の関与が推察される。このことは、CYP1A2誘導剤のヒト曝露によって、ダイオキシン類が肝臓へ長期間残留することを暗示している。

考察

本研究では、2003-04 年に関東地域と愛媛県在住者から採取した臓器・組織の POPs 汚染とその蓄積特性を明らかにした。本研究で検出された POPs 濃度は、他の国に比べて高い値を示したことから、これら汚染物質によるわが国の人体汚染は長期化するものと考えられた。本研究で検出された脂肪組織と肝臓の POPs 濃度は、ほぼ同等であり脂肪依存的な分配がみられた。また、関東地域の試料提供者 2 人について、脂肪組織と肝臓に加え腎臓、膵臓、副腎、乳腺、骨髄そして脳の POPs 分析をおこなったところ、脳を除く全ての臓器・組織でほぼ同等の濃度がみられ、これら汚染物質は脳を除き脂肪依存的に体内分配されていることが判明した。脳のレベルは、他の臓器・組織に比べ 1 桁低値を示し、これは脳血液関門の存在や脳の脂質成分に起因していると考えられた。一方、HCHs や高塩素化 PCDD/DF 同属体は、脂肪組織に比べ肝臓で高い濃度がみられ、肝臓のタンパクなどに結合していることが推察された。ヒトの HCHs やダイオキシン類の肝集積に関する知見はみあたらないことから、今後さらに検体数を増やして考察を深めるとともに、肝集積に関与するリガンドの検証が課題である。

D. 考察

母乳や血液を除いて、ヒト組織中のダイオキシン類に関するデータはきわめて少ない。また、膵臓や骨髄中のダイオキシン類のデータはほとんど存在しない。特に、一般人のデータは少ないし、複数例の複数臓器中データは非常に貴重である。さらに、0.5g 以下という少量の臓器試料から迅速で高精度のダイオキシン類分析を実現できたことにより、ダイオキシン類が及ぼすヒト健康影響に関する研究が進展する条件が整った。また、ダイオキシン類とは異なる影響が危惧されている PCBs 類全般の個別分析が同時に実現できた。今後、これらの化合物による健康影響も研究可能となった。

7 例中 1 例で、骨髄及び腋窩脂肪中ダイオキシン類濃度が、それぞれ、1296 及び 1496 pg-TEQ/g lipid という高濃度を示した。この症例では骨髄及び腋窩脂肪中の脂肪含量が 0.62%及び 1.99%と他の 6 例（平均値 13%及び 51%）に比べて著しく低かった。この症例を除いた 6 例の腋窩脂肪及び肝臓中ダイオキシン類濃度は、それぞれ、117 及び 226 pg-TEQ/g lipid で、以前に調べた 20 例の腸管膜脂肪及び肝臓中濃度(平均値 139 及び 228 pg-TEQ/g lipid) と、ほぼ、同程度の濃度であった。骨髄中ダイオキシン類濃度の測定例はほとんど見られないが、脂肪中濃度で見ると、腋窩脂肪中濃度とほぼ同程度の値であった。このことから、膵臓ガンと臓器中ダイオキシンとの関係は特に強いとは考えられない。

6 例の骨髄及び腋窩脂肪中 PCBs 濃度を脂肪中濃度で比べると非常によく一致した。一方、肝臓中濃度は脂肪中濃度で比べても骨髄及び腋窩脂肪中濃度よりかなり高く、余り良い一致が見られず、肝臓と PCBs の親和性は他の臓器とは異なるかもしれないことが示唆された。

F. 研究発表

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Minh TB, Watanabe M, Kajiwara N, Iwata H, Takahashi S, Subramanian A, Tanabe S, Watanabe S, Yamada T, Hata J Human blood monitoring program in Japan: contamination and bioaccumulation of persistent organochlorines in Japanese humans. Archives of Environmental Contamination and Toxicology (submitted)

G. 知的所有権取得

1．特許取得　なし

2．実用新案登録　なし

3．その他　なし

表1 分析に供試した肝臓および脂肪組織の詳細

地域	No.	性別	年齢	臨床診断
愛媛県	1	男性	25	横紋筋肉腫
	2	男性	34	肝不全、B型肝炎
	3	男性	49	肉腫
	4	男性	69	両側頸部リンパ節転移癌
	5	男性	70	消化管穿孔、カニリ肺炎
	6	男性	79	肺癌
	7	女性	53	間質性肺炎
	8	女性	59	急性リンパ性白血病
	9	女性	62	多発全身性筋萎縮症
	10	女性	71	乳癌、癌性リンパ管症
東京都	1	男性	66	腎不全
	2	男性	79	悪性リンパ腫
	3	男性	81	心筋梗塞
	4	女性	83	肺癌
	5 *	女性	109	老衰
埼玉県	6 **	男性	58	急性骨髄性白血病
千葉県	7	男性	60	肝細胞癌
神奈川県	8	女性	62	乳癌

* 腎臓、膵臓、副腎、乳腺、骨髄、脳も供試
** 腎臓、膵臓、骨髄、脳も供試

表2 愛媛県と関東地域在住者の脂肪組織および肝臓中有機塩素化合物(OCs)濃度 (ng/g脂肪重当り)

－脂肪組織－

地域		Lipid (%)	DDTs	PCBs	HCHs	CHLs	HCB
愛媛県	平均	65	2200	1000	640	270	29
(n = 10)	範囲	10-82	68-9000	99-3100	46-1700	44-730	6.9-84
関東地域	平均	63	3200	1300	1000	310	57
(n = 8)	範囲	43-77	120-8500	450-2700	99-3800	120-560	34-100

－肝臓－

地域		Lipid (%)	DDTs	PCBs	HCHs	CHLs	HCB
愛媛県	平均	4.6	980	540	1300	220	21
(n = 10)	範囲	1.9-6.2	90-2300	62-1300	77-2700	27-720	9.0-46
関東地域	平均	4.4	1800	790	1600	320	43
(n = 8)	範囲	2.9-6.3	79-4900	370-1300	740-3700	110-650	22-69

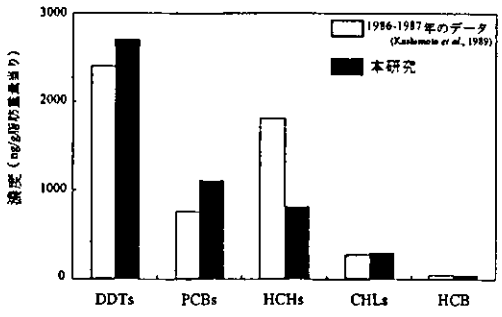

図1 脂肪組織中OCs濃度と文献値との比較

表3 脂肪組織中OCs濃度（ng/g脂肪重当り）の国際比較

国	調査した年	PCBs	DDTs	HCHs	CHLs	HCB	引用文献
日本	2003-04	1100	2700	810	290	41	本研究
ベルギー	2000	840	290	-	-	46	Covaci *et al*., 2002
メキシコ	1997-98	-	5600	160	-	58	Waliszewski *et al*., 1999
ヨルダン	1996	-	3900	1600	-	120	Alawi *et al*., 1999
トルコ	1995-96	-	2100	520	-	33	Cok *et al*.,1998
韓国	1994-95	400	1100	190	-	20	Kang *et al*., 1997
カナダ	1992	-	600	25	25	56	Mes *et al*., 1992
イラン	1991-92	-	2900	770	-	55	Burgaz *et al*., 1995
スペイン	1991	2400	4300	1500	-	3400	Gomez-Catalan *et al*., 1995
ポーランド	1990	1500	15000	250	70	260	Tanabe *et al*., 1993
フィンランド	-	500	580	200	-	-	Smeds *et al*., 2001
スウェーデン	-	1200	790	-	-	56	Weistrand *et al*., 1998

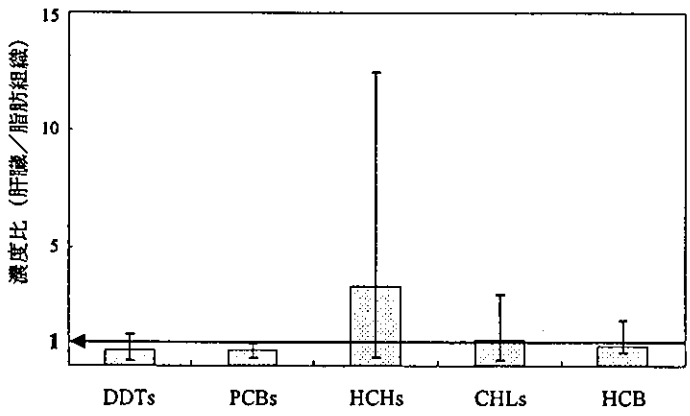

図2 OCsの肝臓／脂肪組織の濃度比

表4 関東地域在住者から採取した臓器・組織中OCs濃度 (μg/g脂肪重当り)

－男性－

臓器・組織	Lipid (%)	DDTs	PCBs	HCHs	CHLs	HCB
脂肪組織	43	1800	1300	99	370	43
肝臓	4.5	670	720	740	260	36
腎臓	3.1	1000	680	180	260	32
膵臓	28	1400	920	91	340	42
骨髄	7.2	1600	1400	110	380	56
脳	9.4	110	110	27	31	10

－女性－

臓器・組織	Lipid (%)	DDTs	PCBs	HCHs	CHLs	HCB
脂肪組織	63	3800	730	690	140	54
肝臓	6.3	1600	880	650	110	45
腎臓	3.4	2100	680	530	160	42
膵臓	27	2200	890	760	140	50
副腎	40	2300	280	720	110	54
乳腺	63	3000	890	570	130	38
骨髄	20	2000	340	710	100	49
脳	7.6	170	130	57	12	8.8

表5　愛媛県在住者の肝臓および脂肪組織中ダイオキシン類濃度(g脂肪重当り)

	肝臓 (n=10)	脂肪組織 (n=10)
	平均 (範囲)	平均 (範囲)
Lipid (%)	4.7 (2.2-8.8)	66 (13-93)
Dioxins		
2,3,7,8-T4CDD	3.6 (0.40-11)	3.4 (0.31-9.8)
1,2,3,7,8-P5CDD	12 (2.2-30)	15 (2.4-44)
1,2,3,4,7,8-H6CDD	5.0 (<0.20-13)	4.7 (1.0-12)
1,2,3,6,7,8-H6CDD	50 (8.1-110)	68 (6.6-220)
1,2,3,7,8,9-H6CDD	8.2 (1.2-25)	8.2 (0.78-21)
1,2,3,4,6,7,8-H7CDD	38 (8.7-120)	9.9 (3.4-19)
O8CDD	1500 (190-7100)	250 (42-500)
Furans		
2,3,7,8-T4CDF	1.4 (<0.10-6.1)	1.7 (0.12-5.5)
1,2,3,7,8-P5CDF	1.4 (<0.10-4.3)	1.1 (<0.10-3.9)
2,3,4,7,8-P5CDF	33 (5.6-110)	28 (4.5-92)
1,2,3,4,7,8-H6CDF	17 (1.5-53)	6.2 (1.4-18)
1,2,3,6,7,8-H6CDF	25 (2.3-93)	7.3 (1.7-22)
1,2,3,7,8,9-H6CDF	<0.20	<0.20
2,3,4,6,7,8-H6CDF	5.2 (0.85-18)	1.4 (0.39-2.4)
1,2,3,4,6,7,8-H7CDF	9.0 (2.2-28)	2.4 (0.55-8.8)
1,2,3,4,7,8,9-H7CDF	1.1 (<0.50-3.5)	<0.50
O8CDF	<1.0	<1.0
Non-ortho PCBs		
3,3',4,4'-T4CB (77)	7.5 (1.1-16)	4.9 (2.0-11)
3,4,4',5-T4CB (81)	5.4 (0.90-21)	7.9 (0.84-36)
3,3',4,4',5-P5CB (126)	130 (9.0-290)	220 (11-760)
3,3',4,4',5,5'-H6CB (169)	80 (17-200)	170 (24-640)
Mono-ortho PCBs		
2,3,3',4,4'-P5CB (105)	4100 (440-13000)	5900 (450-21000)
2,3,4,4',5-P5CB (114)	1400 (260-2900)	2100 (340-4800)
2,3',4,4',5-P5CB (118)	19000 (2000-54000)	29000 (2400-86000)
2',3,4,4',5-P5CB (123)	390 (42-1100)	580 (44-1900)
2,3,3',4,4',5-H6CB (156)	9200 (1400-21000)	14000 (3000-32000)
2,3,3',4,4',5'-H6CB (157)	2200 (370-4500)	3200 (690-7900)
2,3',4,4',5,5'-H6CB (167)	2900 (400-6000)	4900 (520-14000)
2,3,3',4,4',5,5'-H7CB (189)	1100 (95-2500)	2400 (4100-8400)
Total PCDDs	1600 (220-7400)	360 (56-590)
Total PCDFs	94 (14-310)	48 (9.8-150)
Total non*ortho* PCBs	220 (42-500)	410 (50-1400)
Total mono*ortho* PCBs	40000 (7100-97000)	61000 (7900-160000)
PCDDs-TEQs	22 (5.8-50)	26 (3.6-79)
PCDFs-TEQs	22 (4.0-73)	16 (2.7-51)
non-*ortho* PCBs-TEQs	14 (1.2-31)	24 (1.4-82)
mono-*ortho* PCBs-TEQs	8.9 (1.5-19)	13 (2.4-31)
Total TEQs	67 (11-140)	79 (10-240)

表6 脂肪組織中PCDD/Fs濃度の国際比較

国	調査した年	濃度 (pg TEQ/g)	引用文献
日本	2003-2004	42	本研究
韓国	1994-1995	22	Kang *et al*., 1997
スウェーデン	1994-1997	27	Hardell *et al*., 2001
USA	1984-1986	32	Patterson *et al*., 1994
スペイン	1989-1998	36	Schuhmacher *et al*., 1999
フランス	1999	44	Arfi *et al*., 2001
ドイツ	1980s	65	Beck *et al*., 1994
フィンランド	1984	88	Koistinen *et al*., 1995

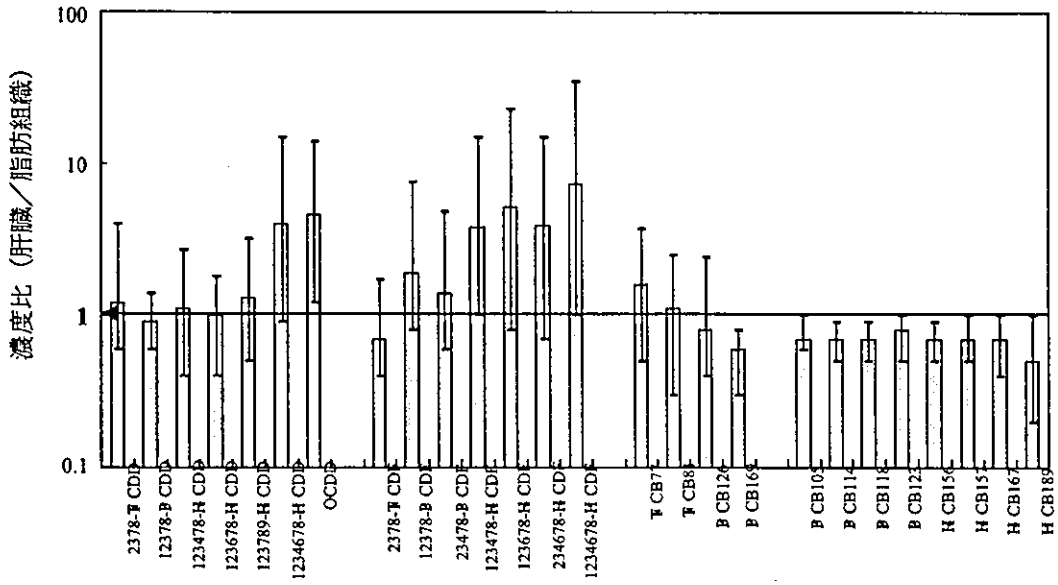

図4　ダイオキシン類の肝臓／脂肪組織の濃度比

別添５

研究成果の刊行に関する一覧表

書籍　　なし

雑誌

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Minh TB, Watanabe M, Kajiwara N, Iwata H, Takahashi S, Subramanian A, Tanabe S, Watanabe S, Yamada T, Hata J	Human blood monitoring program in Japan: contamination and bioaccumulation of persistent organochlorines in Japanese humans.	Archives of Environmental Contamination and Toxicology		In press	2005
国末達也，染矢雅之，渡部真文，豊田卓枝，黒田優子，長山淳哉，田辺信介	残留性有機汚染物質(POPs)による愛媛県在住初産婦の母乳汚染	環境科学会誌	17	37-48	2004

Human Blood Monitoring Program in Japan: Contamination and Bioaccumulation of Persistent Organochlorines in Japanese Humans

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Abstract

Concentrations of persistent organochlorines (OCs) such as polychlorinated biphenyls (PCBs), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane [DDT] and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs), hexachlorobenzene (HCB) and *tris*(4-chlorophenyl)methane [TCPMe] were determined in plasma samples of residents from three sub-metropolitan locations in Japan, Miyako, Saku and Tottori for understanding the geographical variation and specific accumulation. Residues concentrations of PCBs and DDTs were the highest in samples collected in Saku (400 and 370 ng/g lipid wt, respectively) while people from Miyako contained greater CHL residues (70 ng/g lipid wt) than those in other two locations. This contamination pattern reflects the historical usage of OCs in each area. For the first time, concentrations were detected in most of the plasma sample analyzed with the concentration ranged from < 0.1 to 8.1 ng/g lipid wt, which were lower than those in other human tissue previously reported. Larger geographical differences in OC accumulation were observed for PCBs and CHLs, while DDTs and HCHs exhibited little variability. PCB concentrations in people from Saku were higher than those in countries from circumpolar Arctic region but lower than those reported for some populations in the United States and Western European countries. Interestingly, CHL residue concentrations in human blood from Japan is among the highest values reported for the countries examined, suggesting continued elevated exposure to CHLs by Japanese population. Time trend analysis of CHLs in human blood samples from Miyako, Okinawa prefecture showed that CHL residues have decline substantially during the last decade, indicating the effect of the official ban of CHLs in 1986 in Japan. Isomer-specific analysis of PCBs revealed lower proportions of higher chlorinated congeners such as hepta- and octachlorobiphenyls in female than those in males, suggesting a possibility of preferential elimination of higher chlorinated biphenyls in females. The difference in gender dependent accumulation of OC compounds in healthy and diseased persons was suggested. To our knowledge, this is the first report on the specific accumulation of persistent OCs including TCPMe in human blood samples from Japan.

Keywords: chlordane compounds, geographical variation, human blood, Japan, persistent organochlorines, *tris*(4-chlorophenyl)methane

Introduction

During the last few decades, numerous studies have been conducted on the global contamination and toxic effects of persistent organic pollutants (POPs) such as DDTs and PCBs because of their highly bio-accumulative nature and effects on environmental quality, human health and wildlife. Recently, there has been a growing concern that these classic 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 (Colborn *et al.* 1993). Over the last few years, our laboratory has been involved in a research program on human exposure to persistent organochlorines (OCs), which was supported by the Health Sciences Research Grants of Ministry of Health, Labor and Welfare, entitled "Research on Environmental Health - Human Exposure by Endrocrine Disruptors in Japan". In frame of this program, we have extensively investigated the status of recent OC contamination and their bioaccumulation and elimination kinetics in Japanese humans using various archived human tissues (Minh *et al.* 2000a, 2001).

The present study reports the results of the analysis of PCBs and classic organochlorine insecticides such as DDT and its metabolites (DDTs), hexachlorocyclohexane (HCHs), chlordane compounds (CHLs), hexachlorobenzene (HCB) and *tris*(4-chlorophenyl)methane (TCPMe), a newly detected environmental contaminant, in human blood from three sub-metropolitan areas in Japan. This is a continuous study of our long term program on human exposure, in which various archived human tissues were employed for chemical analysis (Minh *et al.* 2000a, 2001). Despite the ban of usage of OC chemicals (except for CHLs, which were banned in 1986) for more than three decades, their long term accumulation and elevated contamination was still observed in Japanese humans (Minh *et al.* 2000a, 2001; Kunisue *et al.* 2004). While a number of studies on human exposure to persistent OCs have been conducted using adipose tissue and breast milk, data for Japanese human blood samples are limited. Breast milk is considered as one of the most important tissues for monitoring of OC exposure

because it is easily to collect and has high lipid content, while adipose tissues proved to be good samples for retrospective monitoring studies. On the other hand, blood may be a sensitive sample for understanding any change in environmental exposure and therefore, may provide insights into the accumulation kinetics of OCs in humans. The advantage of human blood samples over breast milk and adipose tissues is the possibility to conduct epidemiological and bio-accumulation kinetics (e.g. age and sex dependent accumulation) studies, and does not require invasive surgery (Hooper and She, 2003).

In the present study, we examined the concentrations of PCBs, DDTs, CHLs and HCB in plasma samples of residents living in three locations in Japan, Miyako, Saku and Tottori. For the first time, residue levels of TCPMe, a compound has been recently reported as a new endocrine disrupting contaminant (Lascombe et al., 2000), were also determined. The major purpose of this study is to make clear the geographical variations in human exposure of OC compounds using blood plasma samples, and to understand the possible influence of the historical status of OC usage on the degree of exposure in Japanese general population. Extensive comparison of OC residue levels in human blood samples in both local and global scales is made to understand the magnitude of contamination of OCs in Japanese humans. In addition, age and gender dependent accumulation in blood samples was also evaluated to provide insights into the accumulation kinetics of persistent OCs in human body.

Materials and Methods

Samples and sampling locations

Blood samples were obtained in Keio University Hospital, Tokyo. Blood samples were collected in local health center in each location (Miyako, Saku and Nagano) by physicians. Samples were then transported and managed in Keio University Hospital, Tokyo. The chemical analysis was conducted in Center for Marine Environmental Studies, Ehime University. Human blood samples were taken from residents living in Miyako, Okinawa prefecture, Saku,

Nagano prefecture and Tottori, Tottori prefecture, Japan. Miyako is a district in Okinawa prefecture located in the East China Sea, which is about 600 km southwest of Kyushu Island. Saku is a city at the mid-west of Nagano prefecture. Nagano prefecture is located in the center of the mainland of the Japanese Archipelago, and is one of the most famous horticultural producing areas in Japan. In particular, Nagano prefecture is a leading apple, grape, prune and blueberry producer in Japan. Tottori is a capital city of Tottori prefecture, located at the western coats of Japan Sea. This prefecture is also known as one of Japan's foremost agricultural areas. Particularly, Tottori accounts for about half of the national production of pears. More details of three locations, Miyako, Saku and Tottori can be found in the worldwide web (Wikipedia Encyclopedia 2004; Website Nagano Prefecture 2001; NAER Member, Tottori Prefecture 2002). Information of age, body weight and height was obtained through questionnaires provided by the donors. We obtained the informed consent from donors for all the samples analyzed in this study. The communication of the data was also permitted by the Japanese Ministry of Health, Labor and Welfare. Details of samples were given in Table 1.

Chemical Analysis

Chemical analyses of OCs followed the methods that employed in our previous studies dealing with human samples with some necessary modifications (Kitamura *et al.* 2000; Minh *et al.* 2001). Chemical analyses of OCs consist of extraction, lipid removal, fractionation and quantification. Blood sample was collected into a transfusion bag (200 ml) containing heparin sodium solution (SH-207-Terumo, Japan). The lipids were extracted from 50 g plasma with a solution of 9 ml saturated ammonium sulfate and 36 ml of mixture of ethanol: hexane (1:3) solution. The extraction process was repeated several times and the pooled hexane layers were condensed, washed with distilled water, treated with anhydrous sodium sulfate, and evaporated to dryness; and lipid weight was measured. The lipid was stored in a 1 ml capped vial and maintained at -20°C until chemical analysis. These lipid samples were used for chemical

analysis of persistent organic pollutants including PCBs and OC insecticides. For persistent OC analysis, the lipid of plasma was re-constituted to hexane. The solution was then subjected into gel permeation chromatography column (GPC) for removing of fat. Organochlorine compounds were eluted in the second fraction of GPC with 100 ml of mixture of hexane: DCM (50:50 v/v). After concentration, the solution was 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 *tris*(4-chlorophenyl)methanol (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-MS) for quantification.

Organochlorines (except TCPMe and TCPMOH) were quantified by a Hewlett Packard 6890 series GC-ECD (Wilmington, DE) equipped with an auto injector (Hewlett Packard 7683 series). The GC column employed was DB-1 fused silica capillary column (0.25 mm x 30 m; J & W Scientific Inc., Folsom, CA) coated with 100 % dimethylpolysiloxane at 0.25 μm film thickness. The column oven temperature was programmed from 60 to 160°C, held for 10 min, 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 and 280°C, respectively. Helium and nitrogen were used 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. Concentrations of individually resolved peaks of PCB isomers and congeners were summed to obtain total PCB concentrations. For the quantification of TCPMe and TCPMOH, a Hewlett-Packard 6890 series GC-MS coupled with 5973 mass selective detector was employed. Electron Impact Single Ion Monitoring mode (EI-SIM) was used for quantification of TCPMe, TCPMOH and PCB congeners. Data acquisition was

performed by a Hewlett-Packard 5973 data system, in which the cluster ions were monitored at *m/z* 311, 313, 346, 348 for TCPMe and 139, 251, 253, 362, 364 for TCPMOH. Ion m/z 346 and 362 was used for quantification of TCMPe and TCPMOH, respectively. However, due to the low levels of TCPMe and TCPMOH in human blood samples, we monitored signals of 4 ions for each compound to confirm the present of these compounds in blood samples. Recoveries of target analytes through this analytical 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, 94 ± 5.9 % for HCB. Concentrations were not corrected for recovery rates. A procedural blank was analyzed 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, while CHLs include *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane. HCHs include α, β and γ- isomers. In this study, TCPMOH was not detected in any samples, and therefore was excluded from the discussion. Concentrations of OCs were expressed as ng/g on a lipid wt 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) and Marine Mammal Health and Stranding Response Program of the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (Silver Spring, MD). Standard reference material SRM 1945 was analyzed for selected PCB congeners and persistent OCs. Data from our laboratory were in good agreement with those for reference materials. The average of percentage deviation from certified values was 13 % (range 0.5 – 20 %) for OC pesticides and 28 % (range: 1.3 – 57 %) for PCB congeners.

Statistical analysis

Mann-Whitney *U*-test was used to verify the significant differences in concentrations of OCs according to geographical locations; age and sex dependent accumulation. Spearman rank

correlation was used for testing the significant correlation between OC concentrations and age. The statistical analysis was performer by Microsoft Excel 2003 for Windows with various add-in functions for statistics.

Results and Discussion

Status of contamination and geographical variation

Concentrations of OCs in human blood from Miyako, Saku and Tottori are shown in Table 1. PCBs and DDTs were predominant contaminants and their overall mean concentrations are 360 and 300 ng/g lipid wt, respectively. HCB and TCPMe residues were the lowest; about 1 - 2 order of magnitude less than those of PCBs and DDTs. People from Saku contained the highest concentrations of DDTs and PCBs than the other two locations. An individual (sample No. 308) from Saku carried elevated amount of PCBs (990 ng/g lipid wt) and DDTs (2700 ng/g lipid wt).

One of the major objectives of this study is to understand the geographical differences in OCs accumulation in three locations in Japan, and subsequently to evaluate the possible implications of the historical usage pattern of OCs on human exposure in each location. Organochlorine concentrations of 2 groups of males and females in each location were compared using Mann - Whitney U test to verify the geographical differences (Figure 1). People from Saku contained the highest PCB concentration, followed by Tottori and Miyako with overall mean concentrations of 400, 390 and 280 ng/g lipid wt, respectively. In particular, PCB residues in males from Saku were significantly higher than those in males from Miyako ($p < 0.01$); while females from Tottori and Saku accumulated significantly greater residues than females from Miyako ($p < 0.05$) (Figure 1). Similar pattern was observed for DDTs with higher concentrations in humans from Saku than those from Miyako and Tottori, although statistical analyses did not showed any significant differences. Saku is the most extensive apple growing area in Japan and therefore, DDTs might have been used for agricultural purposes

relatively heavier than other two locations. There were no significant differences in concentrations of HCHs and HCB among three locations; however, slightly higher levels of HCHs and HCB were note in Tottori and Saku than in Miyako.

Interestingly, there were apparent geographical differences in CHL residues concentrations, showing higher levels in samples from Miyako in both male ($p < 0.05$) and female groups ($p < 0.001$) (Figure 1). CHL compounds were imported to Japan and used mainly for termite control and wood treatment. It was estimated that a cumulative amount of > 2,000 tons of CHLs was used during the period of 1955 – 1986 (Ministry of the Environment, Japan 2002) (Table 2). In Okinawa prefecture, CHLs were more extensively applied for termite control as compared to other locations in Japan. Thus, our results in human blood samples in residents from Miyako, a district in Okinawa prefecture, clearly reflect the high degree of historical usage of CHLs in this region. In addition, differences in OCs accumulation pattern among three locations also highlighted the implications of extensive usage of CHLs on human exposure to these compounds. In Saku and Tottori, concentrations of CHLs were comparable or lower than those of HCHs, while in Miyako, a specific pattern was observed. In particular, CHL residues in both males and females in Miyako residents were about 2 times greater than those of HCHs (Table 1, Figure 1). Generally, in Japanese humans, concentrations of HCHs were higher than those in CHLs, due to much larger amount of usage in the past (about 400,000 tons during 1950 – 1972, Ministry of the Environment Japan 2002) (Table 2). In our previous surveys on human adipose tissues of residents from Tokyo and nearby areas, we also found relatively higher concentrations of HCHs than those of CHLs (Minh *et al.* 2000a, 2001). Similar accumulation pattern in human blood from Sapporo and Akita, Japan was observed, showing higher accumulation of HCHs as compared to CHLs in Japanese humans (Takasuga *et al.* 2002; Hanaoka *et al.* 2002). The specific accumulation of CHLs in Miyako in the present study deserves continuous monitoring studies for CHLs in this area.

TCPMe was detected in most of the samples analyzed, with the highest concentrations of 8.1 ng/g lipid wt (Table 1). To our knowledge, this is the first report on the detection of TCPMe in human blood samples. Concentrations of TCPMe in males were similar among three locations, while results in female showed significantly higher levels in people from Miyako and Tottori (Figure 1). Our previous studies demonstrated high bioaccumulation potential and slow elimination kinetics of this compound in humans (Minh *et al.* 2000a, 2001). Data on TCPMe in human samples are rather scant. For Japanese humans, concentrations of TCPMe were in the range of 2.7 – 44 (mean: 18 ng/g lipid wt) in adipose tissue, 1.1 – 20 (mean: 7.0 ng/g lipid wt) in liver and < 5 – 62 (mean 17 ng/g lipid wt) in bile (Minh *et al.* 2001). These levels are generally higher than those in blood plasma samples examined in this study. In human breast milk, data are available only for nursing woman in Sweden and Vietnam. Mean concentration of TCPMe in Swedish human milk was 1.6 ng/g lipid wt, which was lower than in Hanoi (3.8 ng/g lipid wt), and Hochiminh (7.2 ng/g lipid wt), Vietnam (Rahman *et al.* 1993; Minh *et al.* 2004). TCPMe has been recently reported to exhibit weak estrogenic properties *in vitro* at the concentration of 3.4 ppb (Lascombe *et al.* 2000). On a wet weight basis, concentrations of TCPMe in most of the autopsied adipose tissues of the diseased patients from Tokyo and nearby areas were higher than the level represented weak estrogenic activity *in vitro* (3.4 ppb). However, residues in human blood from Miyako, Saku and Tottori examined in the present study were still orders of magnitude lower than this critical level. Overall, our result further indicates widespread occurrence of TCPMe in the terrestrial environment with little geographical variability.

The geographical variations of OC contamination in three locations in Japan indicate that PCBs, CHLs and TCPMe tend to have larger variations than other chemicals. This observation suggests the different degrees of exposure to these contaminants among localized areas in Japan. In general population with no specific exposures such as accidental, occupational exposure or special eating habit, two major factors can be considered to explain the differences

in geographical variations of OC accumulation. The first factor is related to the historical status and degree of usage. Lower PCB contamination in Miyako as compared to Saku and Tottori may be due to the less industrial activities in Miyako. In contrast, as we have discussed earlier, elevated CHL residues in humans from Miyako is the result of the extensive usage of CHLs for termite control in Okinawa prefecture. Less geographical variation of DDTs and HCHs can be explained by their widespread and extensive usage of these insecticides throughout Japan. Statistical data from the Ministry of the Environment, Japan showed that approximately 45,000 tons of DDTs and 400,000 tons of HCHs were used during the period from early 1950s to early 1970s (Ministry of the Environment, Japan 2002) (Table 2). It should also be noted that in addition to the use in agriculture, DDT was also exclusively used for epidemic prevention. In Japan, after the World War II, dust formulations of DDT were aerially applied directly on humans for prevention of an epidemic louse-born typhus. In addition, oil solutions were also sprayed on the wall of houses (Ministry of the Environment Japan 2002). As for HCHs, this insecticide was produced and used in Japan in the largest quantity among the OCs studied. Japan is among the top ten countries with highest technical HCH usage and particularly, is the second largest in HCH usage per unit area (amount of usage per arable land area, 0.08 tons/ha) in the world (Li 1999). These facts are plausible explanations for the high and widespread contamination of DDTs and HCHs in Japanese humans. On the other hand, from the physico-chemical properties point of view, we realized that highly bio-accumulative and persistent compounds such as PCBs, CHLs and TCPMe showed larger geographical differences as compared to less lipophilic and more bio-degradable chemicals such as HCHs and HCB. In the marine environment, less geographical variation of HCB was also observed in cetaceans collected from various sites along the Japanese coastal waters (Prudente *et al.* 1997; Minh *et al.* 2000b).

Local and international comparison

To understand the magnitude of contamination in human blood from Miyako, Saku and Tottori, residue levels of OCs in blood samples collected from various locations in Japan and other countries in the world were compiled (Table 3 and 4). There are a number of factors, which can influence the concentrations of OCs in human blood samples. These include the differences in the lipid contents of blood tissues (serum, plasma, and whole blood), analytical techniques, biological factors, status of specific exposure and feeding habit. Whenever possible the lipid-normalized concentrations were cited for comparison. For epidemiological studies, data for control groups were taken into consideration. In our study, volumes of blood samples were also measured and therefore, concentrations on the wet weight basis are available for comparison with the investigations, in which lipid-normalized concentrations were not reported.

Concentrations of some major PCB congeners and total PCBs in human blood samples were given in Table 3. Since the total PCB concentrations may vary greatly due to the differences in number of congeners measured and methods of quantification, data on some major recalcitrant congeners such as CB-118, CB-138, CB-153 and CB-180 were also considered for a more precise comparison. Total PCB concentrations in Saku and Tottori were higher than those in Sapporo, Japan (Takasuga *et al.* 2002). Data on PCB contamination in human blood from Japanese populations are limited, suggesting the need for further extensive monitoring studies. On a global perspective, concentrations of CB-118, 138, 153, 180 and total PCBs in Japanese subjects were comparable to or higher than those people from Canadian and Russian Arctic, Belgium and Wurzburg, Germany (Loffler *et al.* 2000; Pauwels *et al.* 2000; Voorspoels *et al.*, 2002; Walker *et al.*, 2003). The residues are, however, less than those observed in various locations in the United States in Norway, Finland, Sweden, and the Netherlands (Table 3). Congener specific analyses of PCBs in human blood in developing countries are limited. Korrick *et al.* (2001) reported total PCB residue levels (sum of 67 congeners) in maternal serum of women in Anqing, China were 0.2 $\pm$0.1 ng/ml (mean $\pm$ SD),