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CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS OF METHYLSULPHONE METABOLITES OF POLYCHLORINATED BIPHENYLS RETAINED IN HUMAN TISSUES

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SUMMARY

Structures and concentrations of methylsulphone (MSF) metabolites of polychlorinated biphenyls (PCBs) retained in the liver, lung and adipose tissue of a Yusho patient and of a normal person were studied using capillary gas chromatography coupled with electron-capture detection or mass spectrometry. More than 60 isomers of tri-, tetra-, penta- and hexachloro-MSF-biphenyls were detected in the lung of the Yusho patient, and the gas chromatographic peaks coincided with those of 40 authentic isomers in retention times by three separate capillary columns, when they were compared with those of 86 synthesized reference compounds. The main components of MSF-PCBs identified in the Yusho patient were 4-MSF-2,5,4′-tri-, 4-MSF-2,5,2′,4′-tetra-, 4-MSF-2,5,2′,5′-tetra-, 3-MSF-4,5,2′,3′-tetra-, 4-MSF-2,5,2′,3′,4′-penta- and 4-MSF-2,5,2′,4′,5′-pentachlorobiphenyls, estimated to be 0.7–1.5 μg/kg in the lung and 0.3–1.5 μg/kg in the adipose tissue.

INTRODUCTION

Methylsulphone (MSF) derivatives of polychlorinated biphenyls (PCBs) are known to be one of the metabolic products of PCBs, which were first identified in blubber of seals from the Baltic[1]. Since then, a number of studies on the metabolism of PCBs have demonstrated the existence of MSF-PCBs in animals[2–4]. MSF-PCBs were isolated from the excreta of mice or rats treated with a number of tri-, tetra-, penta-, and hexachlorobiphenyls. The metabolic fate of several structurally defined chlorobiphenyls in mice showed that the sulphur-containing metabolites were formed by the mercapturic acid pathway from PCB arene oxide[5–10], apart from hydroxy species, which were considered to be major metabolites of PCBs[11–14]. Recent evidence indicated that some MSF-PCBs accumulated in the bronchial mucosa[4] or uterine luminal fluid[15] of mice given oral doses of certain chlorobiphenyls. The accumulative properties of MSF-PCBs were also observed in the breast milk and adipose tissue of a Japanese woman exposed to PCBs[16,17].

In our previous study[18], methylthio- and methylsulphonyl PCBs were detected and tentatively quantified in the tissues of patients with Yusho, PCB poisoning that

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occurred in western Japan in 1968[19]. Their mass fragmentographic analyses indicated that at least sixteen components of MSF-PCB congeners with three to six chlorine atoms were present in the lung of Yusho patients. It therefore seemed to be necessary to clarify further the isomeric compositions and their concentrations retained in the tissues for understanding the etiology of Yusho and the metabolism of PCBs in human body. In order to attain this aim, the 86 authentic compounds of MSF-PCBs having two to seven chlorine atoms have been synthesized as reference substances and chromatographically characterized in this laboratory[20]. In the present study, gas chromatography with electron-capture detection (GC–ECD) and mass spectrometry (GC–MS) with high-resolution capillary columns were developed for the identification of the suspected metabolites, providing not only the total content of MSF-PCB congeners in the tissues but also the isomer distribution. This paper reports on the structural identification and their quantification of MSF-PCBs accumulated in human tissues by the use of the capillary GC technique with synthesized MSF-PCB standards.

EXPERIMENTAL

Materials and chemicals

Samples of the lung, liver and mesenteric adipose tissue of a deceased Yusho patient and a person who had died from causes other than Yusho were supplied by courtesy of the Department of Pathology, School of Medicine, Fukuoka University, Fukuoka, Japan. The tissues had been preserved in formaldehyde solution after resection. Alumina for column chromatography (neutral, No. 1077, Activity grade I) was purchased from Merck (Darmstadt, F.R.G.). All other chemicals and solvents used were of the purest grade available.

Synthesis of reference compound

The 86 individual MSF-PCB isomers (*i.e.* 2 di-, 10 tri-, 40 tetra-, 20 penta-, 13 hexa- and 1 heptachloro-MSF-biphenyls) and 3-MSF-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl were prepared by three synthetic routes as will be described elsewhere[20]: (1) the diazo coupling reaction of 3-MSF-chloroaniline with chlorobenzene; (2) the nucleophilic substitution of chlorobiphenyl with methanethiolate and successive oxidation of the corresponding methyl sulphide; (3) the diazo couling reaction of chloroaniline with chlorothioanisole and successive oxidation of the methyl sulphide.

Procedure

A portion (17–45 g) of the liver, lung and adipose tissue of a Yusho patient and a normal person spiked with 3-MSF-4-methyl-5-2′,3′,4′,5′-pentachlorobiphenyl (20 ng) as the internal standard (I.S.) were saponified with 100 ml of 1 *N* sodium hydroxide–ethanol solution by refluxing for 1 h. After addition of 200 ml of water, the aqueous alcoholic solution was extracted twice with 50 ml of *n*-hexane. The combined *n*-hexane extracts were rinsed with water and dried over anhydrous sodium sulphate and concentrated to *ca.* 10 ml with a Kuderna–Danish concentrator. The *n*-hexane extract was shaken with an equal volume of concentrated sulphuric acid and two phases separated. The acidic phase was diluted with ice water to 70% sul-

phuric acid and back-extracted twice with another 10-ml portion of *n*-hexane. The combined *n*-hexane extracts were chromatographed on a 3 × 1.5 cm I.D. column of alumina (activated at 130°C for 3 h) and eluted successively with 100 ml of *n*-hexane and 50 ml of *n*-hexane–dichloromethane (1:1). The former eluate was used for PCB analysis. The latter eluate was evaporated to dryness and dissolved in *ca.* 100 μl of acetonitrile. The sample was subjected to a reversed-phase high-performance liquid chromatography (HPLC) and followed by a normal-phase HPLC.

HPLC fractionation

HPLC was performed on a Model ALC-GPC-204 system (Waters Assoc., Milford, MA, U.S.A.) equipped with a Model 3000 pump, a Model U6K universal injector and a Model 440 absorbance detector at 254 nm. The sample was injected onto a reversed-phase column packed with μBondapak C_{18} (10 cm × 8 mm I.D., 10-μm particle size, Waters Assoc.), and fractionated by elution with a stepwise gradient of 50, 80 and 100% (v/v) acetonitrile in glass-distilled water at a flow-rate of 1.4 ml/min for 30 min, respectively. The fraction with retention times of 40–60 min was collected and diluted with water to 30% acetonitrile in water, and then extracted twice with 10 ml of *n*-hexane. The combined *n*-hexane extract was concentrated to *ca.* 200 μl and injected onto a normal-phase column packed with μBondapak CN (10 cm × 8 mm I.D., 10-μm particle size, Waters Assoc.) and fractionated by elution with *n*-hexane–dichloromethane (4:1, v/v) at a flow-rate of 1.0 ml/min. The eluate of the expected MSF-PCB fraction with retention times of 6–12 min was concentrated to a small volume and analysed by GC–MS and GC–ECD.

GC–MS analysis

GC–MS analysis for quantitation of MSF-PCB congeners was carried out with a JEOL JMS-DX300 mass spectrometer (JEOL, Japan) combined with a JMA-DA5000 data system in electron-impact mode. The gas chromatograph was fitted with a 40 m × 0.25 mm I.D. OV-101 fused-silica capillary column (Shimadzu, Japan) coupled directly into the MS source. The oven temperature was isothermal at 180°C for 2 min, programmed to 270°C at 8°C/min and held for 50 min. The carrier gas was helium at a flow-rate of 0.7 ml/min. The following parameters were held constant: injection temperature, 290°C; separator and inlet temperature, 250°C; ion source temperature, 210°C; ionizing energy, 70 eV; ionizing current, 300 μA; accelerating voltage, 3 kV. The resolving power was set at 1000 for the determination. The computer-controlled selected-ion monitoring mode was used for the analysis. The ions monitored were two molecular ions, M^+ and $[M + 2]^+$ of di-, tri-, tetra-, penta-, hexa- and heptachloro-MSF-biphenyls and of 3-MSF-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl.

The I.S. method was applied for the quantification, in which 3-MSF-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl was used as the I.S., since there was no interference with its GC peak by the peaks of MSF-PCBs and other compounds separated from the human tissues.

Quantitative determination by GC–MS was made by comparing the relative peak areas of the MSF derivatives and the I.S. in each of the fragmentograms obtained from the tissues with those of authentic samples of 4-MSF-2,5,4′-tri-, 4-MSF-2,5,2′,5′-tetra-, 4-MSF-2,5,2′,4′,5′-penta- and 4-MSF-2,5,2′,3′,4′,5′-hexachlo-

robiphenyl, respectively, under the same GC–MS conditions. These analyses were based on the assumption that all the MSF-PCB isomers isolated from tissue samples had the same peak-area sensitivity as that of the above authentic compound with the same chlorine number, regardless of the sites of chlorine substitution.

Capillary GC analysis

Capillary GC analysis for individual MSF-PCB isomers was carried out on a Shimadzu GC-7AG gas chromatograph equipped with a ^{63}Ni electron-capture detector. The following capillary columns were used: (1) A 50 m × 0.25 mm I.D. OV-101 fused-silica (Shimadzu): the temperature was isothermal at 180°C for 2 min, programmed to 270°C at 6°C/min; (2) A 30 m × 0.2 mm I.D. Dexsil 410 fused-silica (Shimadzu): the temperature was isothermal at 200°C for 2 min, programmed to 265°C at 4°C/min; (3) A 30 m × 0.25 mm I.D. SP-2250 fused-silica (Supelco, Bellefonte, PA, U.S.A.): the temperature was isothermal at 200°C for 2 min, programmed to 260°C at 4°C/min.

The temperatures of the inlet and detector were maintained at 290°C. Nitrogen was used as the carrier gas at a flow-rate of 0.7 ml/min. Samples were injected with an SPL-7 solventless sampler (Shimadzu) with splitting in the injection (10:1 split ratio).

The amounts of individual MSF isomers were determined by comparing the peak-area ratio of isomer in the sample to I.S. with that of each standard sample under the same conditions as the sample analysis. Each analysis was run at least twice. The concentration of each isomer was expressed as the lowest value among the each determination on three separate columns because of the possible co-elution with unknown isomers. Standard solutions were prepared by dissolving 1 mg of MSF-PCB isomer in 10 ml of diethyl ether and successive dilution with *n*-hexane down to 1 pg/μl.

About 90% of the authentic MSF-PCB congeners and I.S. were recovered from bovine fat (5 g) fortified with 20 ng of them in this procedure (Table I). Their percentage recoveries did not differ significantly, and therefore required no correction for the determination. Calibration plots of two isomers *vs.* I.S. gave straight lines in the range 5–100 ng/g when the amount of I.S. was 20 ng (Fig. 1).

PCBs in the tissues were analysed as described previously[21].

TABLE I

RECOVERY OF MSF-PCB CONGENERS AND INTERNAL STANDARD FROM FORTIFIED BOVINE FAT

Congener	*Recovery (%)*	
	*Mean**	*S.D.*
4-MSF-2,5,2′-trichlorobiphenyl	84.5	6.3
4-MSF-2,5,2′,5′-tetrachlorobiphenyl	91.3	3.5
4-MSF-2,5,2′,4′,5′-pentachlorobiphenyl	87.8	4.2
3-MSF-4,5,6,2′,4′,5′-hexachlorobiphenyl	88.7	5.1
3-MSF-4-methyl-5,2′,3′,4′,5′-pentachlorobiphenyl (I.S.)	90.2	4.2

* Average of four replicate determinations of 20 ng fortification.

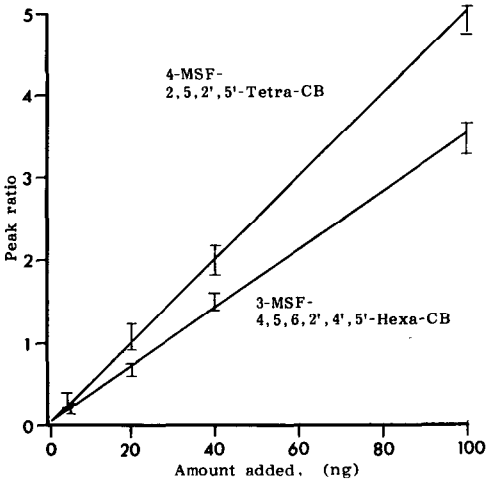

Fig. 1. Plot of the amounts of MSF-PCBs added to control bovine fat *vs.* the peak area ratio to I.S. To bovine fat (5 g) spiked with 20 ng of I.S., 5-100 ng of 4-MSF-2,5,2′,5′-tetrachlorobiphenyl and 3-MSF-4,5,6,2′,4′,5′-hexachlorobiphenyl and analysed as described in the text.

RESULTS

Fig. 2 shows mass fragmentograms of the MSF-PCB fraction from the lung of the Yusho patient; the fraction was spiked with the I.S. and monitored at *m/e* 336, 370, 404, 438 and 418 for $[M + 2]^+$ of tri-, tetra-, penta-, and hexachloro-MSF-biphenyls and of the I.S., respectively.

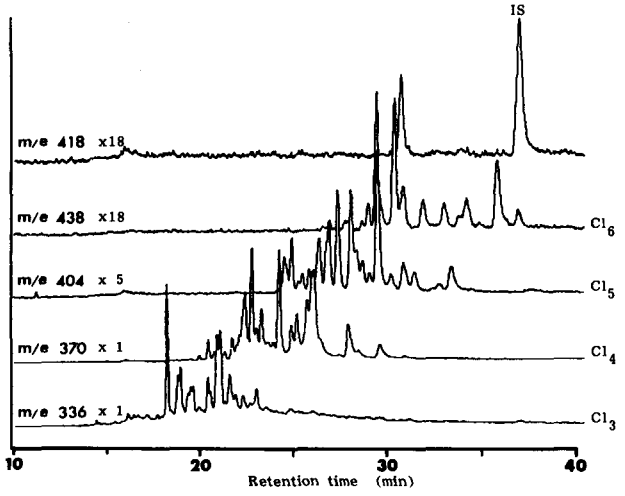

Fig. 2. Mass fragmentogram of the MSF-PCB fraction, obtained from the lung of the Yusho patient, spiked with 20 ng of I.S. (*m/e* 418), monitored at $[M + 2]^+$ ions of MSF-PCBs. The numbers of chlorine atoms substituted on the biphenyl nucleus are indicated. The GC–MS conditions are given in text.

TABLE II

VARIATION OF RELATIVE RESPONSE FACTORS FOR MSF-PCBs (3–6 CHLORINES) ON FUSED-SILICA CAPILLARY GC–MS AND GC–ECD

Congener	*GC–MS**			*GC–ECD**		
	Range	*Mean*	*R.S.D. (%)*	*Range*	*Mean*	*R.S.D. (%)*
3 Cl (7)**	4.38–17.6	9.8	51	0.71–1.20	0.84	22
4 Cl (17)	1.83–11.5	3.9	66	0.75–1.27	0.97	21
5 Cl (8)	0.62–2.59	1.4	43	0.63–1.19	0.94	24
6 Cl (8)	0.25–1.52	0.7	71	0.79–1.59	1.12	31

* All values are relative to 3-MSF-4-methyl-5,2',3',4',5'-pentachlorobiphenyl.
** Values in parentheses are numbers of MSF-PCBs measured.

More than 60 different peaks were observed in these mass fragmentograms with the $[M + 2]^+$ ion, which were synchronized to those of the M^+ ion, with the exception of a few peaks, indicating the presence of tri- to hexachloro-MSF-biphenyls in the lung of the patient. No peaks appeared in the mass fragmentograms monitored at *m/e* 302 and 472 for $[M + 2]^+$ of the di- and hepta-chloro-MSF-biphenyls.

Quantitation of MSF-PCB congeners by GC–MS was based on the comparison of the relative response factor (RRF) for each congener with that for the appropriate standard. Table II shows the variation of RRFs for individual MSF-PCB isomers by GC–MS and GC–ECD. The relative standard deviations (R.S.D.s) for RRF values within a congener obtained with GC–MS ranged from 43 to 71%. These variations were larger than those with GC–ECD, probably owing to the difference of the molecular cluster ion abundance formed. The quantitative results are listed in Table III. When the levels of congeners were compared between the Yusho patient and the normal person, the following differences were observed in the lung and adipose tissue. First, the concentration of the tetrachloro congeners of MSF-PCBs in the patient was the highest, followed in order by penta-, tri- and hexachloro conge-

TABLE III

GC–MS DETERMINATION OF TRI-, TETRA-, PENTA- AND HEXACHLOROBIPHENYL METHYLSULPHONES IN HUMAN TISSUES

Tissue		*Sample weight (g)*	*PCB (μg/kg)*	*Level* (μg/kg) of MSF-PCB*				
				3 Cl	*4 Cl*	*5 Cl*	*6 Cl*	*Total*
Lung	(Yusho)	30	64	1.9	7.7	4.5	1.8	15.9
	(Normal)	45	4	0.6	1.2	0.2	0.04	2.1
Liver	(Yusho)	34	29	0.8	1.4	0.8	0.4	3.4
	(Normal)	30	17	0.9	1.4	0.7	0.2	3.2
Adipose	(Yusho)	17	1700	1.6	4.0	3.7	1.0	10.3
	(Normal)	26	304	1.7	3.2	0.5	0.1	5.7

* Average of duplicate determinations.

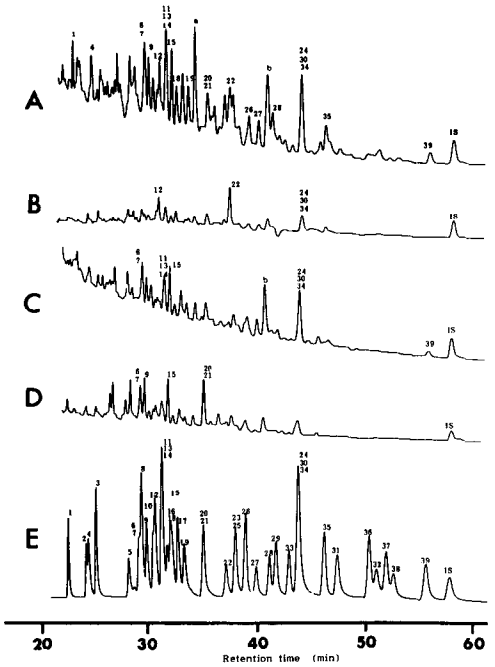

Fig. 3. Gas chromatograms on the OV-101 column of the MSF-PCB fractions from (A) the lung of the Yusho patient, (B) the lung of the normal person, (C) the adipose tissue of the Yusho patient, (D) the adipose tissues of the normal person, and (E) of a mixture of 39 authentic MSF-PCB isomers. The numbered peaks correspond to the isomers listed in Table IV. Peaks a and b were not identified. The GC conditions are given in text.

ners, whereas the level of pentachloro congeners in the normal person was below that of trichloro congeners in each of the tissues. Second, the levels of penta- and hexa-chloro congeners in the lung and adipose tissue of the Yusho patient were more than ten times higher than those of the normal person, respectively, although no significant difference in these components between both livers was found. The levels of total MSF-PCB congeners obtained from the Yusho patient were 16 μg/kg in the lung, 10 μg/kg in the adipose tissue, and 3 μg/kg in the liver, whereas those from the normal person were 2, 5, and 3 μg/kg, respectively.

To identify the individual MSF-PCB isomers in the tissue, the retention times of GC peaks were compared with those of the 86 synthesized reference compounds using capillary GC–ECD. Many peaks in the gas chromatograms were identical with those of 40 authentic compounds in their retention times of three separate columns. Fig. 3 shows the chromatograms of the MSF-PCB fraction obtained from the lung and adipose tissue of the Yusho patient and normal person, and of a mixture of 40 authentic MSF isomers. All the peaks observed in the region of the retention time after 20 min on the OV-101 column were confirmed to be MSF-PCB isomers by GC–MS. The numbers on the major peaks in each chromatogram correspond to the MSF-PCB isomers listed in Table IV. Some peaks overlapped in the chromatograms as shown in Fig. 3E. Peaks a and b in Fig. 3A and C, which were characteristic in

TABLE IV

STRUCTURAL DETERMINATION OF MSF-PCB CONGENERS RETAINED IN THE YUSHO PATIENT (Y) AND THE NORMAL PERSON (N)

Peak No.	*Identified isomer*	*Level* (μg/kg) of MSF-PCBs*					
		Lung		*Adipose*		*Liver*	
		Y	*N*	*Y*	*N*	*Y*	*N*
Trichlorobiphenyls							
1	2-MSF-4,2',5'	0.18	N.D.	0.14	0.03	N.D.	N.D.
2	3-MSF-2,5,2'	0.08	0.02	0.02	N.D.	N.D.	0.05
3	4-MSF-2,5,2'	0.31	0.15	0.21	0.06	0.08	0.08
4	3-MSF-6,2',5'	0.48	0.07	0.11	0.04	0.06	0.06
5	4-MSF-2,5,3'	0.31	0.20	0.25	0.21	0.17	0.46
6	4-MSF-2,5,4'	1.23	0.11	1.08	1.24	0.15	0.19
7	4-MSF-3,2',5'	N.A.	N.A.	N.D.	N.D.	N.A.	N.A.
Tetrachlorobiphenyls							
8	3-MSF-2,5,2',5'	0.18	0.05	0.18	0.12	0.03	0.05
9	3-MSF-4,6,2',5'	0.38	N.D.	0.28	0.13	0.16	0.05
10	3-MSF-4,6,2',4'	0.12	N.D.	0.17	0.03	0.14	0.11
11	3-MSF-4,5,6,2'	N.A.	N.D.	N.A.	N.A.	N.D.	N.D.
12	4-MSF-2,5,2',5'	1.03	0.17	0.25	0.47	0.33	0.24
13	4-MSF-2,5,2',4'	1.43	N.D.	1.25	0.80	0.14	0.06
14	3-MSF-2,5,2',3'	0.91	0.14	0.61	0.50	0.22	0.04
15	3-MSF-4,6,2',3'	0.41	0.09	0.09	0.18	0.06	0.02
16	4-MSF-2,3,6,3'	0.17	0.09	0.05	0.04	0.13	0.13
17	4-MSF-2,5,2',3'	0.21	0.05	0.40	0.43	0.08	0.06
18	2-MSF-4,2',4',5'	0.40	0.16	0.24	0.18	0.12	0.14
19	3-MSF-6,2',3',4'	0.47	0.11	0.12	0.20	0.02	0.02
20	4-MSF-2,3,2',4'	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
21	2-MSF-4,2',3',4'	0.05	0.02	0.22	0.04	0.11	0.11
22	3-MSF-4,5,2',3'	1.01	0.62	0.22	0.24	0.21	0.12
23	4-MSF-2,5,3',4'	0.10	0.02	0.10	0.03	0.11	0.05
24	3-MSF-4,5,3',4'	0.40	0.15	0.14	0.12	0.24	0.05
Pentachlorobiphenyls							
25	3-MSF-2,5,2',4',5'	0.36	0.15	0.45	0.23	0.12	0.17
26	3-MSF-4,5,6,2',5'	0.44	0.08	0.17	0.11	0.06	N.D.
27	4-MSF-2,5,2',4',5'	0.68	0.06	1.16	0.18	0.11	0.08
28	3-MSF-4,5,2',3',6'	0.63	0.05	0.16	0.02	N.D.	0.02
29	3-MSF-2,5,2',3',4'	0.13	N.D.	0.20	0.05	N.D.	N.D.
30	4-MSF-2,5,2',3',4'	1.50	0.14	1.47	0.20	0.14	0.05
31	4-MSF-2,3,2',3',4'	0.10	N.D.	0.02	0.01	N.D.	0.01
32	3-MSF-4,5,2',3',4'	0.19	N.D.	0.11	0.02	0.07	0.01
Hexachlorobiphenyls							
33	3-MSF-2,5,2',3',5',6'	0.10	N.D.	0.05	0.02	0.02	0.01
34	4-MSF-2,5,2',3',5',6'	0.42	0.05	0.09	0.05	0.16	0.08
35	3-MSF-4,5,6,2',3',6'	0.23	0.01	0.09	0.04	0.03	0.01
36	4-MSF-2,3,6,2',3',4'	0.04	N.D.	0.03	N.D.	N.D.	N.D.
37	3-MSF-4,5,6,2',4',5'	0.08	N.D.	0.01	N.D.	N.D.	0.03
38	3-MSF-2,5,2',3',4',5'	0.11	N.D.	0.04	0.02	N.D.	N.D.
39	4-MSF-2,5,2',3',4',5'	0.25	0.01	0.26	0.05	0.02	0.02
40	3-MSF-4,5,6,2',3',4'	0.06	N.D.	N.D.	N.D.	N.D.	N.D.

* Lowest value among the determinations on three separate capillary column (analytical conditions as in text); N.D. = not detected (detection limit, 5 ng/kg); N.A. = not quantitatively analysed.

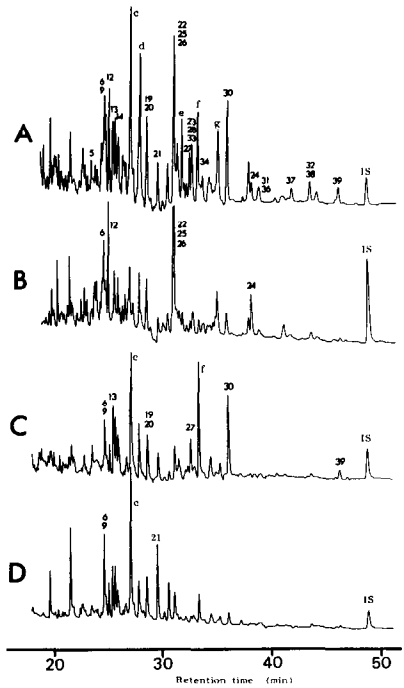

Fig. 4. Gas chromatograms on the Dexsil-410 column of the MSF-PCB fraction from (A) the lung of the Yusho patient, (B) the lung of the normal person, (C) the adipose tissue of the Yusho patient, and (D) the adipose tissue of the normal person. The numbered peaks correspond to the isomers listed in Table IV. Peaks c, d, e, f and g were not identified. The GC conditions are given in text.

the Yusho patient, were confirmed to be tetrachloro-MSF-biphenyls by GC–MS, but were unassigned to any of the 86 synthesized standards by GC–ECD.

The MSF-PCB fractions in the above tissues were also analysed on a Dexsil-410 capillary column, as shown in Fig. 4. Overlapping of some components on the OV-101 column was somewhat improved by this column. For example, peaks of isomer Nos. 11, 13 and 14, or Nos. 24, 30 and 34, which eluted together on OV-101, were separated on the Dexsil-410 column, although other components (Nos. 22, 25 and 26, or Nos. 23, 28 and 33) were eluted together. Of the unknown peaks c, d, e, f and g in Fig. 4A, peaks d, e and g were characteristic of the lung of the Yusho patient, but peaks c and f were observed in adipose tissue of both the patient and the normal person. In the lung of the normal person, the peaks corresponding to isomer Nos. 22 and 24 were relatively high in concentration. Comparison of these chromatograms showed that there was a distinct difference in the peak pattern between the Yusho patient and the normal person and that the former contained more of the MSF-PCB isomers with comparatively longer retention times.

To identify and determine the MSF-PCB isomers that were not separated on the above two columns, the MSF-PCB fraction from the tissues were further chromatographed on a SP-2250 column. Fig. 5 shows chromatograms of the MSF-PCB fraction from the lung, adipose tissue and liver of the Yusho patient on the SP-2250

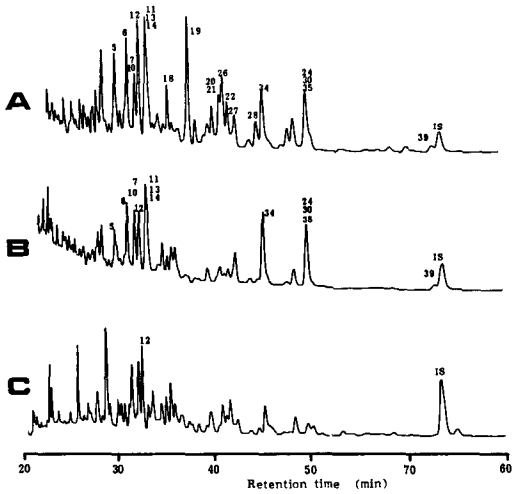

Fig. 5. Gas chromatograms on the SP-2250 column of the MSF-PCB fraction from (A) the lung, (B) the adipose tissue, and (C) the liver of the Yusho patient. The numbered peaks correspond to the isomers listed in Table IV. The GC conditions are given in text.

column. Separation patterns of these metabolites on the SP-2250 were similar to those on the OV-101.

Although the GC pattern of MSF-PCB isomers from the lung of the patient was similar to that of the adipose tissue (Figs. 3 and 4), they were considerably different from that of the liver (Fig. 5). A peak corresponding to component No. 19 may be co-eluting with unknown components, because this component exhibits a minor peak on the other columns. The serious overlapping of isomer Nos. 7, 11 and 20, which were commonly observed on the three columns, made isomeric identification and quantification by GC–ECD very difficult. The 40 components identified in the tissues are listed in Table IV. The RRFs for individual MSF-PCB isomers obtained with GC–ECD ranged from 0.71 to 1.59 with a mean of 0.97 (R.S.D. 24.8%) as shown in Table II. Quantitative determination of the MSF-PCB isomers was based on the individual RRFs on the alternative column. Their concentrations in the tissues are also shown in Table IV, of which the above three isomers were undetermined owing to overlapping of the peaks. Major components of the MSF-PCB metabolites identified in the lung of the Yusho patient were 4-MSF-2,5,4′-tri-, 4-MSF-2,5,2′,4′-tetra-, 4-MSF-2,5,2′,5′-tetra-, 3-MSF-2,5,2′,3′-tetra-, 3-MSF-4,5,2′,3′-tetra-, 4-MSF-2,5,2′,3′,4′-penta- and 4-MSF-2,5,2′,4′,5′-pentachlorobiphenyl, with concentrations estimated at 1.2, 1.4, 1.0, 0.9, 1.0, 1.5 and 0.7 μg/kg, respectively. On the other hand, in the lung of the normal person, the concentration of 3-MSF-4,5,2′,3′-tetrachlorobiphenyl was estimated to be 0.6 μg/kg, but all other isomers were below 0.2 μg/kg. In the adipose tissue of the Yusho patient, 4-MSF-2,5,4′-tri-, 4-MSF-2,5,2′,4′-tetra-, 3-MSF-2,5,2′,3′-tetra-, 4-MSF-2,5,2′,3′,4′-penta- and 4-MSF-2,5,2′,4′,5′-pentachlorobiphenyl were the major components, as in the lung tissue, with concentrations estimated at 1.1, 1.3, 0.6, 1.5 and 1.2 μg/kg, respectively. However, the levels of 4-MSF-2,5,2′,5′-tetra- and 3-MSF-4,5,2′,3′-tetrachlorobiphenyl were relatively low in the adipose tissue, approximately one-fifth of those in the lung

TABLE V

COMPARISON OF THE LEVELS OF 3- AND 4-MSF METABOLITES DERIVED FROM PCB WITH 2,5-DICHLOROPHENYL NUCLEUS IN THE LUNG AND ADIPOSE TISSUE OF THE PATIENT

Parent PCB	*Level (μg/kg) of the metabolites*					
	Lung			*Adipose*		
	4-MSF	*3-MSF*	*4:3**	*4-MSF*	*3-MSF*	*4:3*
Dichlorobiphenyl						
2,5	N.A.	N.A.	–	N.A.	N.A.	–
Trichlorobiphenyls						
2,5,2′	0.31	0.08	3.9	0.21	0.02	10.5
2,5,3′	0.31	N.A.	–	0.25	N.A.	–
2,5,4′	1.23	N.A.	–	1.08	N.A.	–
Tetrachlorobiphenyls						
2,5,2′,3′	0.21	0.91	0.2	0.40	0.61	0.7
2,5,2′,4′	1.43	0.38	3.8	1.25	0.28	4.5
2,5,2′,5′	1.03	0.18	5.7	0.25	0.18	1.4
2,5,2′,6′	N.D.	N.D.	–	N.D.	N.D.	–
2,5,3′,4′	0.10	N.D.	–	0.10	N.D.	–
2,5,3′,5′	N.D.	N.A.	–	N.D.	N.A.	–
2,5,6,3′	0.17	N.D.	–	0.05	N.D.	–
5,6,2′,4′	0.05	N.D.	–	0.06	N.D.	–
Pentachlorobiphenyls						
2,5,2′,3′,4′	1.50	0.13	11.5	1.47	0.20	7.4
2,5,2′,4′,5′	0.68	0.36	1.9	1.16	0.45	2.6
5,6,2′,3′,4′	0.10	N.D.	–	0.02	N.D.	–
Hexachlorobiphenyls						
2,5,2′,3′,4′,5′	0.25	0.11	2.3	0.26	0.04	6.5
2,5,2′,3′,5′,6′	0.42	0.10	4.2	0.09	0.05	1.8
2,5,6,2′,3′,4′	0.04	N.D.	–	0.03	N.D.	–

* Ratio of 4-MSF to 3-MSF.

of the patient. On the other hand, in the normal person, 4-MSF-2,5,4′-tri-, 3-MSF-2,5,2′,3′-tetra- and 4-MSF-2,5,2′,4′-tetrachlorobiphenyl were the major components in adipose tissue, just as observed in the patient, though other isomers were below 0.5 μg/kg. In the liver of the patient, the levels of all the isomers did not differ greatly from those of the normal person and were less than 0.5 μg/kg.

As Yusho patients consumed rice oil that was contaminated with Kanechlor-400, a commercial mixture of PCBs, the 3-MSF- and 4-MSF-PCBs were probably derived from parent PCBs with a 2,5-dichlorophenyl ring, and accumulated in the lung and adipose tissue. The levels and ratios of 4-MSF-PCBs to 3-MSF-PCBs in the Yusho patient were therefore determined and are listed in Table V. The ratios were found to be very high in both tissues, except for 4- and 3-MSF-2,5,2′,3′-tetrachlorobiphenyls, indicating that 4-MSF-substituted PCBs were more readily formed from the PCBs with a 2,5-dichlorophenyl nucleus and accumulated more readily in the tissue than 3-MSF-substituted PCBs. The 2- and 3-MSF-PCBs, formed from

PCBs that had the 4-position blocked with chlorine atoms, were also present in the tissues.

DISCUSSION

As has been shown in earlier studies[18], accumulation and retention of many MSF-PCB congeners were demonstrated in the Yusho patient and also in the normal person. The present GC–MS analyses indicated that the tetrachloro congeners of MSF-PCBs accumulated significantly in the human tissues. Comparison of the MSF-PCB congener levels between the Yusho patient and normal person showed that the more highly chlorinated MSF-PCBs (*i.e.* penta- and hexachloro congeners) were more retained in the lung and adipose tissue of the patient than in those of the normal person. The reason for these variations in the congener level is considered to be as follows. Yusho patients were poisoned by a large quantity of Kanechlor 400, a PCB mixture containing mostly tetrachlorobiphenyls, which were different in composition from those found in common foods and normally ingested[22]. Among the PCBs ingested, most of the less chlorinated biphenyls were rapidly eliminated, whereas the more chlorinated biphenyls were partially metabolized to MSF-PCBs and accumulated in the tissues, thus maintaining the difference in MSF-PCB composition for more than ten years after the outbreak.

The toxicological significance of the existence of MSF-PCBs in the lungs of the Yusho patient is unclear at present. Brandt has found that there are great differences in tissue localization of different PCBs or their metabolites[23]. Their selective accumulation and long retention were observed in the respiratory mucosa of mice injected with certain chlorobiphenyls[24]. The marked accumulation of MSF-PCBs in the lung tissue was confirmed by experiments in mice treated with some bronchial-seeking PCBs[4], 4-MSF-tri-chlorobiphenyl[25] and 2,4′,5-trichlorobiphenyl mercapturic acid[9]. These phenomena noted in the mice are similar to our observations in the Yusho patient exposed to PCBs, although there were no such observations in the normal person. Considering that the victims of the PCB intoxication in Japan exhibited respiratory distress that persisted in most cases for more than ten years, the structural specific accumulation of MSF-PCBs in lung tissue may correlate with the respiratory symptoms in the Yusho patients as suspected by Shigematsu *et al.*[26].

With recent advances in PCB analysis[27,28], high-resolution GC capillaries combined with ECD provided excellent resolution and a better signal-to-noise ratio of the individual isomer. In this study three capillaries with different stationary phases were applied for isomer-specific analysis of MSF-PCBs, which allowed the identification of 40 MSF-PCB isomers in the tissues by comparison with the GC retention data of 86 authentic samples. The quantitative results showed that the most accumulative MSF metabolites were found to be the 4-MSF-substituted PCBs with chlorine atoms in the 2- and 5-positions, in the lung and adipose tissue of the Yusho patient, whereas the 3-MSF substituents were minor or not detectable. The accumulative ratios of the 4-MSF to 3-MSF-PCB isomers expected from parent PCBs are rather high, in ratios from 1.4:1 to 11.5:1 or more in all cases, with one exception in both tissues (Table V). These findings are very similar to the previous results[4], which showed that 4-MSF-PCBs are accumulated in ratios from 1.5:1 to 15:1 in the lungs of rats given oral doses of tri- to pentachlorobiphenyls. In contrast, only the

MSF metabolites derived from 2,5,2',3'-tetrachlorobiphenyl were accumulated as major 3-MSF substituents in both the tissues. It therefore appears that the formation and accumulation of MSF-PCB isomers are somewhat structure dependent. The presence of these isomeric metabolites suggests that the metabolic route probably involves an initial epoxidation step at the 3- and 4-positions in the 2,5-dichlorophenyl nucleus.

The additional important feature on the accumulative structure is the existence of 2- and 3-MSF-PCBs with the 4-mono-, 2,4-di-, 3,4-di- and 2,3,4-trichlorophenyl nucleus as a structural unit. This finding suggests that PCB metabolism in the human body also involves a 2,3-epoxidation in the 4-substituted phenyl nucleus. In fact, 5- and 6-MSF-PCBs were detected from the feces of mice given 2,2',4,4'-tetrachlorobiphenyl[3,29]. Human liver microsomes metabolized the 4,4'-dichlorobiphenyl, which had the *para*-position blocked, to hydroxyl PCBs[30]. In addition, the ease of formation of hydroxyl PCB in rats and pigeons was 4-chloro > 4,4'-dichloro > 2,5,2',5'-tetrachloro > 2,4,5,2',4',5'-hexachlorobiphenyl[12]. It therefore seems unlikely that the unsubstituted *para*-position is required for the metabolism of PCBs. The formation of MSF metabolites from the PCBs lacking adjacent unsubstituted carbon atoms (*e.g.* 2,4,5,2',4',5'-hexachlorobiphenyl) may be negligible, because these PCBs are rather resistant to metabolic breakdown[31,32]. It is likely that most of the MSF metabolites are derived from the PCBs with two adjacent unsubstituted carbon atoms for 2,3- or 3,4-epoxide formation, probably via the mercapturic acid pathway metabolites, as described earlier[5–7].

Although several components of MSF-PCBs in the tissues remain to be identified structurally, the major components were partially delineated. Therefore, toxicological tests for the accumulative MSF-PCB isomers may be helpful for understanding the etiology of the current symptoms of Yusho.

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