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Levels and Gas Chromatographic Patterns of Polychlorinated Biphenyls in the Blood of Patients after PCB Poisoning in Taiwan

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In March 1979, an epidemic of a peculiar skin disease was reported in Taichung and Changhwa of the central Taiwan. In October, the cause of the disease was identified to be the ingestion of rice oil contaminated with polychlorinated biphenyls(PCB). At the end of April 1980, the victims numbered 1900. The magnitude of this PCB poisoning is likely to surpass a similar mass outbreak of "Yusho" in Japan in 1968(KURATSUNE et al. 1972).

Since December 1979, we have been engaged in the analysis of PCB levels in the blood of these PCB-intoxicated patients. We have been using both GC/MS and ECD-Gas Chromatography for the quantification of PCB levels in the blood of patients. We report here the blood PCB levels of sixty-six patients determined by ECD-Gas Chromatography. Quantification of PCB residues by GC/MS method will be published elsewhere.

MATERIALS AND METHODS

Blood samples were collected at the hospital ward and the out-patient clinic of the Department of Dermatology, Veterans General Hospital, Taipei, or at patients' residential areas in Taichung during the period of December 1979 to March 1980. The blood(about 10 ml) drawn from each patient was transferred to a 20-ml glass tube containing 200 USP units of heparin in 0.2 ml solution. For isolation of PCB from the blood sample we used the alkali decomposition method similar to the one used by AKIYAMA et al.(1975). The procedure is as follows: To 10 g of whole blood was added 20 ml of ethanol. Five g of potassium hydroxide was added to this and the whole mixture was refluxed in a steam bath for one hour. After cooling, the content was extracted with 20 ml of redistilled n-hexane for three times. The combined n-hexane extract was washed with 20 ml of water for three times, followed by drying over anhydrous sodium sulfate. The dried extract was concentrated in a Kuderna-Danish evaporator to about 5 ml, then carefully blown with a very mild stream of nitrogen to about 1 ml.

The condensed extract was cleaned up by silica gel column chromatography. A mixture of 3 g of activated silica gel(Wakogel S-1) and 25 ml n-hexane was poured into a 1.7 x 22 cm glass column. After washing the column with about 25 ml of n-hexane, PCB extract was applied to the top of the column, then the column was eluted

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with n-hexane. Discard the first 25 ml, then collect the next 100 ml of eluant. Concentrate the eluant in a Kuderna-Danish evaporator to about 5 ml, then carefully blow with a very mild stream of nitrogen to below 1 ml. The condensed 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 Chromosorb WAW-DMCS, carrier gas nitrogen flow rate was 40 ml/min. The column and detector temperatures were maintained at 220°C and 270°C, respectively.

Quantitation of PCB residues was made by comparing respective area of PCB peaks in the sample with the area of the corresponding peak in the chromatogram of KC-500. For the calculation of PCB quantity in each peak of KC-500, we followed the method presented by UGAWA et al.(1973).

RESULTS AND DISCUSSION

Gas chromatograms of KC-500, KC-400/KC-500(1:1), and PCB residues in the contaminated rice oil are shown in Fig. 1. Three typical chromatograms of PCB residues in the patients' blood are shown in Fig. 2. The peak numbering system in the chromatograms are the same as that used by UGAWA et al.(1973). In the portion of peaks 9 to 25, gas chromatographic pattern of PCB in the contaminated rice oil(Fig. 1-C) is similar to that of KC-400/KC-500 (1:1) (Fig. 1-B). The chlorine content of PCB residues in the rice oil as determined by GC/MS method was about 52-53%, this is between those of KC-400(47.9%) and KC-500(54.6%).

Gas chromatographic patterns of PCB in the blood of patients can be classified into three types, i.e., types A, B, and C. In type A, peaks 15 and 16 are larger than peak 18(see Fig. 2-A), whereas in type B, the reverse is true(see Fig. 2-B). Other than this difference, the relative intensities of other peaks in the two chromatograms are about the same. In type C, pattern of peaks 15 to 25 is similar to that of type A, however, peaks 9 and 11 are much larger than those of either type A or B(see Fig. 2-C). The chlorine numbers of PCB components corresponding to peaks 9,11,15, 16, and 18, as determined by GC/MS, are 4,5,5,5 and 6, and 6, respectively. Examination of GC patterns of a large numbers of blood samples revealed that among PCB components corresponding to peaks 15, 16, and 18, the component of peak 18(a hexachlorobiphenyl) was retained in human body longer than the PCB components of peak 15(a pentachlorobiphenyl) and of peak 16(a mixture of penta- and hexachlorobiphenyls). The faster excretion of PCB components of peaks 15 and 16 than that of peak 18 likely contributes to the formation of type B pattern.

Patients whose blood analyzed by us are mostly from Taichung. The blood PCB patterns of these patients belong to types A and B.

Only five out of sixty six patients are from Changhwa area or have ingested rice oil bought from Changhwa. The blood PCB pattern of these five patients belongs to type C. This suggests that either the rice oil ingested by the patients from Changhwa contained PCB of higher percentages of lower chlorine numbers than the rice oil

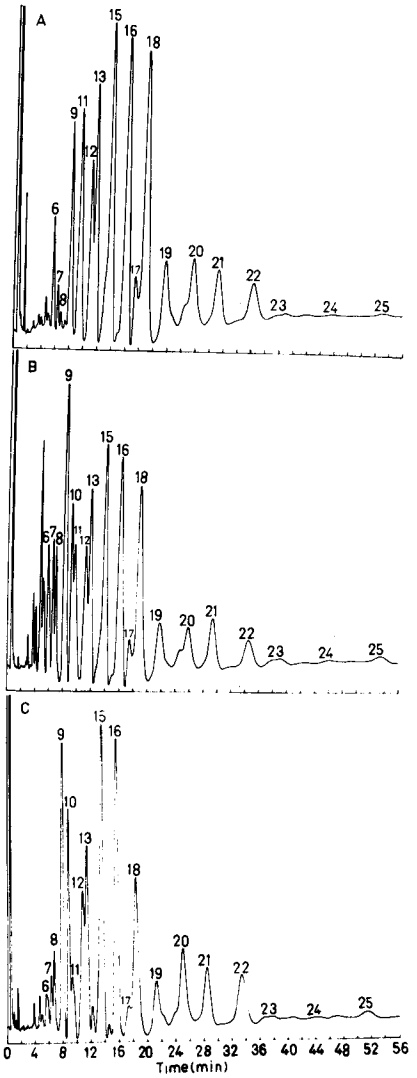

Fig. 1. Gas chromatograms of PCB.
A: KC-500, B: KC-400/KC-500 (1:1), C: Rice oil.

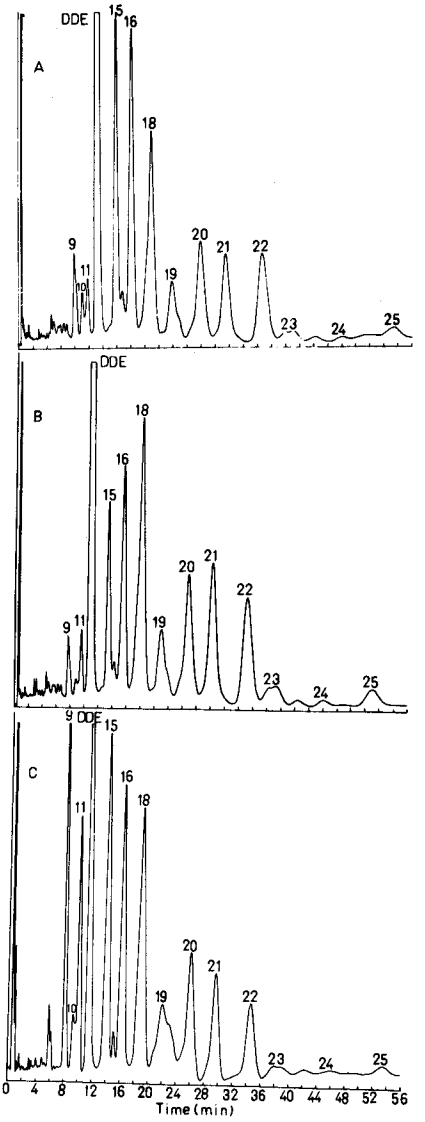

Fig. 2. Gas chromatograms of PCB residues in human blood. A, B, and C: Patterns of types A, B, and C.

TABLE 1. PCB Levels in the Blood of PCB-Intoxicated Patients

No.	Age	Sex	Sampling date	PCB level (ppb)	No.	Age	Sex	Sampling date	PCB level (ppb)
1	22	F	12/21/79	40	34	25	F	2/26/80	22
2	23	F	12/21/79	61	35	17	F	2/26/80	28
3	37	F	12/21/79	40	36	17	F	2/26/80	16
4	22	M	12/21/79	62	37	22	F	2/26/80	17
5	39	M	1/13/80	59	38	21	F	2/26/80	20
6	34	F	1/13/80	62	39	31	F	3/ 4/80	34
7	28	F	1/14/80	52	40	24	F	3/ 4/80	25
8	45	F	1/14/80	35	41	16	F	3/ 4/80	29
9	27	F	1/14/80	66	42	56	F	3/ 5/80	720
10	69	F	1/14/80	50	43	12	F	3/ 7/80	56
11	30	M	1/15/80	50	44	11	M	3/ 7/80	48
12	26	F	1/31/80	37	45	8	M	3/ 7/80	82
13	48	M	1/31/80	19	46	13	M	3/ 7/80	71
14	24	F	1/31/80	21	47	20	F	3/11/80	28
15	20	F	2/ 1/80	41	48	16	F	3/11/80	11
16	24	F	2/ 1/80	33	49	17	F	3/11/80	21
17	31	M	2/ 1/80	37	50	60	M	3/11/80	26
18	31	M	2/ 1/80	67	51	60	M	3/12/80	35
19	24	F	2/ 1/80	41	52	25	M	3/12/80	42
20	21	F	2/ 1/80	31	53	58	M	3/14/80	76
21	30	M	2/ 1/80	27	54	31	M	3/14/80	26
22	18	F	2/ 1/80	31	55	42	M	3/14/80	120
23	29	F	2/ 1/80	17	56	66	F	3/14/80	51
24	26	M	2/ 1/80	58	57	24	M	3/18/80	21
25	48	M	2/ 1/80	83	58	28	M	3/18/80	24
26	B	M	2/ 4/80	15	59	8	M	3/18/80	76
27	B	M	2/ 4/80	21	60	23	M	3/18/80	24
28	25	F	2/ 5/80	71	61	19	F	3/18/80	26
29	20	F	2/13/80	24	62	17	F	3/18/80	21
30	19	F	2/23/80	32	63	19	F	3/18/80	26
31	28	M	2/26/80	34	64	20	F	3/18/80	16
32	20	F	2/26/80	14	65	21	M	3/25/80	21
33	16	M	2/26/80	25	66	56	M	3/28/80	21

M, F, and B designate male, female, and baby(one month old), respectively.

from Taichung, or these patients from Changhwa happened to excrete PCB components of peak 9(a tetrachlorobiphenyl) and peak 11(a pentachlorobiphenyl) slower than normal patients. As a matter of fact, two of the five patients have abnormal liver function which might explain the slower elimination of PCB components of peaks 9 and 11 by these two patients. The gas chromatogram of PCB residues in the blood of one of these two patients is shown in Fig. 2-C.

In the chromatograms of PCB residues in the blood of patients, peaks 12 and 13 overlap with a large peak due to DDE(see Fig. 2). Therefore, peaks 12 and 13 were not included in the calculation of PCB quantity. Fortunately, the exclusion of these two peaks in the quantification of PCB would presumably not lead to a large error. This is based on the data from the selected ion chromatograms in GC/MS analysis which showed that, in most cases, peaks 12 and 13 were relatively small in the blood samples.

Blood PCB concentrations of 66 patients are tabulated in Table 1. They range from 11 to 720 ppb. The mean value is 49 ppb. The high value of 720 ppb(patient no. 42) is much higher than the mean value. If this very high value of 720 is excluded in the calculation of the mean, then the mean value drops to 39 ppb. The very high PCB concentration in the blood of patient no. 42 is at least in part due to his difficulty in metabolizing and subsequen excretion of PCB components from his body. This is supported by clinical data which indicated that the patient's hepatic and renal functions were both abnormal. It may also be due to the ingestion of unusually high quantities of PCB by this patient. The GC pattern of PCB residues in the blood of this patient is shown in Fig. 2-C.

The blood PCB levels of the PCB-intoxicated patients reported in this study are much higher than those of Japanese Yusho patients. For the Yusho patients, the mean PCB value of seventy-two patients was 5.9 ± 4.5 S. D. ppb(KODA and MASUDA 1975). This difference is, to a large extent, presumably due to the difference in time lags between PCB intoxication and blood PCB analysis. For Yusho patients, the blood PCB analysis was done about five years after the ingestion of the toxic rice oil, whereas for our patients, the blood PCB measurement was made about nine months to one year after intoxication. It is expected that the blood PCB level of Yusho patients would drop significantly five years after intoxication. Other factor which is attributable to the higher blood PCB level in our patients than in Japanese Yusho patients may be due to the difference in the degree of intoxication between Chinese and Japanese patients. It should be noted that the PCB ingested by Chinese patients contained larger percentages of high numbers of chlorine(such as 5, 6, and 7) than that ingested by Japanese Yusho patients. These highly chlorinated PCBs, i.e., penta-, hexa-, and heptachlorobiphenyls, will be retained in human body longer than the lower chlorinated PCBs such as tri- and tetrachlorobiphenyls.

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