Role of Polychlorinated Dibenzofuran in Yusho (PCB Poisoning)

TAKASHI KASHIMOTO, Ph.D.
HIDEAKI MIYATA, Ph.D.
SHINJI KUNITA, Ph.D.
Osaka Prefectural Institute of Public Health
1-3-69, Nakamichi, Higashinari-ku
Osaka, Japan

TA-CHENG TUNG, M.D., Ph.D.
SHU-TAO HSU, M.O., Ph.D.
KING-JEN CHANG, M.D.
SHU-YING TANG, M.D.
Department of Biochemistry
National Taiwan University
College of Medicine
1 Jen-Ai Road
Taipei, Taiwan

GEN OHI, M.D., Ph.D.
JUNICHI NAKAGAWA
SHUN-ICHI YAMAMOTO, M.D , Ph D.
Department of Hygiene and Preventive Medicine
Faculty of Medicine
University of Tokyo
7-3-1, Hongo, Bunkyo ku
Tokyo 113, Japan

ABSTRACT. In the blood of 15 patients with Yusho or "polychlorinated biphenyl poisoning" that occurred in 1979 in Taiwan, was found polychlorinated dibenzofurans (14 of 15) and polychlorinated quaterphenyls (15 of 15), as well as polychlorinated biphenyls (15 of 15). The mean concentration ratio of these substances was approximately 1 : 160 : 500. Based on the following evidence, we propose that polychlorinated quaterphenyls were major pathogenic substances in the development of Yusho: (1) Clinical manifestations and course of Yusho patients are disproportionately severe and persistent for the observed blood levels of polychlorinated biphenyls, while patients who were occupationally exposed to pure polychlorinated biphenyls take characteristically mild and benign clinical course despite polychlorinated biphenyl levels often much higher than those noted in Yusho patients; (2) Polychlorinated debenzofurans show a marked tendency to accumulate in the liver, which might explain frequent presence of jaundice and other abdominal symptoms in Yusho, which are, again, not observed in those with occupational polychlorinated biphenyl poisoning; (3) Toxicity of polychlorinated dibenzofurans is a hundred to ten thousand times greater than that of polychlorinated biphenyls and polychlorinated quaterphenyls in animal experiments.

"YUSHO" is a poisoning characterized clinically by acne-like dermal lesions and a variety of constitutional symptoms. In 1968, an outbreak occurred in western parts of Japanese Archipelago and as a result, more than a thousand persons suffered. The outbreak was initially thought to result from polychlorinated biphenyl (PCB) poisoning[1-3] caused by the consumption of rice oil contaminated with Kanechlor 400, a heat transfer medium.[3] Subsequently, the "toxic" rice oil was also found to contain elevated levels of polychlorodibenzofurans (PCDFs)[4]; highly toxic contaminants of PCBs[5]; and polychlorinated quaterphenyls (PCQs)[6]—toxicologically ill-defined, but substances perhaps equally toxic as PCBs.[7,8] Both PCDFs and PCQs can be generated when PCBs are heated.[9] The ratio of PCDFs or PCQs to PCBs can become high during the deodorization process of rice oil under high temperature and reduced pressure.[10] Toxicolog-

MONS 209758

ical contribution of PCDFs in Yusho was further implicated by discovering that the liver of Yusho patients who died from other causes after the outbreak of the poisoning contained PCDFs one thousand times higher than that found in normal populations.[11,12] A recent analysis of the blood of Yusho patients conducted 11 yr after the Yusho outbreak, detected PCQs but not PCDFs, suggesting the role of PCQs as a suitable marker for the past episode of exposure to toxic oils.[13]

During the spring and summer of 1979, cases of peculiar skin disease characterized mainly by acne; cheese-like discharge from the Meibomian gland; pigmentation of the skin, gingiva and the nail beds; and abdominal pains were reported in two prefectures in the middle part of Taiwan, Republic of China.[13] Samples of suspected "toxic" rice oil delivered to us in the autumn were found to contain PCBs. Gas chromatographic patterns of these samples were similar to those found in Japanese "toxic" rice oils. All blood samples from the patients likewise contained PCBs ranging from 54 to 136 ppb (unpublished data). The number of patients exceeded 1,800 at the end of 1980; a detailed epidemiological and clinical report will be made elsewhere.

Of the 15 patients in the present study, PCDFs were found in the blood of all except for one who had mild clinical manifestations. In this study strong evidence suggesting a major role of PCDFs in developing clinical manifestations and prognosis which characterize Yusho (not mere "pure PCB" poisoning) is presented.

METHODS AND MATERIALS

Three samples of "toxic" oil, and 10-ml blood samples were collected from 15 patients with various clinical severities, who were pupils living in the dormitories on the school campus. Every meal was provided by the school. The pupils were estimated to have consumed the contaminated oil in the school chow served during a 4-month period, up to 6 months prior to the time of blood sampling.

Analytical methods for PCBs, PCQs, and PCDFs. Ten milliliters of blood were saponified with 20 ml of 2 *N* KOH ethanol solution for 1 hr under refluxing. After addition of 20 ml water, the saponified sample was extracted with 30 ml of *n*-hexane. The *n*-hexane layer was then washed with 50 ml of water, dried over anhydrous sodium sulfate and concentrated to approximately 5 ml in a K.D. concentrator. The *n*-hexane extract was put on a Florisil column (20 g of 60–100 mesh Florisil, activation at 130°C overnight; 1.8 cm ID) and eluted successively with 130 ml of *n*-hexane, 50 ml of 2% diethyl ether in *n*-hexane, 100 ml of 5% diethyl ether in *n*-hexane, 250 ml of 50% diethyl ether in *n*-hexane, and 250 ml of acetone at a rate of approximately 2 ml/min. The first and second eluates were combined, concentrated, and analyzed for PCBs by gas chromatography (GC) on a Varian Aerograph 2100 machine equipped with ^{63}Ni-ECD, as described previously.[1]

Polychlorinated quaterphenyls eluted in the third fraction were chlorinated exhaustively with antimony-pentachloride, cleaned up on an alumina column to remove impurities, and determined by a Shimadzu GC-6A gas chromatograph fitted with ^{63}Ni-ECD.

Polychlorinated dibenzofurans were eluted in the fourth and fifth fractions from a Florisil column, and after careful evaporation to dryness were subjected to a final clean-up on an alumina column (20 g alumina, Merck Co., Ltd., Art. No. 1077; 1.5 cm ID). To achieve more efficient removal of impurities, 200 ml of 2% methylene chloride in *n*-hexane was used as the first eluate. Polychlorinated dibenzofurans were recovered with 200 ml of 20% methylene chloride in *n*-hexane. The eluate was evaporated to dryness and the residue was dissolved in *n*-hexane and analyzed by GC using a Varian Aerograph 2100 gas chromatograph machine with ^{63}Ni-ECD. Gas chromatograph conditions were as follows: column, 2 m X 2 mm glass column packed with 2% OV-17 on Gas Chrom Q (100/120 mesh); injection, detection, and column temperatures, 255, 320, and 250°C, respectively; carrier gas, N_2 (30 ml/min). Polychlorinated dibenzofurans were estimated by comparing the peak heights of the gas chromatogram with those of PCDF standard, assuming that PCDF isomers with the same number of chlorine atoms all had the same sensitivity, regardless of the sites of chlorine substitution (Fig. 1).

Polychlorinated dibenzofurans in the samples were identified (Fig. 2) by gaschromoto-masspectrographic (GC–MS) methods, using a JEOL-20KP gas chromatograph-JMS-300 mass spectrometer with a computer system. The conditions were as follows: column, 2 m X 2 mm glass column packed with 3% OV-17 on Gas Chrom Q (80/100 mesh); inlet and column temperatures, 300 and 300°C, respectively; ionizing energy, 70eV; acceleration, 3.0 KV, carrier gas, He (2.0 kg /cm^2).

GC-MS Chromatograms at m/z (M^+) and ($M^+ - 63$) of PCDF in "toxic" oil in Taiwan (Fig. 2). The PCDF fraction of the oil was injected into a GC-MS using a JEOL-20KP gas chromatograph-JMS-300 mass spectrometer with a computer system. The GC-MS conditions were as follows: column, 2 m X 2 mm glass column packed with 3% OV-17 on Gas Chrom Q (80/100 mesh); inlet and column temperatures, 300 and 300°C, respectively; ionizing energy, 70 eV; acceleration, 3.0 KV; carrier gas, He (2.0 kg/cm^2).

RESULTS

The gas chromatographic patterns of PCDFs (Fig 1) extracted and purified from toxic oil samples in both countries show fair resemblance in peaks 10 through 16, indicating similarity of constituent isomers between the two. A GC–MS confirmation was also done on each PCDF peak for toxic oil and Taiwanese blood samples. A similar relationship was likewise present between the blood of a Yusho patient (No. 11) in Taiwan and the liver of a Japanese patient. A GC–MS chromatographic analysis of the toxic oil from Taiwan indicates that peaks 5 through 14 represent 4–6 chlorinated dibenzofurans with molecular ion (M^+) and fragmental ion ($M^+ - 63$) resulting from the deletion of COCl (Fig. 2).[5]

The blood concentrations of PCBs, PCQs, and PCDFs found in patients from Taiwan and Japan, Japanese workers occupationally exposed to PCBs, and control Japanese subjects are shown in Table 1. Polychlorinated dibenzofurans were detected in all the Taiwanese patients (6 months after termination of toxic oil ingestion), except for one who had

MONS 209759

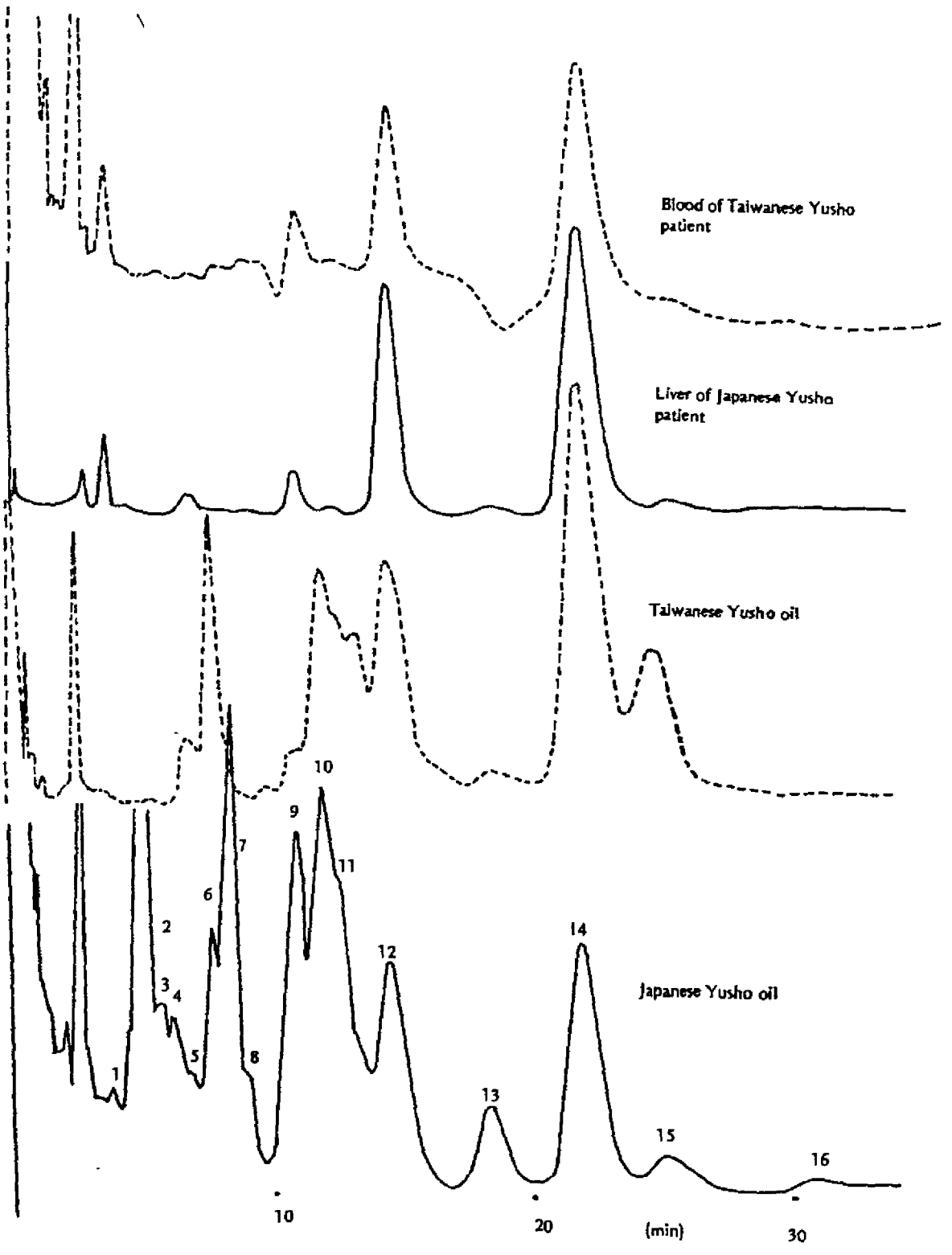

Fig. 1. Gas chromatograms of PCDF fraction: (A) blood of Taiwanese "Yusho" patient (No. 11), (B) liver of Japanese "Yusho" patient, (C) "toxic" oil in Taiwan; (D) "toxic oil in Japan. Compound of each peak shown in gas chromatograms was confirmed as follows by GC-MS analysis: peak No. 2, tetrachlorobiphenyl; peak Nos. 3–7, tetrachlorodibenzofuran; peak Nos. 8, 9, 11, and 12, pentachlorodibenzofuran; peak Nos. 13, 14, and 16, hexachlorodibenzofuran.

MONS 209760

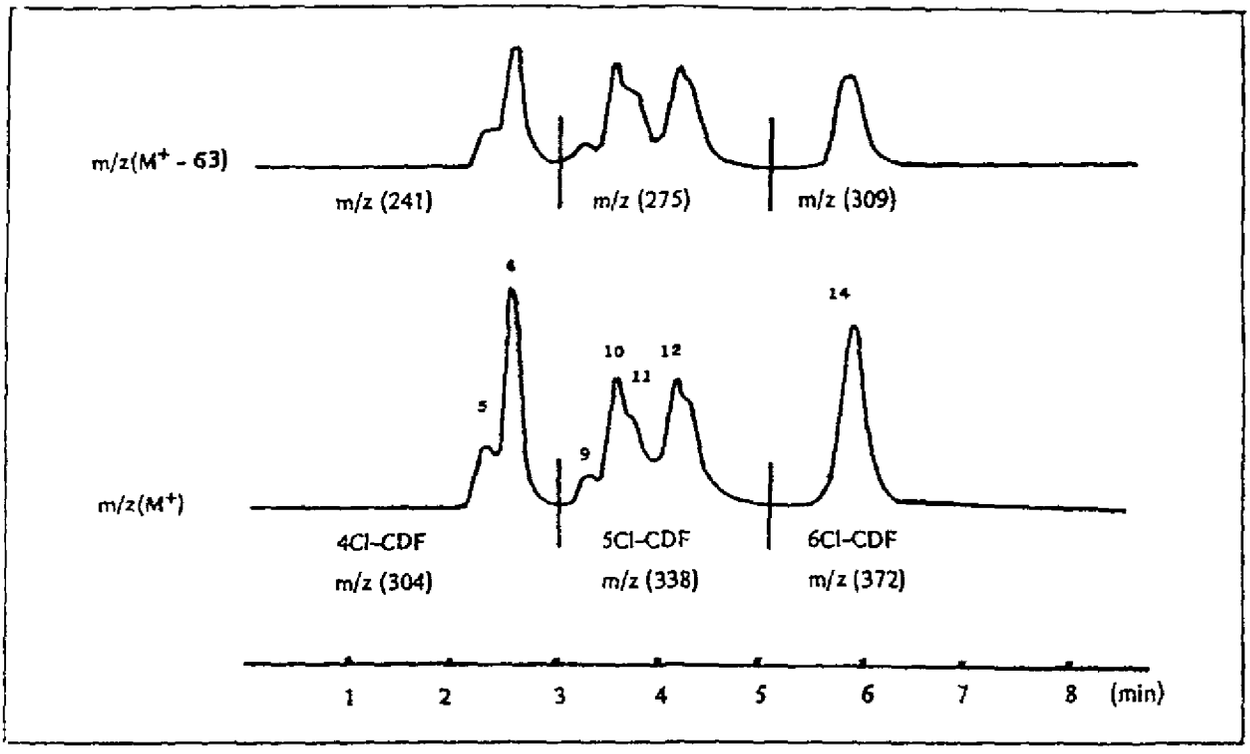

Fig. 2. GC-MS chromatograms at m/z (M^+) and (M^+ - 63) of PCDF in "toxic" oil in Taiwan. Each figure shown in the lower mass chromatogram was equal to the peak number in the gas chromatogram of Figure 1.

the mildest clinical manifestations and corresponding low levels of PCBs and PCQs.

DISCUSSION

Although attempts to identify PCDFs in the blood of Japanese Yusho patients 11 yr after the outbreak failed, PCDF concentrations exceeding those found in the present cases, in relation to PCB levels, would have been found shortly after the outbreak. Japanese toxic oils have a greater PCDF : PCB ratio, and roughly 10 times higher contamination with both substances than Taiwanese toxic oils (Table 1). However, a direct comparison between the two outbreaks with regard to the intake of toxic substances and severity of poisoning is impossible because only some "toxic" lot samples from the Taiwanese incident are available for analysis.

What do the PCDF levels noted in our study signify? We believe that the following evidence strongly suggests that PCDFs were mainly responsible in the pathogenesis of Yusho.

(1) Those individuals occupationally exposed to pure PCBs are usually symptom-free; sometimes mild dermal manifestations such as chloracne accompanied by mild constitutional symptoms occur.[14-16] This occurs despite that blood concentrations of PCBs in individuals 7 yr after leaving their workplace are equal to or greater by one digit than concentrations observed among Taiwanese patients in the current study (Table 1). When occupational exposure to PCBs was terminated, their dermal lesions quickly improved[15] in contrast to persistent clinical signs and symptoms among Yusho patients that have lingered more than 10 yr.[19] It should be again emphasized that most Japanese Yusho patients now have blood PCB levels that barely exceed those of the general population.[12]

(2) PCDFs show strong hepatotropism,[17] as do their analogue[18]—polychlorinated dibenzodioxines. While the PCDF to PCB ratio in the blood of Taiwanese patients is approximately 1 : 500, which is similar to that found in Taiwanese toxic oils (i.e., 1 : 300), the ratio found in the liver of Japanese patients, who succumbed 1 to 9 yr after the incident, is 1 : 5—a value far above the ratios noted in the Japanese toxic oils [1 : 100 in average (Table 1)]. Clinically, no abdominal pains nor jaundice have been described among the three series of workers occupationally exposed to PCBs,[14-16] while 45 to 73% of Japanese Yusho patients experienced abdominal pains[20] and 11% had jaundice accompanied by other abdominal symptoms.[21] Presence of hepatotropic and hepatotoxic[22] PCDFs in Yusho can account for this symptomatic discrepancy.

(3) Although toxicity of PCDFs varies depending on the biological parameters (e.g., chick embryo,[5] liver necrosis,[22] thymic atrophy,[23,24] animal species,[25] etc.) and on the structural difference of isomers,[23,24,26] most of these

MONS 209761

Table 1. PCB, PCQ, and PCDF Levels in Taiwanese and Japanese Patients with "Yusho" Disease, Workers Occupationally Exposed to PCBs, Healthy Persons, and in Toxic Oils Causing "Yusho" Disease

	Sample	No.	Period after Termination of Exposure (yr)	PCB Level (ppb)	PCQ Level (ppb)	PCDF Level (ppb)	PCB : PCQ : PCDF
BLOOD	Yusho patients in Taiwan		0.5	43	14.5	0.072	100 : 33 : 0.16
				54	24.5	0.14	100 : 45 : 0.26
				43	24.0	0.092	100 : 56 : 0.21
				90	17.0	0.11	100 : 19 : 0.12
				54	8.4	0.066	100 : 16 : 0.12
				68	21.0	0.10	100 : 31 : 0.15
				35	11.0	0.17	100 : 31 : 0.17
				4	0.9	N.D.*	100 : 26 : < 0.13
				36	9.0	0.059	100 : 16 : 0.14
				64	15.5	0.069	100 : 24 : 0.11
				188	63.8	0.27	100 : 34 : 0.14
				36	16.9	0.091	100 : 50 : 0.27
				79	24.4	0.086	100 : 31 : 0.11
				37	16.9	0.067	100 : 46 : 0.18
				33	14.4	0.036	100 : 27 : 0.07
		15		60 ± 39‡	19.1 ± 13.0‡	0.14 ± 0.07‡	100 : 32 : 0.16
	Yusho patients in Japan	58	11	5.9 ± 4.4	2.0 ± 2.8	N.D.†	100 : 34 : < 0.17
	Japanese workers occupationally exposed to PCB[17]	17	4	33.3 ± 22.8	N.D.§	—	100 : < 0.06 : —
	Japanese workers occupationally exposed to PCB[18]	19	7	149.6 ± 170.2	—	—	
	Healthy subjects in Japan	60		2.8 ± 1.3	N.D.§	—	100 : < 1 : —
LIVER	Yusho patients in Japan	3	1	225.9	217.5	64.7	100 : 96 : 29
			7	114.4	51.7	6.2	100 : 45 : 5
			9	68.4	27.8	6.1	100 : 39 : 9
				130.7 ± 72.0	98.1 ± 84.6	25.7 ± 27.6	100 : 76 : 20
	Healthy subjects in Japan[19]	4		27.5 ± 15.4	—	0.0061 ± 0.0063	100 : — : 0.02
	Toxic oil in Taiwan	3		43,900 ± 12,400	17,300 ± 5,800	147 ± 33	100 : 39 : 0.32
	Toxic oil in Japan[7]	3		430,000 ± 391,000	630,000 ± 167,000	3,850 ± 2,570	100 : 147 : 0.90

*0.005 ppb detection limit.
†0.01 ppb detection limit.
‡0.02 ppb detection limit.
§Values expressed as mean ± SD.

MONS 209762

toxicological comparisons were made using the PCDF and PCB samples which directly arrived "fresh" and "pure" from manufactures. Utilizing PCDFs and PCBs that were either reproduced under the original conditions (i.e., temperature and coexisting air) at the rice oil production plant, or purified from the "toxic oil"—the causative agent of Japanese Yusho—we observed that the toxicity of PCDFs was hundreds to ten thousand times greater than PCB toxicity in monkey,[8] mouse,[26] and rat.[7] In addition to hepatotropism of PCDFs, the blood PCDF . PCB ratio in the current study alerts one to the toxic contribution of PCDFs in Yusho

(4) Although the toxicologic role of PCQs has been barely defined in Yusho, the present study shows that the toxicity of PCQs is almost equal to that of PCBs.[7,8] Therefore, the blood levels of PCQs observed among Taiwanese Yusho patients suggest that these substances were relatively insignificant in toxicological contribution. Perhaps PCQs are more important in enabling one to differentiate whether the patient has had a past exposure to "Yusho-type" chlorinated hydrocarbons (i.e., PCBs, PCQs, and PCDFs) or to only PCBs.[12]

* * * * * * * * * *

Submitted for publication August 6, 1981; accepted for publication September 13, 1981.

Requests for reprints should be sent to: Gen Ohi, M.D., Ph.D., Department of Hygiene and Preventive Medicine, School of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo ku, Tokyo 113, Japan.

* * * * * * * * * *

REFERENCES

1. Tsukamoto, H ; Makisumi, S.; Hirose, H.; Kojima, T.; Fukumoto, H., Fukumoto, K.; Kuratsune, M.; Nishizumi, M.; Shibata, M.; Nagai, J.; Yae, Y.; Sawada, K.; Yoshimura, H.; Tatsumi, K.; Oguri, K.; Shimeno, H.; Ueno, K.; Kobayashi, H.; Yano, T.; Ito, A., Okada, T.; Inagami, K.; Koga, T.; Tomita, Y.; Koga, T.; Yamada, Y.; Miyaguchi, M.; Sugano, M.; Hori, K.; Takeshina, K.; Manako, K.; Nakamura, Y.; and Shigemori, N. 1969. The chemical studies on detection of toxic compounds in the rice bran oils used by the patients of Yusho. *Fukuoka Acta Medica* (in Japanese) 60: 496–512.
2. Tanabe, H. 1970. Ad hoc study on prevention, diagnosis and treatment of Yusho, Study Report from Japanese Agency of Technology (1970), in Japanese.
3. Kuratsune, M.; Yoshimura, T.; Matsuzaka, J.; and Yamaguchi, A. 1972. Epidemiological study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. *Environ Health Perspect* 1: 119–28.
4. Nagayama, J.; Masuda, Y.; and Kuratsune, M. 1975. Chlorinated dibenzofurans in Kanechlor and rice oil used by patients with "yusho". *Fukuoka Acta Medica* 66: 593–99.
5. Vos, J.G.; Koeman, H.H.; Van der Maas, H.L.; ten Noever de Brauw, M.C.; and de Bos, R. H. 1970. Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial polychlorinated biphenyls. *Food Cosmet Toxicol* 8: 625–33.
6. Miyata, H.; Kashimoto, T.; and Kunita, N. 1978. Detection of unknown organochlorinated compounds in Kanemi rice oils which caused "Kanemi Yusho". *J Food Hyg Soc Japan* 19: 364–70.
7. Hori, S.; Obana, H.; Kashimoto, T.; and Fukuda, Y. 1979. Distribution and biological effect of PCB, PCT and PCQ on rats. *Proc Jap Congress Food Hyg* (in Japanese) November, 1979: 35.
8. Hori, S. 1980. Biological effects of PCBs and related polychlorinated compounds on croo monkeys. *Proc 7th Symp. Pharm Soc Jap* (in Japanese), 66–68.
9. Miyata, H.; Murakami, Y.; and Kashimoto, T. 1978. Determination of polychlorinated quaterphenyl (PCQ) in Kanemi rice oil that caused "Yusho" and investigation on PCQ formation. *J Food Hyg Soc Japan* 19: 417–24.
10. Miyata, H., and Kashimoto, T. 1979. Investigation on organochlorinated compounds formed in Kanemi rice oil that caused "Yusho". *J Food Hyg Soc Jap* 20: 1–9.
11. Miyata, H.; Kashimoto, T.; and Kunita, N. 1977. Detection and determination of polychlorodibenzofurans in normal human tissues and Kanemi Yusho. *J Food Hyg Soc Jap* 18: 260–65.
12. Kashimoto, T ; Miyata, H.; and Kunita, N. The presence of polychlorinated quaterphenyls in the tissues of Yusho victims. *Food Cosmet Toxicol* (in press).
13. Chen, P.H.; Gaw, J.M.; Wong, C.K.; and Chen, C.J. 1980. Levels and Gaschromatographic patterns of polychlorinated biphenyls in the blood of patterns of polychlorinated biphenyls in the blood of patients after PCB poisoning in Taiwan. *Bull Environ Contam Toxicol* 25: 325–29.
14. Hirayama, H. 1978. A study of PCB contamination among industrial workers. *Kurume Med Assoc* (in Japanese) 41: 1–14.
15. Hara, I.; Hirata, M.; Watanabe, I.; Yakushiji, T.; Takahashi, M.; and Nishitani, N. 1979. Health status of the workers occupationally exposed to PCB at a paint manufacturing factory. *Ann Rep Osaka Metropol Inst Pub Health* (in Japanese) 17: 11–21.
16. Ouw, H.K.; Simpson, F.R.; and Siyali, D.S. 1976. Use and health effects of Aroclor 1231, a polychlorinated biphenyl, in an electrical industry. *Arch Environ Health* 31: 189–94.
17. Nagayama, J.; Masuda, Y.; and Kuratsune, M. 1977. Determination of polychlorinated dibenzofurans in tissues of patients with "Yusho". *Food Cosmet Toxicol* 15: 195–98.
18. Firestone, D.; Flide, D.F.; Ress, J.; and Higginbotham, G.R. 1971. Distribution of chick edema factors in chick tissues. *J AOAC* 54: 1293–98.
19. Asahi, S.; Koda, H.; Urabe, H.; and Tositani, S. 1979. Dermatological symptoms of Yusho alterations in this decade. *Fukuoka Acta Medica* (in Japanese) 70: 172–80.
20. Yoshimura, T.; Mori, H.; and Kuratsune, M. 1970. Dose-response relationship between toxic oil intake and symptoms in Yusho patients: Short report. *Jap J Hyg* (in Japanese) 32: 111.
21. Kuratsune, M.; Yoshimura, T.; Matsuzaka, J.; and Yamaguchi, A. 1971. Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls. *HSMHA Health Rep* 86: 1083–91.
22. Vos, J.G., and Koeman, J.H. 1970. Comparative toxicological study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis and tissue residues. *Toxicol Appl Pharmacol* 17: 656–68.
23. Ohishi, S.; Morita, M.; and Fukuda, H. 1978. Comparative toxicity of polychlorinated biphenyls and dibenzofurans in rats. *Toxicol Appl Pharmacol* 43: 12–22.
24. Moore, J.A.; Gupta, B.N.; and Vos, J.G. 1980. Toxicity of 2, 3, 7, 8 tetrachlorodibenzofuran—preliminary results. *Proc National Conf PCB (EPA)* 77–80.
25. Moore, J.A.; McConell, E.E.; Dalgard, D.W.; and Harric, M.W. 1979. Comparative toxicity of their halogenatad dibenzofurans in guinea pigs, mice and rhesus monkeys. *Annals NY Acad Sci.* W. J. Nicholson and J. A. Moore, eds., pp. 151–163.
26. Saeki, S.; Ozawa, N.; and Yoshimura, H. 1977. Synthesis of 2-chloro and 1, 4, 8-trichlorodibenzofuran and their effect on the growth of mice and on the liver microsomal drug metabolizing enzyme systems of rats. *Fukuoka Acta Medica* (in Japanese) 68: 96–103.

MONS 209763