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Accumulation Levels of Organochlorine Pesticides in Human Adipose Tissue and Blood

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Because of their persistence and potential for bioaccumulation, the use of organochlorine pesticides, technical hexachlorocyclohexane (HCH) and pp'-DDT, has been prohibited since 1971 in Japan. Furthermore, chlordane which had been applied for termite control has the potential for bioaccumulation and the use of it has been also prohibited since 1986. These chemicals can enter human body through a food chain or by inhalation of vapours. Widespread contamination of the foods with these chemicals has continued (Sekita et al. 1985; Miyazaki et al. 1986-1, 1986-2) and after application for termite control the chlordane concentration in indoor air has remained at high level for a long time (Louis and Kisselbach 1987). Therefore, it is considered that human pollution with these chemicals has persisted. The results of human adipose tissue surveys for total DDT, β-HCH and dieldrin have been reported (Sasaki et al. 1987). Taguchi and Yakushiji (1988) and Wariishi et al. (1986) determined chlordane in human milk and blood, respectively. However, few data on chlordane residue in human adipose tissue are available in Japan. The aims of the present study were to assess the levels of organo-chlorine chemicals in adipose tissue and blood of Japanese and to examine the relationship between them.

MATERIALS AND METHODS

All solvents used were pesticide analytical reagent grade and anhydrous sodium sulfate was PCB analytical reagent grade. Formic acid was washed with n-hexane. Silica gel, Kieselgel 60 (70-230 mesh, Art. 7734, E. Merck, Darmstsadt) was activated at 450°C for 3 hrs and de-activated with 10 % W/W water for at least 16 hrs. All glasswares used for the analysis were rinsed with n-hexane before use.

The adipose tissue and blood were obtained from patients on surgery operation at Akita and Okinawa prefectures between 1986 and 1988.

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These samples were stored in clean glass bottles and kept in a freezer until analyzed.

Adipose tissue was heated at 120 °C in the autoclave for 20 min. After cooling, the sample was extracted with 10 ml of acetone-hexane (3:17). The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was heated at 90 °C in a dry oven for 1 hr and cooled in the desicator for 40 min. The residual lipid was weighed and a part of the lipid (<500 mg) dissolved in a few ml of hexane was loaded on silica gel column (15 g of de-activated silica gel was dry-packed in 2.2 cm id column). The column was eluted with 130 ml of methylene chloride-petroleum ether (1:4). The eluate was concentrated to 5 ml for GC determination.

Blood was mixed with 5 ml of formic acid, autoclaved in the same way as the adipose tissue and extracted with 2 portions of 10 ml hexane. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue dissolved in hexane was treated on silica gel column chromatography similarly to the lipid of adipose tissue. The eluate was evaporated just to dryness and the residue was dissolved in 10 ml of hexane. The hexane solution was washed with 1 ml of conc. H_2SO_4. The hexane layer was neutralized with $NaHCO_3$ powder and evaporated just to dryness. The residue was dissolved in 1 ml of hexane for GC determination.

For gas chromatographic analyses, a Shimadzu GC-9A gas chromatograph equipped with an electron capture detector and a fused silica capillary column (CBP 10, 0.31 mm id x 25 m, Shimadzu Co.) was used. Operating conditions : injector and detector temperature, 270 °C ; column oven temperature, initial 70 °C for 1 min - heated at 40 °C/min to 190 °C - hold for 4 min - heated at 40 °C/min to 225 °C - hold for 6 min - heated at 40 °C/min to 245 °C - hold for 7 min; carrier gas (He) 1 Kg/cm^2; make up gas (N_2) 50 ml/min; sample injection volume, 2 µl; splitless injection mode; splitter opened after 1 min.

RESULTS AND DISCUSSION

Human tissue samples, especially blood, may contain various microorganisms causing some infectious diseases, so it is desirable to sterilize before analysis. Therefore, the effects of commonly used autoclaving treatment (120 °C for 20 min) on residue levels of organochlorine chemicals in adipose tissue and blood were examined and the results were shown in Table 1.

Most chemicals added to the mixture of blood and formic acid were recovered quantitatively through the procedure but heptachlor was destroyed completely and pp'-DDT was converted to pp'-DDD. PP'-DDT in chicken tissue was reported to convert to pp'-DDD during cooking by either frying, steaming or baking (Herring et al. 1969). pp'-DDT added to butter fat was stable during autoclaving, but pp'-DDT contained in adipose tissues of human and monkey was converted to pp'-DDD.

Table 1. Recoveries of organochlorine chemicals from samples subjected to H_2SO_4 washing and autoclaving treatment.

Chemicals	Recovery (%, mean±sd)				
	H_2SO_4 washing[1]	Autoclaving treatment			
		Blood[2]	Butter[2]	Human's adipose[3]	Monkey's adipose[3]
α-HCH	95	79±10	98±5	395±235	830±860
β-HCH	106	103±3	107±5	72±15	81±7
γ-HCH	103	80±5	100±5	-	-
pp'-DDE	107	97±5	100±5	85±10	85±12
pp'-DDD	110	154±12	102±7	-	1200±610
pp'-DDT	111	8±6	101±6	48±5	72±8
trans-Chlordane	105	101±4	100±5	-	87±11
cis-Chlordane	106	103±3	101±6	-	-
trans-Nonachlor	107	98±4	102±7	90±8	-
cis-Nonachlor	106	102±5	100±6	79±4	-
Oxychlordane	105	94±4	99±5	90±16	90±7
Heptachlor	--	1	99±5	-	-
Heptachlor epoxide	104	104±4	99±5	93±9	-
γ-Chlordene	--	94±6	100±5	-	-
Dieldrin	0	101±6	100±5	-	-

-: not detected; --: not determined

1: Hexane solution containing 0.2 μg of each chemical was subjected to the treatment. Values are means of 2 experiments.

2: Acetone solution containing 0.2 μg of each chemical was added to the blood or butter fat before the treatment with autoclave.

3: Adipose tissue were collected from monkey administered hypodermically with pp'-DDT, trans-chlordane, and β-HCH and from human. Results are expressed as percentage of the chemical concentrations after autoclaving to those before autoclaving.

β-HCH in the adipose tissue converted to α-isomer which was detected in a few samples without heating procedure. Kawahara and Moku (1972) reported the heat decomposition of β-HCH to α-HCH.

pp'-DDT and pp'-DDD were not concerned in this study because of their small contents in the specimens and the reduction in β-HCH was small. Therefore the autoclaving treatment was used in this study. The heating procedure improved the separation of solvent from blood or adipose tissue samples in the extraction.
The cleanup with conc. H_2SO_4 destroyed dieldrin (Table 1).

The analytical results of organochlorine chemicals for adipose tissue and blood are shown in Table 2 and 3, respectively. β-HCH ranging from 0.37 to 2.02 ppm and 0.7 to 5.5 ppb was detected in adipose tissue and blood, respectively. pp'-DDE was found together with β-HCH in all samples. The levels of pp'-DDE was 0.52 - 11.04 ppm and 1.5 - 18.6 ppb in adipose tissue and blood, respectively.

Table 2. Organochlorine chemicals in human adipose tissue

No.	Area	Sex	Age	Concentration (ppm on a fat weight basis)							
				HCH	DDE	t-N	c-N	HEP	OXY	T-C	DIEL
1	A	M	58	1.00	1.41	0.24	0.06	-	0.06	0.36	0.08
2	A	M	59	0.70	2.91	0.14	0.04	0.04	0.04	0.26	0.08
3	A	M	70	0.51	2.71	0.24	0.06	0.03	0.05	0.38	0.10
4	A	M	62	1.50	2.35	0.10	0.03	0.05	0.05	0.25	0.06
5	A	M	65	2.02	2.43	0.50	0.12	0.10	0.15	0.99	0.08
6	A	M	53	0.38	1.05	0.21	0.05	0.04	0.08	0.38	*
7	A	M	54	1.10	1.55	0.44	0.09	0.06	0.13	0.73	0.04
8	A	M	58	0.94	0.86	0.18	0.04	0.05	0.07	0.34	0.04
9	A	M	69	0.77	5.86	0.34	0.08	0.05	0.09	0.56	*
10	A	M	78	0.85	1.27	0.21	0.06	0.11	0.10	0.61	0.02
11	A	F	66	0.56	0.52	0.07	0.01	0.03	0.02	0.13	*
12	A	M	73	0.52	0.79	0.21	0.04	-	0.05	0.30	0.08
13	O	M	19	0.85	0.97	0.63	0.16	0.12	0.22	1.30	0.10
14	O	F	48	0.58	4.77	0.52	0.12	0.06	0.15	0.85	0.16
15	O	F	40	0.44	1.08	0.26	0.05	0.04	0.06	0.41	-
16	O	F	42	1.66	11.04	1.26	0.22	0.25	0.34	2.16	-
17	O	F	46	0.95	0.95	0.49	0.09	0.12	0.17	0.87	0.27
18	O	F	47	0.82	1.78	0.34	0.06	0.07	0.10	0.67	0.15
19	O	M	67	1.43	6.25	0.94	0.16	0.19	0.27	1.62	0.03
20	O	F	49	0.47	1.93	0.54	0.13	0.08	0.15	0.90	0.26
21	O	F	59	0.46	0.79	0.22	0.04	0.02	0.06	0.34	-
22	O	M	54	0.53	1.30	0.37	0.07	0.08	0.08	0.60	0.08
23	O	M	62	0.37	0.65	0.30	0.06	0.04	0.07	0.47	0.04
mean				0.84	2.40	0.38	0.08	0.07	0.11	0.67	0.08
(sd)				(0.44)	(2.50)	(0.27)	(0.05)	(0.06)	(0.08)	(0.48)	(0.08)

Area A:Akita; O:Okinawa, Sex M:male; F:female,
HCH:β-HCH, DDE:pp'-DDE, t-N:trans-nonachlor, c-N:cis-nonachlor,
HEP:heptachlor epoxide, OXY:oxychlordane, T-C:total chlordane,
DIEL:dieldrin
*: undetectable because of H_2SO_4 treatment
-: not detected

The mean value of β-HCH in the adipose tissue was 0.84 ± 0.44 ppm. It was higher than the mean of 0.136 ± 0.47 ppm and 0.065 ± 0.085 ppm in Canadian (Lebel and Williams 1986) and was comparable with the value of 1.17 ± 0.18 ppm in Japanese (Sasaki et al. 1987).

pp'-DDE in adipose tissue was 2.4 ± 2.5 ppm in the mean. It was comparable with 2.56 ± 2.01 ppm and 3.26 ± 2.86 ppm in Canadian (Lebel and Williams 1986) and with 3.07 ± 0.71 ppm in Japanese (Sasaki et al. 1987) and was higher comparing with 0.638 ± 0.531 ppm in Norwegian (Skaare et al. 1988).

Dieldrin ranging from 0.02 to 0.27 ppm was found in all adipose tissue except No. 6, 9 and 11 which were treated with conc. H_2SO_4. Dieldrin in the blood could not be determined, because blood was

Table 3. Organochlorine chemicals in human blood

No.	Area	Sex	Age	Concentration (ppb)							
				HCH	DDE	t-N	c-N	HEP	OXY	T-C	DIEL
1	A	M	58	3.8	4.6	0.38	0.24	-	-	0.62	*
2	A	M	59	1.6	5.7	0.15	-	-	-	0.15	*
3	A	M	70	0.8	5.4	0.16	-	-	-	0.16	*
4	A	M	62	3.1	5.2	-	-	-	-	-	*
5	A	M	65	5.5	8.2	0.61	0.16	-	0.19	0.96	*
6	A	M	53	0.8	1.9	0.21	-	-	-	0.21	*
7	A	M	54	2.5	4.4	0.53	0.30	-	-	0.82	*
8	A	M	58	2.3	2.6	0.18	-	-	-	0.18	*
9	A	M	69	1.5	7.6	0.31	-	-	-	0.31	*
10	A	M	78	1.1	2.6	-	-	-	-	-	*
11	A	F	66	2.6	1.9	-	-	-	-	-	*
12	A	M	73	1.2	1.9	0.23	-	-	-	0.23	*
13	0	M	19	2.0	2.5	0.49	-	-	0.14	0.63	*
14	0	F	48	1.3	11.0	0.68	0.20	0.06	0.07	1.11	*
15	0	F	40	1.4	3.1	0.30	0.08	0.07	-	0.45	*
16	0	F	42	4.2	18.6	1.58	0.33	0.40	0.49	2.80	*
17	0	F	46	2.2	1.9	0.40	0.10	0.15	0.15	0.80	*
18	0	F	47	1.3	4.5	0.26	0.07	0.08	0.09	0.50	*
19	0	M	67	2.8	9.2	0.91	0.20	0.26	0.30	1.67	*
20	0	F	49	1.4	4.1	0.39	0.13	0.08	0.13	0.73	*
21	0	F	59	1.1	2.3	-	-	-	-	-	*
22	0	M	54	0.7	1.7	0.23	0.07	0.07	-	0.37	*
23	0	M	62	1.0	1.5	0.39	0.08	0.05	0.10	0.62	*
mean				2.0	4.9	0.36	0.09	0.05	0.08	0.58	
(sd)				(1.2)	(4.0)	(0.35)	(0.10)	(0.10)	(0.12)	(0.64)	

cf. the note of Table 2.

treated with conc. H_2SO_4. The mean value for dieldrin of 0.08 ppm in this study was comparable to the mean value of 0.13 ppm in Australian (Ahmad et al. 1988), 0.043 ppm and 0.036 ppm in Canadian (Lebel and Williams 1986) and 0.054 ppm in Japanese (Sasaki et al. 1987).

trans-Nonachlor, cis-nonachlor, heptachlor epoxide and oxychlordane were detected in both adipose tissue and blood as 4 major chemicals in chlordane constituents. Other chlordane constituents, trans-chlordane, cis-chlordane, heptachlor and γ-chlordene were detected in a few specimens.

The compositions of chlordane detected in adipose tissue (n=23) were 59 ± 6 % for trans-nonachlor, 13 ± 2 % for cis-nonachlor, 11 ± 6 % for heptachlor epoxide and 17 ± 3 % for oxychlordane and were corresponding with those in blood. These chlordane compositions were extremely different from those of technical chlordane which consists of 24 ± 2 % of trans-chlordane, 19 ± 3 % of cis-chlordane, 7% of heptachlor, 7 ± 3 % of trans-nonachlor and others

Table 4. Correlation coefficients between chemical concentrations in adipose tissue and blood.

	<in Adipose tissue>					
	HCH	DDE	t-N	c-N	HEP	OXY
<in Adipose tissue>						
HCH	-	-	-	-	-	-
DDE	0.49	-	-	-	-	-
t-N	0.50	0.77	-	-	-	-
c-N	0.49	0.70	0.96	-	-	-
HEP	0.59	0.69	0.89	0.85	-	-
OXY	0.55	0.70	0.97	0.96	0.93	-
<in Blood>						
HCH	0.89	-	-	-	-	-
DDE	-	0.95	-	-	-	-
t-N	-	-	0.94	-	-	-
c-N	-	-	-	0.65	-	-
HEP	-	-	-	-	0.84	-
OXY	-	-	-	-	-	0.92

cf. Abbreviations in Table 2.

(Sovocool et al. 1977). The chlordane compositions in adipose tissue and blood in this study were similar to those in human milk reported by Miyazaki et al. (1986-1) and they were comparable to the chlordane compositions of cat adipose tissue but not those of dog adipose tissue reported by Miysazaki et al. (1986-1). Tashiro and Matsumura (1978) reported that trans-nonachlor was metabolized to oxychlordane via trans-chlordane and trans-nonachlor accumulation in human adipose tissue came from the little capability for dechlorination of trans-nonachlor in human liver.

From the blood collected in Okinawa prefecture, 4 chlordane constituents were found. On the contrary, most of the blood from Akita contained none of the chlordanes except trans-nonachlor. Chlordane had been used for the control of termites in Okinawa but not in Akita, and it was regarded as one of the pollutants of eatable fish (Miyazaki et al. 1986-2). The chlordane levels in indoor air and blood of inhabitants after termiticide application to the house were higher comparing with control samples (Wariishi et al. 1986; Louis and Kisselbach 1987). Therefore, the termiticide application was considered to influence to the chlordane levels in Okinawa inhabitants together with ingestion of fish polluted with chlordane.

In this study 23 concomitant adipose tissue and blood were analyzed. The correlation coefficient for chemical concentrations in adipose tissue (ppm on a fat basis) with those in blood (ppb) were 0.89 for β-HCH, 0.95 for pp'-DDE, 0.94 for trans-nonachlor, 0.65 for cis-nonachlor, 0.84 for heptachlor epoxide and 0.92 for oxychlordane (Table 4). Close relationships were observed between the chemical concentrations in adipose tissue and those in blood except cis-nonachlor. Several studies showed that blood

concentrations of HCH isomers, pp'-DDE, polychlorinated biphenyls and polybrominated biphenyls are their adequate estimate of body burden as measured in adipose tissue (Baumann et al. 1980; Wolff 1984). From this work it became evident that in addition to β-HCH and pp'-DDE the concentration of chlordane constituents, trans-nonachlor, heptachlor epoxide and oxychlordane, in adipose tissue were estimated from those in blood.

Table 4 shows correlation coefficients between 6 chemicals in adipose tissue. Significant correlations were found between each chemical related to chlordane, namely trans-nonachlor, cis-nonachlor, heptachlor epoxide and oxychlordane. pp'-DDE correlated with these 4 chemicals at the coefficients ranged from 0.69 to 0.77, but it had little correlated with β-HCH (γ= 0.49). It is generally accepted that accumulation of organochlorine chemicals in the general population refers to foods. From the survey for food contaminants intake with market basket method in Japan it was recognized that pp'-DDE came mainly from marine fish and β-HCH derived from dairy products and meat (Murakami et al. 1981, 1983). Chlordanes were contained in fish and shellfish more than in meat, eggs or dairy products (Sekita et al. 1985). Consequently, human accumulation of chlordane correlated with those of pp'-DDE but not β-HCH.

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