Table 6. Correlations of persistent organochlorine concentrations with age in human blood from Miyako, Saku and Tottori, and in human adipose tissues from Tokyo and nearby areas, Japan[a].

Location, type of human sample	PCBs	DDTs	HCHs	CHLs	HCB	TCPMe
Miyako, human blood						
Spearman rank correlation coefficient, *r*	0.63	0.60	0.39	0.37	-0.06	0.68
Two-tailed *p* value	0.0011*	0.0026*	0.0064	0.081	0.79	0.0024*
Saku, human blood						
Spearman rank correlation coefficient, *r*	0.1	0.01	0.22	0.32	0.03	0.35
Two-tailed *p* value	0.58	0.97	0.22	0.069	0.86	0.05*
Tottori, human blood						
Spearman rank correlation coefficient, *r*	0.41	0.41	0.43	0.23	0.28	0.52
Two-tailed *p* value	0.057	0.056	0.047*	0.30	0.21	0.013*
Tokyo and nearby areas, human adipose tissue						
Spearman rank correlation coefficient, *r*	0.59	0.24	0.64	0.38	0.61	0.65
Two-tailed *p* value	0.0063*	0.31	0.0022*	0.098	0.0046*	0.0019*

[a]Spearman rank correlation was used for statistical analysis.

*Two-tailed p values < 0.05 is considered to be significant good correlation.

Figure Caption

Figure 1. Geographical differences in organochlorine concentrations in human blood from Miyako, Saku and Tottori, Japan. Mann-Whitney's *U* test was used to verify significant differences of OC concentrations among locations. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

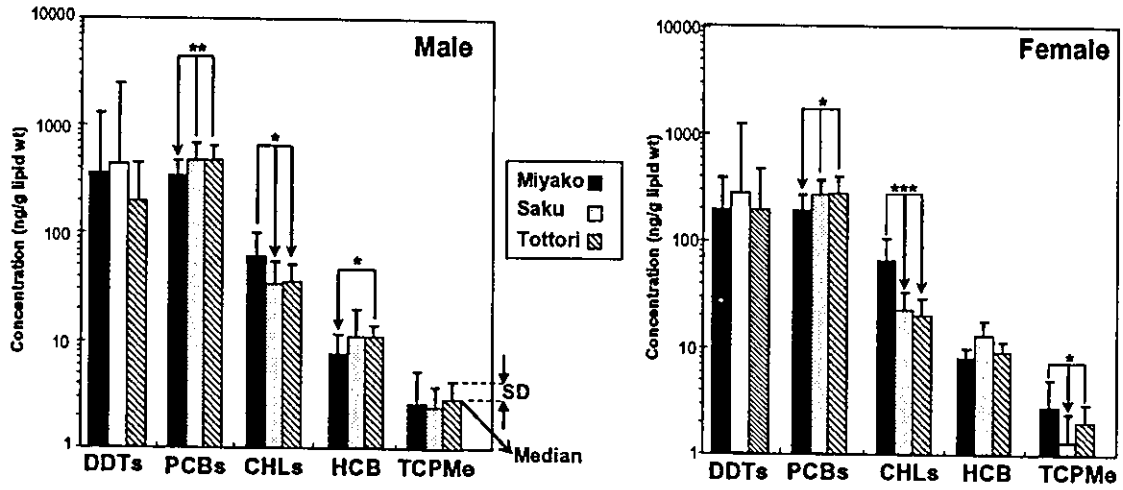
Figure 2. Residue concentrations of persistent organochlorines in human blood and their dietary intakes via foodstuffs in different countries in the world. Data for dietary intakes of Canadians were cited from Van Oostdam *et al.* (1999); Americans from ATSDR (1994, 2000, 2002 and 2003), Chinese from Zhao *et al.* (2002) [for DDTs]. Daily intakes of PCBs and CHLs for Chinese were estimated based on the data of fish and seafood consumption reported by FAO (FAOSTAT Data 2004), and the residue concentrations in fish and mussels reported by Monirith *et al.* (2000, 2003). Intakes for other countries were cited from Kannan *et al.* (1997). See text for further details.

Figure 3. Accumulation pattern and residue concentrations of chlordane compounds in Japanese human blood. Abbreviation: CHL, chlordane; oxyCA, oxychlordane; t-CA, *trans*-chlordane; c-CA, *cis*-chlordane; t-nona: *trans*-nonachlor and c-nona, *cis*-nonachlor. Data were from the following source: Miyako, Saku and Tottori residents, present study; Okinawa residents in 1986-88 survey, Sasaki *et al.* (1991) [For this study, data are available for only three compounds, oxychlordane, *trans*- and *cis*-nonachlor]; pest control operator in Tokushima and controls from Okinawa, Wariishi and Nishiyama (1989); people from Tokushima living in houses with and without chlordane treatment for termite control, Wariishi *et al.* (1986). See text for further details.

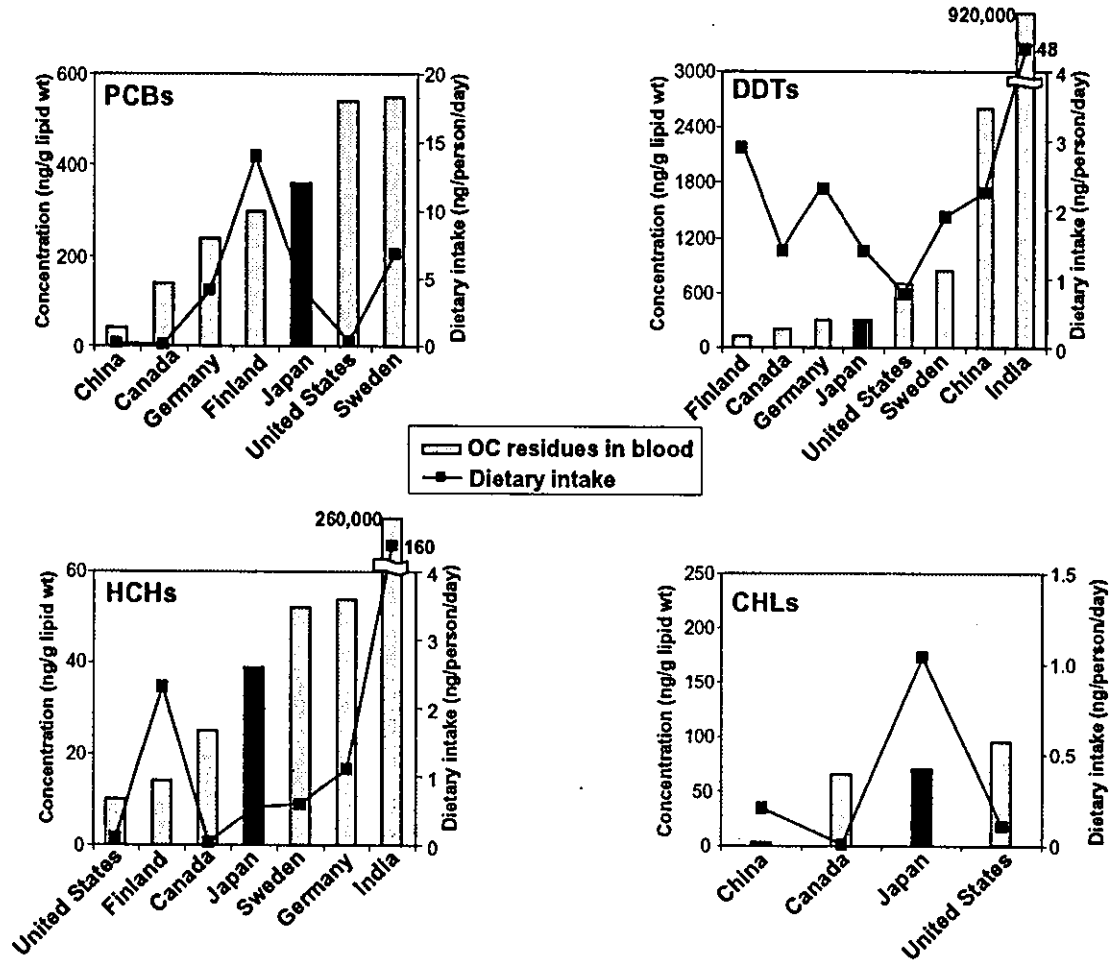
Figure 4. Isomer-specific pattern of PCBs in human blood from Saku, Japan. Vertical bars represent the relative concentrations of CB_i to CB-153, which was treated as 1.0.

Figure 5. Age and sex dependent accumulation of PCBs, DDTs and TCPMe in healthy people from Miyako, Saku and Tottori and in diseased persons from Tokyo and nearby areas. (A) Blood of healthy persons from Miyako, Saku and Tottori. (B) Autopsied adipose tissues of patients died from disease in Tokyo and nearby areas. Data for diseased persons were cited from Minh *et al.* (2001).

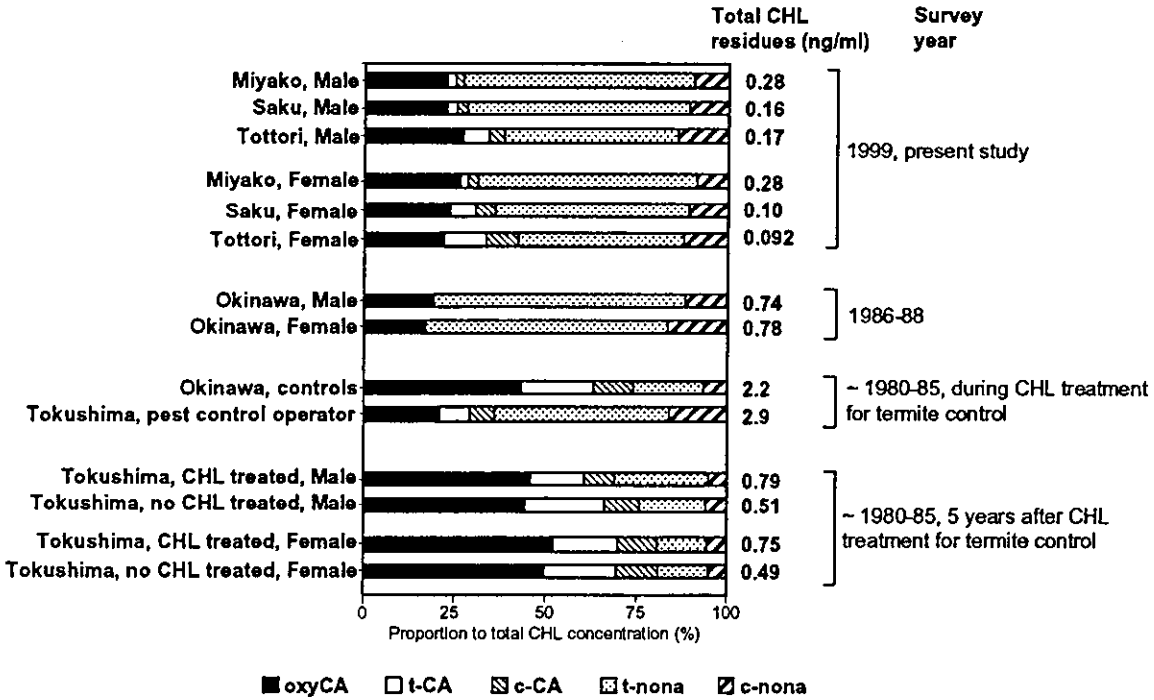
Figure 6. Age and sex dependent accumulation of CHLs, HCHs and HCB in healthy people from Miyako, Saku and Tottori and in diseased persons from Tokyo and nearby areas. (A) Blood of healthy persons from Miyako, Saku and Tottori. (B) Autopsied adipose tissues of patients died from disease in Tokyo and nearby areas. Data for diseased persons were cited from Minh *et al.* (2001).



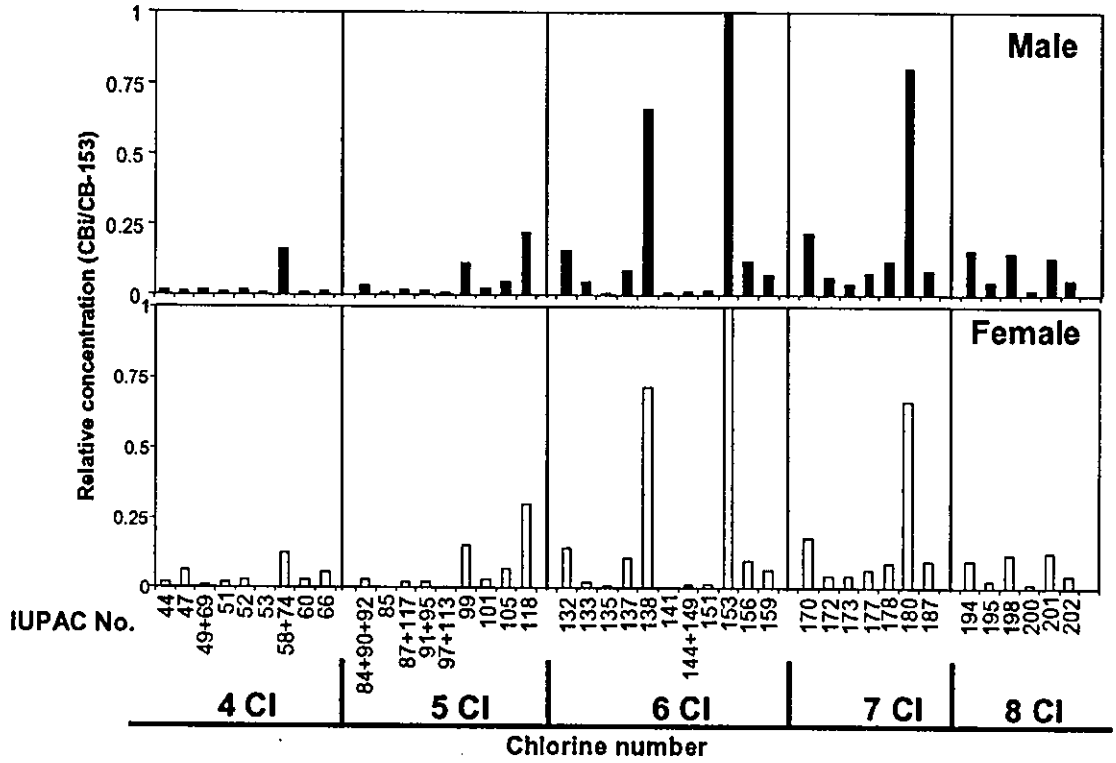
Minh *et al.* Figure 1



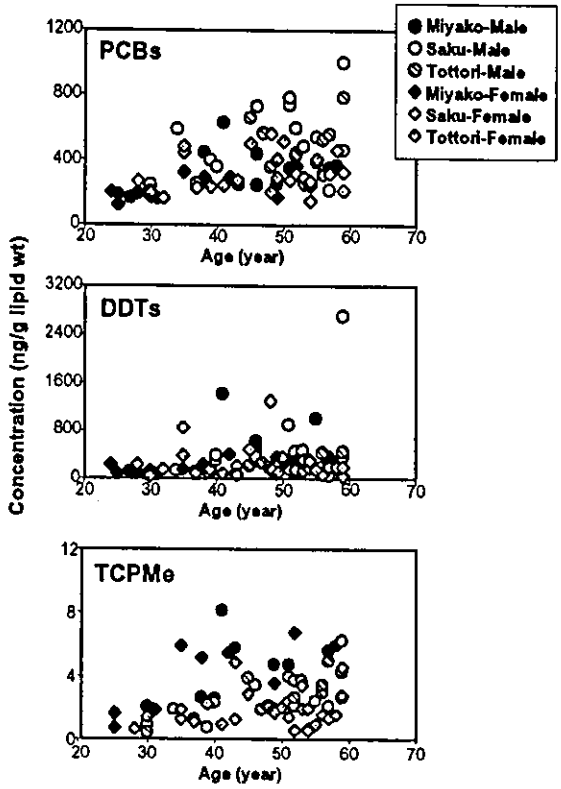
Minh *et al.* Figure 2



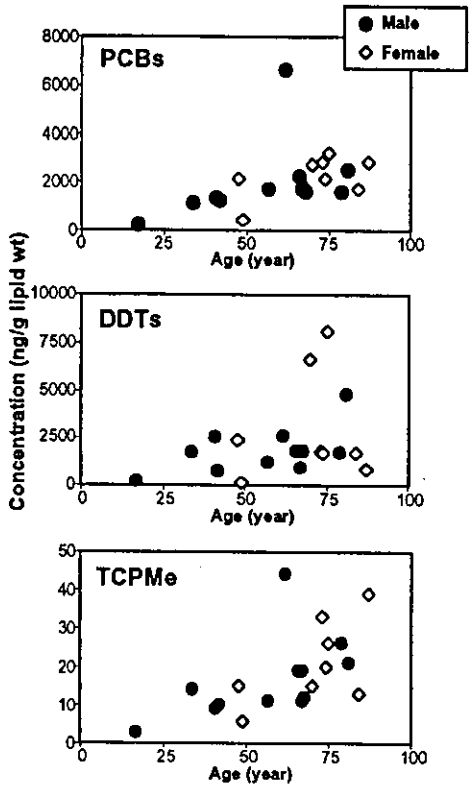
Minh *et al.* Figure 3



Minh *et al.*, Figure 4

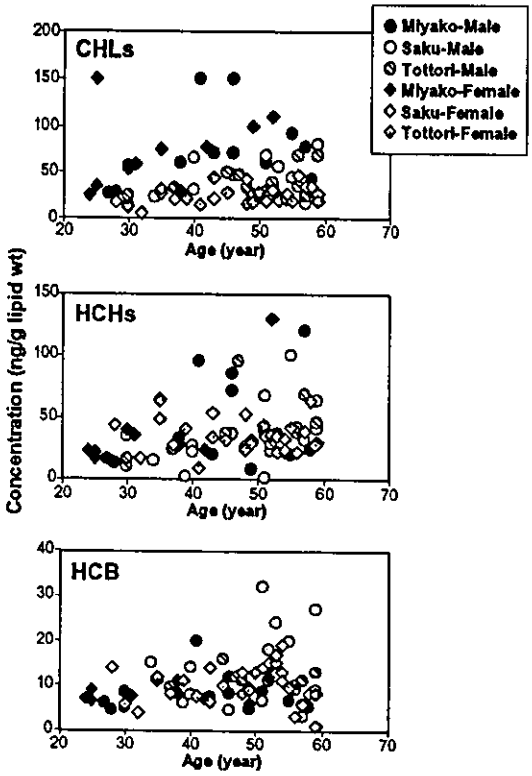


A: blood of healthy persons from Miyako, Saku and Tottori

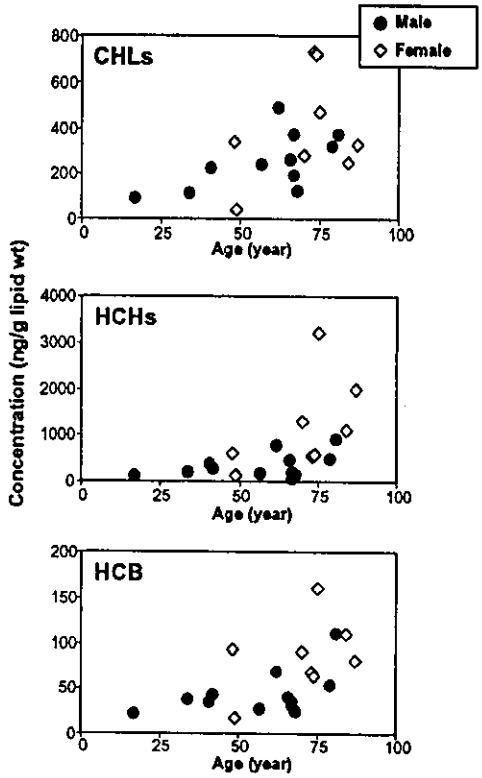


B: adipose tissues of diseased persons from Tokyo and nearby areas

Minh *et al.* Figure 5



A: blood of healthy persons from Miyako, Saku and Tottori



B: adipose tissues of diseased persons from Tokyo and nearby areas

Minh *et al.* Figure 6

Chemosphere (submitted)

Concentration and distribution of dioxins and related compounds in human tissues

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Abstract

Polychlorinated dibenzo-p-dioxin, polychlorinated dibenzofuran and dioxin-like polychlorinated biphenyl concentrations in human blood, lung, liver, bile, pancreas, spleen, kidney and mesentery fat were determined to assess the concentration and distribution of these chemicals in human tissues from 20 donors. The mean TEQ concentrations in the blood, lung, liver, bile, spleen, pancreas, kidney and mesentery fat were 119, 178, 228, 50, 113, 163, 138 and 139 pg-TEQ/g lipid, respectively. Parallel levels were seen in the blood, spleen, kidney and mesentery fat; in the lungs and pancreas, the levels were somewhat higher. Among the organ tissues samples, the highest concentration was observed in the liver and the lowest in the bile. Mean total-TEQ concentration of the liver was about 4.5 times higher than that of bile. Positive correlations were observed among the concentrations of dioxins in various tissues. However, the concentrations in bile were not correlated with any tissues. It is suggested that the distribution behavior of dioxin-like congeners in human tissues varies among tissues and the kinds of congeners ingested. To evaluate the relationship between the accumulation levels of dioxins and their pathophysiological significance or risk, data must be accumulated from a more extensive group of human samples.

Author Keywords: Polychlorinated dibenzo-p-dioxins; Polychlorinated dibenzofurans; Coplanar polychlorinated biphenyls; Human tissues; Distribution; Tissue concentration

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1. Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-ortho coplanar polychlorinated biphenyls (Non-Co-PCBs) and mono-ortho coplanar polychlorinated biphenyls (Mono-Co-PCBs) accumulate in the human body due to their highly lipophilic properties. It is well known that the main intake route of dioxins is from food. A recent Japanese survey showed that the food intake of dioxins were from 0.96-3.22 pg TEQ/kg b.w./day (Tsutsumi et al., 2001; Hori et al., 2001;Toyoda et al., 2002).

In recent years, there has been some concern about the potential health effects of dioxins and related chemicals for accidental exposure i.e. Yusho and Yu-cheng rice-oil poisonings as well as the general population of humans (Kuratsune, 2001; Kitamura et al., 2000). Although an enormous amount of data exists on this subject, most of it is from breast milk and blood, due to the ease of collection (Matsueda et al., 1993;Kang et al.1997); information concerning the concentrations and distribution in various human tissues hardly exists. Therefore, new data concerning various human tissues is required to evaluate the pathophysiological significance of dioxins and related compounds in humans.

We previously determined dioxin-like chemicals in various human tissue levels, including the blood, liver, kidney, fat, muscle and lungs (Iida at al., 1999a). We also reported on the concentration levels in the human liver and adipose tissues from 28 donors (Takenaka at al., 2002).

In this study, we attempted to determined the concentrations of dioxin-like isomers in 8 tissues, including the blood, lungs, liver, bile, spleen, pancreas, kidney and mesentery fat from 20 donors, to estimate the concentration levels and distribution behavior of dioxins and related compounds in various human organ tissues.

2. Materials and Methods

2.1. Human tissue samples.

The human tissue samples were collected from twenty patients who had died at the School of Medicine, Keio University, from 1999-2001. The families of the donors gave their informed consent. The tissues collected were blood, lungs, liver, bile, spleen, pancreas, kidney and mesentery fat. Table 1 shows details of the sample characteristics. The samples were stored at -20 degrees for later analysis. The donor ages ranged from 27 to 90 years; the average age was 65 years.

The human tissue samples including the blood, liver, bile and mesentery fat were analyzed using the method reported by Takenaka et al (2002). In our study series, we improved the analytical method to apply for more small amounts of human tissue samples (Iida et al. 2003; Todaka et al. 2002). Combination of accelerated solvent extraction (ASE), mini-column chromatography and solvent-cut large volume (SCLV) injection system were sufficient for the analysis of trace levels of dioxins in human tissue, so we chosen the method in this study, described bellow.

2.2. Chemicals and apparatus

Mixed standard solution as GC/MS standards for checking the relative response factor were purchased from Wellington Laboratories, Ont., Canada. 13C-labeled-PCDDs, 13C-labeled-PCDFs, and 13C-labeled-PCBs as internal standards were also purchased from Wellington Laboratories, Ont., Canada.

Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgen, UK) was used for packing material in the ASE extraction of human tissue samples.

An active carbon column was prepared as follows: active carbon was purchased from Nacalai Tesque, Kyoto, Japan, refluxed three times with toluene for 1h and dried in vacuo, and then 500 mg of the active carbon was mixed with 500 g of anhydrous sodium sulfate (Wako Pure Chemicals, Tokyo, Japan).

A silver nitrate/silica gel column was purchased from Wako Pure Chemicals, Tokyo, Japan. All other chemicals used were of the analytical grade of dioxin and PCB commercially available.

HRGC/HRMS (Autospec Ultima E, MicroMass Ltd., Manchester, UK) equipped with an SCLV (SGE International, Victoria, Australia) injection system was used for the analysis of dioxins.

A BPX-5 fused silica pre-capillary column (0.25 mm i.d. X 6 m, 0.25 _m film thickness) was used for the solvent-cut, and for the analytical column a BPX-dioxin fused silica capillary column was used (0.15 mm i.d. x 30 m), (SGE International, Victoria, Australia). HT-8 PCB column (0.25 mm i.d. X 60 m, 0.25 _m film thickness, SGE International, Victoria, Australia) was sued for the analysis of Mono-Co-PCBs

An accelerated solvent extractor (ASE-200, Dionex, Sunnyvale, CA), was used for the extraction of the human tissue lipid.

2.3. Sample preparation

Each sample was extracted by an ASE-200, weighed to 5 g accuracy, and mixed with 4 g Isolute. After the mixed sample was loaded into the extraction cell, 13C-labeled-PCDDs, 13C-labeled-PCDFs, and 13C-labeled-PCBs, as internal standards, were added. Acetone and n-hexane (1:4, v/v) were used as extraction solvents. The lipid obtained was dissolved in n-hexane and treated with concentrated sulfuric acid. The separated hexane layer was applied to a silver nitrate/silica gel column (0.5 g) and eluted with 15 ml of n-hexane. The eluted solution was loaded into an active carbon column (0.5 g) after being evaporated to 1 ml and separated into two fractions. The first fraction, containing Mono-Co-PCBs, was eluted with 10 ml of 10% (v/v) dichloromethane /n-hexane.

PCDDs, PCDFs, and Non -Co-PCBs were eluted with 25 ml of toluene as the second fraction. The method employed here requires only a reduced amount of human tissues collected from patients compared with the conventional method. The chromatographic column packing (silver nitrate silica gel, active carbon column, and anhydrous sodium sulfate) used in this experiment was washed in order to reduce blank materials by ASE-200 under the same conditions as the lipid extraction with n-hexane or toluene.

2.4. GC/MS analysis

Concentrations of the PCDDs, PCDFs and Non-Co-PCBs were measured using HRGC/HRMS equipped with an SCLV injection system. The setting conditions in detail were referred to our previous reports (Iida et al., 2003; Todaka et al., 2003). The Mono-Co-PCBs was measured by HRGC/HRMS at 10000 resolution with HT-8 PCB column.

3. Results and Discussion

3.1. Concentration of dioxins in various tissues

There is little information on the concentration and distribution of dioxins and related compounds in various human tissues for the general population (Patterson et al.1994). Schecter et al. (1994) reported the levels of these chemicals in human samples from the general population. Most of the samples were blood or adipose tissues, because of the relative ease of collecting these specimens. To estimate the human risk of these toxic chemicals, it is necessary to have extensive experimental data on the distribution of these compounds in various human tissues (Maruyama et al., 2003). We have been studying the concentration of dioxins and dioxin-like PCBs in various human samples from the general population and from exposed persons, including Yusho patients (Iida et al., 1999b).

In this study, we measured dioxins and dioxin-like PCBs in the blood, lungs, liver, bile, spleen, pancreas, kidney and mesentery fat from 20 donors. Table 2 shows the mean concentrations of toxic congeners which have TEF among the PCDDs, PCDFs, Non-Co-PCBs and Mono-Co- PCBs in eight tissues. The congener patterns of PCDDs, PCDFs and Mono-Co-PCBs in the 8 human tissues are summarized in Figure 1-3. Fig. 4 shows the distribution of concentrations of TEQ in 8 different tissues.

The mean TEQ concentrations in the blood, lung, liver, bile, spleen, pancreas, kidney and mesentery fat were 119, 178, 228, 50, 113, 163, 138 and 139 pg-TEQ/g lipid, respectively. Parallel levels were seen in the blood, spleen, kidney and mesentery fat; in the lungs and pancreas, the levels were somewhat higher. Among the organ tissues samples, the highest concentration was observed in the liver and the lowest in the bile. Mean total-TEQ concentration of the liver was about 4.5 times higher than that of bile.

We have previously reported the concentrations of PCDDs/PCDFs in Japanese human tissues (Iida et al., 1999a). The level in the mesentery fat was 76pg TEQ/g on a lipid basis, which was half that of this study. A recent report on the level of dioxin-like compounds in Japanese human adipose tissues was 11.9±7.4 pg-TEQ/g fat wt (Choi et.al 2002). In this study, the mean level in mesentery fat was 139 pg-TEQ/g fat wt, which was 12 times higher than that of Choi's report. This difference may be due to the differences in the sample group examined, as well as the different sample size and age distribution from the Choi's study and the current one.

The prevalent congener among the PCDDs was OCDD, ranging from 923 to 6837 pg/g lipid; in particular, OCDD was found in considerably higher levels in the liver than in other tissues (Figure 1). Thoma et al.(1999) reported that the tendency for the

concentration ratio in the liver and adipose tissue increased with the higher chlorinated PCDD. However, parallel levels were seen in the blood, spleen, kidney and mesentery fat and the lungs and pancreas for tetra- to hexa- chlorinated dioxins, while bile was at a significantly lower level.

As shown in Figure 2, among PCDFs, 2,3,4,7,8-PeCDF was prevalent, except in the liver sample. 2,3,4,7,8-PeCDF was found in considerably higher levels in the liver than in other tissues.

In the mesentery fat sample, the mean TEQ concentration was 108 pg-TEQ/g lipid (except for mono-ortho-Co-PCBs). This value was 1.6 times higher than that of our previous data (Takenaka et al. 2002) of 66 pg/g lipid for persons of a mean age of 63 years old. In this survey, extremely high levels of dioxin congener were detected from patient number 10 (see Table 1), who was 90 years old. Age-related increases in dioxin concentrations in human samples have been reported (Wittsiepea et al., 2000; Hirakawa et al., 1994); however, whether our observations have a pathophysiological significance or show the effects of age cannot be determined at present.

The prevalent congener in human tissues among the Mono-Co-PCBs was PCB118, which is generally detected at a higher concentration in PCB products and environmental samples, including food. Parallel levels were seen in the blood, lung, liver, pancreas and mesentery fat; however, the levels in the bile, spleen and kidney were somewhat lower. This tendency can be seen for most congeners of Mono-Co-PCBs and Non-Co-PCBs.

3.2 Contribution of each isomer to the total TEQ

Figure 5 shows the toxic contribution of each congener for 8 tissues to the total TEQ.

The average toxic contribution of PCDDs, PCDFs, non- ortho-Co-PCBs and mono-ortho-Co-PCBs in tissue samples was 32±6%, 25 ±7 %, 24±6% and 19 ± 4% to the total TEQ value, respectively. In the liver and spleen samples, the contribution rate of the PCDFs was higher (30%) than in other tissues (20%). The contribution rate varied slightly among tissues, with the rate of non-ortho-Co-PCB in the spleen falling as low as 13%.

3.3. Correlations of concentrations of dioxins in various tissues

We estimated the concentrations of the correlations of toxic congeners among the 8

tissues determined in this study using Spearman's correlation analyses. The data on TEQ are summarized in Table 3. The TEQ concentration among the tissues correlated well with those in each tissues, including the blood, lung, liver, spleen, pancreas, kidney, and mesentery fat ($p<0.05$). However, the TEQ concentrations in bile were not correlated with any tissues.

For the individual congeners, a positive correlation among almost all the congeners was observed in tissues; however, the levels of highly chlorinated PCDDs and PCDFs such as HpCDDs/DFs and OCDD were not correlated. Figure 6 shows a regression curves for the TEQ concentration in the blood and other tissues. From these results, we concluded that the congener levels in the lung, lung, liver, pancreas, spleen and mesentery fat can be predicted from the blood levels.

The dioxin congeners showing significant correlations among the concentrations in various human tissues may indicate similar behavior in the human body. However, bile did not show a significant correlation to other tissues in almost all of the congeners, indicating that it may behave differently in humans.

To evaluate the relationship between the accumulation levels of dioxins and their pathophysiological significance or risk, data must be accumulated from a more extensive group of human samples.

Acknowledgements

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Table and Figures

Table 1

Detalils of samples un the present study

No.	Age	Sex	Case of death
1	72	Male	Lung cancer
2	72	Male	Pneumonia, marrow fibroma
3	65	Male	Liver cancer
4	68	Female	Chest aortic aneurysm burst
5	56	Male	Malignant lymphoma
6	74	Male	Hodgkin disease
7	76	Male	Prostatic cancer
8	61	Male	Postoperative hemorrhagic shock
9	27	Female	Ovarian cancer
10	90	Male	Multiplex cancer (prostatic, stomach ,esophagus cancer)
11	55	Male	Acute myocardial infarction
12	68	Male	Pneumonia, Cancer-related lymphopathia
13	54	Male	Brain tumor
14	71	Male	Acute marrow leukemia
15	73	Male	Lung cancer
16	58	Male	Lung cancer
17	63	Female	Interstitial pneumonia
18	58	Male	Myocardial infarction, Cerebral infarction
19	58	Female	Ovarian cancer
20	80	Male	Pneumonia

Table 2

Concentrations of PCDDs, PCDFs, Non-ortho-coplanar PCBs and Mono-ortho-coplanar PCBs in human tissues

Congeners	Blood (n=18)	Lung (n=18)	Live (n=20)	Bile (n=15)	Spleen (n=18)	Pancreas (n=18)	Kidney (n=20)	Mesentery fat (n=20)
2,3,7,8-TCDD	4.9	8.2	7.8	2.7	5.0	5.8	5.7	5.4
1,2,3,7,8-PeCDD	21	38	35	12	27	25	26	23
1,2,3,4,7,8-HxCDD	9	18	17	3	21	12	18	9
1,2,3,6,7,8-HxCDD	67	110	96	30	92	90	97	73
1,2,3,7,8,9-HxCDD	12	15	20	7	12	10	13	10
1,2,3,4,6,7,8-HpCDD	48	100	170	28	150	55	130	32
OCDD	1300	2200	6800	1100	2000	1200	2200	920
2,3,7,8-TCDF	1.9	3.0	10	1.0	1.1	2.9	2.0	2.6
1,2,3,7,8-PeCDF	1.8	2.3	9.2	1.2	1.3	2.1	2.3	2.1
2,3,4,7,8-PeCDF	43	67	119	19	58	51	55	42
1,2,3,4,7,8-HxCDF	15	23	81	7.9	24	13	21	12
1,2,3,6,7,8-HxCDF	21	27	128	8.7	22	16	21	15
2,3,4,6,7,8-HxCDF	6.8	14	30	ND	22	6.1	16	4.7
1,2,3,7,8,9-HxCDF	5.3	ND	3.6	ND	ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	14	13	119	4.6	20	7.9	16	8.6
1,2,3,4,7,8,9-HpCDF	ND	ND	11	ND	ND	ND	ND	ND
OCDF	ND	4.3	14	ND	ND	ND	ND	ND
344'5-TCB (#81)	20	19	24	6	ND	24	14	21
33'4'4'-TCB (#77)	109	29	50	33	8.4	25	19	20
33'44'5-PenCB (#126)	309	367	545	91	133	472	276	422
33'44'55'-HxCB (169)	217	249	242	76	162	317	189	301
2'344'5-PenCB (#123)	955	931	1211	361	373	1203	626	1458
23'44'5-PenCB (#118)	52116	42611	58955	20505	18215	44238	33899	64235
2344'5-PenCB (#114)	3997	3425	4546	1840	1606	4509	2817	5289
233'44'-PenCB (#105)	11022	9472	12743	4987	4671	10901	7649	14986
23'44'55'-HexCB (#167)	7715	6309	10458	2660	2729	8089	4469	11644
233'44'5-HexCB (#156)	24300	19446	27367	10077	10068	23902	14509	33700
233'44'5'-HexCB (#157)	5349	4431	5662	2550	2223	5367	3219	6784
233'44'55'-HpCB (#189)	2486	2359	3067	991	1336	2779	1659	3766
Total PCDD	1505	2465	7180	1210	2262	1357	2441	1074
Total PCDF	113	157	524	49	154	103	139	92
Total Non-ortho PCBs	654	663	862	206	308	838	498	764
Total Mono-ortho PCBs	107939	88984	124009	43971	41220	100989	68846	141861
T PCDDs-TEQ	35	62	58	19	46	43	46	38
T PCDFs-TEQ	27	41	86	12	36	29	34	25
T Non-ortho PCBs-TEQ	33	39	57	10	15	50	30	45
T Mono-ortho PCBs-TEQ	24	36	26	10	16	40	28	31
Total TEQ	119	178	228	50	113	163	138	139

Table 3
Correlations of concentrations of TEQ in verious human tissues

Human tissues	Blood	Lung	Liver	Bile	Spleen	Pancreas	Kidney
Blood							
Lung	0.821**						
Liver	0.575*	0.843**					
Bile	0.236	0.347	0.211				
Spleen	0.715**	0.921**	0.836**	0.462			
Pancreas	0.777**	0.896**	0.817**	0.477	0.797**		
Kideny	0.724**	0.938**	0.818**	0.407	0.954**	0.826**	
Mesentrey fat	0.763**	0.837**	0.819**	0.315	0.755**	0.933**	0.728**

*:p<0.05, **:p<0.001

Fig. 1 Congener pattern of PCDDs in human tissues