Specific Accumulation and Elimination Kinetics of Tris(4-chlorophenyl)methane, Tris(4-chlorophenyl)methanol, and Other Persistent Organochlorines in Humans from Japan

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We examined human adipose tissue, liver, and bile from humans in Japan to understand the contamination status, specific accumulation, and elimination of two newly identified environmental contaminants, tris(4-chlorophenyl)methane (TCPMe), tris(4-chlorophenyl)methanol (TCPMOH), and other persistent organochlorines such as polychlorinated biphenyls (PCBs), DDT and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), and chlordane compounds (CHLs). TCPMe and TCPMOH concentrations in Japanese human adipose tissue were slightly higher than those reported previously, indicating widespread exposure to these compounds in humans. Elevated residues of PCBs and DDTs are found in adipose tissue and liver. Concentrations in bile strongly correlated with concentrations in adipose fat and liver, which may suggest an equilibration in adipose fat/bile and liver/bile and possible biliary excretion of persistent organochlorines in humans. Composition of the organochlorines accumulated further indicates a metabolic capacity in humans higher than that of marine mammals. We observed age-dependent accumulation for TCPMe, TCPMOH, and other organochlorines, but there were no significant gender differences. *p,p´*-DDE and TCPMe were estimated to have low biliary excretion rate. Elimination potential of persistent organochlorines may be related to their octanol–water partition coefficient. The relationship between excretion rate and octanol–water partition coefficient may be used to predict the biliary excretion potential of some other lipophilic organochlorines such as dioxins and dibenzofurans in humans. The presence of organochlorines in bile suggests that the hepatic excretory system plays a major role in the elimination of xenobiotics in humans. To our knowledge, this is the first study of accumulation and elimination of TCPMe and TCPMOH in humans. *Key words*: age-dependent accumulation, biliary excretion, humans, persistent organochlorines, tissue distribution, tris(4-chlorophenyl) methane, tris(4-chlorophenyl) methanol. *Environ Health Perspect* 109:927–935 (2001). [Online 24 August 2001]

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During the last few decades, numerous studies have been conducted on global contamination by and toxic effects of persistent organochlorines (OCs) such as DDTs, polychlorinated biphenyls (PCBs), and hexachlorocyclohexane isomers (HCHs). These are highly bioaccumulative and have serious effects on environmental quality as well as human health and wildlife. Recently there has been a growing concern that these synthetic chemicals can act as estrogen or androgen mimics and hence disrupt normal endocrine function, possibly leading to various reproductive abnormalities in wildlife and humans (*1*). Among these chemicals, DDT and its metabolites, particularly *o,p´*-DDT and *p,p´*-DDE, are potent estrogen- and androgen-receptor antagonists, respectively (*2*). In addition, other compounds with structures similar to DDT, such as dicofol, also have been reported as environmental endocrine disruptors (*3*). Tris(4-chlorophenyl)methane (TCPMe) and tris(4-chlorophenyl)methanol (TCPMOH) are among the most recently detected environmental contaminants. These compounds have structures similar to DDT and dicofol, respectively, and are thought to be derived mainly from technical DDT (*4–7*). In recent *in vitro* studies, TCPMe and TCPMOH have been shown to possess high binding affinity for both androgen and estrogen receptors (*8,9*). Although the endocrine-disrupting effects of these compounds have not been adequately validated *in vivo*, these findings suggest that TCPMe and TCPMOH may act as estrogen mimics at relatively low concentrations. However, understanding of environmental exposure to these new environmental endocrine disrupters, particularly in humans, is still limited.

Over the last few years, we have extensively investigated the global distribution, transport, behavior, and bioaccumulation of TCPMe and TCPMOH in higher trophic animals, including marine mammals and humans. We have pointed out that these chemicals have strong bioaccumulation potential and exhibit transport behavior similar to that of DDT (*6,7,10,11*). We have also provided the first data on human exposure to TCPMe and TCPMOH and suggested that contamination in humans is expanding, possibly because of exposure to DDT (*12*). These findings may provide basis for assessing risk for humans and wildlife. Nevertheless, for a sound risk evaluation, understanding of bioaccumulation and elimination kinetics of these compounds is needed. No investigation has been conducted so far on this aspect because data are lacking on tissue distribution in humans. In addition, recent epidemiologic studies have suggested an association between certain OCs and breast cancer risk (*13*). Understanding of long-term accumulation of highly persistent OCs such as PCBs and DDTs in humans is necessary for evaluating health risks associated with these chemicals. However, extensive studies on the accumulation and elimination potential of persistent OCs in humans have not been made in recent years.

Having followed the accumulation of TCPMe and TCPMOH in marine mammals and their transport in the marine ecosystem, we have extended our research to human exposure to these chemicals so as to elucidate their specific accumulation (age-and sex-dependent accumulation) and elimination kinetics in humans. In the present study, we examined the concentrations of TCPMe, TCPMOH, and other classic OCs such as DDT and its metabolites (DDTs), PCBs, HCHs, hexachlorobenzene (HCB), and chlordane compounds (CHLs) in adipose tissue, liver, and bile from Japanese subjects. We examined residue levels, tissue distribution, compositions, as well as age- and sex-dependent accumulation of TCPMe, TCPMOH, and other OCs in humans in comparison to those in marine mammals collected from Japanese coastal waters. We provide the most

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recent status of contamination by persistent OCs in Japanese citizens. In addition, we estimated biliary excretion of these compounds on the basis of residue levels in bile. For the first time, we investigated the accumulation and elimination kinetics of TCPMe and TCPMOH and evaluated comparative elimination of OCs on the basis of their physicochemical properties.

Materials and Methods

Samples. We obtained human adipose tissue samples by autopsy in Keio University Hospital, Tokyo, during March–August 1999. We obtained informed consent from bereaved family members for all the samples analyzed in this study. Adipose tissues were wrapped in aluminum foil and stored at –80°C until analysis. These samples were taken from randomly selected patients in Tokyo and some other cities in Japan. Details of cases are shown in Table 1.

Chemical analysis. Chemical analyses of TCPMe and TCPMOH as well as other OCs followed the method previously described (*10,12*). Briefly, approximately 2 g adipose tissue samples were homogenized with anhydrous Na_2SO_4 and extracted using a Soxhlet apparatus (Wako Chemicals, Osaka, Japan) with a mixture of hexane and diethyl ether. We determined fat content gravimetrically from an aliquot of the extract. The extract was then added into a dry Florisil (Wako Chemicals USA, Inc., Richmond, VA, USA) column to remove fat. We eluted organochlorines with 150 mL of 20% water in acetonitrile into a separatory funnel containing hexane and water. After partitioning, the hexane layer was concentrated and then passed through an 8-g activated Florisil column for fractionation. The first fraction eluted with hexane contained PCBs, *p,p´*-DDE, *trans*-nonachlor, and HCB; the second fraction eluted with 20% dichlomethane in hexane contained other organochlorine pesticides and TCPMe. The third fraction eluted with 50% dichloromethane in hexane contained TCPMOH. Each fraction was concentrated and injected into a gas chromatograph with electron capture detector (GC-ECD) and a gas chromatograph with a mass selective detector (GC-MSD) for quantification.

We quantified the organochlorines (except TCPMe and TCPMOH) using a Hewlett Packard 6890 series GC-ECD (Wilmington, DE, USA) equipped with an auto injector (Hewlett Packard 7683 series). The GC column employed was DB-1 fused silica capillary column (0.25 mm × 30 m; J & W Scientific Inc., Folsom, CA, USA) coated with 100% dimethylpolysiloxane at 0.25 µm film thickness. The column oven temperature was programmed from 60°C to 160°C, held for 10 min, and then increased to 260°C at a rate of 20°C/min and held for 20 min. Injector and detector temperatures were set at 260°C and 280°C, respectively. We used helium and nitrogen as carrier and make-up gases, respectively.

We calculated OC concentrations from the peak area of the sample to the corresponding external standard. The PCB standard used for quantification was an equivalent mixture of Kanechlor preparations (KC-300, KC-400, KC-500, KC-600) with known PCB composition and content. We summed concentrations of individually resolved peaks of PCB isomers and congeners to obtain total PCB concentrations. For quantification of TCPMe and TCPMOH, we used a Hewlett-Packard 6890 series GC-MSD coupled with 5973 mass selective detector. We acquired data using a Hewlett-Packard 5973 data system, in which the cluster ions were monitored at m/z 311, 313, 346, and 348 for TCPMe and 139, 251, 253, 362, and 364 for TCPMOH. Recoveries of target analytes through this analytic method were 95 ± 1.1% for TCPMe, 100 ± 2.1% for TCPMOH, 99 ± 2.0 % for PCBs, 95 ± 7.5% for DDTs, 96 ± 7.7% for HCHs, 100 ± 4.7% for CHLs, and 94 ± 5.9% for HCB. Concentrations were not corrected for recovery rates. We analyzed a procedural blank with every set of 6 samples to check for interfering compounds and to correct samples values, if necessary. DDTs represent the sum of *p,p´*-DDT, *p,p´*-DDD, and *p,p´*-DDE, and CHLs include *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane. HCHs include α, β, and γ-isomers. Concentrations of OCs were expressed as ng/g on a lipid weight basis, unless otherwise specified.

For quality assurance and quality control, we participated in the Intercomparison Exercise for Persistent Organochlorine Contaminants in Marine Mammal Blubber organized by the National Institute of Standards and Technology (Gaithersburg, MD, USA) and Marine Mammal Health and Stranding Response Program of the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (Silver Spring, MD, USA). We analyzed standard reference material SRM 1945 for selected PCB congeners and persistent OC insecticides. We obtained reliable results by comparing data from our laboratory with those from standard reference values.

Results and Discussion

Residue levels in human adipose tissue, liver, and bile. Mean, range, and geometric mean of concentrations of TCPMe, TCPMOH, and other OCs in adipose tissue, liver, and bile are shown in Table 2. Residue pattern in adipose tissue was in the order of DDTs > PCBs > HCHs > CHLs > HCB > TCPMe > TCPMOH. The environmental exposure as impurities of other materials may explain why concentrations of TCPMe and TCPMOH are substantially lower than those of other OCs (*4,14*). Two individuals (nos. 6 and 14) had very low lipid content (Table 2), which produced extraordinarily high concentrations of OCs, and these samples were omitted for calculation of mean concentrations. On a lipid weight basis, concentrations of TCPMe and TCPMOH in adipose tissues ranged between 2.7 and 44 (mean = 18) ng/g and < 0.28 and 31 (mean = 12) ng/g, respectively, which were approximately 2 orders of magnitude less than those of DDTs. Concentrations

Table 1. Information on the Japanese human samples analyzed in this study.

Sample no.	Sex	Age (year)	Body weight (kg)	Residence	Cause of death
1	M	17	56.8	Tokyo	Acute lymphoid leukemia
2	M	34	46	Nagano	Colon cancer
3	M	41	63	Tokyo	Malignant lymphoma
4	M	42	59.2	Tokyo	Intracerebral hemorrhage
5	M	57	81.3	Tokyo	Malignant lymphoma
6	M	59	58.2	Tokyo	Pancreatic carcinoma
7	M	62	57	Yokohama	Malignant mesothelioma
8	M	66	61.8	Tokyo	Multiple organ failure
9	M	67	56.8	Tokyo	Lung cancer, prostate cancer
10	M	67	40.4	Tokyo	Lung cancer
11	M	68	NA	Yokohama	Cardiac infarction, prostate cancer
12	M	79	50.2	Chiba	Hepatocellular carcinoma
13	M	81	67.8	Tokyo	Cerebrospinal meningitis
14	F	47	62.2	Tokyo	Gastric cancer
15	F	48	58.6	Tokyo	Lung cancer
16	F	49	58.8	Tokyo	Breast cancer
17	F	70	46.6	Tokyo	Cardiac sarcoidosis
18	F	73	42	Tokyo	Rheumatoid arthritis
19	F	74	51.4	Tokyo	Malignant lymphoma
20	F	75	46.5	Tokyo	Endometrial carcinoma
21	F	84	38.5	Tokyo	Ovarian cancer
22	F	87	45.8	Tokyo	Cerebral infarction

Abbreviations: F, female; M, male; NA, not available.

of TCPMe and TCPMOH reached up to 44 and 31 ng/g lipid weight, respectively, which were slightly higher than those reported previously (*12*). This observation suggests widespread contamination by TCPMe and TCPMOH in humans. Mean concentration of TCPMe (18 ng/g) in human adipose tissues was higher than those found in harbor porpoises, Dall's porpoises, and striped dolphins, but significantly lower than that in Fraser's dolphins; these samples were collected from animals in Japanese coastal waters (*7*). TCPMOH residues in human tissues were lower than those in cetaceans (*7*). This result implies that humans have a higher capacity than marine mammals to metabolize these compounds, which is similar to that reported for other persistent OCs.

On a wet weight basis, concentrations of TCPMe, TCPMOH, and other OCs in liver and in bile were lower than those in adipose tissue. However, because of the low lipid content of liver and bile, concentrations of these tissues are comparable to those in fat when expressed on a lipid weight basis (Table 2). To our knowledge, this is the first report of detection of TCPMe and TCPMOH residues in human liver and bile. Concentrations of TCPMe and TCPMOH in livers ranged between 1.1 and 20 (mean = 7.0) and < 4.0 and 38 (mean = 19) ng/g lipid weight. Residues of these compounds were scarcely reported in liver of higher trophic animals. The only available data are those for small cetaceans stranded along Florida coasts in the United States (*11*). Hepatic concentrations of TCPMe and TCPMOH in Japanese humans were significantly lower than those in livers of bottlenose dolphin (mean = 2,000 and 1,300 ng/g lipid weight), Atlantic spotted dolphin (mean = 190 and 860 ng/g lipid weight), and pygmy sperm whale (24 and 84 ng/g lipid weight), which were collected along Florida coastal waters (*11*). Concentrations of TCPMe in bile ranged between < 5.0 and 62 (mean = 17 ng/g) lipid weight, which were somewhat higher than those in liver. TCPMOH concentrations in bile were below the detection limit. The presence of TCPMe in bile suggests the possible biliary excretion of this compound in humans.

PCB concentrations in adipose tissues ranged from 230 to 6,600 (mean = 2,100) ng/g lipid weight, which were slightly higher than those reported in our previous survey (*12*). More than one-third of the samples analyzed in this study contained PCB levels > 2,000 ng/g with the highest concentration of 6,600 ng/g lipid weight in a male patient (sample 7). These levels are comparable to

Table 2. Concentrations (ng/g lipid weight) of persistent organochlorines in human adipose tissue, liver, and bile from Japan.[a]

Sample	Sex	Age[b]	Fat (%)	TCPMe	TCPMOH	PCBs	*p,p´*-DDE	*p,p´*-DDD	*p,p´*-DDT	DDTs	α-HCH	β-HCH	γ-HCH	HCHs	Oxy	*t*-CA	*c*-CA	*t*-nona	*c*-nona	CHLs	HCB
Adipose tissue																					
1	M	17	52	2.7	< 0.28	230	190	0.88	9	200	0.57	100	0.69	100	25	0.79	< 0.28	58	5.8	90	21
2	M	34	38	14	8.9	1,100	1,700	5.3	32	1,700	4.2	170	8.4	180	29	2.2	1	74	7.6	110	37
3	M	41	86	9.1	7.9	1,300	2,400	3.7	86	2,500	3.5	370	7.1	380	43	45	0.87	110	26	220	34
4	M	42	76	10	5.4	1,200	660	1.8	61	720	1.4	260	2.4	260	41	4.1	0.88	140	25	210	43
5	M	57	81	11	< 0.28	1,700	1,200	3.4	39	1,200	0.72	170	0.7	170	46	0.89	1	160	33	240	27
6	M	59	1.9	520	220	35,000	18,000	13	290	18,000	63	5,200	180	5,400	1,100	79	20	3,700	530	5,400	580
7	M	62	68	44	9.3	6,600	2,500	21	110	2,600	2.5	760	< 0.28	760	78	18	19	250	120	490	68
8	M	66	73	19	26	2,200	1,800	1.6	48	1,800	1	450	0.75	450	38	2.2	0.82	190	32	260	40
9	M	67	62	11	9.4	1,800	920	3.5	42	960	1.4	44	1.9	47	47	1.9	6.6	230	84	370	31
10	M	67	70	19	20	1,700	1,800	3	39	1,800	0.72	190	< 0.28	190	36	2.1	2.8	120	26	190	34
11	M	68	74	12	8.3	1,600	1,800	2.7	30	1,800	2.8	130	5.1	140	23	1.5	0.87	84	15	120	24
12	M	79	64	26	< 0.28	1,600	1,700	1.5	9.1	1,700	1.5	470	< 0.28	470	63	1.4	1	220	39	320	53
13	M	81	72	21	< 0.28	2,500	4,700	13	130	4,800	2.4	900	< 0.28	900	61	1.8	17	170	120	370	110
14	F	47	0.6	95	< 0.28	13,000	5,200	<0.14	150	5,400	67	2,500	730	3,300	250	2,200	< 0.28	650	110	3,200	700
15	F	48	71	15	17	2,100	2,300	2.7	54	2,400	2.9	600	3.9	600	70	58	0.92	180	31	340	93
16	F	49	94	5.7	2.7	400	150	1.2	11	160	0.6	130	< 0.28	130	13	0.77	0.63	24	3.9	42	17
17	F	70	71	15	15	2,700	6,500	6.1	68	6,600	5.2	1,300	5.4	1,300	68	< 0.28	1.54	180	34	280	90
18	F	73	46	33	18	2,800	1,700	2.2	50	1,800	2.8	540	6.7	550	180	1.9	1.2	500	46	730	67
19	F	74	43	20	23	2,100	1,600	2.3	56	1,700	2.6	580	6.5	590	190	6	1.3	470	54	720	63
20	F	75	62	26	23	3,200	7,900	10	140	8,100	8.5	3,200	3.5	3,200	120	77	1.4	230	39	470	160
21	F	84	63	13	7.8	1,700	1,600	4.6	84	1,700	3	1,100	3	1,100	56	11	< 0.28	150	30	250	110
22	F	87	36	39	31	2,800	860	<0.14	21	880	5.8	2,000	1.2	2,000	94	4.2	0.69	210	23	330	81
Mean			65	18	12	2,100				2,300				680						310	60
Range			36–94	2.7–44	< 0.28–31	230–6,600				160–8,100				47–3,200						42–730	17–160
Geometric mean			63	15	5.7	1,700				1,600				400						250	50
Liver																					
1	M	17	14	1.1	< 4.0	240	120	10	14	140	5.6	260	< 4.0	260	25	4.8	7.1	41	35	110	18
4	M	42	12	3.2	15	920	400	7.4	44	450	18	240	< 4.0	260	34	5.3	11	100	17	170	30
6	M	59	2.8	150	96	11,000	6,400	710	1,000	8,100	15	1,700	< 4.0	1,700	540	39	< 4	2,100	3,400	6,100	260
8	M	66	5.6	7.5	11	1,200	630	8.2	17	660	7.8	930	< 4.0	930	34	16	< 4	96	45	190	23
12	M	79	2.5	5.6	28	1,000	600	680	880	2,200	31	440	< 4.0	470	34	< 4	< 4	240	3,400	3,800	38
15	F	48	2.9	7.6	38	1,200	1,000	83	38	1,100	12	1,100	< 4.0	1,100	52	23	< 4	160	41	280	62
18	F	73	3.8	3.9	24	1,200	740	29	53	820	< 4.0	1,100	< 4.0	1,100	140	< 4	< 4	320	39	500	32
20	F	75	10	20	17	2,900	5,600	83	120	5,800	7.3	2,300	< 4.0	2,300	93	7.4	< 4	210	72	380	130
Mean			7.2	7	19	1,200				1,600				920						780	48
Range			2.5–14	1.1–20	< 4–38	240–2,900				140–5,800				260–2,300						110–3,800	18–130
Geometric mean			5.4	5.1	16	1,000				900				700						360	38
Bile																					
1	M	17	0.50	62	< 10	4,600	130	< 10	28	160	< 10	440	< 10	440	100	< 10	< 10	180	54	330	240
4	M	42	0.53	18	< 10	2,300	430	< 10	26	460	83	400	170	650	72	110	< 10	140	26	350	130
6	M	59	0.20	150	< 10	27,000	8,000	< 10	160	8,200	< 10	3,100	< 10	3,100	700	< 10	< 10	1,700	280	2,700	800
8	M	66	0.80	8.8	< 10	2,500	540	13	< 10	550	< 10	390	< 10	390	30	< 10	< 10	86	21	140	81
12	M	79	0.87	8	< 10	2,400	1,700	15	15	1,700	< 10	680	< 10	680	92	< 10	< 10	130	23	250	100
15	F	48	1.5	6	< 10	1,800	730	< 10	< 10	730	46	290	33	370	61	30	< 10	67	15	170	67
18	F	73	0.91	19	< 10	3,100	710	< 10	< 10	710	71	360	52	480	140	64	< 10	270	49	520	120
20	F	75	0.50	< 5	< 10	3,400	1,800	140	< 10	1,900	56	1,200	36	1,300	190	140	84	140	50	600	240
Mean			0.8	17		2,900				880				620						340	140
Range			0.5–1.5	< 5 – 62		1,800–4,600				160–1,900				370–1,300						140–600	81–240
Geometric mean			0.79	12		2,800				680				560						300	130

Abbreviations: *c*-CA, *cis*-chlordane; *c*-nona, *cis*-nonachlor; F, female; M, male; Oxy, oxychlordane; *t*-CA, *trans*-chlordane; *t*-nona, *trans*-nonachlor.
[a]Concentrations of samples 6 and 14 of adipose tissue and 6 of liver and bile were omitted for calculation of mean, range, and geometric mean. [b]In years.

those reported in adipose tissue of patients exposed to PCBs during the Yusho poisoning in Japan in 1968 (*15*). Elevated PCB burdens in humans from Japan observed here is of concern. Our data provide one of the most up-to-date contamination status reports on PCBs in Japanese human adipose tissue, and these elevated residues suggest continuing exposure to PCBs by Japanese humans. In fact, temporal trend investigations conducted in developed nations such as the United States, Canada, the United Kingdom, the Netherlands, and Japan revealed that there was no significant decline in PCB residues in humans (*16–20*). PCB production ceased in Japan in 1972; however, large proportion of PCBs, accounting for approximately > 50% of the cumulative production, still remained in use in older transformers and capacitors (*21*). Matsumoto et al. (*22*) reported that there was no decline in dietary intakes of PCBs in Japanese population until 1985, after production ceased. All of these facts may account for the continuing high exposure to PCBs in Japanese humans. Our recent investigations have also indicated notable PCB pollution in marine mammals collected from various sites along the Japanese coastal waters (*23–25*).

DDTs were the most abundant contaminants among OCs analyzed, with concentrations ranging from 160 to 8,100 (mean = 2,300) ng/g lipid weight in adipose tissue. Interestingly, concentrations of DDTs were significantly higher than those reported recently (*12*). A few patients contained relatively higher DDT levels (e.g., samples 13, 17, 20)—one female patient, who suffered from endometrial carcinoma, carried a DDT level of up to 8,100 ng/g lipid weight. This result indicates long-term persistence of DDT in humans despite the ban of this insecticide enforced in Japan more than 2 decades ago. The long life span of humans has led to long-term accumulation of this persistent insecticide, evidenced by apparently higher residues in older patients. Concentrations of HCHs, CHLs, and HCB in adipose tissue were comparable to those reported previously (*12*). HCH residues in Japanese human adipose tissue analyzed in this study ranged from 47 to 3,200 (mean = 680) ng/g lipid weight. This level was greater than that of CHLs (mean = 310 ng/g lipid weight) and HCB (mean = 60 ng/g lipid weight) (Table 2).

In our previous study (*12*), we compiled recent OC residues in human adipose tissue from various countries to assess the contamination. PCB contamination in Japan is one of the highest among developed nations and substantially greater than in developing countries (*12*). The data obtained from this study show that DDT pollution was even greater. DDT concentrations found in this study were comparable to those in some developing countries where DDT was used in large quantities in the past and had been used until recently. Here, we compiled residue levels of OCs in human liver because the data on OCs in human liver were relatively limited compared to those for adipose tissue (Table 3). Since the data represent those for different years, including the 1970s and 1980s, they may not be directly comparable. Hepatic concentrations of PCBs in Japanese humans were comparable to those reported for European countries such as Finland (*26*), Norway (*27*), and Sweden (*28*), and higher than those in the United States (*31*). This trend is somewhat similar to the results observed for adipose tissue (*12*). Residues of DDTs and HCHs in Japanese livers collected in 1999 are significantly greater than those found in these European countries in the 1980s or the 1990s. The higher concentrations of DDTs in human tissues in recent years highlight the serious impact on human health. Epidemiologic studies [reviewed by Ahlborg et al. (*13*)] also suggested an associa-

Table 3. Comparison of organochlorine residues (ng/g lipid weight) in human liver from different countries.[a]

Country	Year	PCBs	DDT	HCHs	CHLs	HCB	Reference
Japan	1999	1,200	1,600	920	780	48	Present study
Finland	1982–83	1,100	550	290[b]	50[c]	20	(*26*)
Norway	1977	1,900	800[d]	ND	ND	150	(*27*)
Sweden	1997	1,100	840[d]	ND	ND	58	(*28*)
Italy	1989	ND	310[e]	89[f]	ND	ND	(*29*)
Greenland	1992–94	42,000	2,900[g]	390[h]	2,900[i]	750	(*30*)
USA and Canada	1980s	280	3,600	ND	ND	8.7	(*31*)

ND, not determined.
[a]Cited values were rounded to two digits. [b]γ-HCH only. [c]Sum of *trans*-chlordane, *cis*-chlordane, oxychlordane, and *trans*-nonachlor. [d]*p,p´*-DDE only. [e]Sum of *p,p´*-DDE, *p,p´*-DDT and *o,p´*-DDT. [f]Sum of β- and γ-HCH. [g]sum of *p,p´*-DDT and *p,p´*-DDE. [h]β-HCH only. [i]Sum of α-chlordane, γ-chlordane, oxychlordane, *cis*-nonachlor, and *trans*-nonachlor.

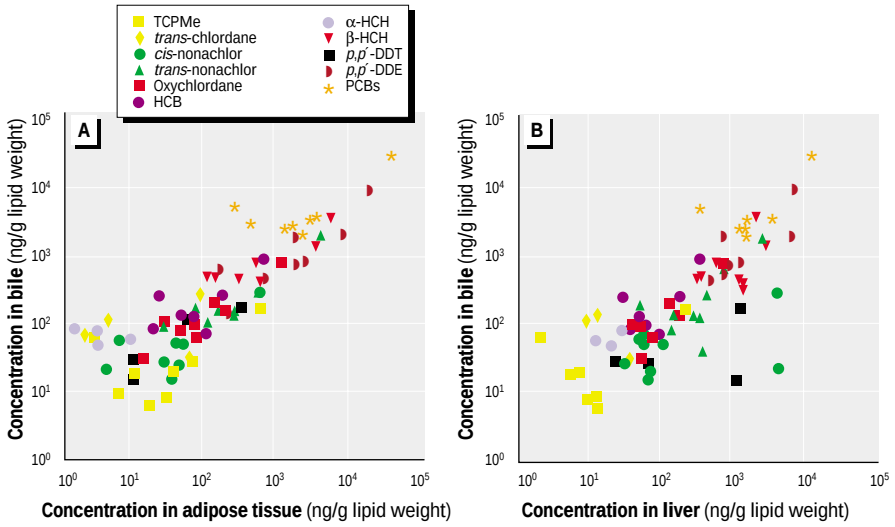

Figure 1. Relationship between OC concentrations in (*A*) adipose tissue/bile [$r = 0.95$ ($p < 0.0001$)] and (*B*) liver/bile [$r = 0.84$ ($p < 0.0001$)] in Japanese humans. Concentration values lower than the detection limit were omitted.

Table 4. Mean concentrations of organochlorines in adipose tissue, liver, and bile and their ratios between these tissues in humans from Japan ($n = 7$).[a]

Concentration (ng/g wet weight)	Lipid (%)	TCPMe	TCPMOH	PCBs	DDTs	HCHs	CHLs	HCB
Adipose tissue	63	12	8.2	1,200	1,500	510	210	43
Liver	7.3	0.49	0.97	86	120	63	29	3.5
Bile	0.8	0.089	< 0.10	21	7.1	4.4	2.4	1.0
Concentration ratio								
Wet weight basis								
Adipose/liver		24	8.5	14	13	8.1	7.2	12
Adipose/bile		130	—	57	210	120	88	43
Liver/bile		5.5	—	4.1	17	14	12	3.5
Lipid weight basis								
Adipose/liver		2.8	0.6	1.6	1.5	0.87	0.45	1.4
Adipose/bile		1.1	—	0.65	2.7	1.3	1.0	0.49
Liver/bile		0.40	—	0.41	1.8	1.5	2.3	0.34

[a]Data for sample 6 omitted for calculation of mean concentrations and concentration ratios.

tion between high concentration of *p,p´*-DDE in human tissues and breast cancer.

Interestingly, HCH residues in human liver from Japan were the highest among the countries surveyed (Table 3). Similar results were also found for adipose tissue; HCH concentrations in Japanese humans were higher than those in most of the developing countries as well as in the United States, Canada, and some western European nations (*12*). In fact, HCH was produced in Japan in the largest quantity among the persistent OCs studied, approximately 400,000 tons until the early 1970s (*20*). HCH isomers are less lipophilic and less persistent than PCBs and DDTs, but their production and use in Japan was much more widespread than the later ones (*20*); therefore, relatively high HCH residues were still found in Japanese human adipose tissue and liver.

Mean concentration of CHLs in adipose tissue is slightly higher than that reported previously (Table 2). Chlordane was banned in 1986 in Japan, and this is probably a plausible explanation for the elevated levels found in Japanese human adipose tissue and liver. Hirai and Tomokuni (*32*) reported that the CHL level in human adipose tissue obtained in 1989 in Japan was 143 ng/g wet weight. Interestingly, CHL levels found in the present study were higher than in the 1989 data. Given these results, we can infer that human exposure to CHLs is unlikely to decline in the near future. Monitoring of CHL residues in humans is therefore required in the future.

Tissue distribution and composition of OC accumulation. To date, data on residue in human liver and particularly in bile are limited. Because of the small number of samples, we did not perform statistical analysis of correlation between tissue concentrations of each OC. However, the relationship of concentrations of all the individual compounds suggested a strong correlation between human adipose/bile and liver/bile (Figure 1). The presence of OCs in bile with significant relationship to concentrations in adipose fat and liver indicates biliary excretion of OCs.

To understand the bioaccumulation kinetics of persistent organochlorines in the human body, we analyzed the concentration ratios between adipose tissue/liver, adipose tissue/bile, and liver/bile (Table 4). We used eight samples for which data for the three tissues were available for this calculation. The data for sample 6, with low lipid content in adipose tissue, liver, and bile samples, was excluded from the discussion. On a wet weight basis, concentrations in adipose tissue were substantially higher than those in liver and bile. For the highly persistent OCs such as DDT and its metabolites, adipose/bile ratios were very high, indicating the low biliary excretion of these compounds. Like DDT, TCPMe presented relatively low levels in bile compared to that in adipose tissue. When expressed on a lipid weight basis, concentration in adipose tissue was slightly higher than or comparable to that in liver and bile. Similar results were also reported for human adipose and liver samples from Sweden (*28*) and Greenland (*30*). Other studies, however, have reported that concentrations in adipose tissue were generally higher than those in liver, on a lipid wt basis (*29*,*31*).

Biotransformation of xenobiotics in humans takes place in liver; the metabolites are then preferentially excreted into bile (*33*). As shown in Figure 2, the greater proportion of *p,p´*-DDE in bile than in liver indicates the transformation of parent compounds to *p,p´*-DDE and subsequent excretion into bile. As for CHLs, considerable enrichment of oxychlordane in bile also suggests the transformation of other chlordane compounds to oxychlordane and preferential excretion of this compound into bile. β-HCH was the most predominant isomer among HCHs; this pattern is usually observed in higher trophic animals such as marine mammals, indicating the persistence of this isomer toward enzymatic degradation (*6*,*23*,*24*). The slightly higher percentage of α- and γ-HCH in bile than in liver may suggest the excretable

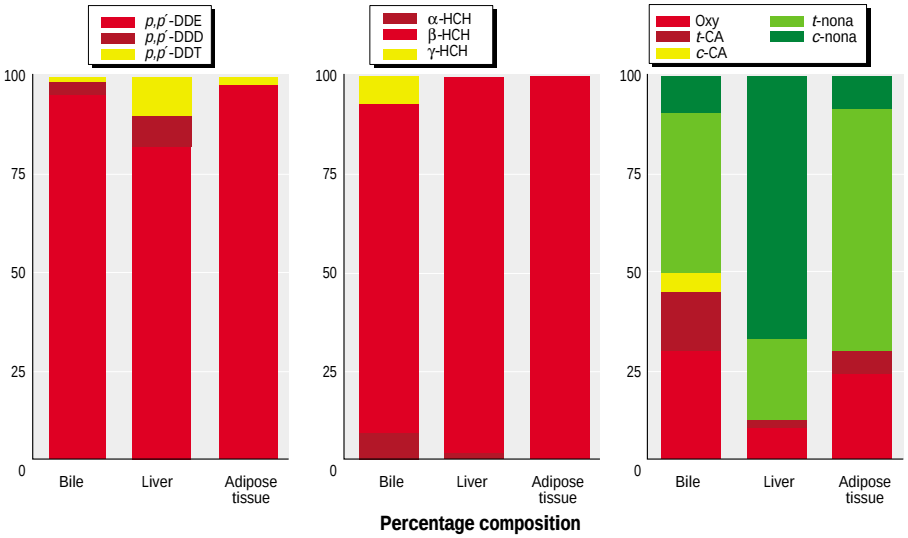

Figure 2. Comparison of compositions of OCs in Japanese human adipose tissue, liver, and bile. Abbreviations: *c*-CA, *cis*-chlordane; *c*-nona, *cis*-nonachlor; Oxy, oxychlordane; *t*-CA, *trans*-chlordane; *t*-nona, *trans*-nonachlor.

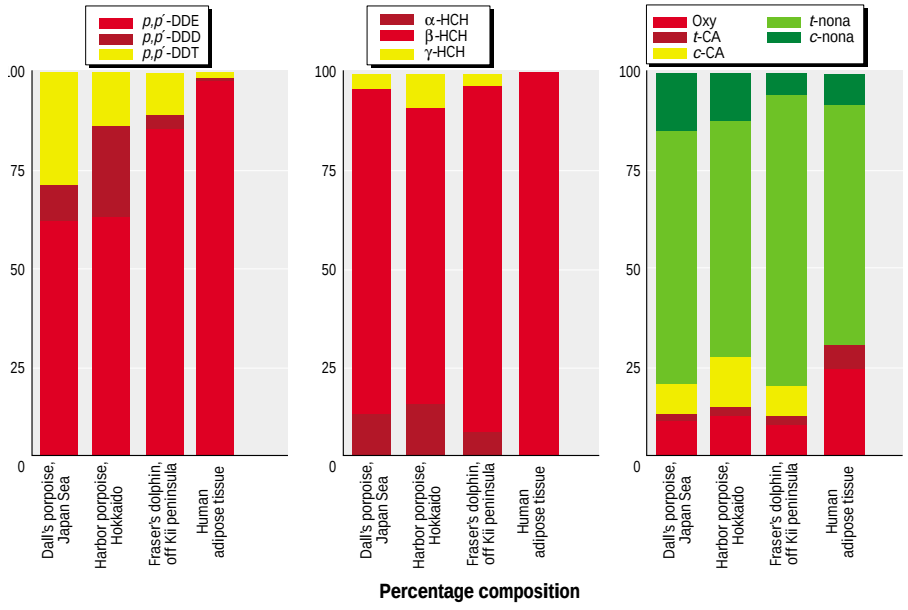

Figure 3. Comparison of compositions of OCs in Japanese human adipose tissue and in the blubber of cetaceans collected from Japanese coastal waters. Data for cetaceans from Minh et al. (*7*). Abbreviations: *c*-CA, *cis*-chlordane; *c*-nona, *cis*-nonachlor; Oxy, oxychlordane; *t*-CA, *trans*-chlordane; *t*-nona, *trans*-nonachlor.

nature of less lipophilic organochlorines through bile. Thus, the composition pattern of OCs in liver and in bile may provide insight into the bioaccumulation kinetics of these compounds in the human body.

To understand the metabolic capacity of OCs in humans and marine mammals, we compared organochlorine compositions in Japanese human adipose tissue and in marine mammals collected from Japanese coastal waters (Figure 3). The percentage of *p,p´*-DDE in total DDTs and of oxychlordane in total CHLs in human tissues was considerably higher than those in Fraser's dolphins, harbor porpoises, and Dall's porpoises collected from various sites along the Japanese coastal waters (*7*), again indicating the higher capacity of humans to metabolize OCs. The proportion of α- and γ-HCH isomers in humans was also substantially smaller than that in cetaceans (*7*), further confirming this notion. Although this fact was suggested in a few earlier studies (*34*,*35*), we believe that our current study provides the most recent data comparing accumulation patterns of persistent OCs in humans and marine mammals from Japan. Our earlier biochemical analysis (*34*) also examined the differences in metabolic capacity of OCs among various higher trophic animals, including humans and marine mammals, by estimating the hepatic phenobarbital (PB) and 3-methylcholanthrene (MC)-induced microsomal enzymes. We revealed that cetaceans had relatively lower MC-type activities and a deficient PB-type enzymes. This specific drug-metabolizing enzyme system may explain accumulation of OCs in marine mammals greater than in humans.

Age- and sex-dependent accumulation. In general, concentrations of TCPMe, TCPMOH, and other OCs increased with age in both males and females (Figure 4). However, there was no significant differences in concentration between males and females at given times. For this reason, we performed regression analysis using data in adipose tissue samples of both male and female. Samples 6 and 14, which had low lipid content (Table 2), were excluded from this analysis. Interestingly, TCPMe concentrations correlated significantly with age compared to other organochlorines. Bioaccumulation characteristics of TCPMe and TCPMOH similar to DDTs were also suggested in other studies (*5*,*7*,*10*). TCPMOH concentrations in humans were less significantly correlated with age, probably because of the low accuracy of analytic data, which is close to the detection limit. Although the number of samples is limited, more significant age-dependent accumulation pattern of TCPMe compared to DDTs and other classic OCs may suggest higher bioaccumulation potential of this compound in humans. Age-dependent accumulation was also observed in other countries such as Netherlands (*19*), Spain (*36*), Italy (*37*), and Korea (*38*).

The present accumulation pattern of persistent OCs with age in Japanese human adipose tissue indicates elevated concentrations, particularly those of DDTs, HCHs, and CHLs, in older persons (Figure 4). Most patients older than 65 contained relatively higher OC burden. Japan is one of the developed nations that used huge quantities of OC insecticides and PCBs during the 1950s and 1960s (*20*). Elevated concentrations of OCs found in older patients imply a high degree of exposure to these chemicals before their ban. In addition, in Japan after the World War II DDT was used directly on humans to eradicate lice.

Furthermore, there were no significant differences in the concentration between males and females (Figure 4). Unlike humans, marine mammals exhibited different age- and sex-dependent accumulation. Our earlier studies have demonstrated that OC accumulation in marine mammals increases with age in males, but in females residues remain relatively constant at lower levels after maturity (*24*,*39*,*40*). In marine mammals, significant transfer of OC burden through lactation in females may explain the substantial differences in accumulation pattern between males and females (*41*). In humans, shorter lactation period, lower lipid content in milk, and smaller number of childbirths may explain why there are fewer sex differences in humans than in marine mammals.

Biliary excretion of persistent organochlorines in humans. In the present study, because OC concentrations in adipose tissue and in bile from the same humans were available, we were able to estimate excretion of persistent OCs. In the body, xenobiotics undergo various processes including absorption, distribution, biotransformation, and elimination. Besides urinary excretion, fecal excretion is the other major pathway for elimination of xenobiotics. Biliary excretion is probably the most important mechanism contributing to fecal excretion (*33*). In the case of persistent OCs, high lipophilicity and persistence enable these compounds to be

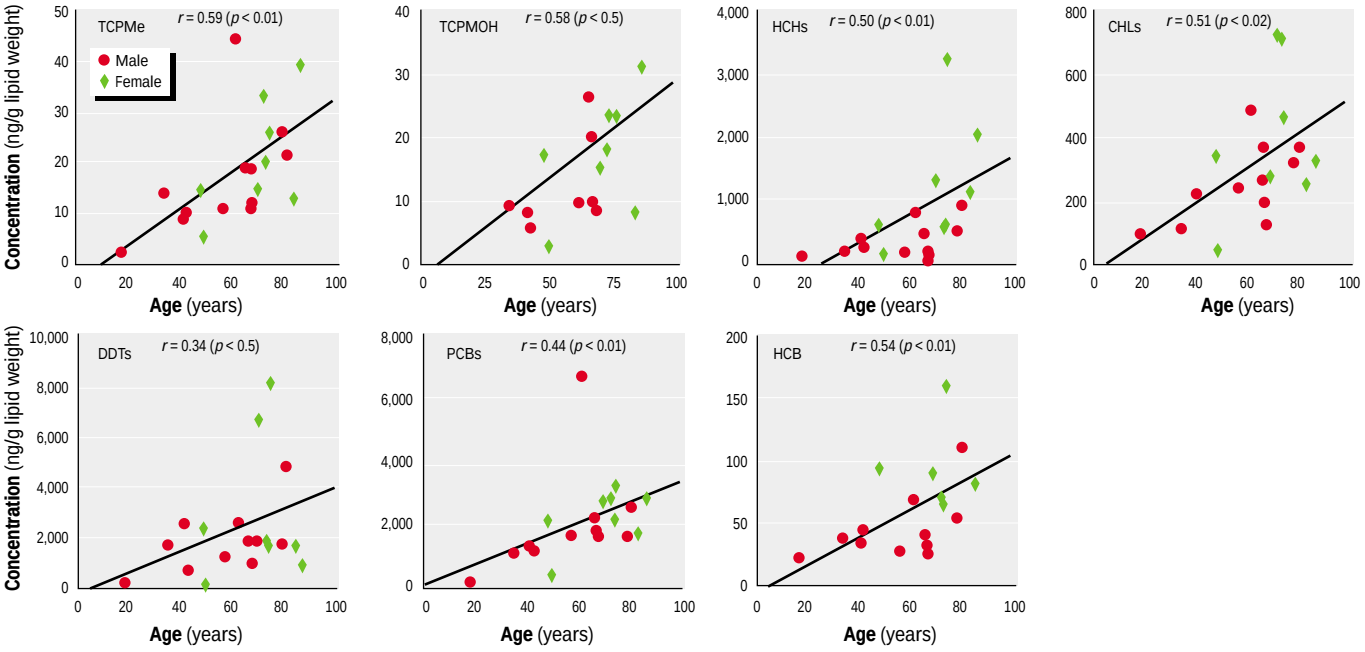

Figure 4. Accumulation pattern of OCs with age in Japanese human adipose tissue. Concentration values lower than the detection limit were omitted.

readily accumulated in adipose tissues. Therefore, we used concentrations in adipose tissue and in bile to estimate the elimination potential.

In this estimation, we used only data for adipose and bile samples taken from the same body. Sample 6, which had low lipid content in both adipose and bile, was omitted from this calculation. We estimated excretion rate on the basis of the amount of contaminants in adipose tissue and the amount in bile that a human body can excrete per day. This approach has been applied in several investigations of pharmacokinetics of persistent OCs, particularly dioxins and related compounds (*42–45*). To estimate the approximate amount of OCs in adipose tissue and in bile from a human body, we used the following assumptions: We assumed that a standard individual weighing 70 kg body weight (bw) contains 10 kg adipose tissue (*43,46*). In this study, mean body weight of 7 individuals used for this calculation is 54 kg (Table 1); thus, mean lipid weight is 7.7 kg. Approximately 700–1,200 g of bile can be extracted per day from an adult body (*47*). Mean lipid content of bile samples analyzed in this study was 0.8% (Table 2). We calculated the amount of toxic contaminants excreted into bile to provide the maximum estimate. Thus, the 1,200 g bile that a human body can excrete per day contain 9.6 g lipid [1,200 g × 0.8%]. Amount (burden) of OCs in adipose depot and in bile thus can be calculated as follows:

Amount in adipose tissue (ng) = concentrations in adipose tissue (ng/g lipid) × 7,700 (g adipose tissue)

Amount in bile (ng) = concentrations in bile (ng/g lipid) × 9.6 (g lipid in bile)

Excretion rate is calculated as:

Amount in bile ÷ amount in adipose tissue × 100 (%)

Concentrations (lipid weight), calculated amount (nanograms), and excretion rate (percent) of persistent OCs are given in Table 5. Organochlorine compounds with concentrations lower than the detection limits such as *p,p´*-DDD, *p,p´*-DDT, α-HCH, γ-HCH, *trans*-chlordane, *cis*-chlordane, and TCPMOH in bile were not subjected to this analysis. Estimated excretion rates of OCs ranged from 0.046 to 0.25%. *p,p´*-DDE showed the lowest elimination rate, followed by β-HCH, TCPMe, chlordane compounds, and PCBs. HCB had the highest excretion rate among OCs examined.

The elimination potential of persistent OCs depends substantially on their persistence in the human body. Therefore, we examined biliary excretion rate in comparison with octanol–water partition coefficient (K_{ow}). Figure 5 shows the relationship between excretion rate and K_{ow}. TCPMe is a lipophilic chemical with the highest K_{ow} value among organochlorine pesticides studied. TCPMe was estimated to have a relatively low excretion rate among OCs examined. The estimated excretion rate for TCPMe is consistent with K_{ow}, indicating high bioaccumulation and relatively low elimination potential of this compound in humans. TCPMe has been suggested to have high bioaccumulation potential in higher trophic animals such as seabirds and marine mammals (*5,10*). Concentrations of TCPMOH were below the detection limit in bile samples; therefore, we did not estimate the excretion rate of this compound. Further studies should examine quantitative information regarding elimination of TCPMOH, because human breast cancer cell proliferation assays (*9,52*) show that this chemical has estrogenic and antiandrogenic effects.

p,p´-DDE with higher value of K_{ow} showed the lowest excretion rate among the OCs examined. An earlier study also indicated that excretion of DDTs is slow—a rate of approximately 1% of stored quantity per day (*53*). CHLs with relatively high K_{ow} were estimated to have low excretion rates, higher than DDT compounds but lower than those observed for HCB. As for HCH isomers, concentrations of α- and γ-HCH were lower than the detection limit in most bile samples (Table 2), which makes it difficult to verify the excretion rate accurately. β-HCH was estimated to have a relatively low excretion rate. In general, HCH isomers, with lower K_{ow}, were considered less lipophilic than other groups of persistent OCs such as PCBs and CHLs. However, in the present study, we observed a lower rate of excretion for β-HCH. This isomer exhibits a high resistance toward enzymatic degradation and has a strong bioaccumulation potential in higher trophic animals. Elevated residues of β-HCH found in adipose tissue in this study may account for the observed low excretion rate. HCB, with an excretion rate of 0.25%, represents the highest values among chemicals investigated. A recent study (*46*) on the fecal excretion of HCB in a population highly exposed to HCB has revealed that this compound eliminated at a rate of 4% based on residues in blood and feces. The authors also evaluated excretion rates of HCB based on estimated adipose fat with very low values (0.029%). Our data probably provide more realistic estimates because the concentrations in adipose tissue and bile were quantitatively measured.

As for PCBs, the excretion rate was estimated at 0.19%. In a study of fecal excretion, congeners 138, 153, and 180 were almost completely absorbed by a breast-fed infant (*54*). The hepatic excretory system is not fully developed in newborns, which may be why some xenobiotics exert greater toxic effects in newborns than in adults (*33*). Several studies dealing with fecal excretion of dioxins and related compounds in breast-fed infants also demonstrated that some dioxin

Table 5. Mean concentrations and estimated amounts in adipose depot and excreted into bile of persistent organochlorines in Japanese humans and their estimated biliary excretion rate.[a]

Compounds	Concentration in adipose tissue (ng/g lipid weight)	Amount in adipose depot (μg)	Concentration in bile (ng/g lipid weight)	Amount excreted into bile (μg)	Biliary excretion rate (%)
TCPMe	19	150	18	0.17	0.11
PCBs	1,900	15,000	2,900	28	0.19
p,p´-DDE	2,300	18,000	860	8.3	0.046
β-HCH	800	6,200	540	5.2	0.084
Oxychlordane	77	590	98	0.94	0.16
trans-nonachlor	220	1,700	140	1.3	0.076
cis-nonachlor	31	240	34	0.33	0.14
HCB	68	520	140	1.3	0.25

[a]Data from sample 6 of adipose tissue and bile were excluded for calculation.

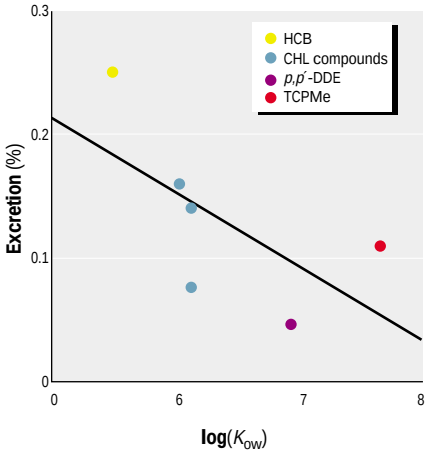

Figure 5. Correlation between biliary excretion rate of persistent OCs and their octanol–water partition coefficient. K_{ow} value of TCPMe from Fisk et al. (*48*); *p,p´*-DDE from Finizio et al. (*49*); HCB from Suntio et al. (*50*); CHL compounds from Kawano et al. (*51*). Data for β-HCH were excluded. Y = 0.061X + 0.52; r =0.64 (p < 0.05).

congeners were readily absorbed in infants (*44,45,54*). However, excretion of dioxins and PCBs is congener-selective (*55*). Further studies on isomer-specific analysis are necessary for understanding congener-selective elimination in humans.

Given the relationship between estimated biliary excretion rate and log(K_{ow}) (Figure 5), the elimination potential of persistent OCs depends on their lipophilic property, which is characterized by K_{ow}. Compounds with high lipophilicity tend to be excreted slowly from the human body. The correlation between excretion rate and log(K_{ow}) found in this study may be used to predict the biliary excretion capacity of other groups of persistent OCs in view of a quantitative structure activity relationship (QSAR) approach. Regression analysis based on quantitative data on residues of persistent OC insecticides as illustrated in Figure 5 may be used to predict the biliary excretion ability of dioxins and dibenzofurans. Because the log(K_{ow}) of dioxin and dibenzofuran compounds range from 4.3 to 8.2 (*56*), data for β-HCH were excluded from Figure 5 because log(K_{ow}) of HCH isomers is less than 4. As an example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the most toxic compound, with log(K_{ow}) of 6.8 (*56*) has a predicted biliary excretion rate of 0.1%. Earlier pharmacokinetics study showed that fecal excretion of 2,3,7,8-TCDD was estimated at 0.33% (*42*). Because biliary excretion is one of the routes contributing to fecal excretion, the value we have suggested here seems acceptable.

We observed no strong correlation between excretion rate and log(K_{ow}) (Figure 5), possibly because of the small number of samples analyzed. The predicted values of excretion rates for dioxins are tentative and should be validated further by continued studies using larger sample sizes. Nevertheless, for the first time, we suggested a way to estimate the biliary excretion for other groups of lipophilic OCs, assuming that there is a significant relationship between K_{ow} and excretion potential of persistent OC insecticides. This approach may be a useful tool for predicting the biliary excretion of lipophilic chemicals such as dioxins and dibenzofurans, because determination of concentrations of these compounds in human bile is difficult or can require expensive and sophisticated analytic techniques.

Disposition of xenobiotics in the human body consists of absorption, distribution, transformation, and elimination; these processes may occur simultaneously. The possible mechanisms of fecal elimination include excretion of nonabsorbed portion, biliary excretion, and intestinal excretion. The mechanism of excretion depends on the chemical (*33*). A few earlier studies suggested that some xenobiotics, including HCB, may excrete into feces through direct transfer to the intestinal content by passive diffusion (*46,57,58*). However, in the present study, we detected persistent OCs in bile, which indicates that the hepatic excretory system still plays a major role in the elimination of xenobiotics in humans. Thus, determination of OCs in bile may also provide useful information regarding elimination of xenobiotics from the human body.

We estimated elimination rates of OCs with assumptions and a small number of samples. Therefore, we considered the excretion rates were tentative. We discussed the elimination potential in view of relative comparison among different OCs. In addition, the biliary excretion rates suggested here are relevant only when the reabsorption is significant. Once a xenobiotic is excreted into the bile and enters the intestine, it can be either reabsorbed or eliminated with feces. Some compounds are conjugated before excretion into bile. However, for lipophilic chemicals such as persistent OCs, their conjugates may be hydrolyzed by intestinal microflora, and thus these compounds become sufficiently lipophilic for reabsorption (*33*). Reabsorption completes the enterohepatic cycle and the repeated enterohepatic cycling leads to long half-lives of persistent OCs in the human body. In addition, our earlier studies (*59,60*) showed relatively high absorption efficiency of PCBs in rat and fish, ranging between 67 and 96%, depending on the number of chlorine-substituted atoms. Thus, the result on excretion rate estimated in this study was probably reliable, given that reabsorption capacity of persistent lipophilic OCs is significant. Furthermore, the excretion rates were estimated only for unchanged compounds. Some OCs may be metabolized; for example, HCB can be metabolized to pentachlorophenol (*61*). With all these factors, we believe our results may provide insight into the pharmacokinetics of OCs in humans, because residues of persistent OCs were quantitatively determined in human adipose fat and bile.

Conclusions

In this study, we analyzed current residue concentrations of two newly detected contaminants, TCPMe and TCPMOH, as well as other classic persistent OCs in Japanese human adipose tissue, liver, and bile to understand their specific accumulation and elimination potential in humans. To our knowledge, this is the first report on the age-dependent accumulation and excretion of TCPMe and TCPMOH in humans. Our data also comprise one of the most recent analyses of concentrations of persistent OCs in Japanese humans. TCPMe and TCPMOH continue to be widespread in humans. Contamination by PCBs, DDTs, HCHs, and CHLs is still a matter of concern as evidenced by relatively higher levels of these compounds in Japanese human tissues compared to those in other developed countries. Accumulation of OCs tends to increase with age, but no gender difference was observed. TCPMe was estimated to have low biliary excretion rate among OCs examined. Elimination potential of persistent OCs may be related to their physico-chemical properties, particularly K_{ow}. The relationship between excretion rate and K_{ow} may help predict biliary excretion of other lipophilic OCs such as dioxins and dibenzofurans. Given that elevated residues of persistent OCs and their relatively slow rate of elimination were still observed in Japanese humans, continued investigations regarding contamination status and toxicokinetics are needed to evaluate risks of these compounds to humans and wildlife.

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