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SOME OF THE RECENT FINDINGS CONCERNING YUSHO

PCB Japan

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Abstract

Analysis of Yusho disease, which was first detected in 1968, has been limited and analyses have produced varying results. Yusho oil has been determined to contain a high level of polychlorinated dibenzofurans (PCDF's). Nagayama et al. found that PCDF levels in the oil were especially high when the oil was contaminated with PCB's used as a heat transfer medium. Their studies also showed that the concentration of PCDF's is much closer to that of PCB's in liver than in adipose tissues in patients with Yusho.

The current clinical state of patients with Yusho is dicussed at length. Subjective symptoms, dermatological findings, serum triglyceride levels, liver conditions, mortality rates, and the effects on children born to mothers with Yusho are all reported.

INTRODUCTION

More than 7 years have passed since the outbreak of an epidemic of Yusho in 1968. According to the latest tabulation, Prof. Omae, current chief of the Study Group for the Therapy of Yusho, reported that a total number of 1,291 patients have been registered as Yusho in 22 prefectures of Western Japan by April 30, 1975 (ref. 1).

We would like to describe some of the recent findings concerning Yusho which we think to be particularly relevant for understanding of toxicity of PCB's. For more detailed information, readers are advised to refer to original papers appearing mainly in the fourth and fifth reports of the study on Yusho and PCB (ref. 3). Most of the patients described in these reports are those living in Fukuoka prefecture. Published information of patients living in other areas are unfortunately very few, so with a few exceptions no reference will be made to them.

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PCB's IN THE BODIES OF PATIENTS WITH YUSHO

1. *Tissues*

First of all, the concentration of PCB's retained in the tissues and fluids of patients with Yusho should be referred to before their current clinical state will be discussed.

Although no accurate estimation is feasible because of the very limited number of analyses so far made, the concentration of PCB's in adipose tissues of patients seems to have been fairly high soon after the occurrence of poisoning—that is, in November 1968, at least 1 month after the discontinued use of the toxic rice oil by patients, as shown in table 1. The corresponding concentrations were considerably lower in 3 patients who died in the next year, 1969. However, no such marked difference could be seen between those who died in 196 and the subsequent decedents, although cases 7 and 10 who died in 1970 or 1975, showed quite low levels of PCB's in adipose tissues. As compared with the figure available from a nationwide survey on residual PCB's in autopsied tissues, the levels noted in the recent decedents are considered to be fairly close to the usual level of ordinary autopsied materials. Similar facts were also noted for PCB concentration in the skin and liver.

2. *Blood*

Since the analysis of PCB's in blood started on after 1972, no figures are available in regard to the blood levels of PCB's in patients in the earlier stage of poisoning. As shown in table 2, however, it is clear that the blood levels of patients had approached the level of ordinary persons already in 1972, although they were still significantly higher than that level. This fact as well as the previous fact of lowered tissue levels of PCB's in recent decedents seem to be rather surprising if we consider also that the majority of patients are still showing various clinical symptoms, as will be discussed later.

3. *Gaschromatographic Patterns of PCB's in the Body of Patients*

Masuda first noted a peculiar common gaschromatographic pattern of PCB fractions isolated from various tissues, blood, and breast milk of patients with Yusho and called the attention of the Study Group for the Therapy of Yusho to it in early 1972. Figure shows a typical example of such a pattern in compari

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Table 1. PCB's concentration in tissues of patients with Yusho and other diseases

Case	Time of death, operation	PCB's (ppm) Skin Whole basis	Skin Fat basis	Adipose tissue Whole basis	Adipose tissue Fat basis	Liver Whole basis	Liver Fat basis	Reference
Case 1 High school boy	Nov. 1968			76 (face) 13 (abdomen)				
Case 2, 3 Adult male, female	Nov. 1968			32, 46 (cheese-like substance from acneform eruptions)				10
Case 4 Boy, 13 yr	July 1969			1.3 (mesentery)	3.7	0.14	9.5	
Case 5 Male, 25 yr	July 1969	1.2	8.7	2.8 (mesentery)	15.1	0.2	10.4	
Case 6 Male, 73 yr	Nov. 1969	1.0	4.4	3.8 (mesentery)	8.4	0.07	3.1	11, 12
Case 7 Female, 48 yr	Dec. 1970	0.6	0.8	0.7 (mesentery)	0.9	0.07	1.3	
Case 8 Male, 46 yr	May 1972	1.8	3.2	4.3	6.5	0.08	8.4	
Case 9 Female, 33 yr	Sept. 1972			1.9 (subcutaneous)	2.9			
Case 10 Male, 72 yr	April 1975			0.19 (mesentery)	0.4	0.04	3.0	14
National survey Males and females 25 - 49 yr	1973	0.04 - 1.7 (n = 54)		0.2 - 4 (n = 47)	0.3 - 6.4 (n = 48)	0.01 - 0.6 (n = 51)	0.02 - 3.1 (n = 30)	13

Table 2. PCB's in blood patients with Yusho, workers and ordinary persons

Material	No. of subjects	Time of examination	PCB's (ppb) Whole basis mean ± S.D.	Range	Reference
Whole blood					
Yusho patients	41	March - August 1973	7	2-26	7
Ordinary persons	37	1972	3	1-7	
Plasma					
Yusho patients	15		6.3 ± 4.0	2-15	
Normal persons	82		3.0 ± 1.3	1- 7	
Whole blood		Jan. 1972			8
Yusho patients	25		4.8 ± 2.9	1-12	
Normal persons	11		2.8 ± 1.5	1- 6	
Whole blood					
Workers[a]	23	1972	364 ± 262	60-920	9

[a]Workers engaged in the production of Kanechlor 200-600 in the air containing 0.05 to 0.2 mg/m^3 of PCB's [Kanechlor-300 + Kanechlor-400 (1:1)]. Two of them showed dermal signs.

with the common one seen in ordinary persons, which is very much close to that of a mixture of Kanechlor 500 + 600 (1 : 1). It is easily notable that the peak 1, which appears immediately after p,p'-DDE in the gas chromatogram in figure 1, is very low in patients with Yusho as compared with ordinary persons, while the peak 5 is much more prominent in Yusho than in ordinary persons. Masuda and his associates observed this peculiar pattern (designated as type "A") in blood of about 60 percent of patients with Yusho, and a somewhat similar pattern (designated as type "B") in about 37 percent (refs. 4,7,11,12).

Takamatsu et al. also observed the same peculiarity in the blood of patients with Yusho (ref. 8). It was further demonstrated that those patients who show the peculiar gas-chromatographic pattern as designated as pattern A by Masuda et al. have a higher average concentration of PCB's in their blood than do other patients (refs. 4,7). Abe et al. (ref. 23) examined in 1974 the PCB residues in the plasma of 30 children born to 18 mothers who had consumed Yusho oil at Goto Islands, Nagasaki prefecture. The specific gas-chromatographic pattern of type A was seen among 24 percent of the children and 44 percent of their mothers, but the peculiarity seemed somewhat less marked as compared with the one seen among patients in Fukuoka prefecture.

The chemical and toxicological nature of the compound or compounds yielding the above mentioned peak 5 must be clarified but have not yet fully been examined. Masuda, however, considers the peak 5 to be 3,4,2',3',4',5'-hexachlorobiphenyl, judging from its retention time on the Apiezon L column developed by Jensen and Sundström (ref. 22).

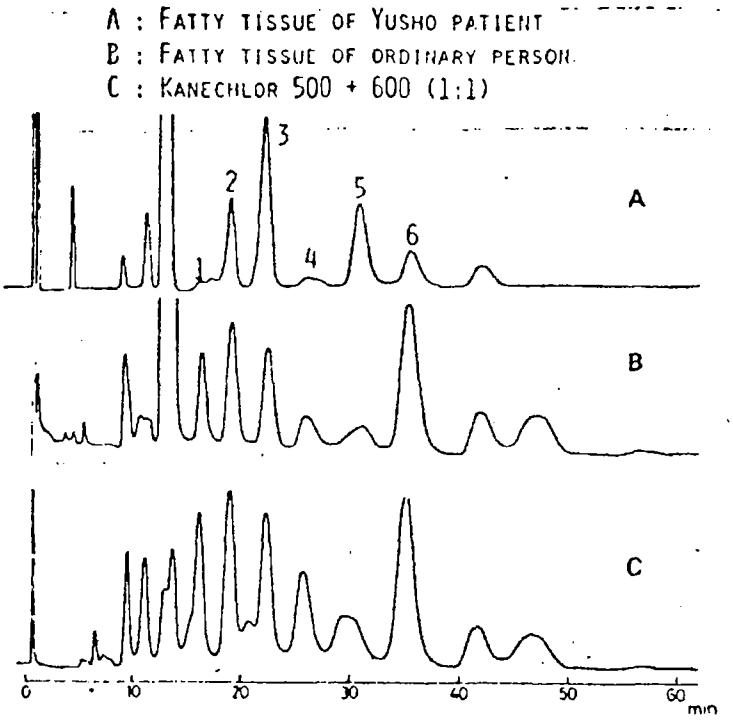

Figure 1. Gaschromatograms (ECD) of PCB's on SE-30.

POLYCHLORINATED DIBENZOFURANS IN KANECHLORS, "YUSHO OIL," AND TISSUES OF PATIENTS

1. *Polychlorinated Dibenzofurans in Kanechlors and "Yusho oil"*

Recently Nagayama et al. analyzed Kanechlors and three samples of toxic "Yusho oil" used by three independent families with Yusho for polychlorinated dibenzofurans (PCDF's) and polychlorinated dibenzo-p-dioxins (PCDD's) (ref. 16). They did a column chromatographic fractionation of PCDF's and PCDD's from a bulk of PCB's by using activated alumina as adsorbent, and n-hexane, n-hexane containing 20 percent carbon tetrachloride, or n-hexane containing 20 percent methylenchloride as eluent. The fractions thus obtained were subjected to gas-chromatographic and mass-spectrometric examination. Quantitative estimation of PCDF's and PCDD's was made by two methods, namely by measuring the gas chromatographic peak heights and by measuring the gas chromatographic peak area of perminated derivatives of these compounds.

Although no PCDD's were found in any of these samples, PCDF's were found in all of them. As shown in table 3, KC-400 contained the highest concentration of PCDF's, about 18 ppm of PCDF's consisting of dichloro- up to pentachloro- dibenzofurans. A peak with the same retention time as that of 2,3,7,8-tetrachlorodibenzofurans, which was kindly provided by Dr. J. G. Vos, was noted in its gas chromatogram. All of the three samples of "Yusho oil" also contained about 5 ppm of PCDF's, the major constituents of which were tetra- and pentachlorodibenzofurans. Here again, the peak with the same retention time as 2,3,7,8-tetrachlorodibenzofuran was noted. Kashimoto also found 1.6 ppm of PCDF's in another batch of "Yusho oil," as shown in table 5 (ref. 18). Table 4 summarized the concentrations of PCDF's in Kanechlor-400, reported by several authors. As clearly noted, there is a fairly large discrepancy in their findings. Whether or not it was due to their analytical procedures or to batch difference cannot be decided at the present time.

Another important fact was disclosed by Nagayama et al. They determined the concentration of PCB's in "Yusho oil" as approximately 1,000 ppm or slightly less than 1,000 ppm, as shown in table 3. Since "Yusho oil" is known to have been contaminated with Kanechlor-400, the ratio of the concentration of PCB's to that of PCDF's in "Yusho oil" is expected to be about 1,000:0.018. The observed ratio, approximately 1,000:5,

Table 3. PCDF's and PCB's in Kanechlors and Yusho oil

Sample		PCDF's (ppm)		PCB's (ppm)	
		Peak height method	Perchlorination method	Peak height method	Perchlorination method
Kanechlor	300	1	1.5	-	-
	400	18	16.6	-	-
	500	4	2.5	-	-
	600	5	2.7	-	-
Yusho oil	A	5	4.4	830	870
	B	4	5.1	900	920
	C	5	5.2	1030	980

Table 4. Reported concentrations of PCDF's in Kanechlor-400

Authors	Year	Concentration (PPM)
Roach et al.[a]	1974	1
Nagayama et al.[b]	1975	18
Kashimoto et al.[c]	1975	33

[a]Ref. 17.
[b]Ref. 16.
[c]Ref. 18.

was thus about 250 times higher than expected (table 5). The reasons for this great discrepancy are not clear yet. It should be noted, however, that the sample of Kanechlor-400 analyzed was an "unused" one, while the Kanechlor-400 present in "Yusho oil" was "used" as heat transfer medium. This fact suggests a practically important possibility that Kanechlor-400 and probably other commercial PCB's, too, will increase their PCDF concentrations when used as heat transfer medium.

2. *PCDF's in Tissues of Patients with Yusho*

Nagayama et al. (ref. 24) further examined the tissues of patients with Yusho for their possible content of PCDF's. Table 6 summarized their findings. Adipos tissues and liver from two ordinary persons who died c accidents contained no detectable amount (< 0.1 ppi of PCDF's, but those from three patients with Yus who died in 1969 or 1972 were all shown to conta PCDF's. Figures 2 and 3 show their gas-chromatograc and mass spectra. The concentration on a whole bas was 0.009 ppm on average for adipose tissues and 0.0 ppm for liver. This seems to be a rather surprising fa because in the case of PCB's the concentration on whole basis is usually much lower in liver than in adipo tissues. When compared on fat basis, another interesti fact was noted that PCDF concentration was much hig er in liver than in adipose tissue. Although the numb

Table 5. Concentrations of PCB's and PCDF's and their ratios in various materials

Materials		PCB's (ppm)	PCDF's (ppm)	PCB's / PCDF's	Reference
Kanechlor-400		1,000,000	ca. 20	50,000	16
Yusho oil	A[a]	ca. 1,000	5	200	
	B[b]	134	1.6	84	18
Patient with Yusho	Adipose tissue	1.3	0.009	144	24
	Liver	0.05	0.013	4	

[a] Samples of the rice oil produced on February 5 or 6, 1968.

[b] A sample of the rice oil produced on February 10, 1968.

Table 6. PCB's and PCDF's in tissues of patients with Yusho and ordinary persons

Subjects	Tissue	Case No.	Time of death	PCB's (ppm) Whole basis	PCB's (ppm) Fat basis	PCDF's (ppm) Whole basis	PCDF's (ppm) Fat basis	Ratio PCB's/RCDF's Whole	Ratio PCB's/RCDF's Fat
Yusho patients	Adipose	1	1969	1.4	3.4	0.013	0.03	108	113
		2	1969	1.3	8.5	0.006	0.04	217	213
		3	1972	1.2	2.1	0.007	0.01	171	210
		Avg.		1.3	4.7	0.009	0.003	144	157
	Liver	1	1969	0.05	4.7	0.025	2.3	2	2
		2	1969	0.06	5.6	0.010	1.1	6	5
		3	1972	0.03	3.5	0.003	0.3	10	12
		Avg.		0.05	4.6	0.013	1.2	4	4
Ordinary persons	Adipose	1	1975	1.0	1.4	ND	ND		
		2	1975	0.4	0.7	ND	ND		
	Liver	1	1975	0.08	1.3	ND	ND		
		2	1975	0.02	1.0	ND	ND		

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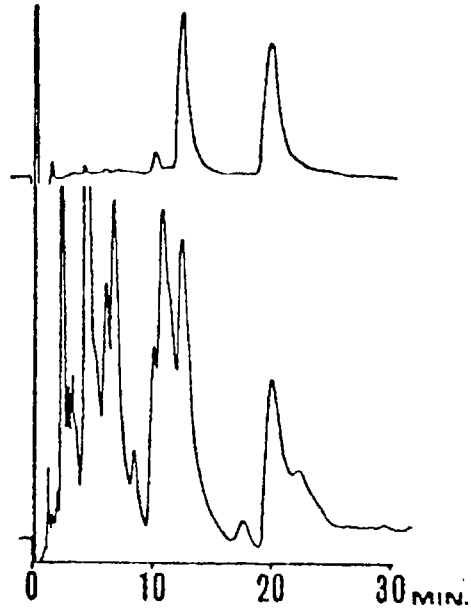

Figure 2. Gaschromatograms of PCDF fractions from liver of patient with Yusho and from "Yusho oil."

of analyses made is quite limited and nothing can be said with certainty, this fact seems to deserve attention. As shown in table 6, these different behaviors in tissue distribution of the compounds caused a remarkable difference in ratio of PCB's to PCDF's between adipose tissues and liver. It was thus demonstrated that the concentration of PCDF's is much closer to that of PCB's in liver than in adipose tissues in patients with Yusho. The PCDF's identified in liver were mainly penta- and hexachlorodibenzofurans, containing only a trace of tetrachloroisomers.

CURRENT CLINICAL STATE OF PATIENTS WITH YUSHO

In 1974, Prof. Urabe (ref. 2), former chief of the Study Group, reported that the dermal and mucosal signs that were most marked at the incipient stage of the poisoning had gradually been improved, while symptoms such as general fatigue, poor appetite, in constant abdominal pain, heavy headedness and headache, feeling of numbness and pain at the limbs, and cough and expectoration of sputum, all of which are considered to be due to some internal disturbances, have become more prominent year by year. In view of these tendencies together with the discovery of a characteristic gas-chromatographic pattern of PCB's remaining in the blood and tissues of patients, the diagnostic criteria for Yusho was revised in 1972 (ref. 2), as shown in table 7. As compared with the former one, the revised criteria describe briefly the dermal and mucosal lesions but newly refer to other noncutaneous objective signs and findings from several laboratory tests. It should be noted, however, that the new criteria do not refer to any specific liver function tests.

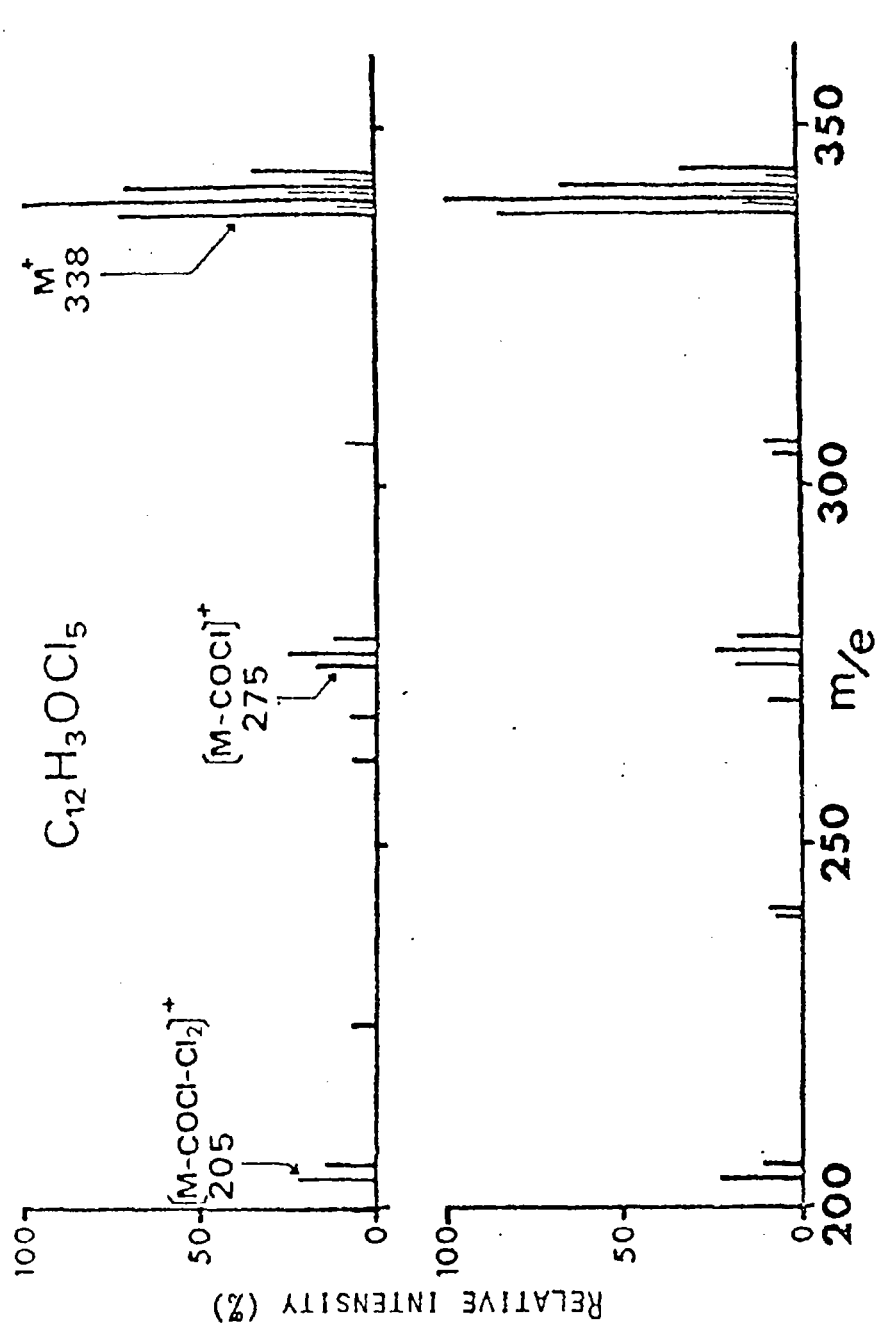

Figure 3. GC-MS of PCDF's fraction from liver of patient with Yusho (upper) and of synthesized PCDF's (lower).

Table 7. The diagnostic criteria for Yusho
(revised in October, 1972).

Yusho is considered as an acute or subacute poisoning with PCB. The general symptoms currently seen are retarded growth, neuroendocrine disturbances, phenomenon of enzyme induction, disturbances in the respiratory system, and abnormal lipid metabolism. As the local symptoms, acneform eruption and pigmentation as cutaneomucosal lesions and ocular symptoms are seen.

1. Conditions of attack
   Fact of ingestion of Kanemi rice oil contaminated with PCB and familial occurrence seen in most cases.

2. General symptoms
   a. Subjective symptoms
      1) general fatigue
      2) heavy headedness and headache
      3) inconstant abdominal pain
      4) feeling of numbness and pain at the limbs
      5) swelling and pain at the joints
      6) cough and sputum
      7) changes in menstruation

   b. Objective symptom
      1) bronchitis-like symptom
      2) sensory neuropathy
      3) bursitis
      4) inhibition in growth and abnormal teeth in children
      5) Small-For-Dates baby and pigmentation of the entire skin of newborns

   c. Results from clinical examination
      1) abnormal properties and concentration of PCB in blood
      2) increase of neutral lipids in blood
      3) anemia, lymphocytosis, hypoalbuminemia
      4) reduced velocity of the sensory nerve conduction and adrenocortical hypofunction

3. Cutaneomucosal signs
   a. Acneform eruption
      Black comedones and acneform eruptions which are seen at the face, buttocks, and other intertriginous sites and their suppurative tendency.

   b. Pigmentation
      Pigmentation of the face, palpebral conjunctiva, gingiva, and nails of the fingers and toes.

   c. Ocular signs
      Swelling and hypersecretion of the Meibomian gland and palpebral edema.

(Translation was made by Kuratsune)

1. *Subjective Symptoms*

Table 8 shows that a considerable portion of the patients are still suffering from various subjective symptoms in recent years. Kōda and Masuda examined their possible association with PCB concentrations in blood, finding no positive association at all (ref. 4). Umeda also reported on various symptoms due to disturbances of higher nervous activities (e.g., forgetfulness) complained of by most patients, but no definite association between such symptoms and the blood levels of PCB's was observed (ref. 5).

2. *Dermatological Findings*

Kōda and Masuda examined 72 patients with Yusho for dermatological signs and PCB levels in the blood from April 1973 to March 1974 (ref. 4). As shown in table 9, the majority of patients were still suffering from skin lesions such as pigmentation, deformation of nail, and hypersecretion of Meibomian gland even 5 years after the poisoning. They also demonstrated another important fact that group A, consisting of patients whose blood shows the gas-chromatographic pattern A, had significantly higher prevalences of dermatologic signs such as pigmentation, acneform eruption, and deformed nails than did group B, which consisted of patients showing no such typical gas-chromatographic pattern.

Since group A had a higher average concentration of PCB's than group B, the dermal lesions seen among current patients, contrary to the subjective symptoms, seem to be causally associated with the current level or pattern of PCB's remaining in their blood. However, no conclusion could be readily made in this regard. First of all, the current excess of PCB's in the blood of patients is not remarkable in degree and is almost negligible as compared with the enormous elevation seen among the occupationally exposed workers, who nevertheless showed a rather low prevalence of dermal symptoms (table 2).

It seems rather hard, therefore, to explain the persisting dermal lesions by elevated PCB levels in blood alone. The chemical peculiarity of such PCB's and the presence of PCDF's in the bodies of current patients seem to be particularly important in this connection. However, our present knowledge does not allow us to continue discussion of the matter along this line without speculation. Furthermore, an entirely different explanation might also be possible. The skin lesions currently seen may merely be the persisting original skin lesions, the severity of which must have been determined primarily by the amount of intake of PCB's; such intake must in turn be reflected by the current PCB levels in blood of patients. According to this explanation, the

Table 8. Frequency of subjective symptoms complained by patients with Yusho from 1973 to 1974

Symptoms	Proportion[a] %
Fatigue	51.4
Headache	41.7
Phymata in articular region	8.3
Fever	2.8
Cough and sputum	56.9
Digestive disorder	40.3
Numbness of extremities	33.3
Menstrual disturbance	26.9
	(7/26)

[a]Calculated by Kuratsune from original figures published by Kōda and Masuda (ref. 4).

Table 9. Prevalence of dermatological and other signs among patients with Yusho from April 1973 to to March 1974, in connection with concentration and gaschromatographic pattern of PCB's in blood

Signs		Prevalence (%)[c] Group A (43 cases)	Group B (26 cases)	Group C (33 cases)	Total (72 cases)
Pigmentation	Skin	51.2[b]	0	0	30.6
	Palpebra	72.1[b]	19.2[b]	0	50.0
	Gingiba	95.3[b]	57.7[b]	66.6	80.6
	Nail	74.4[b]	34.6[b]	0	56.9
Acneform eruption		34.9[b]	0[b]	0	20.8
Comedo		34.9	23.1	0	29.2
Infection of skin		32.6	11.5	0	23.6
Deformation of nail		65.1[a]	38.5[a]	0	52.8
Alopecia		0	3.8	0	1.4
Disorder in teeth		18.6	7.7	0	13.9
Dypersecretion of Meibomian gland		93.0	80.8	100.0	88.9
PCB's in blood	Conc. (ppb) Avg. + S.D.	7.2 ± 4.9	4.3 ± 3.1	1.7 ± 0.2	5.9 ± 4.5
	Pattern	A[d]	B[d]	C[d]	

[a]Significant ($P < 0.05$) difference.

[b]Significant ($P < 0.01$) difference.

[c]Calculated by Kuratsune from figures published by Kōda and Masuda (ref. 4).

[d]"A" means the characteristic gaschromatographic pattern of PCB's remaining in the body of most patients with Yusho. "B" means gas chromatographic patterns somewhat similar to "A". "C" means patterns indistinguishable from those of normal persons.

observed association of the dermal lesions with current blood levels of PCB's is considered as a phenomenal one but not as a causal one. In order to evaluate these different possibilities, it seems essential to examine chemically and toxicologically the PCB's and PCDF's still remaining in patients' bodies.

3. *Serum Triglyceride*

One of the most dominant objective signs seen the incipient stage of Yusho was a markedly increas concentration of serum triglyceride. Okumura and l associates reported recently the results of their extensi followup study on 40 patients who were examined f

um triglyceride at least once a year successively for 6 ars from 1969 to 1974 (ref. 19). As shown in table 10, a group of 14 male patients has shown no significant change in serum triglyceride levels since 1969, still maintaining levels as high as 160 ± 118 mg/100 ml even in 1974. For 26 female patients, however, a significant decrease was seen in 1973 and 1974 when compared with the levels in the previous years. However, 42 percent of them still showed higher levels than 110 mg/100 ml in 1974.

Okumura et al. (ref. 20) examined the possible association between serum triglyceride levels and PCB concentrations in blood in patients. As shown in table 11, they observed a significantly higher mean level of serum triglyceride in a group of patients who showed the characteristic gas chromatographic pattern A, as compared with other patients who did not show such a typical pattern. They also observed a significantly positive correlation between serum triglyceride levels and PCB concentrations in blood ($r = 0.485$).

Table 10. Results of followup study on serum triglyceride levels in patients with Yusho[A]

Subjects	PCB's pattern	No. cases	Age mean	PCB's in blood (ppb)	Triglyceride mg/100 ml	Reference
Patients with Yusho	A	20	31.9	8.6 ± 5.2[b]	134 ± 60.0[a]	
	B	14				
	C	2	21.4	3.8 ± 2.2[b]	91 ± 39.8[b]	20
Controls	C	37	34.5	2.8 ± 1.6		
Controls					74 ± 29	19.21

[a] $P < 0.05$.
[b] $P < 0.005$.

Table 11. PCB's concentrations in blood and serum triglyceride levels in patients with Yusho in 1973

Patients			Triglyceride (mg/100 ml) Mean ± S.D.					
Sex	Age	No.	1969	1970	1971	1972	1973	1974
Male	11 - 73	14	159 ± 57	166 ± 55	169 ± 60	174 ± 69	164 ± 68	160 ± 118
Female	7 - 59	26	155 ± 75	161 ± 70	155 ± 80	153 ± 63	129 ± 50[b]	111 ± 56[b]

[a] Cited from a report by Okumura et al. (ref. 19).
[b] Significantly lower than in 1969, 1970, 1971, and 1972 ($P < 0.05$).

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Here again, a similar question can be raised in regard to such observed correlation, as already discussed in connection with the dermal lesions. Are the current elevated levels of PCB's in blood and their peculiarities in gas-chromatographic patterns causally connected with the abnormally high serum triglyceride levels found in patients? Since, as mentioned, the female patients started to decrease in serum triglyceride concentration in recent years, a followup examination of PCB's in their blood might give a good clue to answer the above question.

4. *Liver of Patients with Yusho*

Both PCB's and PCDF's are well-known toxic agents to the liver. PCDF's seem to be particularly toxic because a single oral administration of PCDF's as small as about 1 mg/kg could kill rabbits by severe liver necrosis (refs. 28,29). Therefore, it is reasonable to expect that patients with Yusho would have a severe liver damage. Okumura et al. (ref. 30) performed detailed medical examinations on 24 patients soon after the onset but, unexpectedly, obtained no objective findings to indicate definite liver disorders. No patients presented jaundice and only three of them had palpable livers. However, an electron microscopic examination of liver biopsy specimens conducted on a patient in February 1969 revealed a marked hypertrophy of smooth endoplasmic reticulum, indicating stimulated enzyme induction in the liver (ref. 31).

Okumura examined 38 patients with various subjective symptoms for serum enzymes, including isozymes from 1971 to 1972 (ref. 32). An increase in a fraction of lactate dehydrogenase (LDH-5) and high titers in thymol turbidity tests were observed in some of the severe cases but no definite evidence for liver disorders was obtained. Recently Hirayama et al. (ref. 33) examined 121 adult patients with Yusho and 257 healthy adult controls for serum bilirubin, demonstrating a significant lower average concentration in the patient group than in the control. They also showed significantly negative correlations between serum bilirubin and blood PCB's in concentration ($r = -0.349$, $p<0.025$) and similarly between serum bilirubin and serum triglyceride ($r = -0.215$, $p<0.05$). They considered that a lowered concentration of serum bilirubin in patients seemed mainly due to an accelerated bilirubin disposal from the blood.

Hirayama et al. investigated 125 patients for Australia antigen and antibody by the immunoelectro-osmophoresis in 1971 (ref. 34). The antigen was positive in three, while the antibody was negative in all of them, indicating no difference at all in the prevalences between the patients and healthy controls. This finding seems to be important in connection with the future risk of cancer which patients might experience.

In view of all these findings, liver function tests currently available do not readily detect serious liver lesions in the patients, but it is highly desirable that adequate caution will continuously be paid to this well-known target organ of chlorinated hydrocarbons.

CHILDREN BORN TO MOTHERS WITH YUSHO

The birth of unusual babies from mothers who took "Yusho oil" during pregnancy is already well known (refs. 25,26). Their clinical features were dark brown pigmentation of the mucous membrane and the entire skin, gingival hyperplasis with pigmentation, a tendency to be small for the date, eruption of teeth at birth, hypersecretation of the Meibomian gland, and edema of the orbital area. Pigmentation of the skin disappeared in 2 to 5 months, followed by growth similar to that of normal babies.

It seems noteworthy, however, that babies with the dark brown pigmented skin continued to be born for a few years after the intake of "Yusho oil" was discontinued by mothers. Yoshimura reported on nine babies with such skin who were born to mothers with Yusho in Nagasaki prefecture from 1969 to 1972 (ref. 27). Three of such babies had been delivered by a patient from 1969 to 1971. Abe et al. (ref. 23) recently reported on PCB levels in the plasma of 30 children (aged 0–7) born to 18 mothers with Yusho in Nagasaki prefecture. Their examination was made in 1974. As shown in table 12, the PCB levels of these children were significantly higher than those of ordinary children but lower than the levels of their mothers. Their gas-chromatographic patterns of PCB's in plasma were already referred to earlier in this paper. Children fed on breast milk from mothers with Yusho tended to show a higher plasma concentration of PCB's than those who were not fed on such milk.

Yoshimura also reported an interesting case, where a baby was thought to have suffered from Yusho due exclusively to intake of PCB's through breast milk from a woman with Yusho (ref. 27). Very few data are available in regard to the concentration of PCB's in breast milk of mothers with Yusho. Masuda et al. found 0.03 – 0.06 ppm of PCB's in 5 samples of breast milk collected from a woman with Yusho within 5 days after delivery in 1973 (ref. 12). Masuda also found about 0.03 ppm of PCB's in another sample of breast milk collected a few days after a woman with Yusho delivered a baby with no dermal signs (ref. 14). The PCB levels in breast milk from patients with Yusho were therefore just within the normal range. However, the gas-chromatographic patterns of these samples were quite unique, the same as that characteristic for Yusho.

Table 12. Concentration of PCB's in plasma of children born to mothers with Yusho

Subjects	PCB's in plasma (ppb)			Reference
	Subjects	Range	Mean ± S.D.	
Mothers with Yusho	18	3-33	11.2 ± 7.32	
Children born to above mothers	30	1-20	6.7 ± 4.28	23
Ordinary children	14	1-8	3.7 ± 1.97	

Table 13. Deaths seen among patients with Yusho[A]

Cause of death	Number
Malignant neoplasms	9
Stomach cancer	2
Stomach cancer + liver cancer	1
Liver cancer + liver cirrhosis	1[b]
Lung cancer	1
Lung Tumor	1
Breast cancer	1
Malignant lymphoma	2
Cerebrovascular lesion	3[b]
Amyloidosis	1[b]
Osteodystrophia fibrosa	1[b]
Myocardial degeneration + pericarditis	1[b]
Status thymicolymphaticus	1
Liver cirrhosis	1
Suicide	1
Senility	1
Traffic accidents	3
TOTAL	22

[a]Cited from a report by Urage 1974 (ref. 2).

[b]Autopsied cases.

MORTALITY OF PATIENTS WITH YUSHO

Omae reported in 1975 that 29 deaths occurred among 1,291 patients with Yusho up to April 30, 1975 (ref. 1). Causes of these deaths were not given, however. Urabe also reported on 22 deaths seen among 1,200 patients until September 13, 1973, and referred to their causes (ref. 2). As shown in table 13, 9 of 22 deaths were caused by malignant neoplasms, suggesting a possible excess of deaths from cancer. Since some essential information needed for epidemiological analysis is not available to us, no further reference can be made with certainty to such a possibility at the present time.

CONCLUSION

As mentioned earlier, we demonstrated the presence of PCDF's in "Yusho oil" at a much higher concentration than expected. We also showed that PCDF's are relatively more concentrated in liver. Although neither the chemical nature nor the toxicity of PCDF's contained in "Yusho oil" and in the bodies of patients are known yet, our findings clearly indicate the necessity to pay greater attention to PCDF's for clarification of the nature of Yusho. Furthermore, our studies suggested the possible formation of PCDF's from PCB's when used as heat transfer medium. Beside this, another possibility that PCDF's might be formed by heating PCB's with peroxides, which are well known to be formed during heating cooking oils, should also be investigated.

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DISCUSSION

MR. ALLEN GREY (IIT Research Institute, Chicago, Illinois): Do you have any feel whether dibenzofurans concentrating in the body are more rapid in metabolism than the result of polychlorinated biphenyls?

DR. KURATSUNE: Unfortunately I have no definite idea. It is a more potent compound than PCB, I guess. I really cannot say.

DR. ORVILLE PAYNTER (EPA, Washington, D.C): Are there any reproductive problems or irregularities continuing for many years after the ingestion of these materials?

DR. KURATSUNE: There is some disturbance of menstruation. There have been some disturbances and there have been some related problems.

MS. DEBORAH A. BARSOTTI (University of Wisconsin, Madison, Wisconsin): Were there are doings that suspected any widespread gastic ulcerations or an erosion in the patients—gastric ulcers?

DR. KURATSUNE: No, I do not think so. Some of the patients had very persistent disorders of the intestines, disorders of the digestive system, but I do not know if they were suffering as a result of this or not.

VOICE: Any residual changes in the sebaceous gland during the autopsy?

DR. KURATSUNE: No, I do not know very much about it.

VOICE: I would like to ask one more question. Have you been able to specifically identify any of the dibenzofurans other than those in the body?

DR. KURATSUNE: You are asking if we could identify any of the dibenzofurans? No.