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化学物質リスク研究事業

内分泌かく乱物質・ダイオキシン類の小児、成人の汚染実態および暴露に関する調査研究

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内分泌かく乱物質・ダイオキシン類の小児・成人の汚染実態及び暴露に関する調査研究

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研究要旨　　ダイオキシン・PCB等の有害化学物質が、日本人の体内にどのように蓄積しているかを明らかにし、その蓄積が臓器等の機能や疾患に与える影響を評価することを目的として、病理解剖症例を用いてヒト各臓器における暴露状況を把握し、化学物質が人体にどのように分布しているかを調査した。これまでに剖検例のべ108例におけるダイオキシン等の化学物質の蓄積状況を、脂肪組織、肝、血液、胆汁、中枢神経、乳腺、腎、脾、膵、肺、副腎について測定した。その結果、日本人における暴露のバックグラウンドレベルが判明し、各臓器での加齢との相関関係や腸肝循環の実態を明らかとなった。また同一症例において血液、肝、胆汁での濃度を測定できたことから、これらの化学物質の胆汁からの排泄の存在と体内での蓄積との関係が初めて明確になった。一方、ダイオキシン類の高い蓄積を示す膵癌症例を1例見出したため、膵癌症例について検討したが、非癌症例との比較において有意な差は見出されなかった。また東京近郊在住の人における蓄積状況と比較する目的で、愛媛在住の人についてその剖検例の肝臓および脂肪組織（10症例）を測定した。その結果、両者に有意な差はなかったが、異性体別でみると1,2,3,6,7,8-H6CDDの濃度が高く、愛媛県での母乳調査と同様の傾向であった。一方、本研究で、少量のヒト組織からの抽出の簡便化・迅速化が必須であったため、新たなダイオキシン類の迅速な抽出方法を検討し、その開発に成功した。

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A.研究目的

ダイオキシン等の化学物質が、日本人にどのように蓄積しているかを明らかにし、その蓄積が臓器等の機能や疾患に与える影響を評価することを目的とする。このダイオキシン・PCB 類は、大量暴露後の追跡調査によるヒト発ガン性や動物実験等で強い毒性を有することが明らかになっている。しかし、我が国のような低濃度慢性的暴露が続いた場合のヒト生体への影響はまだ明らかにされていない。そこで、本研究では剖検例を用いて、各臓器の暴露状況を把握し、ダイオキシン・PCB 類が人体にどのように分布しているかを調査した。また近年、臭素系難燃剤を含む廃棄物の焼却に伴って、ダイオキシン類と同様な生体作用と毒性をもつ臭素系ダイオキシン類が発生していることが明らかにされ、その人体汚染が注目されている。この臭素系ダイオキシン類のうち、Polybrominated Diphenyl

Ether （PBDE）は内分泌撹乱物質としての作用の他、中枢神経系への作用、免疫系への作用なども報告されているため、本物質のヒト体内での蓄積状況も調査した。また新たな環境汚染物質として注目されている *tris* (4-chlorophenyl) methane (TCPMe)および *tris* (4-chlorophenyl) methanol (TCPMOH)と、他の有機塩素化合物（PCB、DDT、HCH、HCB、クロルデン化合物 [CHL]）についても測定した。これらの調査は、我が国における人体への化学物質の暴露量の実態を明らかにするだけでなく、世界においても貴重なヒト臓器・組織内における蓄積分布データを提供するものであり、特定の疾患・病態との関連性についての研究の基礎ともなることから、将来の有害化学物質対策を目指す厚生労働行政へ貢献しうるものと考えた。

B.研究方法

252 の剖検症例について、主要臓器（項部脂肪組織（褐色脂肪に相当）、腋窩脂肪組織、腸間膜脂肪組織、腹壁脂肪組織、下垂体、脳、肝、脾、腎、膵、胃粘膜、上行結腸粘膜、乳腺、骨髄）、血液、胆汁を採取し、臨床データ、病理解剖診断についてファイリングした。平成 17 年 3 月までに測定した臓器は、のべ 108 症例の肝、脂肪、血液、胆汁、各 28 症例腎臓、膵臓、脾臓、各 10 症例の中枢神経（大脳）、乳腺である。測定した化学物質は、ダイオキシン類、臭素系ダイオキシン（polybrominated diphenyl ether (PBDE)）、ブチル化スズ化合物（モノブチルスズ、ジブチルスズ、トリブチルスズ）、有機塩素化合物（PCB類、DDTs、ヘキサクロロヘキサン(HCH)、ヘキサクロロベンゼン(HCB)、クロルデン(CHL)、tris (4-chlorophenyl) methane(TCPMe)、tris (4-chlorophenyl) methanol(TCPMOH))、重金属（水銀、鉛、カドミウム）である。測定は、脂質抽出、クリーンアップ後、高分解能ガスクロマトグラフ、二重収束型質量分析計あるいは GC/MS で行った。

（倫理変への配慮）

剖検にあたっては、研究対象者に対する人権擁護上の配慮および研究により研究対象者が受ける利益・不利益等の説明を遺族に対して行い、インフォームドコンセントを得て、御遺族の同意の署名を剖検承諾書にいただいた。またヒトゲノム・遺伝子解析研究については、ヒトゲノム・遺伝子解析研究に関する倫理指針（平成 13 年 3 月 29 日文部科学省・厚生労働省・経済産業省告示第 1 号）を遵守した。

C.研究結果

PCB 類：剖検症例の肝 (59 検体)、腸間膜脂肪 (54 検体）および腹壁脂肪（54 検体）における mono-ortho PCB（8 種類）と di-ortho PCB（2 種類）を測定したところ、脂肪重量あたりの mono-ortho PCB 平均値は TEQ 表記で、それぞれ 9.0、19.2 および 20.6pg/g であり、肝は脂肪組織の約 1/2 であった。絶対値では、肝、腸間膜脂肪および腹壁脂肪の脂肪重量あたりの mono-ortho PCB は、478-3,366、17,357-171,919 および 20,022-186,417ppt であった。di-ortho PCB も同様に TEQ 表記で、11.36、24.79 および 20.59 であり、mono-orthoPCB と同じように肝は脂肪組織の約 1/2 であった。測定した 12 種類の PCB のそれぞれの相対比は、肝、腸間膜脂肪および腹壁脂肪いずれも同じ傾向を示した。今回、得られた mono-ortho PCB と di-ortho PCB の値は、以前、報告された血液脂肪中の測定値とほぼ同じレベルであった。また血液（120 件）及び胆汁（42 件）では、血中 Mono-ortho-PCBs 濃度は、肝臓、脂肪組織中の Mono ortho-PCBs 濃度と同様に、2,3',4,4',5 PenCB (#118) の濃度が最も高く、平均 159±156ppt であった。次に高かった 2,3,3',4,4',5-HexCB (#156) の濃度は平均 74±52ppt であった。胆汁中の Mono-ortho-PCBs 濃度も血液中と同様に、それぞれ#118 の濃度が平均 347±194ppt で、#156 の濃度は平均 171±99ppt で高かった。また胆汁中の総 Mono-ortho-

PCBs濃度は血液中の約2倍であった。

ダイオキシン類：59 検体の分析結果では血液、胆汁のダイオキシン濃度はそれぞれ 42.6±24.3 pg TEQ /gfat、43.4±30.8 pg TEQ /gfat でほぼ同様の濃度であった。肝臓中は 127.2±53.4 pg TEQ /gfat で血液、胆汁に比べ約 3 倍の濃度であった。また血液中と胆汁中の濃度がよく相関すること、肝では脂肪重量あたりの濃度が血液中、胆汁中濃度よりも高いことが明らかとなった。胆汁からの排泄量は、ダイオキシンの異性体種類により差異が認められた。 OCDD が一番高濃度であり PeCB、HxCB、 1,2,3,6,7,8HxCDD、TeCB 、 2,3,4,7,8-PeCDD 、1,2,3,4,6,7,8HpCDD、がそれに続いた。各異性体パターンは血液にほぼ似ているが HxCDF、HpCDF では血液の約 1/2 の濃度、HxCDD では血液の約 2/3 の濃度、HpCDD、OCDD で約 1/2 の濃度、TeCB では約 1/2 の濃度でその他の異性体はほぼ同等の濃度であった。肝臓では、PCDDs、コプラナーＰＣＢｓ ともに、全般的に濃度が高く、 OCDD, PeCB, HxCB、1,2,3,4,6,7,8-ＨｐＣＤＤ、1,2,3,6,7,8HxCDD、TeCB 、 2,3,4,7,8-PeCDD と続いた。1,2,3,4,6,7,8-HpCDF は胆汁の約 20 倍、血液の 10 倍の濃度があり、1,2,3,4,7,8,9-HpCDF は胆汁の 10 倍、血液の 8 倍、OCDD に関しては血液の約 5 倍、胆汁の約 9 倍の濃度であった。腎臓、脾臓、膵臓、肺では、TEQ 濃度で、それぞれ 138、113、163 及び 178 pg-TEQ/g 脂肪(平均値)の蓄積が認められ、同族体ごとの蓄積パターンは他の臓器と同様であった。Whole ベースにすると平均総ダイオキシン類濃度はそれぞれ 1.1、1.2、33 及び 1.6 pg-TEQ/g whole base であり、膵臓中の濃度が他の臓器と比較して 20～30 倍高濃度であるが、脂肪ベースで比較した場合、ほぼ同じ濃度レベルとなった。中枢神経および乳腺中のダイオキシン類は、中枢神経では肝より 1/4-10 程度の低いレベル、乳腺では脂肪組織と同レベルからやや高いレベルで蓄積していた。年齢、性別と化学物質の蓄積の相関を検討したところ、年齢の増加に伴ってダイオキシン・PCB の蓄積が増加することが明らかとなった。ダイオキシン類の蓄積増加率を概算すると PCDD が 16.3pgTEQ/gfat, PCDD が 9.0pgTEQ/gfat, Co-PCB が 15.0pg/gfat となったが、性差はなかった。臭素系ダイオキシン (polybrominated diphenyl ether (PBDE) の 25 の異性体 (BDE-#17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 119, 126, 138, 153, 154, 155, 166)) は、血液、胆汁、肝臓、脂肪組織でそれぞれ、4,087±4,428、2、953±2,916、4,001±3,191、5399±4826 pg/g 脂肪であった。測定した 25 の異性体のうち、2,2',4,4'-tetraBDE(#47)、2,2',4,4',5,5'-hexaBDE(#153) の濃度が高く全体の 70%を占めた。心血と胆汁の濃度の相関および心血と肝組織中の濃度の相関係数はそれぞれ 0.64, 0.60 であり、ダイオキシン類と同様に胆汁からの排泄のあることが示された。

有機スズ化合物、重金属：モノブチルスズ、ジブチルスズおよびトリブチルスズは、肝臓湿重量 (27 例) あたりそれぞれ 6.0～28、6.5～71、検出限界以下～3.4 ng cation/g であった。また、胆汁 (17 例) では、それぞれ、6.0～21、1.0～13、検出限界以下～3.0 ng cation/g であった。肝臓 (18 例) における、水銀は 0.08 以下-1.49mg/g 乾燥重量まで、鉛は 0.095-1.38mg/g 乾燥重量、カドミウムは 1.05-22.6mg/g 乾燥重量であった。

有機塩素化合物：脂肪組織中 (45 例) の残留パターンは、DDT＞PCB＞HCH[1]＞CHL[2]＞HCB[3]＞TCPMe[4]＞TCPMOH[5]の順であった。脂肪組織中の TCPMe と TCPMOH 濃度は、脂肪重当りでそれぞれ 2.7～44 (平均 18) ng/g と 0.28～31 (平均 12) ng/g であり、DDT 濃度よりほぼ 2 桁低値であった。肝臓の TCPMe と TCPMOH 濃度は、1.1～20 (平均 7.0)

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[1] ヘキサクロロヘキサン
[2] クロルデン
[3] ヘキサクロロベンゼン
[4] tris (4-chlorophenyl) methane
[5] tris (4-chlorophenyl) methanol

と<4.0～38（平均 19）ng/g lipid wt.であった。胆汁中のTCPMe濃度は、<5.0～62（平均17）ng/g lipid wt.であり、肝臓よりいくぶん高値を示したが、TCPMOH 濃度は検出限界以下であった。脂肪中DDT濃度は160～8100(平均2300)ng/g lipid wt.であり、分析した有機塩素化合物の中で最も高いレベルであった。脂肪組織のHCH、CHLそしてHCB 濃度は、以前の報告と同レベルであった。脂肪組織のHCH濃度は47～3200(平均680) ng/g lipid wt.であり、CHL（平均310 ng/g lipid wt.）やHCB（平均 60 ng/g lipid wt.）より高値を示した。

膵癌症例における蓄積：今回測定した症例のなかで、平均値の数倍～10倍以上の化学物質類（ダイオキシン、PCB、塩素系化合物）の蓄積した症例（膵癌症例 1 例）が見いだされた。この膵癌組織において癌遺伝子 H-ras, K-ras 変異を検索した結果、H-rasには変異は見られない一方、K-rasにおいて、コドン 12, 61 にこれまでに報告のない新たな変異を見出した。そこで、膵癌症例 7 例について、血液、肝、脂肪組織での蓄積状況を検討した。その結果、年齢・性をマッチさせた非癌患者との比較では、有意な差は見出されなかった。

東京都近辺と愛媛県在住者での蓄積の比較

東京都近辺在住者と愛媛県在住者の剖検例（それぞれ 7 症例、10 症例）において、化学物質の蓄積に差異があるか調査した。その結果、脂肪組織、肝において、その蓄積に有意な差異はなかった。以前、愛媛県での初産婦母乳において、ダイオキシン、PCB 類の濃度が有意に高かったとの報告があり、また愛媛県での近海魚の摂取が多いことから、今回の調査に至ったが、相違がないことが判明した。しかし、異性体別でみると 1,2,3,6,7,8-H6CDD の濃度が高く、愛媛県での母乳調査と同様の傾向が存在した。

ヒト臓器・組織での化学物質類の測定技術の改良

少量のヒト組織から内分泌撹乱物質を測定することを可能にするため、高速溶媒抽出器、大量注入装置、ミニチュア化カラムクリーンアップ系を用いた微量抽出分析法を開発した。同一検体 20 例で各臓器についてこれらの測定方法を比較検討したところ、これまでの通常の測定方法と比較して、同等レベルで測定可能であることが判明した。このことから、これまで保存試料が少ないため分析が困難であった試料についても、そのダイオキシン類の濃度分布を、0.5g 以下の少量の生体試料から正確に測定しうることが可能になったことを示すものである。

D.考察

同一剖検症例における血液、肝、胆汁における化学物質の濃度を測定することで、ヒトにおけるこれらの物質の代謝経路の一端が明らかになった。この結果は、経口接種される化学物質の体内での代謝・吸収や腸肝循環の動態解明への貴重なデータとなると考える。またダイオキシン類、PCB、有機塩素系化合物、有機スズ化合物が、高濃度の蓄積を認めた悪性腫瘍症例が見いだされ、新たな癌遺伝子 K-Ras の点突然変異を発見したことは、これらの化学物質が関与する悪性腫瘍の存在の可能性を十分に考慮しながら今後、検討する必要があることを認識させられた。今後とも経時的に日本人における化学物質の暴露状況をモニターする必要性があると考えられた。

E.結論

我が国における人体への化学物質の蓄積状況を明らかにし、臓器・組織内濃度分布を計測し、データベースを作製した。このデータは、将来の有害化学物質対策のための基盤となるものと考える。

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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),