Detection and Determination of Polychlorodibenzofurans in Normal Human Tissues and Kanemi Rice Oils Caused "Kanemi Yusho"

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Hideaki MIYATA, Takashi KASHIMOTO and Nobuharu KUNITA

(Osaka Prefectural Institute of Public Health:
3-69, Nakamichi 1-chome, Higashinari-ku, Osaka)

Because of their chemical stability and their lipophilic nature, it is possible that polychlorodibenzofurans (PCDFs) released into the environment could accumulate in human tissue via food sources. Omentums, livers and a mamma of normal human were used for the PCDFs analysis. PCDFs were detected and determined by means of GC-MS fragmentography. Pentachlorodibenzofuran was detected in three out of five omentums, one of four livers and in a mamma sample at the detection limit of 5 ppt. Their levels ranged from n.d. to 45.4 ppt. Tetra- and hexa-chlorodibenzofuran were not detected.

At the same time, analysis of PCB and PCDF was made in seven samples of Kanemi rice oil which caused "Kanemi Yusho." The concentration of PCB in the oil were 0.9 to 1,029 ppm and the concentration of PCDF were 0.0021 to 5.4 ppm, respectively. The ratios of PCDF vs. PCB concentration in Kanemi rice oils were extraordinary high in comparison with that of commercially available PCB preparation containing 40% chlorine (KC-400). In view of its strong toxicity, it seems very likely that PCDF is a compound which caused "Kanemi Yusho" together with PCB.

1. Introduction

PCDF is very highly toxic to chick embryos,¹⁾ rats²⁾ and rabbits,³⁾ and induces a hepatic microsomal oxygenating enzyme activity 170 times stronger than KC-400.⁴⁾ PCDF was produced as by-product in the industrial purification of PCB. The concentrations of PCDF were 0.8 to 33 ppm in various commercial PCB (Kanechlor, Aroclor, Clophen and Phenoclor).⁵⁻⁷⁾ Because of its chemical stability and its lipophilic nature, the possibility that PCDF released into the environment and accumulate in human body through food sources cannot be disregarded.

In 1972, Zitko *et al.*⁸⁾ tried to determine PCDF and polychlorodibenzo-*p*-dioxin in fish and birds which had inhabited in the area of Fundy Gulf in Canada, but could not find these compounds at analytical sensitivity of 1 ppm.

In this study, PCDF in normal human tissues was determined by means of GC-MS fragmentography which is specific and offers an analytical sensitivity of 5 ppt.

In 1975, Kuratsune *et al.*⁹⁾ reported that PCDF was detected at a level of 4.5 to 5 ppm in

Kanemi rice oil which caused "Kanemi Yusho." Moreover it was found that PCDF accumulated in livers and fatty tissues of people died of "Kanemi Yusho."^{*1}

So, it is recently said that PCDF might be one of the causative compounds of "Kanemi Yusho."

This paper described the concentrations and components of PCDF and PCB in seven samples of Kanemi rice oil which caused "Kanemi Yusho," and referred to the ratio of PCDF vs. PCB concentrations in the oils.

2. Materials and Method

2.1. Samples

Normal human tissues

Omentums and a mamma were obtained from people who underwent operations for September to November 1975. Livers were obtained from people who died accidentally for January to March 1976.

^{*1} Nagayama, J., Masuda, Y. and Kuratsune, M.: Abstract of Papers, 34th Meeting of Japanese Society of Public Health, Yokohama, October, 1975, p. 214.

Kanemi rice oils which caused "Kanemi Yusho"

Kanemi rice oil-1 and 2 (K-1 and K-2, respectively) were obtained from Dr.M. Ueta, Kochi Prefectural Institute of Public Health. K-1 was manufactured on February 10th 1968, and its lot no. was 16793. K-2 was manufactured on March 12th 1968, and its lot no. was 32516. Kanemi rice oil-3,4 and 5 (K-3, K-4 and K-5, respectively) were obtained from Dr.M. Kuratsune, Department of Public Health, Faculty of Medicine, Kyushu University. Kanemi rice oil-6 and 7 (K-6 and K-7, respectively) were obtained from Dr.M. Takamatsu, Department of Public Health, Faculty of Medicine, Kurume University. Although K-3 to K-7 were manufactured in February or March 1968, their detailed production date and lot no. were unknown.

2.2 Reagents

Polychlorodibenzofuran preparation containing 46.3 to 56.8% chlorine were obtained from Wako Pure Chemical Co. Ltd. Florisil of 60 to 80 mesh was activated overnight at 130°C. Alumina was obtained from Merck Co. Ltd., Art. 1077. Acetone, methylene dichloride, ethanol, ethyl ether, *n*-hexane and anhydrous sodium sulfate were reagent grade for PCB analysis of Wako Pure Chemical Co. Ltd. Potassium hydroxide was also reagent grade, of Wako Pure Chemical Co. Ltd.

2.3. Analytical method

Ten to twenty grams of samples were saponified in 300 ml of 1.5 *N* alcoholic potassium hydroxide solution by refluxing for 1 hr. One hundred ml of water was added to the saponified sample, then the aqueous solution was extracted with 3×150 ml of *n*-hexane. The hexane extract was washed with 2×200 ml of water, dried over anhydrous sodium sulfate and concentrated to about 5 ml.

Fifteen grams of Florisil (bottom layer) and 5 g of anhydrous sodium sulfate (top layer) were packed with *n*-hexane in a 2 cm i.d. glass column. Before the application of sample to the column, Florisil column was prewashed with 50 ml of *n*-hexane. The concentrated hexane extract was added into the column, and the wall of the column was rinsed with 2 ml of *n*-hexane. The column was then eluted with the following four eluants successively at a rate of about 2 ml/min: 100 ml of *n*-hexane, 100 ml of 5% ethyl in *n*-

hexane solution, 200 ml of 50% ethyl ether in *n*-hexane solution and 200 ml of acetone. The third and fourth fraction were combined, concentrated to about 5 ml, and dried under a jet of air at 30°C. The residue was dissolved with 3ml of 3% methylene chloride in *n*-hexane solution, and put into alumina column.

Twenty grams of alumina (bottom layer) and 5 g of anhydrous sodium sulfate (top layer) were packed with *n*-hexane in a 2 cm i.d. glass column. Before the application of sample was prewashed the column with 50 ml of *n*-hexane. Sample was then put onto the column and eluted successively with 200 ml of 3% methylene chloride in *n*-hexane solution and 200 ml of 20% methylene chloride in *n*-hexane solution. Small quantity of PCB contaminated in the third and fourth eluant from above mentioned Florisil column was removed by 3% methylene chloride in *n*-hexane eluant. The 3% methylene chloride in *n*-hexane fraction was added to the first and second fractions from the Florisil column. After the combined fraction was concentrated, PCB was determined by ECD-GC. The concentration of PCB was calculated with the method of Kashimoto *et al.*⁹⁾ Gas chromatography used was Shimadzu GC-6A equipped with ⁶³Ni-ECD. GC condition: 2 m×3 mm glass column packed with 2% OV-1 on Gaschrom Q (100~120 mesh); Injection and detection temp., 228°C; column temp., 198°C; carrier gas, N₂ (35 ml/min).

Twenty percent methylene chloride in *n*-hexane fraction was concentrated, and analyzed for PCDF by ECD-GC and GC-MS. PCDF in Kanemi rice oils was confirmed by GC-MS and determined by ECD-GC. The condition of GC-MS and ECD-GC was described previously⁵⁾.

As the concentration of PCDF in human tissues was extraordinary low level, PCDF was confirmed and determined with the method of GC-MS fragmentography. GC-MS analysis was carried out on a Shimadzu LKB-9000 GC-MS equipped with MID. GC-MS condition: 2 m×3 mm glass column packed with OV-17 on Gaschrom Q (100~120 mesh); column temp., programmed from 240 to 280°C (3°C/min); ionizing voltage, 20 eV; accel. voltage, 3.5 kV; chamber temp., 280°C.

3. Result

3.1. PCB and PCDF in normal human tissues

The concentrations of PCB were 0.67 to 1.72

Table 1. Tetra-, Penta- and Hexachlorodibenzofuran in Normal Human Tissues

Sample	Sex	Age	Conc. of PCDF (ppt) ^{a)}			Conc. of PCB (ppm)*	Ratio ^{b)}
			Tetra-	Penta-	Hexa-		
Omentum	Male	59	n.d.	13.8	n.d.	0.67	1 : 48,551
Omentum	Male	46	n.d.	31.6	n.d.	1.16	1 : 36,708
Omentum	Female	65	n.d.	n.d.	n.d.	0.60	
Omentum	Female	63	n.d.	n.d.	n.d.	0.92	
Omentum	Female	75	n.d.	45.4	n.d.	1.72	1 : 39,427
Liver	Male	34	n.d.	17.2	n.d.	0.052	1 : 3,023
Liver	Male	57	n.d.	n.d.	n.d.	0.029	
Liver	Male	57	n.d.	n.d.	n.d.	0.017	
Liver	Female	43	n.d.	n.d.	n.d.	0.012	
Mamma	Female	42	n.d.	19.7	n.d.	1.25	1 : 63,451

a) Whole base

b) Ratio of PCDF vs. PCB concentration

ppm in omentums, 0.012 to 0.015 ppm in livers and 1.25 ppm in a mamma sample as shown in Table 1.

Tetra-, penta- and hexachlorodibenzofurans were determined by GC-MS. Tetra- and hexachlorodibenzofurans were not detected at analytical sensitivity of 5 ppt. However pentachlorodibenzofuran (P-PCDF) was found in three of five omentums, in one of four livers and in a mamma sample as shown in Table 1. The concentrations of P-PCDF were n.d. to 45.4 ppt in omentums, n.d. to 17.2 ppt in livers, and 19.7 ppt in a mamma sample.

Figure 1 shows mass fragmentograms of *m/e* 338, 340 and 342 of the PCDF fraction from samples and of the P-PCDF standard. *m/e* 338, 340 and 342 were M^+ , (M^++2) and (M^++4) of P-PCDF, respectively.

P-PCDF in omentums and livers was composed of two isomers, but that in a mamma was composed of only one. As shown in Fig. 1, the retention times of these two isomers were not different from that of P-PCDF standard.

3.2. PCB and PCDE in Kanemi rice oils

The concentrations of PCB in Kanemi rice oils were 0.9 to 1,029 ppm as shown Table 2. The component ratios of PCB in the oils were shown in Table 3.

The component ratios of PCB in Kanemi rice oils in comparison with those of KC-400 were found to be that di- to tetrachlorobiphenyls decreased and penta- to octachlorobiphenyls in-

Fig. 1. Mass fragmentograms of pentachlorodibenzofuran standard and the PCDF fraction from normal human tissues

creased.

The concentration of PCDF in Kanemi rice

oils ranged 0.0021 to 5.4 ppm as shown in Table 2. Gas chromatograms of the PCDF fraction in Kanemi rice oils and KC-400 were shown in Fig. 2, and compounds corresponding to each peak were identified by GC-MS as shown in Table 4. Tri- to hexachlorodibenzofurans were found in Kanemi rice oils. More chlorinated benzofurans than tetrachlorodibenzofuran increased in comparison with PCDF in KC-400.

The mass spectra of peaks 7 and 13 in Fig. 3-(K-1) were given in Fig. 3 (a and b, respectively). Peaks 7 and 13 were identified as tetra- and pentachlorodibenzofuran, respectively. Tetrachlorodibenzofuran fragments were as follows (cf. Fig. 3-a): The molecular ion (M^+) was found at m/e 304, loss of COCl yield the peaks at m/e 241, subsequently elimination of double Cl resulted in m/e 171. The P-PCDF fragments were identified in an analogous fashion

as described above.

4. Discussion

In 1972, Zitko *et al.*⁸⁾ could not detect any PCDF in fish and birds which inhabited in the area of Fundy Gulf in Canada. Likewise, in 1975, Kuratsune *et al.*^{*1} also could not find any PCDF in normal human tissues. The reason why they failed to detect PCDF attributed mainly to the low analytical sensitivity.

In view of above results, it is obvious that

Fig. 2. Gas chromatograms of the PCDF fraction from Kanechlor 400 and Kanemi rice oil (K-1)

K-1, its production date was February 10, 1968, and its lot no. was 16793

Table 2. Concentration of PCDF and PCB in Kanemi Rice Oils Which Caused "Kanemi Yusho"

Sample	Concentration of PCB (ppm)	Concentration of PCDF (ppm)	Ratio ^{a)}
KC-400		33	1 : 30,303
K-1	154	2.2	1 : 70
K-2	0.9	0.0021	1 : 492
K-3	1,029	5.3	1 : 194
K-4	1,025	4.9	1 : 209
K-5	1,023	5.4	1 : 189
K-6	2.7	0.0031	1 : 871
K-7	2.7	0.0021	1 : 1,286

^{a)} Ratio of PCDF PCB concentration

Table 3. Component Ratio of PCB in Kanemi Rice Oils

Cln ^{a)}	Sample							
	KC-400	K-1	K-2	K-3	K-4	K-5	K-6	K-7
2	0.74	1.44	1.42	1.26	2.31	1.63	1.81	0.18
3	18.32	4.35	13.39	7.56	8.20	8.19	9.03	9.90
4	47.92	23.45	43.26	36.03	37.30	36.60	42.09	42.93
5	23.02	40.25	29.48	36.02	32.45	35.10	32.54	32.59
6	7.99	18.35	9.65	13.26	13.91	13.14	11.01	11.08
7	1.71	9.68	2.53	5.28	5.20	4.85	3.15	2.75
8	0.12	2.53	0.36	0.66	0.68	0.40	0.50	0.40
9	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00

^{a)} Number of chlorine atom per PCB molecule

Table 4. Compounds of Chromatograph Peaks of the PCDF Fraction from KC-400 and Kanemi Rice Oils

Peak No.	Sample	
	KC-400	Kanemi rice oil
1	2C1 PCDF ^{a)} , DBP	3C1 PCB
2	3C1 PCB ^{a)} , 4C1 PCB	3C1 PCDF
3	3C1 PCDF	4C1 PCB
4	3C1 PCDF	4C1 PCDF
5	3C1 PCDF	4C1 PCDF
6	3C1 PCDF, 3C1 PCB, 4C1 PCB ^{a)}	4C1 PCDF
7	4C1 PCDF ^{a)} , 4C1 PCB	4C1 PCDF
8	4C1 PCDF ^{a)} , 4C1 PCB	4C1 PCDF ^{a)} , 5C1 PCB
9	4C1 PCDF	5C1 PCDF
10	4C1 PCDF	5C1 PCDF
11	4C1 PCDF ^{a)} , 4C1 PCB	?
12	4C1 PCDF, 5C1 PCDF, M. 5C1 PCN	5C1 PCDF
13	4C1 PCDF, 5C1 PCDF, 6C1 PCN	5C1 PCDF
14	5C1 PCDF ^{a)} , M. 5C1 PCN	6C1 PCDF
15	5C1 PCDF	6C1 PCDF
16	5C1 PCDF, M. 5C1 PCN	?
17	7C1 PCN	6C1 PCDF
18	6C1 PCDF	
19	6C1 PCDF	
20	6C1 PCDF	

^{a)} Main component: M., monomethyl; PCN, polychloronaphthale; PCN, polychloronaphthalene

the limited components of PCDFs were accumulated in human bodies through food sources.

On February 5th 1968, the transfer medium (KC-400) leaked out into Kanemi rice oil in the deodorization tank. The tank was maintained at about 230°C and 4 mmHg pressure. A high pressure steam was jetted into the tank in order to deodorize the oil. Kanemi rice oil, which was contaminated with KC-400, caused "Kanemi Yusho" in 1968 in Japan.

In this study, the concentrations of PCB and PCDF in Kanemi rice oils ranged 0.9 to 1,029 ppm and 0.0021 to 5.4 ppm, respectively. Thus variation of the concentration attributed to the production dates. The component ratios of PCB in Kanemi rice oils were different from that of KC-400; *e.g.* the component ratios of more chlorinated biphenyls than tetrachlorobiphenyl in-

Fig. 3. Mass spectra of peaks 7 and 13 in Fig. 2-(K-1)

creased in comparison with that of KC-400. Similar results were observed on the components of PCDF in Kanemi rice oils. After KC-400 leaked out into the deodorization tank, such change of the component ratios of PCB and PCDF in Kanemi rice oils might occur in the tank.

The ratios of PCDF *vs.* PCB concentration in Kanemi rice oils were extraordinary high in comparison with that of KC-400. This reason may be explained as follows. "While KC-400 was used as transfer medium, PCDF would be formed from KC-400.^{*2,*3} After the KC-400 leaked out into Kanemi rice oil in the deodorization tank, PCB and PCDF would evaporate from the tank. However, the evaporation rate of PCB from the tank would be higher than that of PCDF. Consequently the ratios of PCDF *vs.* PCB concentration in the oil would become extraordinary high."

PCDF was found in fatty tissues and livers of people dead of "Kanemi Yusho," and the ratios of PCDF *vs.* PCB concentration were

^{*2} Morita, M., Nakagawa, J., Akiyama, K. and Mimura, K.: Abstract of Papers, 31th Meeting of Food Hygienic Society of Japan, Tokyo, May, 1976, p. 35.

^{*3} Miyata, H., Kashimoto, T. and Kunita, N.: Abstract of Papers, 32th Meeting of Food Hygienic Society of Japan, Okayama, October, 1976, p. 37.

extraordinary high; *i.e.*, the ratios in livers were 0.1 to 0.5, and in fatty tissues were 0.005 to 0.009*¹. Moreover, PCDF shows very high acute toxicity and strong induction of drug metabolizing enzyme.

In view of the above facts, it is very likely that PCDF is a compound which caused "Kanemi Yusho" together with PCB.

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