,8-hepta-CDF were 2.4, 2.6 and 2.9 years in Yucheng and 13.4, 12.0 and 6.0 years in Yusho, respectively.

concentration of 2,3,4,7,8-penta-CDF was 1,350 ppb in the rice oil consumed by Yusho patients (Table 4.2) and the average total intake of the rice oil per capita was 688 ml (Table 4.3). Then 854 μg of 2,3,4,7,8-penta-CDF was contained in the 688 ml rice oil. The total intake of this congener was calculated to be 14 μg/kg, assuming the body weight of the patients to be 60 kg. This dose exceeded by more than ten times the enzyme inducing dose of 1 μg/kg in rats. Kashimoto et al. (1981b) proposed that PCDFs were the major pathogenic substances in the development of Yusho, since the toxic PCDFs accumulated in the tissues and liver of Yusho patients but not in those of workers with occupational PCB poisoning. In a statistical study of the correlation between PCB, PCQ or PCDF concentrations in the adipose tissue and the clinical findings, such as headache, acne-like eruptions, Meibomian gland disorders and others, in Yusho patients, the PCDF concentration in the subcutaneous adipose tissue and the total score of clinical findings showed the highest correlation coefficient in female patients (Nakagawa and Takahashi, 1991).

The relative toxicities of PCDD and PCDF congeners to 2,3,7,8-tetra-CDD (TEQ factor) have been estimated at many research organization (Barnes et al., 1986; Ahlborg et al., 1988; Kutz et al., 1990). Using international TEQ factors for PCDDs and PCDFs (Kutz et al., 1990) and WHO TEQ factors for PCBs (Ahlborg et al., 1994), the toxic contributions of the PCDDs, PCDFs and PCBs retained in a Yusho patient were calculated and are shown in Table 4.7. Eighty-nine and

seventy-six percent of the total TEQ concentrations were considered to be due to the PCDFs in the adipose tissue and in the blood, respectively, while 76% and 65% of the total toxicity were attributed to a single congener of 2,3,4,7,8-penta-CDF. However, in the serum of control subjects, 3,3′,4,4′,5-penta-CB was considered to contribute the most to the total toxicity (23%), well surpassing the contributions by other congeners of PCDDs, PCDFs and PCBs.

4.3.3. PCQs and PCTs

PCQs in the tissue of Yusho patients mainly consisted of hepta, octa and nona chlorinated compounds and showed very broad peaks on their gas chromatograms (Kashimoto et al., 1981a). The quantification of such minute amounts of PCQs eventually became feasible by perchlorination of the PCQ mixture to six skeletal isomers of octadecachloroquaterphenyls (Maeda and Kashimoto, 1978). The concentrations of PCQs in the tissue of Yusho patients were thus determined by the method. Table 4.10 shows the concentrations of PCQs and PCBs in the adipose tissue, liver, blood, buccal mucosa, hair and skin lipid of the Yusho patients and controls. In typical Yusho patients, who had type A PCBs, the PCQ concentrations in the tissue and blood were approximately the same or two to four times lower than the PCB levels. The concentrations of PCQs seemed to decrease with time, as the high concentration of PCQs (2,400 ppb) in the adipose tissue in 1969 was not observed in the same tissue in 1972 or 1986 when the level of PCQs in the tissue was on average 207 ppb. However, the PCQ concentrations in the tissue and blood of patients were always much higher than the corresponding concentrations in the normal controls up to the present time. In 1986, 18 years after the onset, the PCQ levels in the adipose tissue and blood of patients were still more than 100 times higher than the corresponding levels in the controls. The levels of PCQs in the blood of the control persons were mostly lower than the detection limit of 0.02 ppb. In contrast, the PCB levels in Yusho patients were always two to three times higher than those of the controls. When the patients were classified into three types, consisting of A (Typical Yusho), B and C (Similar to control) based on their gas chromatographic peak patterns of PCBs, the concentrations of both PCQs and PCBs in the blood descended in accordance with the types (A, B and C) as shown in Table 4.10. The coefficients of correlations between the concentrations of PCQs and PCBs were higher than 0.8 and those between the PCQ concentrations and the PCB peak pattern were higher than 0.6 from 1973 to 1980 (Kataoka et al., 1983). Japanese workers who had been occupationally exposed to PCBs did not show any detectable levels of PCQs in the blood although their PCB levels were as high as 33 ppb (Kashimoto et al., 1981b). Since the PCQ concentrations were reflective of the amount of rice oil intake, the blood PCQ levels have thus been adopted as one of

Table 4.10. The Concentrations of PCBs and PCQs in the Tissue and Blood of Yusho Patients

	Tissue	Time of sampling	Type of PCB	Concentration (whole basis, ppb)	
				PCBs	PCQs
1	Adipose	1969	A	5,091	2,400
	Liver		A	226	218
	Adipose	1972	A	6,091	1,444
	Liver		A	69	144
	Intestine	1975	A	3,472	1,770
	Liver		A	114	52
	Intestine	1977	A	3,630	1,125
	Liver		A	68	27
	Intestine	1977	B	1,273	25
	Liver		C	18	1
2	Blood (n = 29)	1979	A	7.3 ± 4.5	3.04 ± 2.11
	Blood (n = 15)		B	5.4 ± 3.6	1.39 ± 1.34
	Blood (n = 8)		C	2.7 ± 1.2	0.28 ± 0.19
3	Blood (n = 56)	1979		5.6 ± 4.4	2.0 ± 2.0
4	Blood (n = 11)	1979	A	6.2 ± 4.9	0.09 – 5.85
	Blood (n = 20)		B/C	2.7 ± 1.4	< 0.02 – 0.42
5	Blood (n = 31)	1979	A	9.6 ± 6.4	2.9 ± 2.3
	Blood (n = 4)		B	4.7 ± 5.2	2.0 ± 3.4
	Blood (n = 29)		C	2.6 ± 1.1	0.02 ± 0.03
6	Blood (n = 10)	1979		5.3 ± 3.4	3.9 ± 2.7
7	Blood (n = 91)	1979		0.6 – 18	< 0.02 – 3.2
8	Blood (n = 194)	1982		5.2	0.50
9	Buccal mucosa (n = 27)	1983		279 ± 41	66 ± 13
	Blood (n = 25)			7.2 ± 0.82	0.79 ± 0.13
10	Blood (n = 230)	1983		5.1 ± 3.9	0.65 ± 0.98
	Blood (n = 199)	1984		4.3 ± 3.0	0.57 ± 0.83
11	Adipose (n = 11)	1984		1,579 ± 657	207 ± 112
	Blood (n = 10)			6.45 ± 2.38	1.39 ± 0.64
12	Adipose (n = 11)	1986		1,579 ± 627	207 ± 106
	Blood (n = 32)			5.36 ± 2.51	1.34 ± 1.11
	Hair (n = 13)			28.9 ± 18.1	0.53 ± 0.36
13	Blood (n = 27)	1988/89		6.41 ± 3.17	0.61 ± 0.52
	Hair			25.9 ± 19.3	0.44 ± 0.38
14	Blood (n = 124)	1988		5.4 ± 5.0	0.34 ± 0.46
	Blood (n = 135)	1989		4.6 ± 3.3	0.54 ± 0.62
	Blood (n = 150)	1990		4.5 ± 2.8	0.47 ± 0.52
15	Blood (n = 22)	1990		5.4 ± 2.5	0.65 ± 0.55
	Skin lipid			581 ± 325	29 ± 12.9
	Blood (n = 16)	1991		6.6 ± 2.8	1.31 ± 0.86
	Skin lipid			676 ± 309	25.9 ± 11.7

Table 4.10. (Continued)

	Tissue	Time of sampling	Type of PCB	Concentration (whole basis, ppb) PCBs	PCQs
	Blood (n = 23)	1992		4.8 ± 2.5	0.57 ± 0.36
	Skin lipid			863 ± 463	53.4 ± 23.7
Control					
1	Adipose (n = 3)	1978		248 – 1,478	1.3 – 2.7
	Liver (n = 3)			18 – 71	0.6 – 0.8
2	Blood (n = 29)	1979		2.3 ± 1.5	< 0.02
3	Blood (n = 60)	1979		2.0 ± 1.3	< 0.02
4	Blood (n = 18)	1979		3.3 ± 1.2	< 0.02
5	Blood (n = 23)	1979		2.9 ± 1.0	0.02 ± 0.03
6	Blood (n = 10)	1979		3.4 ± 1.3	< 0.02
9	Buccal mucosa (n = 7)	1983		64.9 ± 16	< 4
	Blood (n = 7)			1.86 ± 0.13	< 0.02
11	Adipose (n = 10)	1984		410 ± 280	1.74 ± 1.27
	Blood			1.2 ± 0.63	< 0.02
12	Adipose (n = 40)	1986		778 ± 670	1.4 ± 0.96
	Blood (n = 32)			2.43 ± 1.74	< 0.02
	Hair (n = 19)			8.06 ± 5.60	< 0.1
13	Blood (n = 22)	1988/89		2.25 ± 0.92	< 0.02
	Hair			9.41 ± 5.55	< 0.10
15	Blood (n = 20)	1990/91		2.1 ± 0.7	< 0.02
	Skin lipid			324 ± 104	< 10

1,2: Kashimoto et al. (1981a)
3: Kashimoto et al. (1981b)
4: Takamatsu et al. (1981)
5: Iida et al. (1981)
6: Iida et al. (1985b)
7: Baba et al. (1979)
8: Hiraki et al. (1982)
9: Okumura et al. (1987)
10: Masuda et al. (1984)
11: Ohgami et al. (1987b)
12: Ohgami et al. (1989)
13: Ohgami et al. (1991)
14: Rikioka et al. (1990)
15: Ohgami et al. (1993)

the criteria for the diagnosis of Yusho together with the level and type of PCBs (Kuratsune et al., 1987).

PCQs increased the hepatic microsomal drug-metabolizing enzyme activity and decreased the adrenal corticoid level in rats. However, the degree of these activities was far less than that of PCBs or PCDFs (Hori et al., 1986). Some skeletal congeners of PCQs administered at a dose of 10 mg/rat depressed the DT-diaphorase activity and 4,4′-PCQ showed significant atrophy of the thymus in rats (Takenaka et al., 1985). In a group of female cynomolgus monkeys given PCQ at a dose 5 mg/day for 20 weeks, immunosuppression, enlarged liver, and an excessive enlargement of liver cells were observed. However, the monkey fed PCQs at 0.5

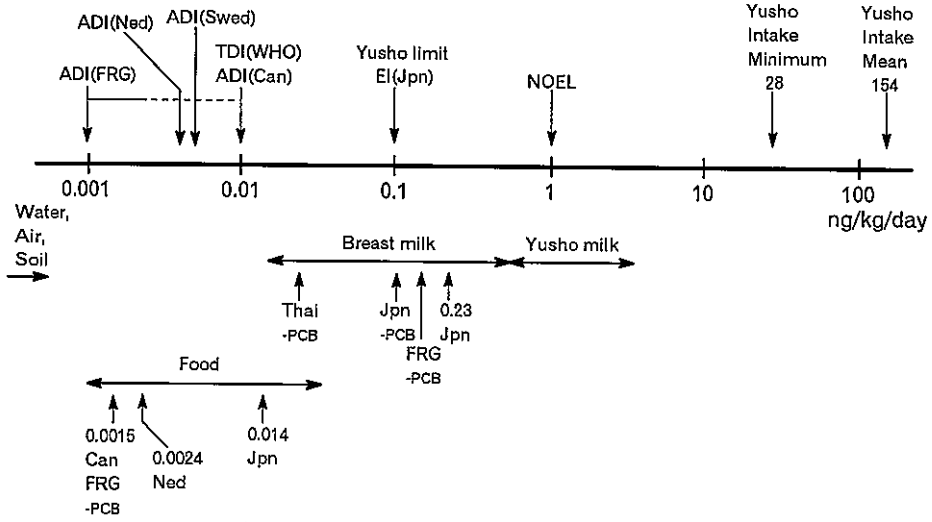
mg/day showed no changes in comparison to the control group (Hori et al., 1982). As PCQs have been found to be much less toxic than PCBs in rats and monkeys (Kunita et al., 1985), the major toxic agents for Yusho were considered to be PCDFs but not PCQs, even though PCQs were actually ingested by the patients with rice oil and retained in their bodies for more than 20 years.

PCTs were identified in the blood of 10 Yusho patients in Fukuoka, with the mean concentration and SD measuring 0.62 ± 0.59 ppb and ranging from 0.16 to 2.2 ppb. These levels were a little less than those in 10 controls, which were 1.2 ± 0.78 ppb, and ranged from 0.2 to 2.69 ppb (Iida et al., 1985a).

4.4. Risk Assessment of PCDD/PCDFs and PCBs from Yusho

The daily TEQ intakes by PCDD/PCDFs have been estimated in Japan (Ono et al., 1987), Canada (Birmingham et al., 1989a; Birmingham et al., 1989b), Germany (Beck et al., 1992; Fürst et al., 1990), Italy (Di Domenico, 1990) and Netherlands (Theelen et al., 1993), and were calculated to be 1.3, 1.52/1.2, 1.3/1.2, 3.8–7.0 and 1 pg/kg/day, respectively. More than 90% of the total TEQ intakes were from foods and TEQ sources other than foods, such as air, water, soil and others, accounted for less than 10% of the total daily intakes. Takayama et al. (1991b) analyzed fish taken off the Japanese coast and fish on the market for PCDDs, PCDFs and coplanar PCBs, and found that the concentrations were 1.60–4.3, 2.40–7.6 and 290–1,500 ppt for the coastal fish and 0.04–1.5, 0.01–3.7 and 0.4–170 ppt for the fish on the market, respectively. The total TEQ levels (mean $\pm$ SD) of PCDD/PCDFs and coplanar PCBs were estimated to be 0.87 ± 0.28 and 9.4 ± 7.3 ppt respectively for the coastal fish and 0.33 ± 0.25 and 0.22 ± 0.24 ppt for the fish on the market. It is noteworthy that the TEQ level of coplanar PCBs was greater than that of PCDD/PCDFs. The intake of PCDDs, PCDFs and coplanar PCBs through foods were investigated by examining about one hundred kinds of foods in Osaka (Takayama et al., 1991a; Miyata, 1991). The personal daily intakes of PCDDs, PCDFs and coplanar PCBs through foods were estimated to be 2.41, 2.16 and 51 ng, respectively, while the personal daily TEQ intakes from the three groups of toxic chemicals were calculated to be 40, 135 and 660 pg, respectively. It is noteworthy that the ingestion of fish and shellfish was responsible for 60% of the total TEQ exposure from food. The daily TEQ intakes per kg body weight were calculated to be 3 and 11 pg/kg/day from PCDD/PCDFs and coplanar PCBs, respectively, assuming a typical body weight of 60 kg. The Japanese intake of TEQ from coplanar PCBs (11 pg/kg/day) was much higher than that of the Netherlanders, whose TEQ intakes from coplanar PCBs were estimated to be 1.4 and 2.5 pg/kg/day as a median and a 95 percentile, respectively (Theelen et al.,

1993). The World Health Organization (WHO) (Yrjäheikki, 1989) investigated the PCDD/PCDF levels in the breast milk collected from various countries, finding the TEQ levels to range from 5 to 40 ng/kg fat. Relatively high levels of TEQ were determined in the breast milk from Central European countries and South Vietnam, followed by Japan, Nordic countries, Canada and USA and then by East European countries, Southeast Asian countries and New Zealand. Breast milk-fed babies in the world were estimated to consume 24–185 pg/kg/day of TEQ, with a daily consumption of breast milk assumed to be 150 ml/kg body weight. TEQ concentrations from PCDD/PCDFs in 728 breast milk samples in Germany were determined to be 30.6 pg/g fat on average, ranging from 5.6 to 87.1 pg/g fat (Somogy and Beck, 1993). The daily intakes of TEQ by the babies were estimated from 138 to 392 pg/kg, when the fat content in the milk was presumed to be 3%. These values of TEQ, however, were estimated from only PCDDs and PCDFs, but did not include PCB toxicities. Some Japanese babies were thus estimated to ingest higher levels of TEQ (100–530 pg/kg/day) from PCDD/PCDFs and coplanar PCBs through breast feeding, more than 60% being attributed to TEQ of coplanar PCBs. As the breast milk from Yusho patients was estimated to contain TEQ up to 539 pg/g fat, attributing 82% of the toxicity from PCDFs, a baby might consume as much as 3.3 ng/kg/day of TEQ through the breast milk from a Yusho mother, if the baby consumed 150 ml/kg/day of breast milk (Matsueda et al., 1993). Acceptable daily intake (ADI) (Barns, 1989; Ahlborg, 1992) or tolerable daily intake (TDI) (Ahlborg et al., 1992) for 2,3,7,8-tetra-CDD (1–10 pg/kg/day) was established by the Netherlands, Federal Republic of Germany, Canada, Sweden and WHO, based on from 100–1,000 times the safety margin range of the no-effect level (NOEL) of 1 ng/kg/day in animal experiments (Kociba and Schwetz, 1982). When the dioxin risk of municipal incinerators was first evaluated by the Japanese government in 1984, the dioxin intake of 100 pg/kg/day was used as an evaluating indicator (EI) for assessing the incinerators (Suzuki et al., 1984). The values of ADI, TDI, EI and the personal intakes of TEQ are illustrated in Fig. 4.6. The Yusho patient's average and minimum daily intake of TEQ are also calculated in Fig. 4.6 to better understand the difference in the TEQ intakes of Yusho patients and normal persons. The Yusho limit is estimated to be 0.1 ng/kg/day, because, by the daily intake of 0.1 ng/kg for a normal 60-year lifetime, the total intake of TEQ would eventually attains a minimum Yusho intake of 0.11 mg (Table 4.3). If the intake was less than that, the TEQ accumulation in a person would never exceed the minimum Yusho intake in a lifetime. When the average daily and the minimum intakes of TEQ by Yusho patients (154 and 28 ng/kg/day, respectively) are compared with the average daily intakes of general population (1–19 pg/kg/day), there is a difference of more than three orders of magnitude. However, as the periods of ingestion differ greatly for



-PCB: This figure does not include the TEQ concentration from PCBs.

Fig. 4.6. The Regulation and Personal Intake of TEQ

the two groups, 71 and 135 days for Yusho patients and lifelong for the general population, the TEQ levels of PCBs, PCDFs and PCDDs remaining in the Yusho patients were only 3–200 times higher than those of control persons, 185–2,000 pg/kg fat of TEQ in the serum and adipose of Yusho patients (Table 4.7) versus 10–60 pg/g fat of TEQ for the breast milk of general population (Somogy and Beck, 1993). In 1991, 23 years after the onset, the total TEQ level in the blood of a Yusho patient, whose blood PCB was of the typical Yusho type, was only three times higher than that of the control serum, although his level of PCDFs was 12 times higher than the control one (Table 4.7). When the intakes of nursing babies are compared to those of Yusho patients, the intakes of TEQ by breast milk-fed babies of the general population, 530 pg/kg/day at the highest, are 53 or more times lower than that of Yusho patients, 28 ng/kg/day at the smallest intake. Moreover, the feeding periods of the toxic chemicals are very close to each other in the two groups, being several months for babies of general population and from one to five months for Yusho patients. As the intake of TEQ of 28 ng/kg/day level was the lowest dose to cause Yusho, the intake of one or two orders of magnitude lower than this level might cause mild Yusho symptoms, such as the signs mediated by the receptor binding and enzyme induction caused by exposure to a baby. Besides the severe dermal and ocular symptoms, Yusho patients have also demonstrated from various other symptoms. Most symptoms were observed in the early stages of Yusho, while significantly elevated levels of serum thyroxins (Murai et al., 1987), serum triglyceride (Okumura et al., 1974; Hirota et al., 1993) and lymphocyte

AHH (Nagayama et al., 1987) persisted in the Yusho patients for as long as 15–20 years after the initial exposure to PCBs and PCDFs. Pluim et al. (1993) investigated the effect of PCDD/PCDFs on thyroid hormone concentrations in humans. Thirty-eight healthy breast-fed infants were divided into two groups according to the PCDD/PCDF concentrations in the milk fat of their mothers. The total thyroxin concentrations in the blood were found to be significantly higher in the high exposure group at birth and at the ages of 1 and 11 weeks. The plasma total thyroxin elevation in newborn infants is postulated to be the result of an effect of the thyroxin hormone regulation system. The TEQ intake of the baby of high exposure group was estimated to be 170 pg/kg/day from the PCDD/PCDF concentrations in the milk fat of the high exposure group. This TEQ intake is smaller than the minimum intake of Yusho (28 ng/kg/day) by about two orders of magnitude, and was actually found to induce thyroxin hormone elevation in newborn infants.

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