

# PCBs, PCQs and PCDFs in Blood of Yusho and Yu-Cheng Patients

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Individual blood samples obtained from Yusho and Yu-Cheng patients who had been poisoned by ingesting contaminated cooking oils, from workers occupationally exposed to polychlorinated biphenyls (PCBs) and from unexposed individuals were analyzed for PCBs, polychlorinated quaterphenyls (PCQs) and polychlorinated dibenzofurans (PCDFs) by gas chromatography and mass spectrometry.

PCBs were found in the blood of all samples. PCQs were detected in the blood of 54 of 56 living Yusho patients 11 years after the outbreak, and in all Yu-Cheng patients 6 months following poisoning. These facts indicate that the presence of PCQs in the blood was a good mark of past ingestion of the toxic oil.

In the Yu-Cheng cases, PCDFs as well as PCBs and PCQs were detected in all blood samples. These identified isomers have been reported to be remarkably highly toxic compounds, i.e., both the 2,3,7,8-tetrachlorinated and 2,3,4,7,8-pentachlorinated compounds are toxicologically hundreds to thousands of times more toxic than PCB.

In view of the high toxicity of PCDFs found in the Yu-Cheng patients' blood, we must deduce that they are the primary causal agents of Yusho as well as of the Yu-Cheng incident.

## Introduction

There are great differences in the species and composition of chlorinated compounds producing symptoms in Yusho patients and workers occupationally exposed to polychlorinated biphenyls (PCBs). The toxic oil that the Yusho patients ingested had high ratios of polychlorinated quaterphenyls (PCQs) to PCBs and polychlorinated dibenzofurans (PCDFs) to PCBs. Moreover, workers occupationally exposed to PCBs were rapidly cured of chloracne by changing their jobs (1), whereas some Yusho patients still suffer from chloracne despite their lowered blood PCB levels even today, 15 years following poisoning (2-4).

The recent occurrence of poisoning in Taiwan (Yu-Cheng) in 1979 due to the same cause as Yusho has provided us with facts enabling us to clarify and understand better the earlier Yusho outbreak, which occurred in 1968. In investigating the cause of Yusho we

decided to analyze for PCB-related compounds in the patients' blood, and in 1980 we established a method for microanalysis of PCQs and PCDFs in addition to PCBs in the blood (3). This analysis was carried out on the blood of Yusho patients 11 years after the outbreak, on workers occupationally exposed to PCBs, on unaffected persons, and on Yu-Cheng patients 1 to 18 months following ingestion of the poisoned oil.

In this study a major causal agent for both Yusho and Yu-Cheng, which are symptomatically quite distinct from occupational PCB poisoning, was present.

## Experimental

### Materials

Blood samples were collected from 113 Yu-Cheng patients between 1979 and 1981. Of those who were living in the dormitory on a school campus 23 were teachers and were over 21 years old. The remaining 90 patients were all students. Most of their meals had been provided by the school. Blood samples were also obtained from 56 Japanese Yusho patients in 1979, from 69 Japanese workers between 1979 and 1981 whose jobs entailed charging transformers with fresh PCB prepara-

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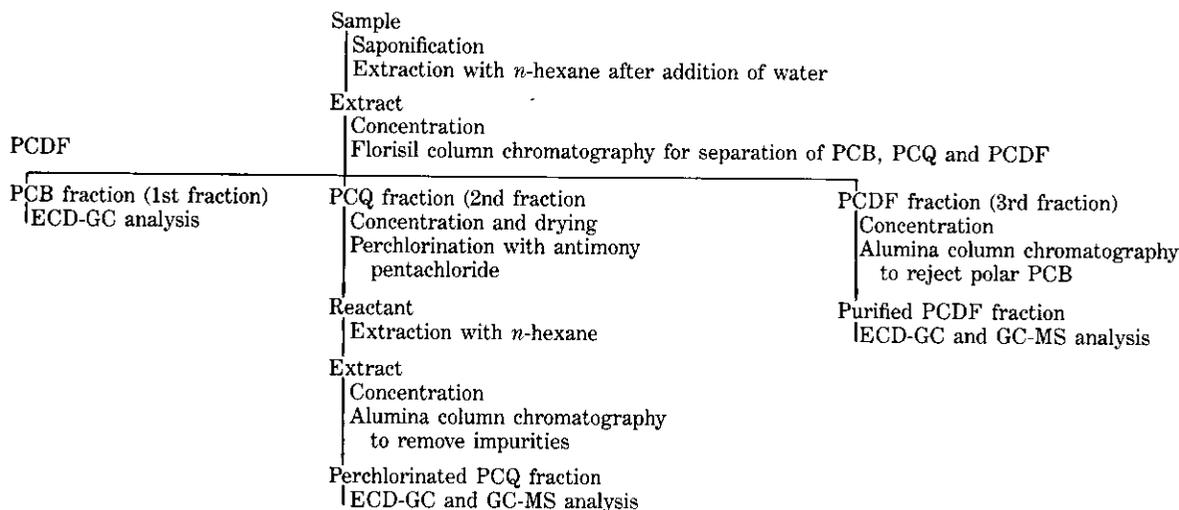


FIGURE 1. Analytical procedure for PCB, PCQ and PCDF in human blood

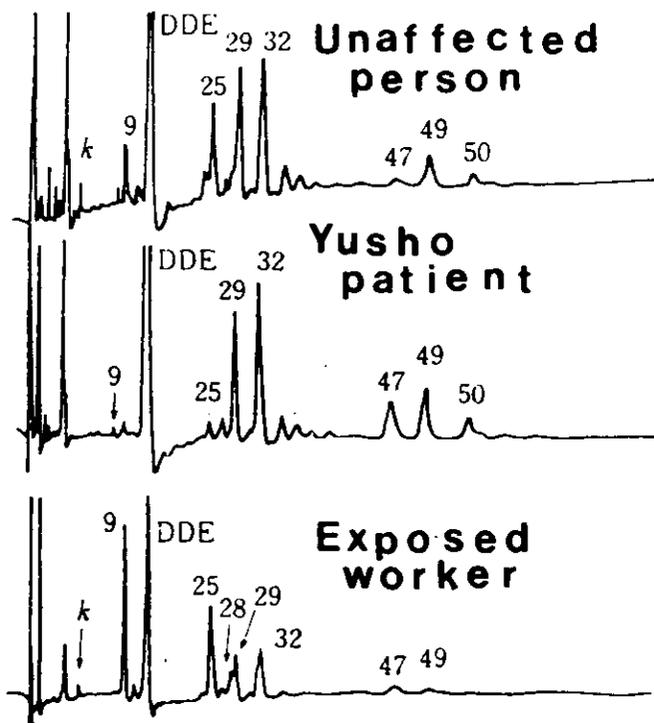


FIGURE 2. Typical gas chromatographic patterns on Apiezon L column of PCBs in the blood of a Yusho patient, a worker occupationally exposed to PCBs and an unaffected person. Blood samples are of from a worker 9 years after discontinuation of handling PCBs (Kanechlor 400) in a condenser factory, from a Yusho patient 11 years following poisoning and from an unaffected person in Osaka.

tions (Kanechlor 300, 400 and 500 containing 42%, 48% and 54% chlorine, respectively, at the Kanegafuchi Chemical Ind. Co., Japan), from three Japanese workers in 1979 who had engaged in reclaiming PCB preparations used as a heat exchanger, and from Japanese unaffected persons between 1979 and 1980.

## Analytical Procedure

Figure 1 shows a scheme of microanalysis for PCBs, PCQs and PCDFs in individual 10 mL blood samples. The compounds in each fraction were identified by GC-MS spectrometry. The recovery of PCBs by this method was 82.5% in 10 ppb, PCQs was 92.3% in 2.5 ppb and PCDFs was 79.2% in 0.25 ppb, respectively (5).

## Results and Discussion.

### Typical Gas Chromatographic Patterns of Blood PCBs

As illustrated in Figure 2, a worker exposed to PCB, a Yusho patient and a healthy control, respectively, all showed characteristic gas chromatographic pattern of PCBs in the blood. The pattern of the Yusho patient is a so-called type A gas chromatographic pattern (6) that is quite different from that observed in both unaffected persons and exposed workers. This peculiar pattern has been found to be characteristic of severe cases of Yusho. Compared to the other two groups, the relative intensity of peaks *k*, 9, 25 and 28, on the Apiezon column are lower in the blood from the Yusho patient, whereas that of peak 47 is higher. It has been established that the pattern persists and is still observed in most of the surviving patients even today.

### Gas Chromatographic Pattern of PCBs in Yu-Cheng Patients' Blood

The chromatographic pattern of Yu-Cheng patients, 6 months after poisoning, included pattern A, but also included many variations (14). As a general rule, the gas chromatograms of Yu-Cheng patients with skin signs (7) developed into pattern A during the first 6 months, but that of the patients with no skin signs took

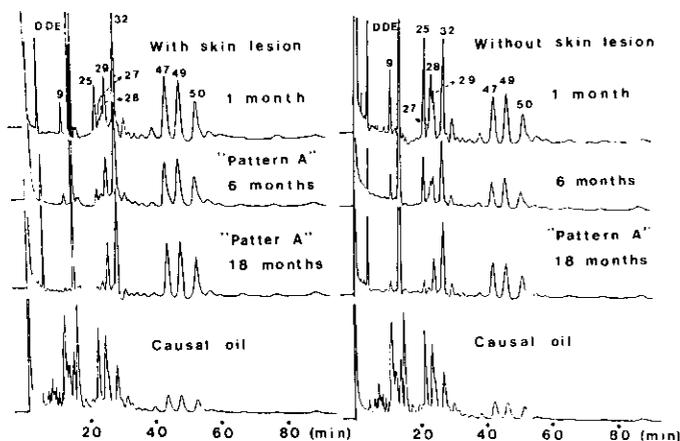


FIGURE 3. Gas chromatograms of PCBs in the blood of two individuals with Yu-Cheng. Glass column, 5 m  $\times$  2.6 mm containing 2% Apiezon L on Gaschrom Q (100–120 mesh); 240°C.

18 months to develop into pattern A. (Fig. 3) The presence of larger quantities of PCDF in the blood of the patient showing skin signs is considered to be the main factor responsible for the comparatively rapid change of the chromatogram toward the pattern A. It is possible that the chromatograms in most patients will change to the peculiar pattern A as seen in the Japanese case, in the near future.

In order to determine the cause of the change into pattern A, animal experiments were done (8). When Kanechlor (KC) 400 or the PCBs from Yusho oil alone were administered to mice, there occurred a remarkable elimination of lower chlorinated congeners (fewer than 5 Cl) from the mice, in the same manner as in the workers occupationally exposed to KC-400. However, the residual PCBs showed different gas chromatographic patterns from the so-called pattern A characteristic of Yusho patients. On the other hand, after simultaneous dosing of KC-400 and PCDFs separated from the toxic oil, or of both the separated PCBs and the separated PCDFs which were mixed in a proportion similar to that found in the original oil, the blood from the mice showed chromatograms of the residual PCBs resembling pattern A (8,9). This was further investigated by another animal experiment (10). Consequently, we clarified that the liver drug-metabolizing enzymes induced more potently in the presence of PCBs and small amounts of the PCDFs accelerated the selective elimination of some of highly bioaccumulative PCB isomers retained in the rat (11). This peculiar pattern was produced as a consequence. Therefore, the presence of PCDF is considered to be the main factor responsible for pattern A.

### Ratio of Blood PCBs in Males and Females

Figure 4 shows the component of each gas chromatographic peak of PCBs in the blood of Yu-Cheng patients. Peaks 9 and 15 with the OV-1 column are the same as

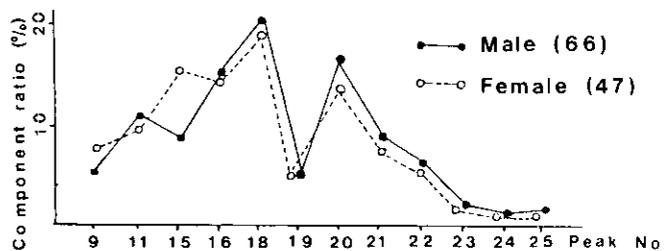


FIGURE 4. Component ratio of PCBs in the blood of patients with Yu-Cheng 6 months after oil ingestion (2% OV-1 column).

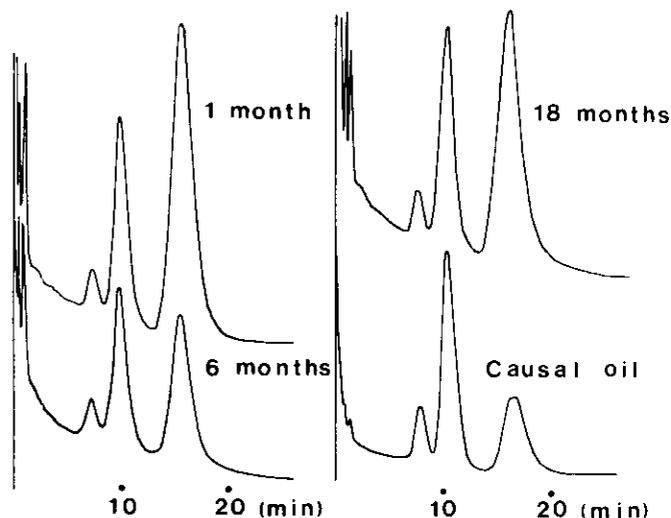


FIGURE 5. Gas chromatograms of PCQs in the blood of a patient 1, 6 and 18 months after oil ingestion. Glass column, 0.5 m  $\times$  2.6 mm containing 2% SE-54 on Gaschrom Q, 100–120 mesh; 290°C.

peaks 9 and 25 with the Apiezon column. Six months after the termination of the oil ingestion, a difference between sexes was noticed in relation to the ratio of the residual components, particularly at the points of peaks 9 and 15 components, both of which have been reported to be readily eliminatable isomers in animal experiments (8,9). However, at the end of 12 months, both males and females contained similar compositions of the blood PCBs which showed the type A gas chromatographic pattern. This suggests that males might have a stronger ability to metabolize these isomers than do females.

This finding is considered to agree with other data in which female rats (12), minks (13) and monkey (14) were reported to be more sensitive to PCBs and the related compounds than were the males.

### Gas Chromatographic Pattern of PCQs in the Blood of a Yu-Cheng Patient

PCQs were detected in the blood of all Yusho and Yu-Cheng patients and a few exposed workers. Figure 5 shows the gas chromatographic patterns of perchlorinated PCQs from the blood of a Yu-Cheng patient 1 to 18 months after exposure and from a sample of the oil involved in a Yu-Cheng. In general, the height of the

third peak in the chromatograms of the blood samples tends to increase in comparison with that of the oil (2). The gas chromatographic analysis revealed also that during the period of from 1 to 6 months following intoxication, the patients had various types of PCQs in their blood. However, we believe that PCQ chromatograms of Yu-Cheng patients will turn out to be the same as those of the Japanese patients in the near future. This suggests that not only the positions and number of chlorine atoms but also the skeletal structure of PCQs retained in the blood are related to the period after the termination of ingestion of the oil. A similar phenomenon on the chromatograms of PCQs in blood was

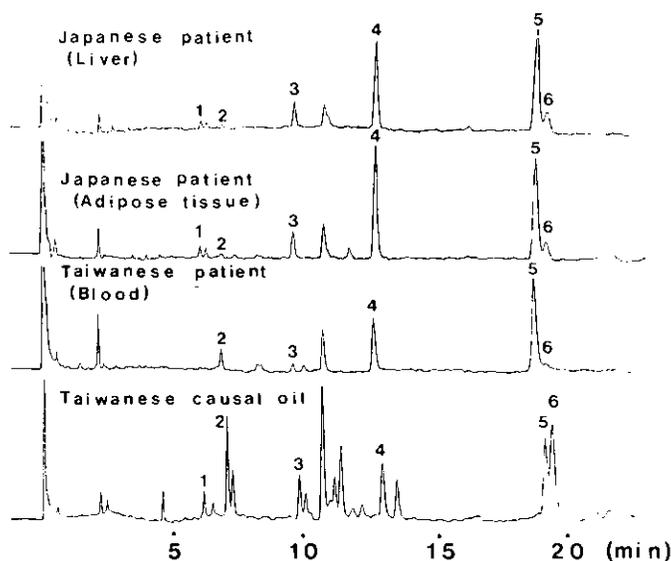


FIGURE 6. Gas chromatograms of PCDFs in various materials: (peak 1) 2,3,6,8-tetrachloro-DF; (peak 2) 1,2,4,7,8-pentachloro-DF; (peak 3) 2,3,4,7,8-chloro-DF penta; (peak 5) 1,2,3,4,7,8-hexachloro-DF; and (peak 6) 1,2,3,6,7,8-hexachloro-DF. OV-17 capillary column, 25 m; 235°C.

confirmed by dosing mice (15,16), rats and monkeys (11) with PCQs.

## PCDFs in Various Materials

PCDFs were found to be present in the liver and adipose tissue of deceased patients with Yusho and in samples of the oil involved in both the Yusho and Yu-Cheng incidents. Figure 6 shows gas chromatograms on a 20 m OV-17 capillary column of PCDFs in samples from a Yu-Cheng patient and a Yusho patient.

A limited number of PCDF isomers were detected in the Yu-Cheng patient's blood sample in comparison with that in the original toxic oil. In addition, there was a close structural similarity among these isomers in all samples. Each peak component in the chromatogram was identified by the coincidences of the retention time and mass spectrum of these peaks with those of authentic compounds on both OV-17 and OV-1 columns. Peaks 5 and 6 in Figure 6 are speculated to be 1,2,3,4,7,8-hexachlorinated- and 1,2,3,6,7,8-hexachlorinated dibenzofuran isomers, respectively, according to Rappe et al. (17). These isomers have been reported to be very highly toxic: both the 2,3,7,8-tetrachloro- and 2,3,4,7,8-pentachlorodibenzofurans are toxicologically hundreds to thousands of times more active than PCBs (11,18).

## Concentrations of PCBs, PCQs and PCDFs in the Blood

As shown in Table 1, PCBs were found in the blood of all groups. PCQs were not detected in the blood of workers occupationally exposed to unused PCBs despite the fact that PCB levels were as much as 7-fold higher than those of the Yusho patients or unexposed individuals. PCQs were, however, detected in all three workers

Table 1. Concentrations of PCBs, PCQs and PCDFs in the blood of Yusho patients, workers occupationally exposed to PCBs, unexposed individuals and Yu-Cheng patients.

	No.	Period after termination of exposure, yr.	Degree of dermatological severity	Concentration (mean $\pm$ SD), ppb		
				PCBs	PCQs	PCDFs
Yusho patients <sup>a</sup>	56	11		6 $\pm$ 4	2.0 $\pm$ 2.0	ND*
Workers occupationally exposed to unused PCBs <sup>b</sup>	69	9		45 $\pm$ 49	ND <sup>†</sup>	MD*
Workers occupationally exposed to used PCBs <sup>c</sup>	3	9		19 $\pm$ 11	0.9 $\pm$ 0.9	ND*
Unexposed individuals	60			2 $\pm$ 1	ND <sup>†</sup>	ND*
Yu-Cheng patients	5 (3)	0.5	None <sup>d</sup>	12 $\pm$ 6	1.7 $\pm$ 1.1	0.24 $\pm$ 0.018
(4 groups)	57 (24)	0.5	I + II	36 $\pm$ 14	7.9 $\pm$ 3.7	0.062 $\pm$ 0.024
	24 (14)	0.5	III	41 $\pm$ 15	8.2 $\pm$ 3.5	0.079 $\pm$ 0.030
	27 (24)	0.5	IV	50 $\pm$ 17	11.0 $\pm$ 5.2	0.100 $\pm$ 0.040
Total	118 (67)	0.5		39 $\pm$ 17	8.6 $\pm$ 4.8	0.076 $\pm$ 0.038

<sup>a</sup>Most of them are officially recognized Yusho patients but some are not.

<sup>b</sup>Workers who engaged in charging fresh PCB preparation into condensers in a factory.

<sup>c</sup>Workers who engaged in reclaiming PCB used as heat exchanger.

<sup>d</sup>Figure in parentheses shows the number of the blood samples analyzed for PCQs and PCDFs.

<sup>e</sup>Classification according the report of Goto and Higuchi. (7).

\* <0.01 ppb.

<sup>†</sup> <0.02 ppb.

who had engaged in reclaiming PCB products used as a heat exchanger. The latter constituted a special case because of their exposure to used PCB products which are deduced from our previous analytical data to contain PCQs and PCDFs.

PCQs were detected in the blood of 54 of 56 living Yusho patients 11 years after the outbreak and in all Yu-Cheng patients 6 months following poisoning. Compared with unaffected persons in Japan, even the group of Yu-Cheng patients without any clinical signs had over 100 times the blood PCQ levels in spite of the PCB level being only 6 times higher. These facts indicate that the presence of PCQs in the blood are a good mark of past ingestion of the toxic oil.

As seen in the Taiwanese cases, the remarkably highly toxic PCDFs as well as PCBs and PCQs were detected in all patients' blood samples, and their respective levels increased with the degree of severity of the clinical signs. The correlation between the blood PCDF level and skin signs was closest when compared with PCB or PCQ, showing a coefficient of correlator of to be 0.565 ( $p < 0.001$ ) (14). We failed to detect PCDFs in the blood of Yusho patients in Japan. However, it is quite certain that in the much earlier stage of poisoning they would have had PCDFs easily detectable in the blood because Yu-Cheng patients who had ingested rice oil containing lower levels of PCDFs than did Japanese Yusho patients definitely had PCDFs in the blood when examined within 18 months of the poisoning, and because the total amount (3.4 mg) (19) of PCDFs ingested by them was close to that (3.84 mg) ingested (20) by Yu-Cheng patients. It is also well known that PCDFs appear in various tissues of Yusho patients 1 to 9 years after the poisoning (2). These facts indicate that not only Yu-Cheng patients but also Japanese Yusho patients were heavily exposed to PCDFs and retained them for a long period of time in their body.

On the other hand, as already explained, the workers we studied who had handled fresh PCBs, had fairly high blood PCB levels (45 ppb on average) but no detectable amounts of PCQs and PCDFs 9 years after the termination of exposure. In an early stage of exposure, they showed only mild clinical signs, despite blood PCB levels as high as 100 to 1000 ppb, and the mild dermal lesions seen in some of them quickly disappeared after discontinuation of PCB handling (21). As is well known, such a phenomenon has been noted among Yusho and Yu-Cheng patients in an extreme form. Their daily intake of PCBs had been reported to be 4 to 12 mg over a few years which is close to that (7.9 mg) of Yusho patients (19).

All these facts indicate that an exposure to pure PCBs may not induce the severe lesions characteristic of Yusho or Yu-Cheng patients, although a possible effect of the different routes of entrance of PCBs on their toxicity should not be overlooked.

Taking all the results described above into account, PCQs as well as PCBs might not be responsible for the

development of Yusho because PCQs were confirmed not to produce significant effects during animal experiments (11,16).

In view of the high toxicity of PCDFs found in the Yu-Cheng patients' blood, we must deduce that these compounds are the primary causal agents of Yusho as well as of Yu-Cheng.

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