Occurrence of *Tris*(4-chlorophenyl)methane, *Tris*(4-chlorophenyl)methanol and Some Other Persistent Organochlorines in Japanese Human Adipose Tissue

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Introduction

In recent years, beside numerous investigations on global pollution and toxic effects of classic man-made chemicals, the discovery of new environmental contaminants has received considerable attention. *Tris*(4-chlorophenyl)methane (TCPMe) and *tris*(4-chlorophenyl)methanol (TCPMOH) are among the most recently detected contaminants in environmental samples. Although the point sources of these compounds are unknown, their environmental contamination is widespread (*1*). Recent investigations have indicated high bioaccumulation potential of TCPMe and TCPMOH in marine food chain, comparable to that of 1,1,1-trichloro-2,2-bis(*p*-chlorphenyl)ethane (DDT), a well known insecticide (*2,3*).

Because TCPMe and TCPMOH are structurally similar to the pesticides DDT and dicofol, respectively, and the endocrine disrupting effects of these chemicals have been well documented, TCPMe and TCPMOH may pose toxic threat to humans and wildlife similar to that observed for DDT. Some toxicological studies imply that TCPMOH induces hepatic enzymes and poses anti-androgenic effects (*4,5*). An *in vivo* test in rat revealed that TCPMOH caused an increased 4-hydroxylation of estradiol activity in male (*6*). More recently, TCPMOH has been indicated to have strong binding affinity to androgen receptor, however, the compound did not show any estrogenic or antiestrogenic activity based on MCF-7 cell proliferation assay (*7*).

While few investigations have been conducted in aquatic mammals, very little is known about the exposure of terrestrial animals including humans to TCPMe and TCPMOH. To our knowledge, only one investigation has reported the occurence of TCPMe and TCPMOH in human milk from Sweden and Italy (*8*). However, in that study, due to the lack of analytical standards, the concentrations of TCPMe and TCPMOH were estimated based on the chromatographic response of 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC No. 169) as an internal standard and therefore, the results might be less accurate. For this reason, actual exposure of humans to TCPMe and TCPMOH is still unknown due to the lack of residue data. Determination of TCPMe and TCPMOH residues in human samples may lead to a further understanding of accumulation features as well as provide a basis for risk assessment to human health posed by these compounds.

Abstract

Tris(4-chlorophenyl)methane (TCPMe) and *tris*(4-chlorophenyl)methanol (TCPMOH) are among the most recently identified environmental contaminants. Despite their widespread contamination in the marine environment, human exposure to these compounds remains relatively unknown. The present study determined the concentrations of TCPMe, TCPMOH and other persistent organochlorines such as polychlorinated biphenyls (PCBs), DDT and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB) and chlordane compounds (CHLs) in human adipose tissue from Japan. TCPMe and TCPMOH were detected in all adipose samples analyzed, with the concentrations ranged from 2.5 - 21 and 1.1 - 18 ng/g lipid wt, respectively. Concentrations of TCPMe and TCPMOH in humans were less than those reported in marine mammals, suggesting the possibility of metabolism and elimination of these compounds by humans. Significant correlation between TCPMe and TCPMOH with DDTs concentrations in human adipose tissues suggested that exposure to DDT is the source of TCPMe and TCPMOH in humans. The age and sex dependent accumulation of TCPMe and TCPMOH as well as other organochlorines was less pronounced. Results for other organochlorines indicated that recent contamination status of PCBs in human samples from Japan was more serious than that in developing countries, whereas DDT contamination is lower. Greater concentrations of CHLs in human adipose tissue from Japan than those from other countries suggest the necessity of continuous monitoring of CHLs in humans in Japan. To our knowledge, this is the first study on the accumulation of TCPMe and TCPMOH in human adipose tissue.

In the present study, we report for the first time the quantitative data of TCPMe and TCPMOH residues in Japanese human adipose tissue. Bioaccumulation features of these compounds in humans were discussed in comparison with other classic persistent organochlorines (OCs) such as DDT and its metabolites (DDTs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB) and chlordane compounds (CHLs). Existing data from our recent investigations on TCPMe and TCPMOH residues in fishes and marine mammals collected from Japanese coastal waters were compiled and interpreted to understand the bioaccumulation potential and metabolic capacity in marine and terrestrial animals. In addition, accumulation of other classic OCs was also examined and an attempt is made to compare OC residues of Japanese human adipose tissue with those from other countries in the world to understand the status of contamination.

Materials and Methods

Samples. Human adipose tissue samples were obtained from patients autopsied in Keio University Hospital (Tokyo, Japan) during the period of May - August, 1998. The informed consents were taken from bereaved family for all the samples analyzed in this study. Adipose tissues were wrapped in aluminum foil and stored at -80°C until analysis. The details of cases were shown in Table 1.

Chemical analysis. Chemical analyses of TCPMe and TCPMOH as well as other OCs were followed the method previously described (*3*). Briefly, about 2 g of adipose tissue samples were homogenized with anhydrous Na_2SO_4 and extracted using a Soxhlet apparatus with a mixture of hexane and diethyl ether. Fat content was gravimetrically determined from an aliquot of the extract. The extract was then added into a dry Florisil column to remove fat. Organochlorines were eluted with 150 ml of 20 % water in acetonitrile to a separatory funnel containing hexane and water. After partitioning, the hexane layer was concentrated and then passed through a 8 g of activated Florisil column for fractionation. The first fraction eluted with hexane contained PCBs, *p,p'*-DDE, *trans*-nonachlor and HCB, while 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.

Organochlorines (except TCPMe and TCPMOH) were quantified by a GC-ECD (Hewlett Packard 5890 Series II) equipped with a moving needle-type injection port. The GC column employed was DB-1 (J & W Scientific Inc., USA) fused silica capillary (0.25 mm x 30 m) 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. Organochlorine concentrations were calculated 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 PCBs isomers and congeners were summed to obtain total PCB concentrations. For the quantification of TCPMe and TCPMOH, a GC-MSD (Hewlett-Packard 5890 series II GC coupled with 5972 mass selective detector) was employed. Data acquisition was performed by a Hewlett-Packard 5972C 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. 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.

Results and Discussion

Bioaccumulation characteristics of TCPMe and TCPMOH in humans. Concentrations of TCPMe, TCPMOH and other OCs are given in Table 2. Residue pattern was in the order of PCBs > DDTs > HCHs > CHLs > HCB > TCPMe > TCPMOH. This observation was somewhat similar to that revealed in our recent investigations on the

occurrence of TCPMe and TCPMOH in marine mammals (*3,9*). TCPMe and TCPMOH have so far been considered as impurities in technical DDT and dicofol (*10,11*) as well as high polymers and agrochemical products (*1*). The environmental exposure as impurities from other materials may explain the relatively lower concentrations of TCPMe and TCPMOH as compared to other persistent OCs. Concentrations of TCPMe and TCPMOH ranged between 2.5 - 21 and 1.1 - 18 (ng/g lipid wt), respectively, which were about 2 orders of magnitude lower than those of DDTs (ranged between 95 - 1200 ng/g lipid wt). The rather high concentrations of TCPMe and TCPMOH were found in a 87 year-old male patient (Sample No. 8) and a 53 year-old female patient (Sample No. 11) (Table 2). In these male and female patients, relatively higher levels of DDTs and PCBs were also recorded. TCPMe and TCPMOH concentrations found in Japanese human adipose tissue were about 6 and 3 times greater than those estimated in human milk from Sweden and Italy, respectively (*8*). However, residue levels in human samples were much more lower than those reported in various marine mammals and sea birds from several locations such as Canada, United States, Japan, Hong Kong and European regions (*1-3,9,12*).

In order to understand the metabolic capacity to TCPMe and TCPMOH in humans, data on these compounds in humans were compared with those in fishes and marine mammals (seals and cetaceans) which were collected from Japanese coastal waters. As shown in Fig.1, TCPMe concentrations in human adipose tissues were higher than those reported in fishes and comparable to those in larga seal, harbour porpoise, Dall's porpoise and striped dolphin, but significantly lower than those in Fraser's dolphin. However, TCPMOH residue levels in humans were about 10 times lower than those in most marine mammals examined. This result implies that contamination and bioaccumulation of TCPMe and TCPMOH has extended over a wide range of higher trophic animals not only in the marine ecosystem but also in the terrestrial environment. In general, residues in humans were comparable or apparently lower than those in marine mammals. This may be due to the differences in the capacity to metabolize xenobiotics between humans and marine mammals. Our earlier studies have pointed out that terrestrial mammals have higher drug-metabolizing enzyme activities as compared to marine mammals (*14-16*). Moreover, apparently lower concentrations of toxic

chemicals such as polychlorinated biphenyls including coplanar congeners were found in terrestrial mammals including humans than in marine ones (*15*), which may suggest higher ability to degrade toxic contaminants in humans.

In this study, there was no age and sex dependence accumulation of TCPMe, TCPMOH as well as other organochlorines (Table 2). We could not find any significant trend of organochlorine concentrations with age. This result was different to that reported in human adipose tissues from Spain (*17*), the Netherlands (*18*), Italy (*19*) and Korea (*20*), which showed increased OC concentrations with age. The less pronounced age and sex dependent accumulation found in this study might be due to small number of samples analyzed in this study, which make it difficult to discuss about the age trend in humans conclusively.

Our results in occurrence of TCPMe and TCPMOH in human adipose tissues suggest possible widespread contamination by these compounds in terrestrial environment. To date, the point sources of TCPMe and TCPMOH were still unknown. Jarman et al. (*1*) suggested that these compounds may be derived from synthetic high polymers and acrylic fibers. On the other hand, TCPMe and TCPMOH were also suggested as impurities in technical DDT and dicofol, respectively (*10,11*). However, no investigation has reported on the residues of TCPMe and/or TCPMOH in synthetic polymers and agrochemicals.

The only available evidence of the source of these compounds was technical DDT, in which TCPMe was detected at trace levels (*10*). In the present study, the relationship of concentrations of TCPMe/TCPMOH and DDTs was examined to understand the possible source of these compounds detected in human samples. Significant correlation was found between TCPMe/TCPMOH and DDTs concentrations (Fig. 2). Significant correlations were also observed in other biological samples previously reported, which includes harbour seals from Pudget Sound (*21*), eggs of birds and marine mammals from the Canadian Arctic and the United States (*1*), Caspian seals from Russia (*3*), coastal cetaceans species from Hong Kong (*9*), various fish species, harbour porpoises and white-tailed sea eagles from the Baltic sea (*2*). Considering such links between environmental occurrence of TCPMe, TCPMOH and DDTs in various marine and terrestrial biota, it seems likely that technical DDT may be a plausible source for the presence of TCPMe and TCPMOH in humans.

The toxicological impacts of TCPMe and TCPMOH residues on humans are currently unknown. Proliferation of MFM-223 human breast cancer cell was induced following the exposure to TCPMOH at the concentration of 5 μmol/L (about 1.8 ppm) *in vitro* (*5*). Concentrations of TCPMe and TCPMOH measured in fat rich tissue such as human adipose and milk were, however, about 2 orders of magnitude lower than those represented anti-androgenic effect. A recent study on the possible endocrine disrupting effect of TCPMOH has revealed that this compound showed high affinity for androgen receptor binding, comparable to that of *p,p'*-DDE, a major breakdown product of insecticide DDT (*7*). Considering these toxicological observations, high bioaccumulation potential of TCPMe and TCPMOH in food chain and widespread contamination in marine environment as well as their occurrence in human samples, further studies are necessary to obtain a better understanding of possible risk for wildlife and human health.

Contamination by other persistent organochlorines. PCBs are the most abundant contaminants in human adipose tissues from Japan. Concentrations of PCBs ranged from 560 to 3200 ng/g lipid wt (mean : 1700 ng/g lipid wt) (Table 2). As mentioned earlier, the sex and age dependent accumulation was less pronounced in these samples. The highest concentration of PCBs was found in an 73-year old male patient, who suffered from lung cancer. In general, mean concentrations of PCBs in males (1900 ng/g lipid wt) were higher than those in females (1400 ng/g lipid wt), however, no significant difference was noticed. Our earlier survey on temporal trend of organochlorines in human adipose tissue from Japan indicated that the decline of PCBs during the period of 1975 - 1985 was extremely slow (*22*). Considering the data of recent human exposure to PCBs in the present study, PCB concentrations were comparable to those reported in 1985. This fact implies that the exposure to PCBs in humans from Japan is still prevailing.

DDT levels found in human adipose tissue samples were in the range of 95 - 1200 ng/g lipid wt (mean : 780 ng/g lipid wt). *p,p'*-DDE was the most predominant metabolite (accounting for 96 % of the total DDTs), indicative of high capacity of humans to metabolize DDT compounds. Similar to PCBs, concentrations in males were higher than those in females, but no age trend was observed. Our data provide one of the most recent

contamination status of DDTs in humans adipose from Japan, and the results indicate that DDT residues have declined substantially as compared with those reported in 1985 (22). However, it should be noted that humans have a long life span, leading to a long-term accumulation of persistent OCs with high lipophilicity and less biodegradability like PCBs and DDTs.

Concentrations of HCHs detected in human adipose tissues ranged from 60 to 600 ng/g lipid wt, ranked next to DDTs. β-HCH was the most abundant isomer, accounting for 99 % of total HCHs. Chlordane compounds were detected at relatively high concentrations, ranging from 96 to 710 ng/g lipid wt. The composition of chlordane compounds was in the order of *trans*-nonachlor (64 %) > oxychlordane (23 %) > *cis*-nonachlor (11 %) > *trans*-chlordane (1.4 %) > *cis*-chlordane (0.6 %). HCB was recorded at the lowest levels among classic OCs, ranging from 30 - 60 ng/g lipid wt.

In order to understand the extent of recent OC contamination in human adipose tissue from Japan, organochlorine residues reported for several countries in the world were compared. It is, however, noted that the cited data were not fully representative of a nation-wide contamination status (Table 3). In general, PCB concentrations in Japanese human adipose tissue were comparable to those reported in developed nations in North America and somewhat lower than those from the Netherlands and Spain. However, these levels were significantly greater than those found in some Asian countries such as South Vietnam and Korea. This trend implies that the extent of PCB contamination in humans was more serious in developed nations, where the production and usage of PCBs for industrial purposes are deemed heavier than those in developing countries. However, for DDT compounds, recent residue levels in Japan were apparently lower than those reported in other countries, particularly in developing countries such as Vietnam, Turkey, Jordan, Iran, Poland and Mexico. The use of DDT has been banned in almost developed nations up to the early 1970s, whereas DDT was still used for vector control in some tropical developing countries (*36*). HCH concentrations were in the range of those reported in the Netherlands, Poland, but lower than those in Spain and the United States. HCHs were banned in Japan in 1971 (*22*). HCB concentrations in human from Japan were lower than in many other countries. It