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Polychlorinated Biphenyls and Polychlorinated Dibenzofurans in the Toxic Rice-Bran Oil that Caused PCB Poisoning in Taichung

P. H. Chen, K. T. Chang, and Y. D. Lu

Department of Biochemistry, National Yang Ming Medical College, Taipei, Taiwan, R.O.C.

In March 1979, an epidemic of a peculiar skin disease broke out in Taichung and Changhwa in Central Taiwan. The cause of the disease was later identified to be the ingestion of rice-bran oil contaminated with polychlorinated biphenyls (CHEN et al. 1980). At the end of 1980, the total number of reported cases was about 2000.

A similar mass outbreak of Yusho (oil disease) occurred in Western Japan in 1968 (KURATSUNE et al. 1972). In 1969, Japanese scientists reported that the toxic rice oil which caused Yusho was contaminated with polychlorinated biphenyls (TSUKAMOTO et al. 1969). A few years later, the oil was also found to be contaminated with a smaller quantity of polychlorinated dibenzofurans (NAGAYAMA et al. 1975) and a relatively large amount of polychlorinated quaterphenyls (MIYATA et al. 1978, KAMPS et al. 1978, MASUDA et al. 1979). In this study, the levels of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs) in the toxic rice oils which caused PCB poisoning in Taichung were determined by ECD-Gas Chromatography and Gas Chromatography/Mass Spectrometry. The presence of polychlorinated quaterphenyls (PCQs) and polychlorinated terphenyls (PCTs) in the Chinese toxic rice oil has been published elsewhere (CHEN et al. 1981).

MATERIALS AND METHODS

Toxic rice-bran oil samples were obtained from six different sources in Taichung. One of these samples was collected at the rice oil retail store by the Food and Drug Bureau of the Republic of China. The other five samples were obtained from school and factory cafeterias and the families of the intoxicated patients in Taichung. The cafeterias and families all bought the toxic rice oils from the oil retailer mentioned above.

The alkali decomposition method similar to the one used by the Japanese workers (NAGAYAMA et al. 1976) was employed for the isolation of PCB and PCDF from the rice oil. This method includes the saponification of the oil with an alkali followed by extraction of PCB and PCDF with n-hexane. The condensed extract was cleaned up

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by silica gel column chromatography using n-hexane as an eluting solvent. The purified extract was analyzed by ECD-Gas Chromatography for PCB. The gas chromatograph used was a Shimadzu GC-6AM equipped with ^{63}Ni Electron Capture Detector. The column used was a 2.5 m x 2.6 mm i.d. glass column packed with 5% SE-30 on 80/100 mesh Chromosorb WAW-DMCS. Quantification of PCB was made by comparing the respective area of PCB peaks in the sample with the area of the corresponding peak in the chromatogram of a PCB standard (Kanechlor-500). The method presented by UGAWA et al. (1973) was used for the calculation of PCB quantity in each peak of Kanechlor-500 (KC-500).

The procedure for the separation of PCDF from PCB is as follows: The condensed eluate from the silica gel column chromatographic clean-up was placed on a column (1.2 x 22 cm) packed with 12 g of basic alumina. The column was first eluted with 115 mL of n-hexane containing 2% methylene chloride, then with 100 mL of n-hexane containing 25% methylene chloride. PCDF was expected to be in the eluate containing 100 mL of 25% methylene chloride in n-hexane. The eluate of PCDF fraction was concentrated in a Kuderna-Danish evaporator to about 5 mL, then was blown carefully with a very mild stream of nitrogen to below 0.5 mL. The concentrated eluate was analyzed for PCDF with a computerized GC/MS (HP 5985A) operating in the selected ion monitoring mode to enhance the instrument's sensitivity. The column used for GC/MS analysis was a 6 ft x 2 mm i.d. glass column packed with 3% OV-17 on 100/120 Supelcoport.

RESULTS AND DISCUSSION

Gas chromatograms of a mixture of KC-400 and KC-500 in a 1 to 1 ratio and PCBs in the contaminated rice-bran oil are shown in Fig. 1. As shown in this figure, in the portion of the chromatogram from peak 9 to peak 25, GC pattern of PCBs in the toxic rice

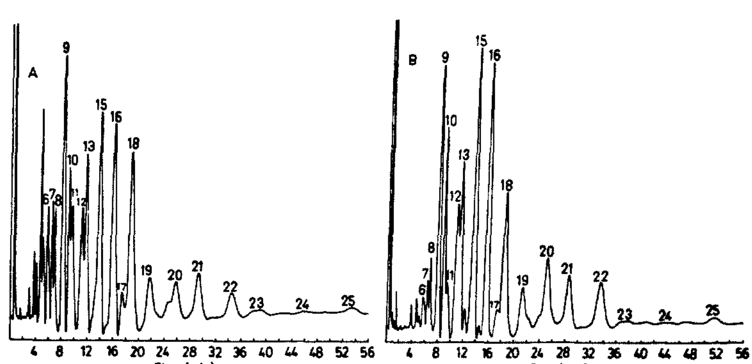

Fig. 1. Gas chromatograms of PCB. A: KC-400/KC-500 (1:1), B: Toxic rice-bran oil.

oil is similar to that of KC-400/KC-500 (1:1); however, in the front portion of the chromatogram, peaks are smaller in the rice oil. This decrease in intensity of early eluting peaks in the rice oil is probably due to the evaporative loss of the more volatile PCB components during heating at a high temperature. This preferential loss of more volatile components is based on the presumption that PCB in the contaminated rice oil came from the leak of the heat transfer medium during the manufacturing process in which PCB was presumably used as a heat transfer medium. The similarity of GC pattern of PCBs in the rice oil with that of KC-400/KC-500 (1:1) does not mean that the toxic rice oil was actually contaminated with KC-400 and KC-500, it might be contaminated with other PCB preparations which give a similar GC pattern. It should be pointed out that GC/MS analysis showed a significant difference in the composition of peak 9 in Fig. 1 between PCBs in the toxic rice oil and KC-400/KC-500 (1:1). In the rice oil, peak 9 is predominantly due to a tetrachlorobiphenyl with a minor amount (5-10%) of a pentachlorobiphenyl (see Fig. 2), whereas in KC-400/KC-500 (1:1) the percentage of the pentachlorobiphenyl in peak 9 is substantially higher.

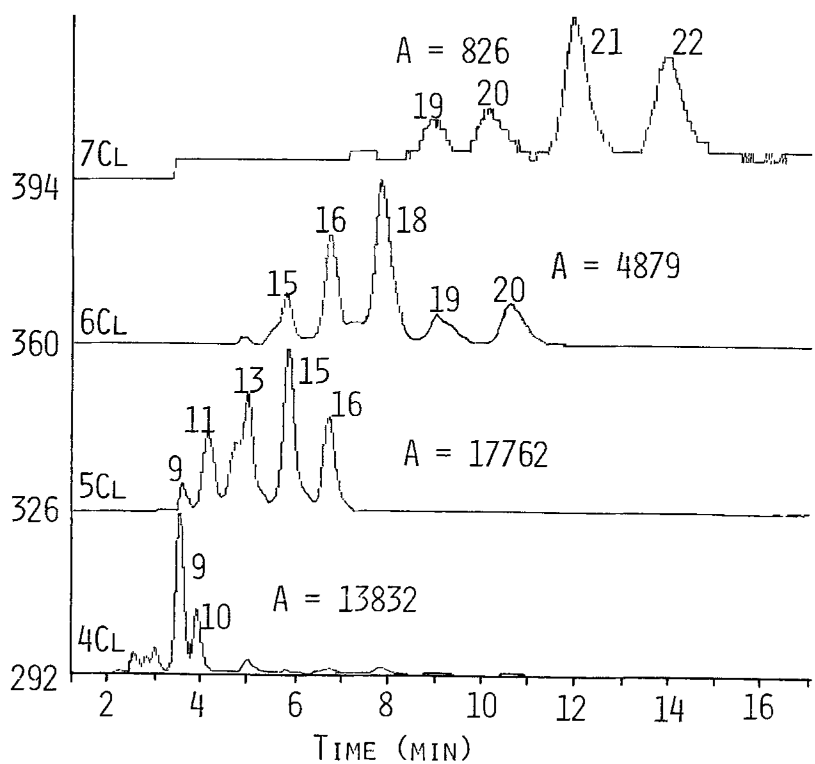

Fig. 2. Selected ion chromatogram of PCBs in the toxic rice-bran oil.

GC/MS analysis showed that the percentage distribution of PCB components in the toxic rice oil according to numbers of chlorine atoms is about 47%, 30%, 16%, 6%, and 1% for pentachloro-, tetra-chloro-, hexachloro-, heptachloro-, and trichloro-biphenyls, respectively. This indicates that the toxic rice oil which caused PCB poisoning in Taichung contained larger percentages of high numbers of chlorine atoms than the rice oil which caused Yusho in Japan, for the Japanese rice oil was known to be contaminated with KC-400 which contained mainly tetrachlorobiphenyls. Since it is difficult to excrete PCBs with five or more chlorine atoms from human body, it is reasonable to expect that PCB components ingested by the Chinese patients in Taichung will be retained in the body longer than that of the Japanese Yusho patients.

GC/MS operating in selected ion monitoring (SIM) mode was used for the identification and quantification of PCDFs in the rice-bran oil. As shown in Fig. 3, the ions at m/e's 270, 306, 340, and 374, which are the molecular ions for PCDFs with 3, 4, 5, and 6 chlorine atoms, respectively, were chosen for monitoring PCDFs. Quantification of PCDFs in the rice oil was made by comparing the area of PCDF peaks in each selected ion tracing for the sample with the area of the peak(s) in the same selected ion tracing for the PCDF standard. Fig. 3 and Fig. 4 show the selected ion chromatograms of PCDFs in the rice oil and PCDFs standard, respectively. The results of GC/MS analysis showed, as is evident in Fig. 3, that PCDFs in the toxic rice-bran oil contained mostly 5 and 4 chlorine atoms with a minor amount of 6 and 3 chlorine atoms.

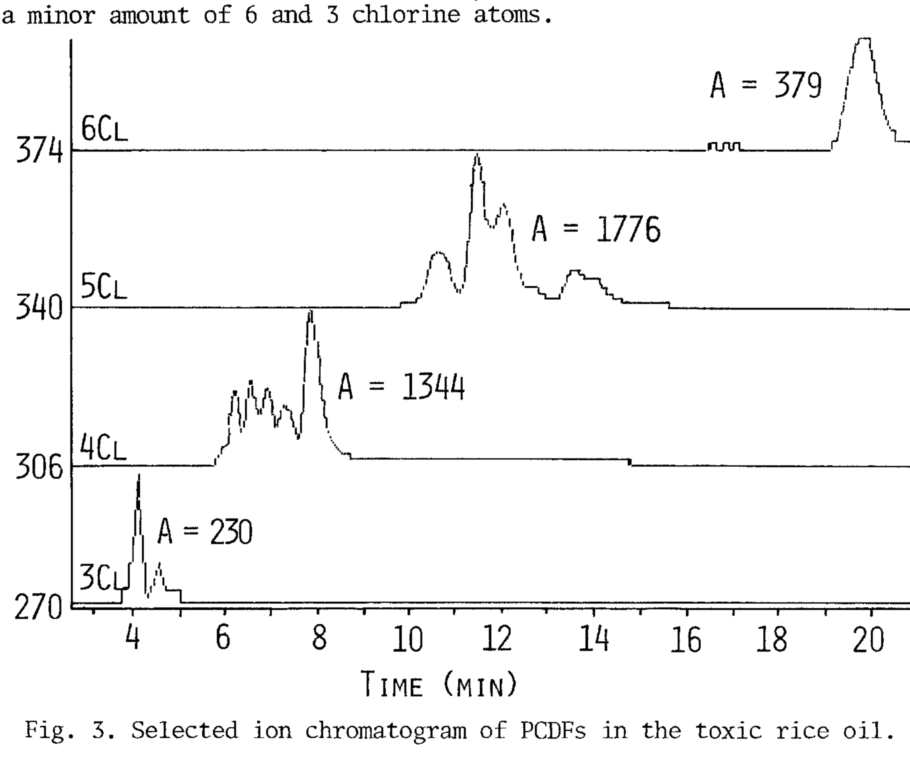

Fig. 3. Selected ion chromatogram of PCDFs in the toxic rice oil.

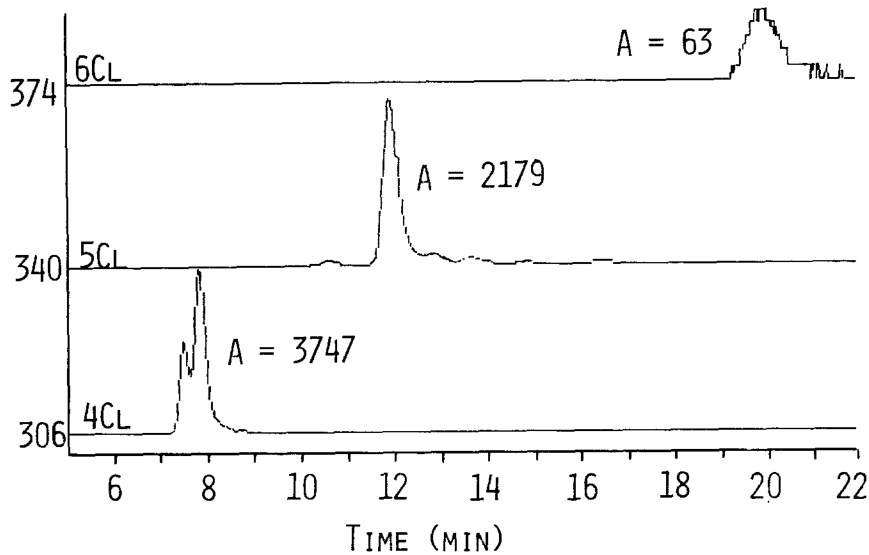

Fig. 4. Selected ion chromatogram of PCDFs standard.

The concentrations of PCB and PCDF in the toxic rice-bran oils which caused PCB poisoning in Taichung are tabulated in TABLE 1. This table shows that with the exception of sample A which has a high PCB level of 405 ppm, the other five samples contain PCB in a range of 53 to 99 ppm. Sample A was given to us by the Food and Drug Bureau (FDB) of the National Health Administration of the Republic of China. According to the FDB's report, this sample contained the highest level of PCB among the thirty-nine samples collected by them at the rice oil retail store where the intoxicated patients in Taichung bought the toxic rice oil. The other five oil

TABLE 1. The Concentrations of PCB and PCDF in the Toxic Rice-Bran Oils Which Caused PCB Poisoning in Taichung

Sample	PCB, ppm	PCDF, ppm	PCB/PCDF
A	405	1.68	241
B	53	0.180	294
C	99	0.399	248
D	78	0.250	312
E	77	0.209	368
F	65	0.297	219

samples obtained from factory and school cafeterias and the families of PCB-intoxicated patients in Taichung contained much lower levels of PCB than sample A. If PCB levels of these five samples are representative of the toxic rice oils ingested by the patients in Taichung, then PCB levels in the toxic rice oils consumed by the patients in Taichung ought to be much lower than that of the Japanese toxic rice oil which in general contained about 830-1030 ppm of PCB (NAGAYAMA et al. 1975). This does not necessarily mean that the Chinese patients in Taichung ingested less PCB than the Japanese Yusho patients because the Chinese patients consumed much more toxic rice oil than the Japanese patients. The estimated amount of PCB ingested by the Chinese patients in Taichung is published in a Special Issue of PCB Poisoning in Taiwan (LAN et al. 1981). The level of PCB in the toxic oil consumed by the patients in Changhwa is not known because no toxic oil sample has ever been available for analysis. However, based on the severity of clinical symtoms for the patients in Changhwa and the blood PCB analysis which showed that the patients in Changhwa had in general higher PCB levels than those in Taichung, one can presume that the toxic oil ingested by the patients in Changhwa contained higher levels of PCB than that consumed by the patients in Taichung. Fortunately, the number of patients in Changhwa is about one half of that in Taichung.

The levels of PCDF in the toxic rice-bran oils determined with GC/MS are mostly below 0.5 ppm with an exception of sample A which is 1.7 ppm (see TABLE 1). These PCDF levels are also lower than that of the Japanese toxic rice oil which is about 5 ppm (NAGAYAMA et al. 1975).

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REFERENCES

CHEN, P. H., J. M. GAW, C. K. WONG, and C. J. CHEN: Bull. Environm. Contam. Toxicol. 25, 325 (1980).

CHEN, P. H., Y. D. LU, M. H. YANG, and J. S. CHEN: Clinical Medicine (Taipei) 7, 77 (1981).

KAMPS, L. R., W. J. TROTTER, S. J. YOUNG, et al.: Bull. Environm. Contam. Toxicol. 20, 589 (1978).

KURATSUNE, M., T. YOSHIMURA, J. MATSUZAKA, and A. YAMAGUCHI: Environ. Health Perspect. No. 1, 119 (1972).

LAN, C. F., P. H. CHEN, L. L. HSIEH, and Y. H. CHEN: Clinical Medicine (Taipei) 7, 96 (1981).

MASUDA, Y. and M. KURATSUNE: Fukuoka Acta Med. 70, 229 (1979).
MIYATA, H., Y. MURAKAMI, and T. KASHIMOTO: J. Food Hyg. Soc. JAPAN 19, 417 (1978).
NAGAYAMA, J., Y. MASUDA, and M. KURATSUNE: Fukuoka Acta Med. 66, 593 (1975).
NAGAYAMA, J., M. KURATSUNE, and Y. MASUDA: Bull. Environm. Contam. Toxicol. 15, 9 (1976).
TSUKAMATO, H., S. MAKISUMI, H. YOSHIMURA, et al.: Fukuoka Acta Med. 60, 496 (1969).