

Detailed Examination of Polychlorinated Dibenzofurans in PCB Preparations and Kanemi Yusho oil

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Polychlorinated dibenzofurans (PCDFs) are known to be highly toxic substances. (HOFMANN 1958) Through chick embryo bioassays, Vos et al. (1970) identified two kind of PCDFs as toxic components in PCBs. Subsequent investigations have revealed that several PCDFs are present in a number of PCB preparations. (BOWES et al. 1973, ROACH and POMERANTZ 1974, NAGAYAMA et al. 1976) However, only limited informations are available on the isomer components of this toxic material.

PCDFs were recently reported to be present in Yusho oil which gave rise to a large scale of human intoxication. (NAGAYAMA et al. 1976) "Yusho", namely oil disease, is a peculiar skin disease characterized by clinical manifestations as follicular accentuation, acneform eruption, pigmentation of the skin and nail and hypersecretion of Meibonian gland. The cause has been considered to be PCB poisoning (Kuratsune et al. 1972) but it was recently suggested that the toxic role of PCDFs was not negligible in the causation of Yusho. Hence, detailed characterization of PCDF components seemed to be necessary in Yusho oil.

The present paper will describe detailed analytical result of PCDF components as to the numbers and compositions in Yusho oil and sixteen PCB preparations.

Materials and Method

Samples Yusho oil was the product of 12th Feb. 1968. PCB samples examined were Kanechlor KC300, KC400, KC500, KC600, Aroclor T64, T241, T1242, T1248, T1254, T1260, Clophen A30, A40, A50, Phenoclor DP4, DP5, DP6. All PCB samples were not specified in lot number. Used PCB sample was Aroclor T1248 used as a heat transfer agent in chemical factory during two years. Operation condition was not specified.

Analysis PCB sample (1g) was dissolved in 2 ml of n-hexane and chromatographed over Florisil column. The size of the column was 1 cm in diameter and 30 cm in length. 20 grams of Florisil (80/100) preactivated at the temperature of 630°C for four hours, was packed. After the first absorption, 200 ml of n-hexane was passed the column in order to remove most of the PCBs and then 50 ml of hexane acetone mixture (95:5) was passed. PCDFs were recovered from the column by eluting with 100 ml of acetone. The recovery was more than 90 %. The acetone fraction was condensed

to the volume of 500 μ l with Kundera-Danish condenser and nitrogen gas flashing. An aliquot of the condensed solution was injected to gaschromatography-mass spectrometer (Shimadzu-LKB 9000). The following condition was employed.

Column Dexil 3% on Chromosorb WAW DCMS(100/120) 3mm \times 2m

Carrier gas He 30ml/min. Ion. Vol. 70eV

Temperatures. Column 270 $^{\circ}$ C Injection 300 $^{\circ}$ C

Separator 300 $^{\circ}$ C Ion source 310 $^{\circ}$ C

Mass spectra were taken at every five second with a computer system (MS-PAC). 192 spectra were obtained from 2 to 18 minutes after injection and the selected mass numbers were plotted on section papers for qualitative and quantitative analysis. Quantification was made on the assumption that each PCDF specimen was ionized to the same extent by the electron of 70eV regardless to the difference of the degree or the positions of chlorine substitution. Amounts of PCDFs were calculated by comparing their peak areas with those of known amount of the standard sample.

Result and Discussion

Mass fragmentgrams of chlorinated dibenzofurans in commercial PCBs and in Yusho oil are shown in Fig. 1 along with our standard sample. Standard sample was synthesized through chlorination of dibenzofuran by chlorine gas under the presence of ferric chloride and iodine. Average chlorine number was 4.7.

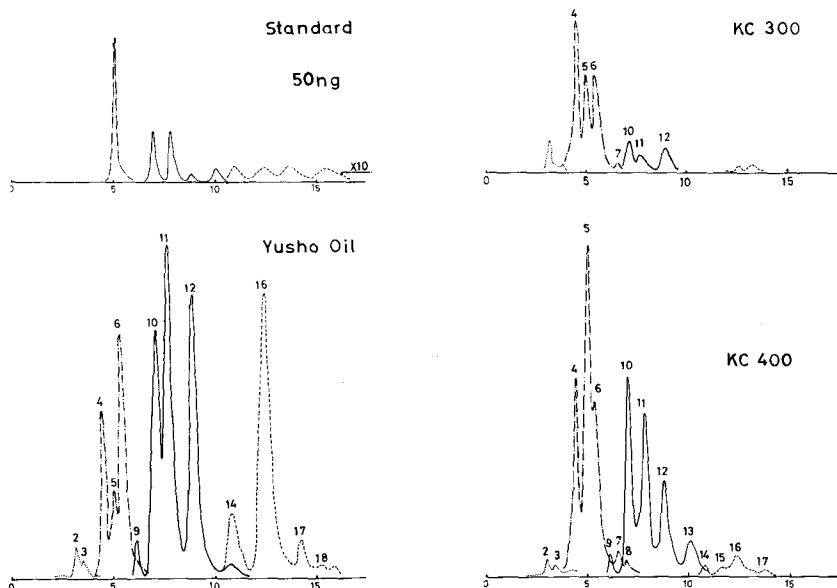


Fig.1. Mass fragmentgrams of PCDFs in PCBs and Yusho oil monitored by parent peaks(270, 304, 338, 372).

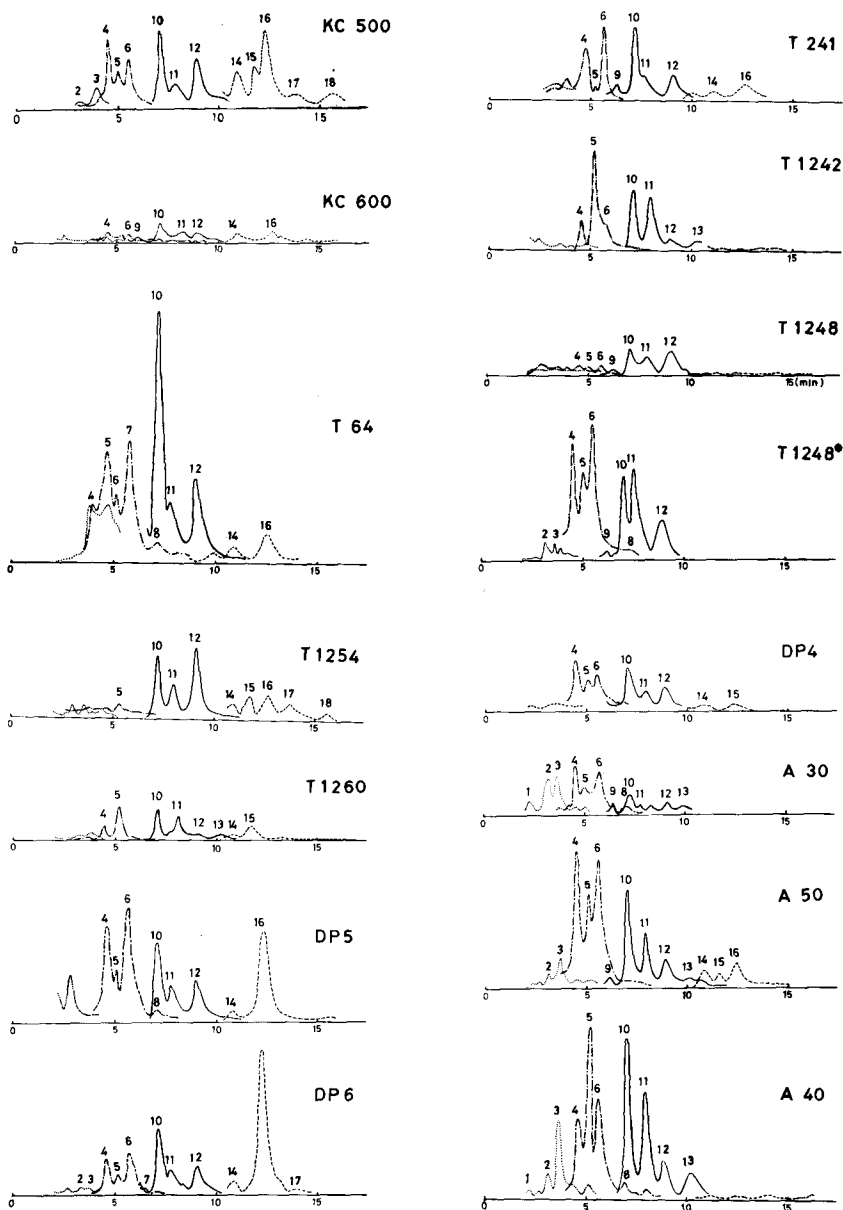
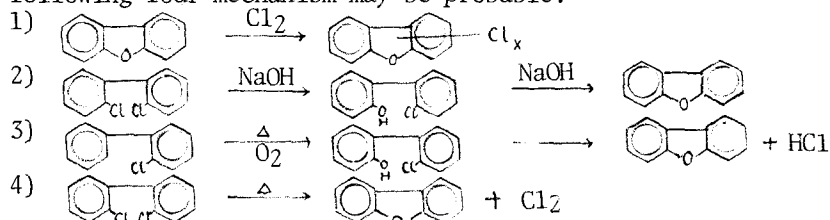


Fig. 1 Continued.

Eighteen PCDF isomers (three trichloro-, five tetrachloro-, five pentachloro- and five hexachlorodibenzofurans) were demonstrable in these samples. Peak 6 was tentatively assigned to 2,3,7,8-tetrachlorodibenzofuran from the coincidence of the retention time with the authentic sample. Other isomers were not identified in their chlorine positions. It is worthy to

note that almost all PCBs have similar PCDF isomers. PCDF concentrations are listed in Table 1.

In regard to the occurrence of PCDF in PCB preparations, the following four mechanism may be probable.



Reaction (1) means that dibenzofuran contained as an impurity in technical grade biphenyl is chlorinated to PCDFs in the process of PCB synthesis. Gaschromatographic pattern of our standard sample, however, is fairly different from that of PCDFs in PCB preparations. Therefore, mechanism (1) may not be the major route of PCDFs. Vos et al (1970) suggested the mechanism 2) as a possible route. However, gaschromatographic pattern of PCDFs produced by heating PCB(Aroclor 1248) with sodium hydroxide were different from that of PCDFs in PCB preparations. Thus, the mechanism (2) may also not a major route for the formation of PCDFs. On the other hand, mechanisms (3) and (4) seem more probable because used PCB has a profound PCDFs with similar isomerism.

When PCBs (Aroclor 1248) were sealed with air in glass tube and heated at 300°C for two weeks, increased amount of PCDFs were found. Produced PCDFs had a similar gaschromatographic pattern as shown in Fig.1. At the same time, the formation of hydrogen chloride and polychlorinated biphenyls was observed. This reaction was catalysed by transition metals or their salts. Detailed investigation is now in progress concerning to this reaction.

PCDFs were concentrated in Yusho oil. The ratio PCDF/PCB reaches to about 1.1% which may be five hundred times higher than that originally contained in KC 400. Nagayama et al. and Miyata et al(private communication) recently identified PCDFs independently in Yusho oil and reported the PCDF/PCB ratio as 0.5% and 1.2% respectively. Both results are comparable to our result. PCBs which had been originally used for the heat transfer agent was KC 400, however, the gaschromatographic pattern of PCBs in Yusho oil was rather resembled to that of KC 500. This indicates that lower chlorinated biphenyls were removed by evaporation in the deodouration process of vacuum distillation to result the concentration of PCDFs which are less volatile than PCBs. Furthermore severe temperature conditions employed in the company might give rise to the profound formation of PCDFs. Metals presented in the heat transfer tubing (stainless steel) might catalysed the reaction.

Yusho disease is a chronic intoxication caused by the consumption of rice bran oil (Yusho oil) contaminated by heat transfer oil (KC400). Yusho patients are considered to have ingested about 2 grams of PCBs in average. Assuming that the

Table 1 PCDF concentrations in Yusho oil and PCB samples (ppm)

	Yusho Oil	DP4	DP5	DP6	KC300	KC400	KC500	KC600
1	-	-	-	-	-	-	-	-
2	0.02	-	-	0.1	-	0.2	-	-
3	-	-	-	0.1	-	0.1	0.2	-
4	0.15	0.7	2.0	0.7	2.6	3.4	0.7	0.1
5	0.09	0.3	0.3	0.4	1.8	6.9	0.3	-
6	0.28	0.7	2.2	0.9	2.2	1.6	0.7	0.1
7	-	-	0.1	0.1	0.1	0.2	-	-
8	-	-	-	-	-	0.1	-	-
9	0.03	-	-	-	-	0.3	-	0.1
10	0.53	0.9	1.4	1.5	0.6	4.5	0.1	0.2
11	0.33	0.3	0.5	0.5	0.4	3.9	0.3	0.1
12	0.42	0.4	0.8	0.6	0.6	0.9	0.7	0.1
13	0.02	-	-	-	-	0.8	-	-
14	-	0.2	0.1	0.3	-	0.2	0.5	0.2
15	0.12	0.3	2.5	5.1	-	0.1	0.5	-
16	0.64	-	-	0.1	-	0.4	1.4	0.2
17	0.04	-	-	0.1	-	0.2	0.2	-
18	0.01	-	-	-	-	-	0.5	-
Tri-	0.02	-	-	0.2	-	0.3	0.2	-
Tetra-	0.52	1.7	4.6	2.1	6.7	12.2	1.7	0.2
Penta-	1.33	1.6	2.7	2.6	1.6	10.4	1.1	0.5
Hexa-	0.81	0.5	2.6	5.6	-	0.9	3.1	0.4
Total	2.68	3.8	9.9	10.5	8.3	23.8	6.1	1.1

* Used PCB

Peaks 1-3,4-8,9-13 and 14-18 correspond to trichloro-, tetrachloro-, pentachloro- and hexachlorodibenzofurans, respectively.

ratio PCDF/PCB is one percent, the total intake of PCDFs reaches to 20mg for average patients. Although there is no way of knowing the toxicity of PCDFs in association with the various symptoms of the Yusho disease, there is a possibility that PCDFs were potentially contributed to the Yusho disease.

It is also noteworthy that a crude rice bran oil of the same origin as the Yusho oil had given rise to a large scale of chick death several months before the outbreak of human intoxication. More than five hundred thousand chicks were died in similar symptoms of chick edema disease through the intake of food prescribed with the crude rice bran oil. (KOHANAWA et al. 1969) Chick edema disease is known to be caused by several

Continued (ppm)

T64	T241	T1242	T1248	T1248*	T1254	T1260	A30	A40	A50
-	-	-	-	-	-	-	0.3	0.1	-
-	-	-	-	0.2	-	-	0.6	0.3	0.2
-	-	-	-	0.1	-	-	0.7	1.1	0.5
1.7	1.1	0.3	0.2	1.9	-	0.2	0.7	0.5	3.0
0.3	0.2	1.8	0.1	1.1	0.1	0.6	0.4	2.7	1.7
2.4	1.1	0.2	0.2	2.7	-	-	1.0	2.1	3.6
0.3	-	-	-	-	-	-	-	-	-
0.1	-	-	-	0.1	-	-	0.2	0.1	-
-	0.2	-	0.2	0.1	-	-	0.2	-	0.2
6.3	1.5	1.0	0.7	1.8	1.3	0.5	0.5	3.1	2.2
0.8	0.6	1.0	0.6	2.3	0.7	0.2	0.1	2.2	1.0
2.3	0.4	0.1	0.8	1.4	1.6	0.1	0.1	0.7	0.6
-	-	0.1	-	-	-	0.1	0.1	0.9	0.1
0.5	0.2	-	-	-	0.3	0.1	-	-	0.7
-	-	-	-	0.2	0.5	0.4	-	-	0.3
1.5	0.6	-	-	0.5	0.7	-	-	-	0.8
-	-	-	-	-	0.3	-	-	-	-
-	-	-	-	-	0.1	-	-	-	-
-	-	-	-	0.3	-	-	1.6	1.5	0.7
4.8	2.4	2.3	0.5	5.8	0.1	0.8	2.3	5.4	8.3
9.4	2.7	2.2	2.3	5.6	3.6	0.9	1.0	6.9	4.1
2.0	0.8	-	-	0.7	1.9	0.5	-	-	1.8
16.2	5.9	4.5	2.8	12.4	5.6	2.2	4.9	13.8	14.9

chlorinated dibenzodioxines. (CANTRELL 1967) We failed to find a detectable amount of chlorinated dibenzodioxines in the rice bran oil. Polychlorinated dibenzofurans might have played a serious role to the large scale chick death in Japan.

There is no evidence that PCDFs are present in ecosystems. It is reported that octachlorodibenzofuran is less rapidly absorbed from food than PCBs. (Zikto and Choi 1973) Di- and Tri- and tetrachlorodibenzofuran were not detectable in juvenile atlantic salmon fed with PCDF containing food. While, some isomers of PCDFs were noticed to be very persistent in the body of mouse to which PCDFs were administered intraperitoneally. Detailed investigation seems to be necessary to elucidate the

distribution and the biological impact in the environment of these highly toxic substances if we consider that at least several ppms of PCDFs were discharged to the environment concomittantly with PCBs.

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