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Chlorinated Dibenzofurans in Kanechlors and Rice Oils Used by Patients with Yusho

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It was reported that the causal agent of the epidemic of Yusho which occurred in the Western Japan in 1968 was polychlorinated biphenyls (PCBs)[1]. According to the animal experiments by Vos and his associates marked differences in toxicity were seen among foreign commercial brands of PCBs[2]. They reported that the more toxic brands made in France and West Germany contained 20 and 5 ppm of polychlorinated dibenzofurans (PCDF), respectively, while the less toxic one made in the United States no such contaminants[3]. It seems quite possible that the toxicity of commercial PCBs is greatly affected by their relatively small amount of contaminants such as PCDF[4-6]. On the other hand, it has been well known that 'chick edema factor', the causal agent of an extensive poisoning of chickens which killed more than a million chicks in the United States in 1957, is polychlorinated dibenzo-p-dioxins (PCDD)[7,8], and that these compounds are extremely toxic and acnegenic to man, too[9,10].

All these facts raise a question whether or not PCDF or PCDD are involved in Yusho in any sense. Thus, it is without doubt needed to investigate the possible contamination by these compounds of Kanechlor-400, a commercial brand of PCBs of Japanese make which is known to have caused Yusho epidemic. In view of the fact that very few studies have been made in this respect, we analyzed various types of Kanechlor and samples of the toxic rice oil used by patients with Yusho for PCDD and PCDF. This paper describes the main results of such analysis.

Materials and Methods

1. Materials analyzed

1) Kanechlor-300, 400, 500, and 600: Kanechlor-300 and -500 were those donated Kanegafuchi Chemical Industry Co., Ltd. in 1968 and Kanechlor-400 and -600 were supplied by the Ministry of Health and Welfare in 1972.

2) Rice oil used by patients with Yusho ("Yusho oil"): 3 samples of rice oil used by 3 independent families with Yusho in 1968. These samples had been kept in glass bottles with glass stoppers at room temperature in our laboratory until 1973 when the analysis was made.

2. Chemicals

1) n-Hexane, ethanol, water, potassium hydroxide, anhydrous sodium sulphate, and silica gel: Purified as described previously[11].

2) Carbon tetrachloride and methylen chloride: Reagent grade, distilled with a fractional distillation column.

3) Activated alumina: Alumina for column-chromatography (about 300 mesh), Wako Pure Chemical Industry, Ltd., activated by heating at 150°C for 12 hours before every use.

4) PCDF and PCDD: Synthesized by chlorination of dibenzofurans or by conden-

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sation of 2, 4, 5-trichlorophenol". PCDF consisted mainly of tetra- and penta-chloro dibenzofurans, while PCDD mainly of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin.

5) Octachlorodibenzofuran and octachlorodibenzo-p-dioxin: Purchased from Analabs Inc., U.S.A.

3. Apparatus

1) Gas chromatograph (ECD-GC): Beckman GC 72-5 with a detecter of electron capture type, using a glass column (4 mm × 2 m) containing chromosorb W AW DMCS (100-120 mesh) coated with 2% SE-30. Column temperature was 200°C and helium was used as carrier gas, flow rate 30 ml/min.

2) Gas chromatograph-mass spectrometer (GC-MS): Type D-100, GC-MS, Japan Electron Optic Laboratory. Column used was similar to the one used for gas chromatography. Ionization energy was 25eV.

4. Analytical methods

The alkaline decomposition method described in the official standard analytical methods for PCBs established by the Ministry of Health and Welfare was applied for the isolation of PCBs fraction from samples of Yusho oil (5-10g). Isolation of PCDF and PCDD from the PCBs fraction mentioned above or from Kanechlor samples (0.1-0.5 g) was made in the following ways. Each material was placed with 20 ml of n-hexane onto a column (1 × 7.5 cm) of activated alumina and then eluted with 120 ml of n-hexane containing 20% carbon tetrachloride, 10 ml of n-hexane, and then with 30 ml of n-hexane containing 20% methylenchloride. PCDF and PCDD were expected to be contained in the final eluate. If their separation from PCBs was not adequate, the above chromatographic fractionation was repeated or another similar column chromatography was tried using a shorter column of activated alumina (1 × 2.5 cm) and a half volume of each of the eluents mentioned above. The eluate consisting of n-hexane containing 20% methylen chloride thus collected was evaporated by a rotary evaporator, then dissolved in n-hexane and subjected to ECD-GC and GC-MS analysis.

Determination of PCDF and PCDD was made by two methods, namely by measuring the gas chromatographic peak heights and by measuring the gas chromatographic peak area after perchlorination of these compounds. The former consists of comparison of each of the peaks of gas chromatogram confirmed as those of PCDF or PCDD by GC-MS with the corresponding one of the synthesized PCDF or PCDD used as standard, assuming that all the PCDF or PCDD have the same peak height sensitivity regardless of the number of chlorine and the site of substitution by chlorine. The latter consisted of chlorination by the BMC reagent[11] of the evaporation residue of the fraction containing PCDF and PCDD to yield octachlorodibenzofuran and octachlorodibenzo-p-dioxin, followed by the gas chromatographic determination of such octachloro-derivatives.

For determination of PCBs, the peak height calculation method and the perchlorination method, described in the official analytical methods for PCBs set by the Ministry of Health and Welfare, were used.

Results

The gas chromatograms of PCDF fractions separated from Kanechlor-400 and -500 are shown in Fig. 1, while the gas chromatogram of the corresponding fraction from Yusho oil in Fig. 2. Fig. 3 and 4 show the mass-spectra of tetra- and penta-chlorodibenzofurans isolated from these PCDF fractions, together with those of the standard PCDF.

Table 1 summerizes the results of analysis by ECD-GC and GC-MS, indicating that from dichloro to heptachlorodibenzofurans were contained in the samples analyzed. The Kanechlors designated larger numbers, starting from 300 to 600, contained the

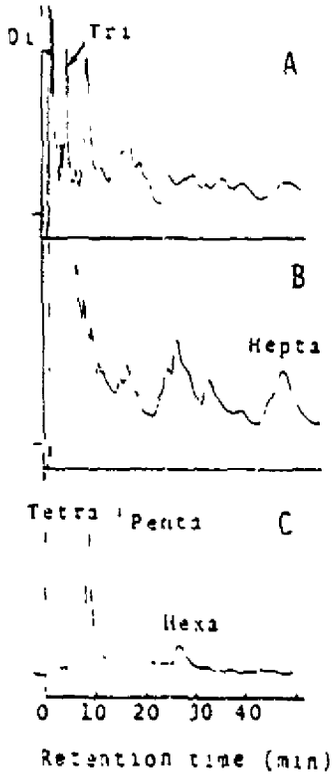

Fig. 1 Gas chromatogram of PCDF fraction
A Kanechlor-400
B. Kanechlor-500.
C Authentic specimen

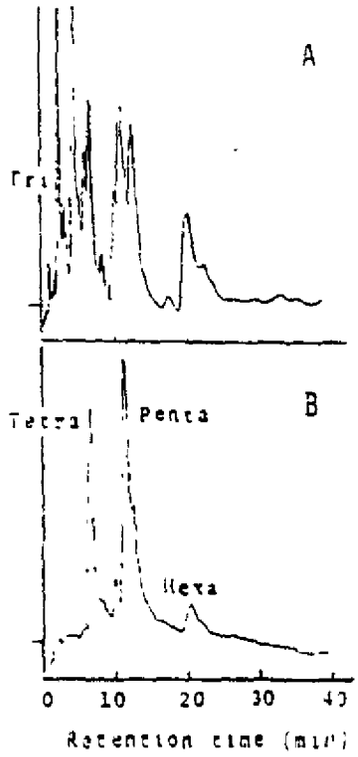

Fig. 2. Gas chromatogram of PCDF fraction.
A, Rice oil (Yusho oil).
B Authentic specimen

Table 1. Concentration of chlorinated dibenzofurans in Kanechlors and "Yusho oil"

Samples		Chlorodibenzofurans						Concentration (ppm)	
		Di	Tri	Tetra	Penta	Hexa	Hepta	a	b
Kanechlors	300			−	−			1	1.5
	400	+	++	++	+			18	17
	500				−	−	±	4	2.5
	600			−	−	−	++	5	3
"Yusho oil"	A		−	−	−	−		5	4.5
	B		−	−	−	−		4	5
	C		−	±	−	−		5	5

a calculated from peak heights, b: calculated by perchlorination method.

higher chlorinated isomers of dibenzofuran. The concentrations of PCDF in Kanechlor-300, -400, -500, and -600 were 1, 18, 4, and 5 ppm by the peak height calculation method and 1.5, 17, 2.5, and 3 ppm by the perchlorination method, respectively. Thus,

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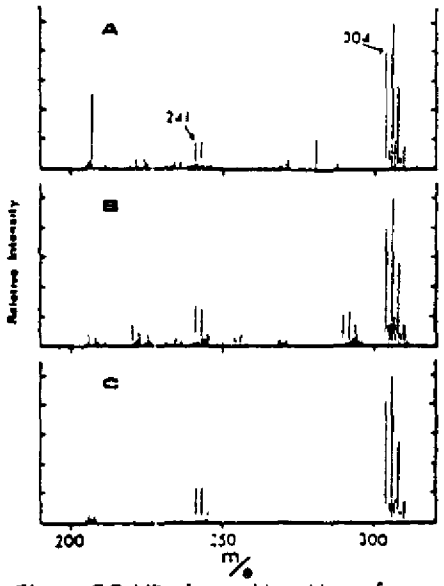

Fig. 3. GC-MS of tetrachlorodibenzofuran.
A. Kanechlor-400.
B. Rice oil ("Yusho oil").
C. Authentic specimen

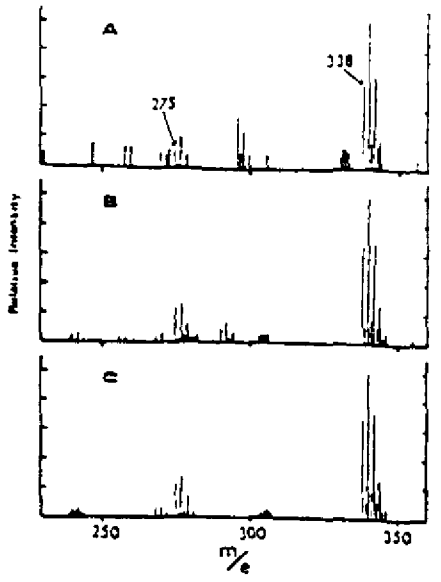

Fig. 4 GC-MS of pentachlorodibenzofuran
A. Kanechlor-400.
B Rice oil ("Yusho oil").
C. Authentic specimen

Table 2. Concentration of PCBs in "Yusho oil"

"Yusho oil"	Concentration (ppm)	
	a	b
A	830	870
B	900	920
C	1,030	980

a. calculated from peak heights
b. calculated by perchlorination method

two methods gave similar results, demonstrating that Kanechlor-400 contains a considerably higher concentration of PCDF than the other types.

Three samples of Yusho oil showed similar gas chromatographic patterns, indicating that the PCDF contained in the oil consisted mainly of tetra- and penta-chlorodibenzofurans together with a small amount of tri- and hexa-chlorodibenzofurans. Both the peak height calculation method and the perchlorination method gave the same figure, 5 ppm of PCDF.

Contrary to the above findings in regard to PCDF, PCDD was not detected in any of the materials examined.

As shown in Table 2, the samples of Yusho oil contained 800-1,000 ppm of PCBs and both calculations gave very close figures.

In additional experiments, 10 g of a vegetable oil containing 10 mg of Kanechlor-400 was subjected to a series of analytical procedures, the same as those employed in the above analysis, including the alkaline decomposition process maintained for 6 hours.

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PCDF found from the oil thus treated was about 0.2 μg, just as much as expected from the PCDF content of 10 mg of Kanechlor-400. Similar blank experiments were repeated 3 times, yielding practically the same results. Therefore, any possible transformation of PCBs to PCDF during the analytical procedures could be denied.

The recovery ratio of 1 μg of PCDF added to 10 g of a vegetable oil was determined to be approximately 90%.

Discussion

Analysis of a trace amount of PCDF and PCDD is generally known to be fairly difficult. As mentioned, it is not conceivable that the PCDF found in our analysis were the artificial products due to the analytical procedures employed. In addition, the recovery ratio was also fairly high. Therefore, we can say with confidence that Kanechlors and "Yusho oil" contain PCDF.

It seems to be noteworthy that the concentrations of PCDF in Kanechlor-400 and in Yusho oil, as determined by the present analysts, are considerably higher than the figures so far reported. Roach *et al.*, found about 1 ppm of PCDF in Kanechlor-400[11]. According to Vos, Bowes, Space Science Laboratory, University of California, could demonstrate the presence of PCDF in Kanechlor samples and in a sample of "Yusho oil". The figures for the formers have not yet been supplied but the figure for the latter approximately 1 ppm, have been provided us[10]. Thus, the informations so far accumulated do not very precisely coincide with each other, but they agree at least in that both Kanechlors and "Yusho oil" contain PCDF.

Since "Yusho oil" which is known to have been contaminated with Kanechlor-400 contained 5 ppm of PCDF and 1,000 ppm of PCBs, the ratio of the concentration of PCDF to that of PCBs is 5 : 1,000, much larger than the expected ratio 0.01 : 1,000 which can be calculated from our figure concerning PCDF concentration in Kanechlor-400. The reasons for this great discrepancy are not well understood, but it should be noted that the sample of Kanechlor-400 we analyzed was an "unused" one, while the Kanechlor-400 present in "Yusho oil" must be "used" one as heat transfer medium.

Very few studies have been made in which the toxicity of PCDF was directly compared with that of PCBs. Araki observed that PCDF was about 10 times stronger than Kanechlor-400 in the activity of hepatic enzyme induction[12]. Nishizumi and Masuda, however, could not observe any marked difference in acnegenicity between PCBs and PCDF when applied to the skin of the ears of rabbits. The ratio of the concentration of PCBs needed to exert a certain degree of acnegenicity to the corresponding concentration of PCDF needed to exert the same degree of acnegenicity was shown to be 1 : 0.3[13].

There are several studies, however, in which "Yusho oil" and Kanechlor-400 were compared in toxicity. Ikeda reported that "Yusho oil" was twice or more toxic when fed to broilers, than expected by the concentration of Kanechlor-400 contained in the oil[14,15]. In feeding experiment with CF-1 mice, it was shown that both a diet added "Yusho oil" to attain about 110 ppm of PCBs in the diet and a diet containing 500 ppm of Kanechlor-400 exerted an approximately similar toxicity. The former diet, however, seemed to affect more the skin and liver than the latter and the authors suggested a possibility that "Yusho oil" might contain some chlorinated compounds which were more toxic than Kanechlor-400[16]. In a feeding experiment with chicken, Goto *et al.* observed that a diet containing 5% of "Yusho oil" showed similar toxic effects to those induced by feeding a diet containing 400 ppm of Kanechlor-400. If the concentration of PCBs in the "Yusho oil" used could be assumed to be about 2,000 ppm, the concentration of PCBs in the diet added "Yusho oil" would be about 100 ppm, considerably

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lower than that of the diet added Kanechlor-400[10].

Furthermore, there seems to be a great difference in acute toxicity between PCDF and Kanechlors. LD_{50} of Kanechlor-400, when fed to mice and rats, was 1.1-1.9 g/kg and that of Kanechlor-300 0.6 g/kg when fed to rabbits[1]. PCDF, on the contrary, killed rabbits due to severe liver necrosis after a single oral administration of as little as about 1 mg/kg[12].

Toxicity of PCBs and possibly of PCDF must greatly vary from isomer to isomer. Rational comparison of toxicities of PCBs and PCDF does not seem to be easy. However, in view of all these past findings described above, PCDF seems to be considerably more toxic than PCBs. The possible role played by PCDF in Yusho should not be dismissed.

Summary

In order to answer the question whether or not polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD) which are more toxic than PCBs are involved in causation of Yusho, various types of Kanechlor, PCBs of Japanese make, and 3 samples of "Yusho oil" used by Yusho families were analyzed for these compounds.

Kanechlor-300, -400, -500, and -600 were shown to contain 1, 18, 4, and 5 ppm of PCDF, respectively, as calculated by gas chromatographic peak heights and 1.5, 17, 2.5, and 3 ppm of PCDF, as calculated by the perchlorination method. Thus, Kanechlor -400 was most contaminated with PCDF. "Yusho oil" samples also contained about 5 ppm of PCDF, much higher than expected. PCDD, however, was not detected in any materials analyzed. The possible role of PCDF in causation of Yusho was suggested.

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