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*Department of Environmental Health, Kurame University School of Medicine, Kurume 830, Japan

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CHARACTERISTICS OF PCB LEVEL AND GASCHROMATOGRAM PATTERN IN BLOOD PLASMA OF YUSHO PATIENTS AMONG FISHERMEN

As we reported elsewhere, PCB levels in the blood plasma of Yusho patients among the fishers in Tamanoura-cho of Nagasaki Prefecture were higher than those of the patients among non-fishers in Fukuoka Prefecture. Our hypothesis in the present study is that the high PCB level in the fishers' Yusho is caused by their taking PCB-contaminated fishes in large amount.

PCB level and the gaschromatogram pattern were measured among the following five groups:

1) healthy non-fishers, 2) healthy fishers whose fishing-grounds were same as the fishers' in Tamanoura-cho, 3) Yusho patients among the fishers in Tamanoura-cho, 4) Yusho patients among non-fishers, and 5) workers exposed to KC-600 in a factory producing paints for ship bottom.

From the results, the following were revealed:

1) The PCB level and the gaschromatogram pattern of Yusho patients among the fishers were like the sum of healty fishers and Yusho patients among non-fishers. 2) The PCB level and the gaschromatogram pattern of the healthy fishers were similar to those of the workers exposed to KC-600.

The concentration and peak patterns of PCB in human blood differ among localities reflecting the degree of environmental pollution of residential areas due to PCB and the kinds of food they eat. In Fukuoka prefecture and Goto Island in Nagasaki Prefecture, there was a clear difference noted in PCB in the blood between healthy individuals and patients affected by rice oil (hereafter, Yusho patients). In particular, Yusho patients among fishermen in the Goto Tamanoura area (hereafter, Yusho fishermen) showed considerably higher PCB concentrations in their blood than Yusho patients in Fukuoka Prefecture. In order to resolve this problem, analyses were made by comparing the situations among fishermen in the area where no [Kanemi] Yusho pollution has been demonstrated, and the blood characteristics among individuals who were working with KC-600, which has been known to have properties similar to those of PCB remaining in the fishermen's blood.

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Subjects and methods

1. Subjects

(1) Yusho patients

Unless otherwise stated, Yusho patients are those in the Fukuoka Prefecture. A total of 23 Yusho patients, all Fukuoka Prefecture residents, who were diagnosed by members of the Prefectural Health Department through a general survey, as type A patient, were subjected to analyses (blood sampling: January, 1975 - January, 1978).

(2) Yusho fisherman patients

Among fishermen in Goto Tamanouracho, Nagasaki Prefecture, who took [Kanemi] rice oil containing PCB, 17 patients who showed A-type in blood PCB gas chromatogram were examined (blood samplings: July 1977).

(3) Fishermen in Nagasaki

28 deep sea fishermen in Goto Island, Nagasaki Prefecture, who did not take any [Kanemi] rice oil were examined (blood samplings: July 1975)

(4) Fishermen in Miyazaki

40 deep sea fishermen in Hinamishi, Miyazaki Prefecture, were examined (blood samplings: December, 1975)

(5) Control group

28 healthy individuals residing in farming villages and cities in the Chikugo area, Fukuoka Prefecture, were examined (blood samplings: July, 1975 - May, 1977).

(6) Individuals contaminated by KC-600

7 individuals who had engaged in working with preparation of boat-bottom coatings in a factory in Osaka and had been contaminated with KC-600 (blood samplings: November, 1976).

2. Method of analysis of PCB in the blood plasma

Blood plasma was tested employing a modification of Jensen's method. Namely, acetonitrile, formic acid and n-hexane were added

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to 4 ml of blood plasma and, after shaking well, the mixture was centrifuged to separate the hexane phase. The lower (bottom) phase was then re-extracted with n-hexane and, by adding fuming sulfuric acid to the hexane phase, the n-hexane was separated after mixing them by shaking the mixture upside down, then it was condensed to 1 ml. The condensed solution of n-hexane was introduced into 2 g of silica gel column and, by eluting with n-hexane and ethylether, n-hexane (1:9 v/v), it was separated into PCB, PP' - DDE and β-BHC, PP'-DDE, PP'-DDT. The eluted solution was condensed to a certain appropriate amount and was subjected to gas chromatography. 1.5% SE-30 and 2% Apiezon L were used as bulking agents and KC-500 and KC-500:600 = 1:1 were used as standard materials. Peak number was determined as follows. In the analysis with SE-30, the peak immediately after the PP'-DDE was assigned as peak 1, and numbering was continued thereafter as peak 2, 3, -- 7. In the Apiezon L analysis, the peak corresponding to peak 1 in SE-30 was assigned as peak 25 and numbering was continued thereafter as peak 27, 29 -- 59.

Results

1. PCB concentration in the blood plasma

PCB concentrations in the blood plasma of fishermen, Yusho patients, Yusho fishermen patients, control group and those polluted with KC-600 are shown in Table 1 and Figure 1.

The mean value of PCB concentration in the blood plasma of fishermen in Nagasaki and Miyazaki was 10.9 ± 4.50 ppb and 12.2 ± 8.49 ppb, respectively, and it was 11.1 ± 11.60 ppb for Yusho patients, and these values were higher than the mean value of the control group, 5.3 ± 2.60 ppb. The PCB concentration in the plasma of Yusho fishermen patients, who were actually engaged in fishing works and took [Kanemi] rice oil, was found to be 22.9 ± 11.37 ppb, which was about twice those of fishermen and Yusho patients.

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The PCB concentration in the plasma of individuals contaminated by KC-600, who were also polluted with PCB during preparation of boat-bottom coatings, varied widely as 26-252 ppb depending upon the degree of contamination.

2. Peak pattern by SE-30 column

Percentage of each peak of each subject with respect to peak 2 of the gas chromatogram pattern by SE-30 is shown in Table 1 and Figure 2.

The fishermen in Nagasaki and Miyazaki, who were engaged in deep sea fishing, showed a lower peak 1 and a higher peak 4 and 5 and these were significantly different from those of the control group. Yusho patients showed a lower peak 1 and higher peaks 3, 5, 6 and 7 -- a characteristic pattern of Yusho patients. On the other hand, Yusho fisherman patients showed peaks 1 and 5 at nearly the same level of those of Yusho patients but they showed especially higher levels of peaks 4 and 6, which were the peaks characteristically higher for Yusho patients and fishermen than for the control group.

Characteristic peaks are shown in Figures 3 and 4 in relation to peak 1. In Figure 3, the relationship between peak 5, a characteristic peak for Yusho patients, and peak 1 was plotted. The Yusho group and the Yusho fishermen group showed similar distributions and there was a clear difference between the control group and the fisherman group. The relationship between peak 6, a characteristic peak for fishermen, and peak 1 is shown in Figure 4. Each subject showed a different, characteristic distribution.

Figure 5 shows comparisons of peak patterns among Nagasaki fishermen, Miyazaki fishermen and KC-600 contaminated patients. High degrees of similarity were seen in the peak patterns among those 3 groups. Especially, there was no significant difference in any of the peak patterns between Nagasaki and Miyazaki fishermen, and the results from the Nagasaki fishermen was used in comparing it with other groups in Figure 2.

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3. Peak pattern with an Apiezon L column

Comparisons in the gas chromatogram pattern, using an Apiezon L column, among Yusho patients, Yusho fisherman patients, the control group and KC-600 contaminated individuals are shown in Figure 6.

With respect to the peaks characteristic of Yusho patients, peak 25 was low, whereas peaks 27, 32, 47, 49, 50 and 52 were high. Among them, increases in peaks 32, 47 and 50 were notable.

For all of the peaks characteristic of Yusho patients, except for peak 32, namely, for peaks 25, 27, 47, 49, 50 and 52, Yusho fisherman patients also showed the same tendency to increase or decrease as the Yusho patients, although to somewhat different degrees. These changes were not so large as in the Yusho patients, but male individuals showed significant differences from the control group.

Peaks 25, 36 and 49 were noted as the characteristic peaks common between Yusho fisherman patients and KC-600 polluted individuals. Among them, peaks 25 and 49 are the characteristic peaks for Yusho patients also and those 3 groups showed nearly the same CB %. With respect to peak 49, in maleindividuals, the CB % was higher for Yusho fisherman patients and KC-600 contaminated individuals than for Yusho patients.

Peak 36 was characteristically high for Yusho fisherman patients and KC-600 contaminated individuals only, and their levels were significantly higher than those of Yusho patients and the control group.

Discussion

In 1974, Abe et al. diagnosed individuals residing in Tamanoura-cho, Goto, Nagasaki Prefecture, and reported PCB in the blood of Yusho fisherman patients [1]. They reported that PCB concentrations in the blood of Yusho fisherman patients were considerably higher than those of Yusho patients residing in Fukuoka and also they noted differences in the peak patterns. In the

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present study, investigations were made which also included fishermen, Yusho patients and a control group and in addition employed an Apiezon L column.

In general, fishermen show higher PCB concentrations in the blood than the usual city residents or farmers and this has been explained by reasoning that fishermen handle and consume a large number of fish containing high concentrations of PCB [1, 6]. In 1976, the authors investigated the relationship between the PCB value in the blood of fishermen and the number of fish caught and the results indicated a clear correlation between them with individuals eating more fish showing higher values of PCB in the blood [4].

In this study, a survey was made for fishermen in Goto Island, but for those fishermen who did not handle or consume [Kanemi] rice oil in Nagasaki and Miyazaki, and it was found that they showed levels of PCB in the blood about twice as high as those in the control group.

Also, the peak patterns seen in the SE-30 column were very similar between these two groups of fishermen and both groups showed no significant difference not only in any of the peaks but also in PCB concentrations in the blood. Those Nagasaki and Miyasaki fishermen both were engaged in ocean (Micronesian Islands in Pacific Ocean) or sea (near Goto and Tanegashima Islands) fishing and the kinds of PCB taken by the above two groups are speculated to be the same. The peak patterns of KC-600 contaminated individuals are also very similar to those of the fishermen and the route of boat bottom coating (KC-600 → fishes → fishermen) is possibly contributing to the relationship between them [5]. On the other hand, with respect to the PCB taken from fishes by Yusho fisherman patients, it is quite possible that they were contaminated through the same route since the fishing area was the same.

When PCB concentrations in the plasma are compared among fishermen, Yusho patients, Yusho fisherman patients and the control group, Yusho patients and fishermen show nearly the same level

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of concentration, whereas those of Yusho fisherman patients are about twice as high as those of the above groups and the value was close to the sum of values of both fishermen and Yusho.

From the viewpoint of peak pattern, with respect to peak 5, in which Nagasaki fishermen and controls show nearly the same height, Yusho fishermen show nearly the same value as that of Yusho patients. With respect to peaks 4, 6 and 7, in which both Yusho patients and fishermen show higher peaks than those of controls, Yusho fishermen patients show even higher peaks, especially in peak 6. According to the report by Watanabe et al. [7], peak 1 and peak 5 of KC-600 contain only little cumulative components. As mentioned earlier, if one assumes that fishermen engaged in ocean and sea fishing are PCB polluted in the same way as KC-600 contaminated individuals, it is likely that the peak pattern in Yusho fisherman patients is a result of the addition of the fishermen's pattern to that of the Yusho patients with respect to the cumulative peaks 4, 6 and 7, only.

From both the concentration and the peak pattern, the properties of PCB remaining in Yusho fisherman patients seem to reflect simultaneous effects of Yusho patients and fishermen.

Next, the results of the Apiezon L column show that peak 25 is low for both Yusho patients and Yusho fisherman patients. This peak is the characteristic one for Yusho [8]. Hori et al. [2] reported that when mice were treated with PCDF together with PCB isolated from the oil causing Yusho, the height of peaks 9, 25 and 28 decreased and in this respect Yusho fisherman patients show the characteristics of Yusho patients.

As for the other peaks characteristic of Yusho patients, it has been reported that peaks 47, 49 and 50 become high [3, 8]. The results of the present authors also showed that the heights of peaks 47, 49, 50 and 52 increased and biphenyl polychlorides with chlorine numbers 6-8 increased relatively.

For all of the peaks characteristic to Yusho, except for peak 32, there is a tendency for Yusho fisherman patients to show a similar pattern to Yusho patients although not with lesser de-

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grees, and Yusho fisherman patients also showed the Yusho characteristics in Apiezon L column.

Peak 36 is the characteristic peak for Yusho fishermen patients and KC-600 poisoned individuals. Watanabe et al. [8] studied the component of PCB in the blood of Yusho patients and professional workers handling PCB using the Apiezon L column, and pointed out that the difference between Yusho patients and KC-600 workers, both of whom show very similar symptoms, was that Yusho patients showed an unusually high peak 47 (48), whereas KC-600 workers showed unusually high peaks 36, 37 and 49, and, based on this, they suggested the possibility of distinguishing them. The fact that peak 36 was unusually high for Yusho fisherman patients with a relative CB % nearly the same as that of KC-600 contaminated individuals, and that both Yusho fisherman patients and KC-600 contaminated individuals showed peak 49 higher than the other peaks and that it was higher than that of male Yusho patients suggest that Yusho fisherman patients are taking in KC-600 besides the PCB originating from rice oil. And this seems to support the idea that, from the similarity in peak pattern with the SE-30 column, fishermen and Yusho fisherman patients are likewise contaminated with PCB just as the KC-600 contaminated individuals.

In this way, although it was not as clear as the case with an EF-30 column, the Apiezon L column also suggested that the peak pattern of Yusho fishermen patients has characteristics of both Yusho patients and KC-600 contaminated individuals.

From the results described above, the Yusho patients among fishermen can be distinguished, whether or not they are Yusho patients if not only quantitative but also qualitative analyses of PCB remaining in the blood are made simultaneously.

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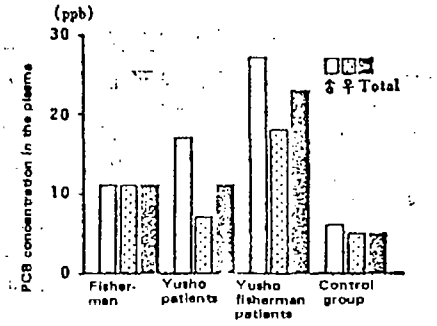
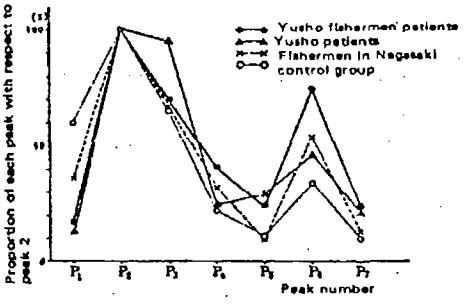


Figure 1. PCB concentrations in the plasma of fishermen, Yusho patients, Yusho fishermen patients and the control group

Figure 2. PCB peak patterns (SE-30 column) for Yusho fishermen patients, Yusho patients, fishermen in Nagasaki and the control group.



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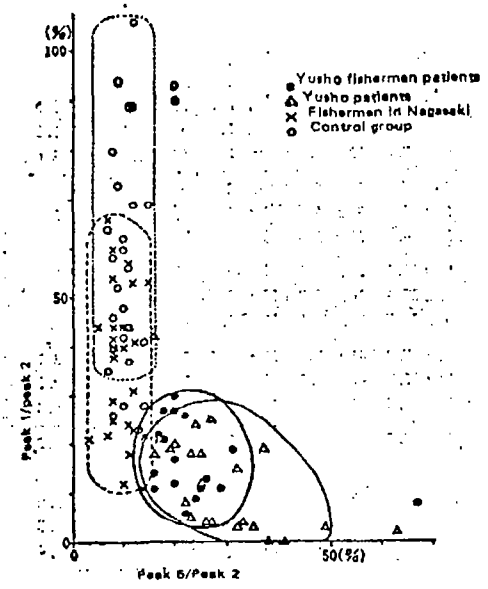
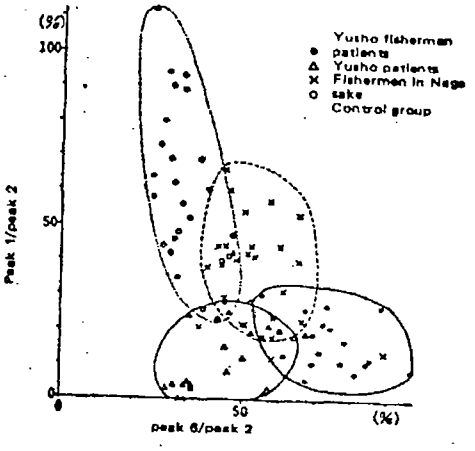


Figure 3. Distributions of relative heights of peaks (peak 1/peak 2 and peak 5/peak 2) with gas chromatogram of PCB in the plasma

Figure 4. Distributions of relative heights of peaks (peak 1/peak 2 and peak 6/peak 2) with gas chromatogram of PCB in the plasma.



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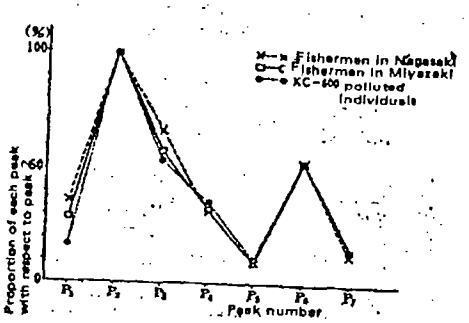

Figure 5. PCB peak patterns (SE-30 column) in the fishermen in Nagasaki and in Miyazaki and in KC-600 contaminated individuals.

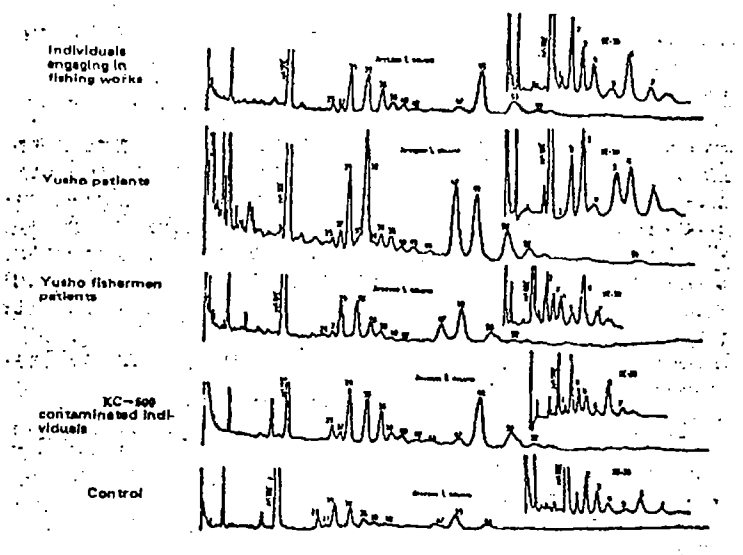

Figure 6. Gas chromatograms of PCB in the plasma

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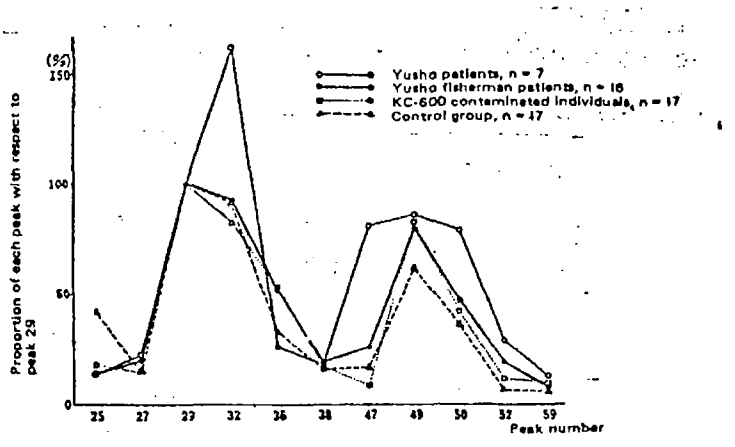

Figure 7. PCB peak pattern (Apiezon L column) in Yusho patients, Yusho fishermen patients, KC-600 contaminated individuals and control group.

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Table I.

Concentration of PCB in the plasma of each subject and PCB peak patterns.

	Sex	n	PCB level (ppb)	1/2(%)	3/2(%)	4/2(%)	5/2(%)	6/2(%)	7/2(%)
Fishermen in Nagasaki	M	13	10.9±4.49	25± 9.5	66± 9.5	33± 5.4	10± 2.8	57±12.0	15± 3.5
	F	15	11.0±4.68	46±10.9	69± 6.4	31± 2.2	9± 2.4	51± 8.3	12± 3.3
	Total	28	10.9±4.50	36±14.9	67± 7.8	32± 4.1	10± 2.6	54±10.5	13± 3.6
Fishermen in Miyazaki	M	20	17.6±8.66	20± 9	60± 5	36± 5	10± 2	59± 7	17± 2
	F	20	6.9±3.54	38±12	56± 5	30± 5	8± 2	49± 7	13± 2
	Total	40	12.2±8.49	29±13.7	58± 4.9	33± 5.4	9± 2.1	54± 8.3	15± 2.6
Yusho patients	M	10	16.5±15.49	7± 6.5	98±27.1	21± 9.3	32±13.8	47±11.3	23± 8.2
	F	13	6.9± 4.84	16±11.6	93±27.4	26± 8.7	27± 8.7	46±12.2	19± 3.9
	Total	23	11.1±11.60	13±10.6	95±26.8	24± 9.0	29±11.2	46±11.5	21± 6.2
Yusho fishermen patients	M	10	26.6±12.31	16± 8.3	66± 7.0	41± 7.6	22± 4.5	75±12.7	22± 3.7
	F	7	17.6±7.85	18± 6.7	75±12.4	40± 5.3	28±17.7	75± 6.8	25± 9.2
	Total	17	22.9±11.37	17± 7.5	70±10.4	41± 6.6	24±11.8	75±10.3	24± 6.4
KC-600 contaminated individuals	M	7	26 ~252	17±11.3	54± 3.7	36± 8.3	11± 2.1	54± 7.7	15± 2.2
control	M	13	6.1±3.24	47±23.8	61± 6.7	23± 6.3	10± 2.6	37± 9.3	11± 3.0
	F	15	4.6±1.68	70±18.4	68± 5.8	22± 2.5	12± 3.7	33± 4.9	10± 1.8
	Total	28	5.3±2.60	59±23.9	65± 7.1	22± 4.6	11± 3.3	34± 7.4	11± 2.4

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