Determination of Chlorinated Dibenzofurans in Kanechlors and "Yusho Oil"

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Polychlorinated biphenyls (PCB) are widely distributed in the environment, especially in the tissues of fish, wild lives and man. They are also known as the causal agent of a mass food poisoning which occured among more than 1,000 persons in western Japan in 1968. The disease induced by this poisoning is called "Yusho", namely oil disease, because the patients were proved to have consumed a commercial rice oil contaminated with a large amount of Kanechlor-400 (KC-400), a brand of PCB of Japanese make (TSUKAMOTO et al., 1969). Feeding experiment using broilers demonstrated, however, that the rice oil taken by patients with Yusho were twice or more toxic than expected from the amount of KC-400 presented in the rice oil (IKEDA, 1972). This suggested that the "Yusho oil" must contain some more toxic substance or substances besides PCB. VOS et al. (1970), on the other hand, found by the chick embryo assay that toxicity of commercial PCB preparations is greatly affected by their contaminants, polychlorinated dibenzofurans (PCDF). Bauer et al. (1961) also demonstrated that structurally related polychlorinated dibenzodioxins (PCDD) were extremely toxic and acnegenic to man. Approximately 1 ppm of PCDF was estimated to contain in KC-400 (ROACH and POMERANTZ, 1974).

All these facts necessitated an analysis of Kanechlors and the rice oil taken by patients with Yusho for their possible content of PCDF and PCDD.

EXPERIMENTAL

In order to separate PCB, PCDF and PCDD from the "Yusho oil", 5 - 10 g of the oil was saponificated with ethanol containing an excess amount of sodium hydroxide for 1 hour at 80°C and the n-hexane extract of the reaction mixture was chromatographed on a column of silica gel (Wakogel S-1, activated by heating at 130°C for 3 hours) with 100 ml of n-hexane. The eluate was concentrated to a small volume.

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This concentrated eluate and samples of KC-300, 400, 500 and 600 (0.1 - 0.5 g) were chromatographed respectively, on a column of alumina (300 mesh, Wako Pure Chemical Ind. Ltd., 5 g) activated by heating at 150°C for 12 hours, using as eluents, first 20 ml of n-hexane, then 120 ml of n-hexane containing 20 % carbon tetrachloride, 10 ml of n-hexane and finally 30 ml of n-hexane containing 20 % methylene chloride. The last eluate was evaporated to dryness, dissolved in n-hexane and subjected to gas chromatography with an electron capture detector (Beckman GC 72-5) and gas chromatography/mass spectrometry (JEOL D-100) for qualitative and quantitative determination of PCDF and PCDD. When the separation of PCDF and PCDD from PCB was not adequate, a similar chromatographic fractionation of the last eluate was repeated using a smaller amount of alumina (2 g) and a smaller volume of the same solvent systems, prior to the determination of PCDF and PCDD. Amount of individual di-, tri-, tetra-, penta-, hexa- and hepta-chlorodibenzofurans was calculated from respective peak heights comparing with those of tetra-, penta- and hexa-chlorodibenzofurans which were synthesized by chlorination of dibenzofuran. In this determination, the individual peaks were confirmed by GC-MS and their heights were assumed to have the same sensitivity. The total amount of PCDF was also determined by the perchlorination method. The PCDF from each sample were chlorinated to octachlorodibenzofuran (OCDF) with BMC reagents (HUTZINGER et al., 1972). The amount of OCDF was determined by ECD-GC comparing with standard OCDF (Analabs, Inc.) and converted to the amount of tetra-, hexa- or hepta-chlorodibenzofuran which was the main component of PCDF in the sample.

Possible formation of PCDF from PCB during the saponification process was denied by comparing the determined amount of PCDF in KC-400 with that of PCDF in a sample of saponified vegetable oil containing KC-400. The recovery of PCDF in the whole analytical procedure was examined by analyzing 10 g of vegetable oil containing 1 μg of PCDF, finding more than 90 %.

RESULTS AND DISCUSSION

Presence of PCDF was demonstrated in all samples of Kanechlors and "Yusho oils" analyzed, as shown in Table 1 and Figure 1, but PCDD was not detected. As might be expected, Kanechlors with higher chlorine contents such as KC-500 and 600 contained more chlorinated dibenzofurans than those with lower chlorine contents. The concentration of total PCDF in KC-400

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.

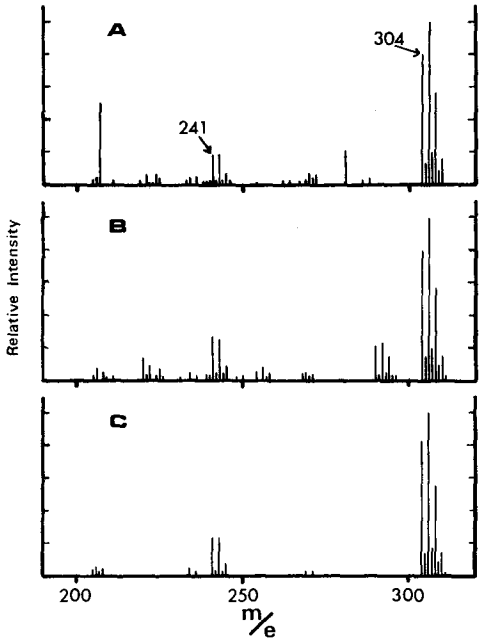

Figure 1. GC-MS of Tetrachlorodibenzofuran
A: Kanechlor-400, B: "Yusho oil",
C: Authentic specimen.

was estimated to be 18 ppm, the highest of the Kanechlors tested. GC peak patterns of PCDF present in three samples of the "Yusho oil" were very similar each other consisting of tri- to hexa-chlorodibenzofurans and their concentrations were 5 ppm. The PCB concentration in the "Yusho oil" analyzed by us was about 1,000 ppm. Therefore, PCDF concentration in the PCB in the oil was calculated to be 5,000 ppm. In other words, PCDF in the oil seemed to have been 250 times concentrated as compared with its concentration in KC-400. VOS et al. (1970) showed in their chick embryo assays that PCDF was far more toxic than PCB. According to ARAKI's (1974) animal experiment, hepatic enzyme-inducing activity of PCDF was 170 times that of KC-400. In view of all these facts, the authers consider that the toxic role of this specific type of contaminants in the "Yusho oil" should not be dismissed in the causation of Yusho, even if their quantity was small in the "Yusho oil".

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