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Review Article

Measurement of Dioxins in Human Blood: Improvement of Analytical Method

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Abstract: We have improved the method of measuring dioxins in the blood of Yusho patients; with this improved method, it is possible to take measurements quickly and precisely, using as little as 5g of blood. The specific points of improvement of this method are the extraction of lipids from blood by accelerated solvent extractor (ASE), the miniaturization of column cleanup for the purification of dioxins, and the use of a solvent cut large volume (SCLV) injection system with high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Using this improved method of analysis, we are currently investigating dioxins in the blood of 400 Yusho patients collected in Japan in 2002. We here describe the details of our announcement at Dioxin 2002 Symposium about the improvement of this analysis method, and discuss several other modifications".

Key words: PCDDs, PCDFs, Coplanar PCBs, Yusho, Blood, Accelerated solvent extraction (ASE), Solvent cut large volume (SCLV) injection system, HRGC/HRMS

Introduction

In order to understand the influence of dioxins on human health, it is necessary to investigate the body burden produced by these chemicals. We have previously reported a significant correlation between the dioxin concentrations in blood and that in tissues (subcutaneous adipose tissue, mesentery fat, liver, kidney, muscles, brain, spleen), and have noted that the dioxin concentrations in blood lipids have almost the same level as the concentrations in fat tissues[2, 3]. Dioxins in the blood of municipal incinerator plant laborers and people who live near factories have been investigated in Japan for several years[4, 5]. However, this research has been limited since it was necessary to extract at least 100 ml of blood for evaluation. In order to conduct extensive research on human contamination by dioxins, an analytical method which can accurately detect dioxins from much smaller samples of blood is required.

Additionally, such a method would be helpful for measuring dioxins in patients who cannot safely provide large samples of blood. We have been studying concentrations of dioxins (PCDDs, PCDFs and coplanar PCBs) in blood samples from Yusho patients afflicted with disease due to PCDF poisoning that occurred in western Japan in 1968. Since most of those afflicted at that time are now over 60 years old, they can safely supply only small volumes of blood for determination of dioxin concentrations. Therefore, in order to reduce the physical burden on patients, it is necessary to develop a highly sensitive analytic method that can accurately evaluate dioxin concentrations from blood samples of only 5 g. Furthermore, the pretreatment procedure of dioxins in the blood is very complicated and time-consuming. In this study, we developed an analytic method for measuring dioxin concentrations in blood samples as small as 5 g using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) equipped with a solvent cut

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Table 1. Conditions of HRGC for determination of dioxins

Gas chromatography:	HP6890 (Hewlett Packard)
Injector port temperature:	300°C
Pre-column:	BPX5 (6 m × 0.25 mm I.D., 0.25 μm film thickness, SGE)
Analityeal column:	BPX5 (30 m × 0.15 mm I.D., 0.15 μm film thickness, SGE)
Ramp of oven temperature:	80°C (0 min) → 20°C/min → 320°C (5 min) → 70°C/min → 180°C (1 min) → 5°C/min → 320°C (5 min)

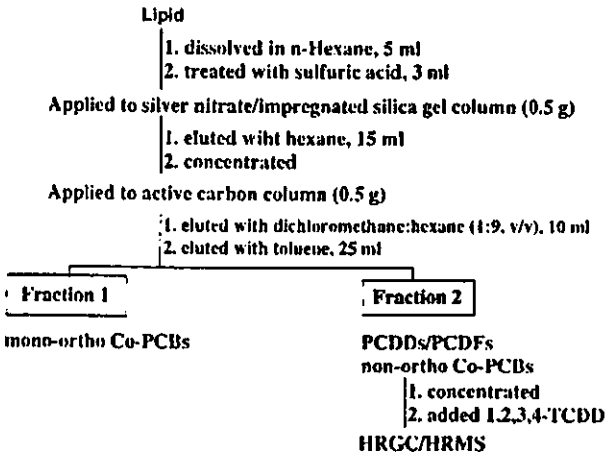

Fig. 1. Purification method of PCDDs, PCDFs and Co-PCBs in the blood.

large volume (SCLV) injection system as a large volume injection technique, and examined efficient methods of speeding up the pretreatment procedure for blood samples and reducing background levels such that they do not affect the measurement of dioxins. Using this method, we measured dioxins in blood samples collected in 2001 from 78 Yusho patients who live in Fukuoka[1].

Experiments

Lipid was extracted from the blood samples by an accelerated solvent extractor (ASE-200, Dionex, Sunnyvale, CA). Each blood sample was accurately weighed to 5 g and mixed with 4 g Isolute (International Sorbent Technology Ltd., Hengoed, Mid Glamorgan, UK). After the extraction cell was filled, the ^{13}C-labeled internal standards were added and extracted by ASE for 10 minutes × 2 times with acetone/hexane (1:4, v/v) under pressure (2000 psi) and temperature (150°C). The extract was concentrated to dryness after treatment with anhydrous sodium sulfate, and the lipid contents were determined gravimetrically. An extracted lipid was used to carry out cleanup at a scale one-fourth that of the conventional method (Fig. 1). The column packing (silver nitrate silica gel, active carbon and anhydrous sodium sulfate) used in this experiment were washed by ASE with hexane or toluene. Dioxin concentrations were measured using HRGC/HRMS equipped with an SCLV injection system (SGE Australia). The setting conditions are summarized in Tables 1, 2.

Table 2. Conditions of HRMS for detemination of dioxins

Mass spectrometer:	AutoSpec-Ultima E (Micromass UK)
Determination mode:	SIM
Interface temperature:	280°C
Ion source temperature:	270°C
Trap current:	750 μA
Electron energy:	34.3 eV
Resolution:	10000
Accelerating voltage:	8000 V
Lock mass:	PFK

Results

1) The sensitivity of GC/MS with the SCLV injection system was increased to 10 times the level of the classical method (Fig. 2, 3).

2) When the lipid was extracted from the blood by ASE with acetone/hexane (1:4, v/v) as the solvent, the lipid contents and dioxin concentrations were nearly equal to those found by the conventional method (Table 3).

3) Using the present method, the concentrations of dioxin isomers in the 5 g blood samples from 8 normal subjects were almost equal to those found by the conventional method using 20 g samples of the same blood (Fig. 4).

4) The present method demonstrated high reproducibility based on experiments conducted using the same blood samples (Fig. 5).

5) The improvement of the pretreatment method made it possible to sufficiently reduce background levels such that they do not affect the measurement of dioxins (Table 4).

6) The recovery of the ^{13}C-labeled internal standards was 60% overall when the dioxin concentrations in the blood of 78 Yusho patients were measured (Table 5).

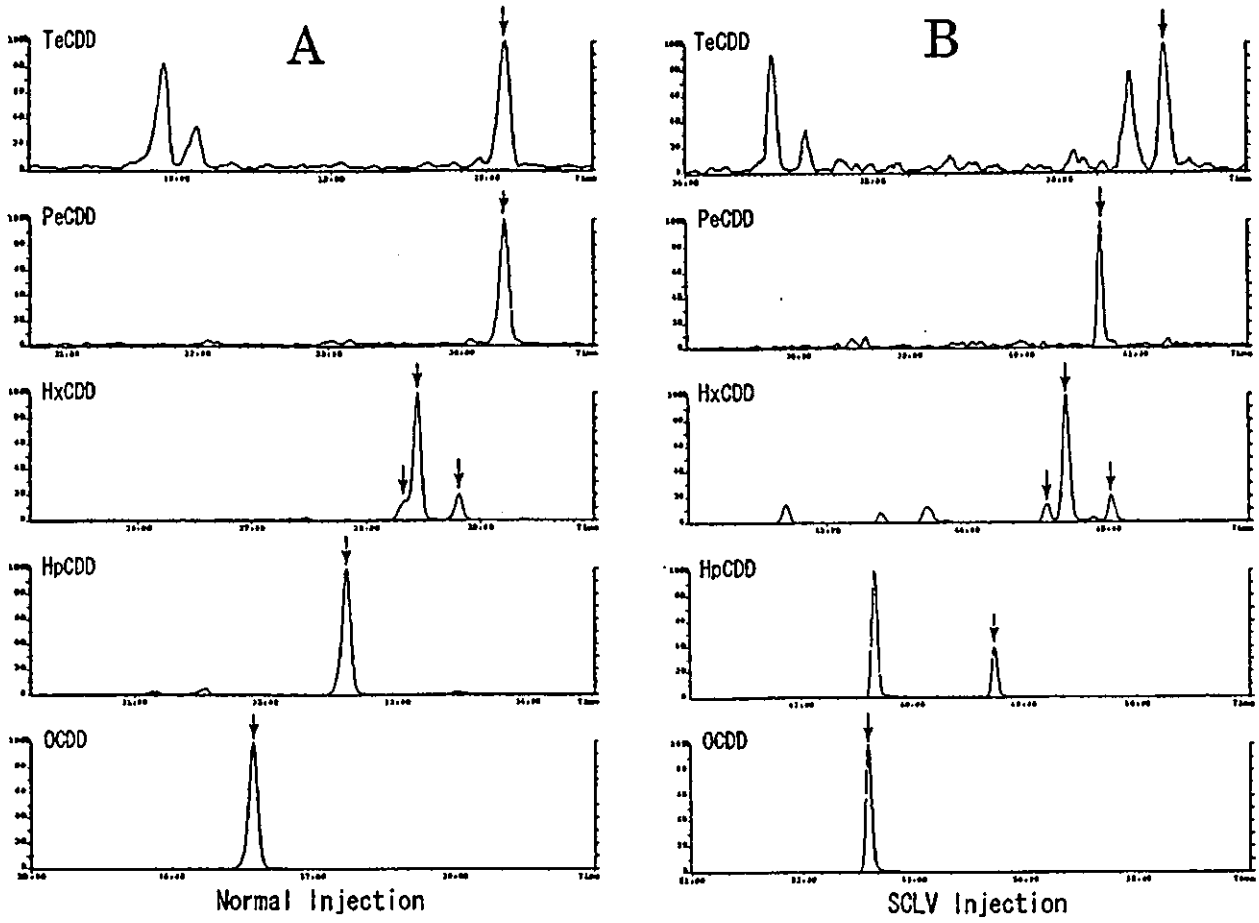

Fig. 2. HRGC/HRMS chromatogams of PCDDs in human blood.
A: Normal injection 2 μl/10 μl (equivalent to 10 g blood). B: SCLV injection 5 μl/10 μl (equivalent to 2.5 g blood).

Discussion

These results indicate that the present method is the most effective for accurately measuring dioxin concentrations using a blood volume of only 5 g. Moreover, this method allows many samples to be treated in a short period of time with high reproducibility in comparison with the conventional method.

We would like to offer additional details about out method. As stated above, our analytic method provides high sensitivity detection of dioxins by ASE extraction, miniaturization of column cleanup, and the adaptation to GC/MS of the SCLV system. All of the above are existing technologies related to dioxin analysis, and indeed, sample analysis of the environment by ASE extraction has previously been reported[6-9]. Miyata *et al.*[10] have reported on miniaturization of column cleanup, and similarly, Matsumura *et al.*[11] and Masuzaki *et al.*[12] discussed the application of the SCLV system for dioxin measurement in human blood. We believe that our method is not only rapid but also highly effective and sensitive, and is therefore of great use in examining and following up Yusho patients. For these patients in particular, it is necessary that GC/MS should be able to detect very small quantities of dioxins (1–10 fg) in 5 g of blood samples, and that the blank level should be fully low compared with the dioxin level in the blood.

First, we equipped GC/MS with SCLV injection system in order to achieve the greatest possible detection sensitivity by GC/MS. By this method, a sample injection volume of 10 μl in 15 μl or 5 μl in 10 μl was attained; that is, an injection volume of 1/2 to 2/3 of a sample is possible, 5 to 10 times the sample injection volume obtained using the traditional method in which a sample injection volume of 1–2 μl in 20 μl is used. This is almost the same as a 2 μl manual injection in a concentrated sample of 5 μl. There is great merit even in this case, in which an automatic injection system can be

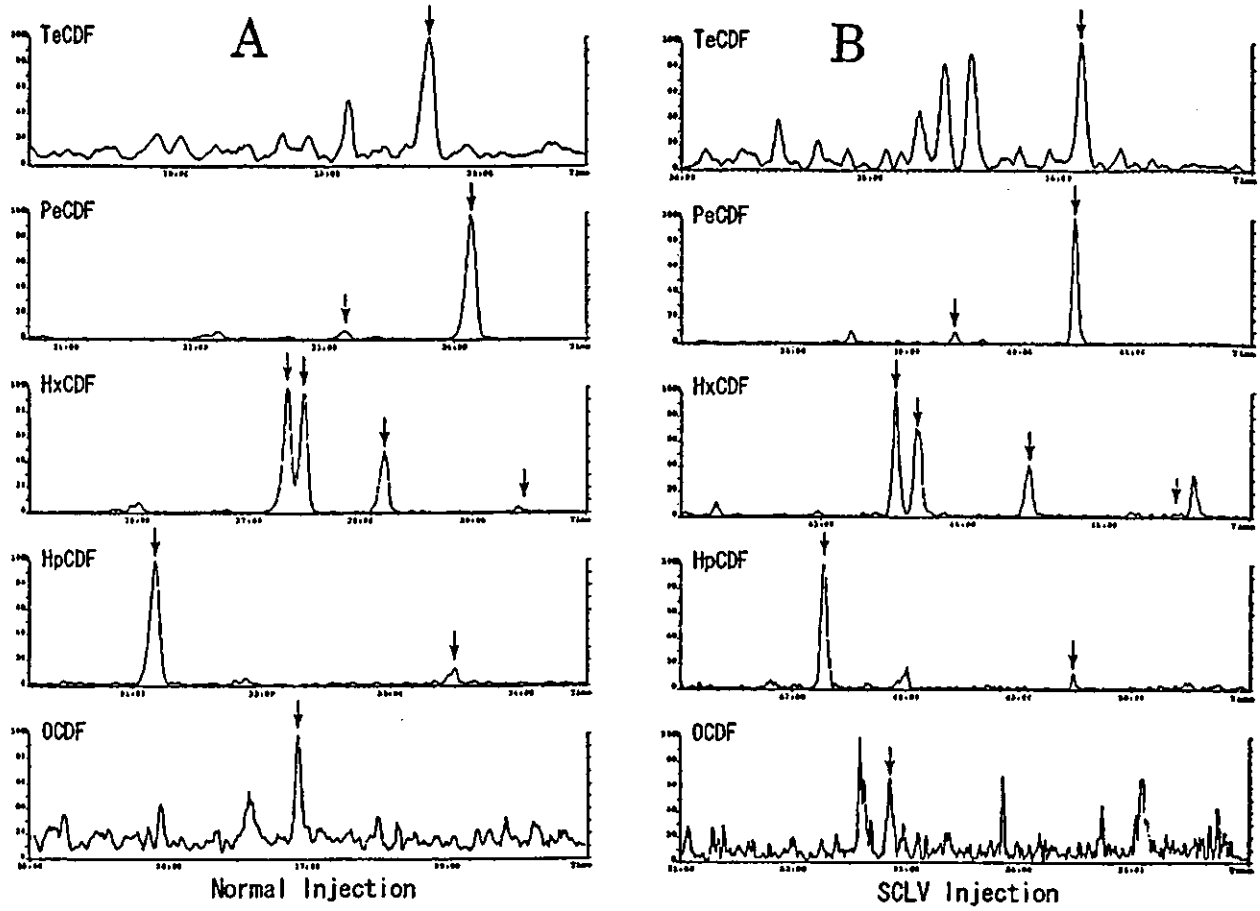

Fig. 3. HRGC/HRMS chromatogams of PCDFs in human blood.
A: Normal injection 2 μl/10 μl (equivalent to 10 g blood). B: SCLV injection 5 μl/10 μl (equivalent to 2.5 g blood).

used. Furthermore, since almost all solvents can be removed, the sample injection volume to an analysis column may be very small, and, since most matrices can be removed not only for a solvent but also for a remarkable coexistence substance in a pre-column, a narrow bore (0.1 to 0.15 mm) and thin film thickness column (0.1 to 0.15 μm) can be used. Removing the solvents and matrices makes it possible to obtain a very sharp peak and raises the S/N ratio. Therefore, the MS state can be kept very stable by removing solvents and matrices in a pre-column stage. Moreover, by using the small analysis column of the inner diameter, career gas is kept low and the inside of the MS can be maintained as a high vacuum. It is reasonable to expect that high sensitivity can thus be maintained by maintaining a high vacuum with the improvement of the S/N ratio as stated above.

A blank level can result from one of three sources: mixed contaminants which originate primarily in a reagent, contaminants adhering to the instruments, and contaminants found in the analysis operation environment. Reagents of guaranteed high purity are now available for dioxin analysis; additionally, reagents and other substances which are used as a column filling are able to perform washing and purification effectively in a solvent using the ASE or a Soxhlet extraction. We can reasonably reduce the contamination to take place using this method due to the miniature size of one-fourth of the amount formerly used during cleanup (in this procedure, the net of silver nitrate and active carbon to be used are only 50 mg and 0.5 mg, respectively).

Concerning the instrument blank, the contaminants adhering to the inside of glassware instruments were decreased by miniaturizing the glassware implements. All glassware implements removed the organic matter by overnight dipping in chromic acid mixture. It was used by acetone and hexane just before use.

In order to reduce the blank due to contamination from analysis operation, we prepared the special laboratory for human blood samples which distinguished from the laboratories concerns environmental samples (sediments,

Table 3. Extraction of dioxins from blood samples by ASE-200

Congeners	Concentration (pg/whole blood)						
	Classical method	Acetone:Hexane (2:1)		Acetone:Hexane (1:4)		Hexane	
		Mean	SD	Mean	SD	Mean	SD
2.3.7.8-TCDD	0.0070	0.0053	0.002	0.0063	0.001	0.0053	0.003
1.2.3.7.8-PeCDD	0.024	0.031	0.004	0.026	0.004	0.024	0.004
1.2.3.4.7.8-HxCDD	0.009	0.012	0.001	0.011	0.004	0.010	0.001
1.2.3.6.7.8-HxCDD	0.096	0.094	0.005	0.094	0.003	0.088	0.007
1.2.3.7.8.9-HxCDD	0.0200	0.0193	0.0023	0.0153	0.0025	0.0177	0.0031
1.2.3.4.6.7.8-HpCDD	0.0920	0.1253	0.0061	0.1043	0.0045	0.0950	0.0026
OCDD	0.8440	1.6460	0.0235	1.1757	0.0662	0.9287	0.0151
2.3.7.8-TCDF	0.0070	0.0080	0.0010	0.0063	0.0006	0.0060	0.0000
1,2.3.7.8-PeCDF	0.0040	0.0040	0.0010	0.0040	0.0010	0.0033	0.0006
2,3,4.7.8-PeCDF	0.0570	0.0553	0.0042	0.0553	0.0085	0.0503	0.0012
1,2.3,4.7.8-HxCDF	0.0310	0.0280	0.0010	0.0287	0.0045	0.0243	0.0021
1,2.3.6.7.8-HxCDF	0.0200	0.0227	0.0021	0.0230	0.0056	0.0193	0.0021
2.3.4.6.7.8-HxCDF	0.0100	0.0090	0.0000	0.0090	0.0026	0.0077	0.0006
1,2.3,7.8.9-HxCDF	N.D.	N.D.		N.D.		N.D.	
1.2.3.4.6.7.8-HpCDF	0.0140	0.0167	0.0015	0.0143	0.0015	0.0130	0.0010
1.2.3.4.7.8.9-HpCDF	N.D.	N.D.		N.D.		N.D.	
OCDF	N.D.	N.D.		N.D.		N.D.	
3'4'5-TCB(#81)	0.017	0.019	0.004	0.020	0.004	0.014	0.002
3'3'4'-TCB(#77)	0.067	0.105	0.026	0.083	0.002	0.046	0.003
3'3'4'5-PenCB(#126)	0.243	0.237	0.005	0.223	0.004	0.205	0.004
3'3'4'5'-HxCB(#169)	0.157	0.159	0.005	0.161	0.007	0.166	0.003
Total PCDDs	1.092	1.933	0.021	1.433	0.074	1.168	0.014
Total PCDFs	0.143	0.144	0.006	0.141	0.027	0.124	0.002
Total PCDDs/PCDFs	1.235	2.077	0.022	1.574	0.101	1.292	0.014
Total Coplanar PCBs	0.484	0.520	0.034	0.488	0.011	0.431	0.010
Total concentration	1.719	2.597	0.019	2.062	0.109	1.724	0.014
Lipid (%)	0.290	0.290	0.017	0.297	0.006	0.117	0.015

N.D.: not detected.

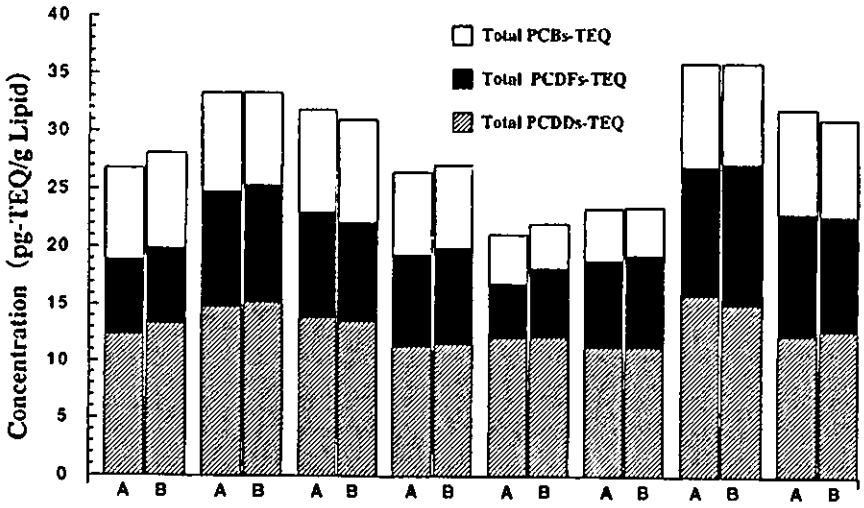

Fig. 4. Comparison of dioxins concentration in the blood by the conventional method (A) and the present method (B).

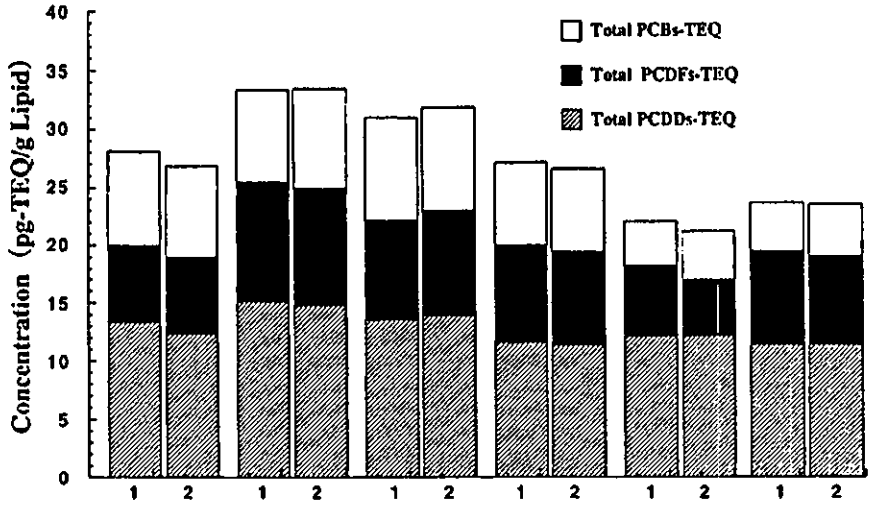

Fig. 5. Reproducibility test of the present method.

Table 4. Background level of dioxins in the present method

Congeners	pg/g Whole			pg/g Lipid		
	Blank-1	Blank-2	Mean	Blank-1	Blank-2	Mean
2,3,7,8-TCDD	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3,7,8-PeCDD	0.0018	N.D.	0.0018	0.60	N.D.	0.60
1,2,3,4,7,8-HxCDD	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3,6,7,8-HxCDD	0.0004	0.0022	0.0013	0.13	0.73	0.43
1,2,3,7,8,9-HxCDD	0.0010	0.0014	0.0012	0.33	0.47	0.40
1,2,3,4,6,7,8-HpCDD	0.0020	0.0034	0.0027	0.67	1.13	0.90
OCDD	0.0140	0.0190	0.0165	4.67	6.33	5.50
2,3,7,8-TCDF	0.0034	0.0022	0.0028	1.13	0.73	0.93
1,2,3,7,8-PeCDF	0.0024	0.0010	0.0017	0.80	0.33	0.57
2,3,4,7,8-PeCDF	0.0022	0.0048	0.0035	0.73	1.60	1.17
1,2,3,4,7,8-HxCDF	0.0048	0.0080	0.0064	1.60	2.67	2.13
1,2,3,6,7,8-HxCDF	0.0010	0.0012	0.0011	0.33	0.40	0.37
2,3,4,6,7,8-HxCDF	0.0006	N.D.	0.0006	0.20	N.D.	0.20
1,2,3,7,8,9-HxCDF	0.0004	N.D.	0.0004	0.13	N.D.	0.13
1,2,3,4,6,7,8-HpCDF	0.0010	0.0018	0.0014	0.33	0.60	0.47
1,2,3,4,7,8,9-HpCDF	0.0010	0.0004	0.0007	0.33	0.13	0.23
OCDF	0.0008	0.0010	0.0009	0.27	0.33	0.30
3,4,4',5-TCB(#81)	0.0038	0.0052	0.0045	1.27	1.73	1.50
3,3',4,4'-TCB(#77)	0.0170	0.0200	0.0185	5.67	6.67	6.17
3,3',4,4',5-PenCB(#126)	0.0028	0.0070	0.0049	0.93	2.33	1.63
3,3',4,4',5,5'-HxCB(#169)	0.0024	0.0006	0.0015	0.80	0.20	0.50

The lipid basis indicated the level estimated the lipid content as 0.3%. N.D.: not detected.

fly ash, soil, waste, etc.). The extraction operation was completed by the sealing system using an ASE extraction machine, so the contaminants mixed during lipid extraction decreased.

Our current research focuses on measuring the dioxins in the blood of approximately 400 Yusho patients sampled throughout Japan in 2002. Because the number of cases to be analyzed increased 5 times over the previous year when the study area was smaller, the analyzing method was improved as follows, and the following countermeasures were devised:

1) HRGC/HRMS equipped with an SCLV system was

Table 5. Recoveries of dioxins by the present method

Congeners	Recovery (n=19)	
	Mean	SD
^{13}C-2,3,7,8-TCDD	69.0	3.3
^{13}C-1,2,3,7,8-PeCDD	81.4	7.1
^{13}C-1,2,3,4,7,8-HxCDD	92.5	11.2
^{13}C-1,2,3,6,7,8-HxCDD	85.0	7.4
^{13}C-1,2,3,7,8,9-HxCDD	85.8	5.6
^{13}C-1,2,3,4,6,7,8-HpCDD	87.6	7.1
^{13}C-OCDD	81.7	12.0
^{13}C-2,3,7,8-TCDF	91.6	3.9
^{13}C-1,2,3,7,8-PeCDF	78.2	4.8
^{13}C-2,3,4,7,8-PeCDF	76.1	5.2
^{13}C-1,2,3,4,7,8-HxCDF	81.2	5.9
^{13}C-1,2,3,6,7,8-HxCDF	79.0	4.5
^{13}C-2,3,4,6,7,8-HxCDF	95.9	4.8
^{13}C-1,2,3,7,8,9-HxCDF	95.8	7.8
^{13}C-1,2,3,4,6,7,8-HpCDF	86.6	6.3
^{13}C-1,2,3,4,7,8,9-HpCDF	89.2	13.9
^{13}C-OCDF	87.7	18.5
^{13}C-3,3',4,5'-TCB (#81)	66.3	2.9
^{13}C-3,3',4,4'-TCB (#77)	63.9	2.3
^{13}C-3,3',4,4',5-PenCB (#126)	68.2	2.9
^{13}C-3,3',4,4',5,5'-HxCB (#169)	73.5	5.6

installed exclusively for blood analysis.

2) The silver nitrate silica gel column and the activated carbon column were linked directly.

3) After freeze-drying blood, lipid in blood was extracted by ASE at 2,000 psi and 150°C, and using acetone/hexane (1:3, v/v). The extraction liquid was able to be produced the required concentrations and dryness, and it was also possible to obtain the lipid content. Since dryness by the anhydrous sodium sulfate of the extraction liquid was excluded, disturbance of the contaminant mixed from a reagent and an instrument was decreased.

4) The analysis laboratory for blood was used exclusively for this purpose and the possibility of contaminants intruding during operation was therefore reduced.

Instead of washing glassware implements with a chromic acid mixture, they were washed with detergent before being heated to 450°C overnight, cooled and used for analysis. This washing method is able to remove contaminants from the glassware implements, therefore it not only eliminates the danger posed by the chromic acid mixture, but also makes unnecessary the washing procedure with hexane and acetone.

Considerations for Future Research

As shown in Table 3, when HpCDD and OCDD in blood are extracted by ASE, they show high concentrations compared with those obtained by the conventional method. Although the cause of this is not clear, it is possible that these compounds are not fully extracted from blood by the traditional method. Since the toxicity equivalency factors (TEF) of these compounds are small at 0.01 and 0.0001, respectively, they do not have significant influence in the total toxicity equivalent (TEQ) with this phenomenon. Nevertheless, it remains to be clarified in the future. It is important to introduce an automatic cleanup system for blood dioxin analysis because it is critical to develop a quick, effective and reliable method for measuring dioxins in blood.

The proposed method of analyzing dioxins in blood is expected to be useful not only in research on Yusho but also to investigate the other cases of patients affected by dioxin contamination. We have already used this method to measure dioxins in serum samples from patients who experienced a toxic hot spot in Vietnam. We have also measured the dioxins in the umbilical cord blood (20 g) of four subjects by this method, and found that the average value of 2, 3, 7, 8-TCDD concentrations was 0.0015 pg/g with a range of 0.001–0.002, and that these values were 1/9–1/2 the values found for each mother's body blood[13]. Greater sensitivity of GC/MS instruments is necessary in order to determine the dioxins in umbilical cord blood with higher accuracy. We believe that it is possible to obtain 2 to 3 times greater sensitivity of GC/MS by adjusting the column conditions (inner diameter, film thickness, etc.) of GC, and the conditions of MS.

Moreover, we have previously reported that the SCLV injection system can be used with a polar SP2330 column, and that dioxins can be separated using a similar method[14]. Such a method would also be applicable not only to blood dioxin analysis but also to analysis of food and environmental samples[15].

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Polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and non-*ortho*, mono-*ortho* chlorine substituted biphenyls in Japanese human liver and adipose tissue

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Polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and non-*ortho*, mono-*ortho* chlorine substituted biphenyls in Japanese human liver and adipose tissue

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Abstract

We measured PCDDs/DFs levels in Japanese human livers and adipose tissues in 1999, and TEQ were calculated with WHO TEF. The mean total levels of PCDDs/DFs in livers and adipose tissues were 57 pg TEQ/g on a lipid basis and 49 pg TEQ/g on a lipid basis, respectively. 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF and 1,2,3,4,6,7,8-HpCDF concentrations in livers considerably differed from those in 1989 ($p < 0.05$). The mean non-*ortho*-chlorine substituted biphenyls levels showed 20 pg TEQ/g on a lipid basis and 17 pg TEQ/g on a lipid basis in livers and adipose tissues, respectively. In livers, the mean of 3,3′,4,4′-TCB concentrations was 131 pg/g on a lipid basis, and 7.7-fold higher than that in 1989. The mean total mono-*ortho*-chlorine substituted biphenyls level was 13.0 pg TEQ/g on a lipid basis in livers and 21.6 pg TEQ/g on a lipid basis in adipose tissues. 3,3′,4,4′,5-PeCB and 3,3′,4,4′,5,5′-HxCB levels decreased in adipose tissues, and 3,3′,4,4′,5-PeCB level only decreased in livers. PCDDs, PCDFs, and mono- and non-*ortho*-chlorine substituted biphenyls levels may have decreased in livers and adipose tissues because of a governmental policy on dioxins discharge for the decade. Then, we estimated the correlations of PCDDs, PCDFs and the related compound levels between livers and adipose tissues. The correlative PCDDs congeners may have had a similar behavior to that between liver and adipose tissue. On the contrary, most PCDFs isomers may have different behavior between liver and adipose tissue, while 2′,3,4,4′,5-PeCB (IUPAC No. 123) may also have a different behavior between liver and adipose tissue.

Keywords: PCDDs; PCDFs; PCBs; High-resolution GC/MS analysis; Human liver; Human adipose tissue

1. Introduction

The current production of waste is a social issue, because polychlorinated dibenzo-*p*-dioxins (PCDDs) and related compounds are produced from the burning of waste containing plastics etc. These chlorinated

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compounds are recognized as environmental pollutants that do not decompose readily in the environment after industrial use and disposal, and have polluted the air, water, soil, plants and animals in the global environment (De Rosa et al., 1996; Visranathan, 1996). Human beings are at the top of the food chain, and human tissues contain high levels of environmental pollutants that tend to bioaccumulate within the food chain. Some toxic congeners in these compounds show variable toxicities depending on the animal species. PCDDs and related compounds exhibit various toxic actions, and these actions are induced by very small amounts of these compounds (Patterson et al., 1993; Gray et al., 1998). General human health is affected by these congeners when they become highly accumulated in the body. Therefore, many reports have published the residual levels of PCDDs and polychlorinated dibenzofurans (PCDFs) and related compounds in human tissues collected from different countries in the world (Guy et al., 1990; Schecter et al., 1990, 1994; Gonzalez et al., 1993; Beck et al., 1994; Patterson et al., 1994; Orban et al., 1994; Koistinen et al., 1995; Ayotte et al., 1997; Kang et al., 1997; Schuhmacher et al., 1999). In Germany, the mean human blood levels of PC DDs and related compounds in 1998 was one half of those in 1989 (Wittsiepe et al., 2000). We also investigated the levels of these compounds in the tissues of Japanese normal subjects died by various accidents, and discussed the correlations between the concentrations of these compounds in blood and in various organs in the normal subjects. As for tissues collected from the normal subjects in 1989, the liver showed the highest mean 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalency quantity (TEQ) concentration (270 pg TEQ/g on a lipid basis). In the adipose tissues, the mean PCDDs and related compounds' TEQ concentration showed 76 pg TEQ/g on a lipid basis (Iida et al., 1999).

Several laws, regulations and measures were made, and have been enforced for some years in Japan (Ministry of the Environment, Government of Japan, 1999a,b, June). Then, we investigated recent levels of PCDDs and related compounds in livers and adipose tissues, and compared with those determined in 1989.

2. Experiment

2.1. Chemicals

Native PCDDs, native PCDFs and native polychlorinated biphenyls (PCBs) as authentic standards were purchased from Wellington Laboratories, Ont., Canada. [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs, and [$^{13}C_{12}$]-PCBs as internal standards were also purchased from Wellington Laboratories, Ont., Canada. An active carbon column was prepared as follows: active carbon was purchased from Nacalai Tesque, Kyoto, Japan, refluxed three times with toluene for 1 h, and dried in vacuo, 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 prepared as follows: Kiesel Gel 60 (70–230 mesh) was purchased from Merck, Darmstadt, Germany, and heated at 180 °C overnight. Fifty ml of 40% (w/v) silver nitrate solution (Wako Pure Chemicals, Tokyo, Japan) was added to 200 g of Kiesel Gel 60, and evaporated to dryness at 50 °C. Distilled water used in this experiment was treated with *n*-hexane. All other chemicals used were of the analytical grade of PCB and phthalate commercially available.

2.2. Samples

Twenty-eight paired livers and mesenteric adipose tissues of patients with various illnesses (12 women and 16 men aged from 19 to 87) were collected from 1998 to 1999. Table 1 shows the sample characteristics. We obtained the informed consent from bereaved family members for all the samples analyzed in this study. All samples were wrapped with aluminum foil, and stored in freezer at −20 °C until the analysis of PCDDs and related compounds.

2.3. Sample preparation

Each gram of sample specimens was approximately weighed from livers or adipose tissues of Japanese subjects. [$^{13}C_{12}$]-PCDDs, [$^{13}C_{12}$]-PCDFs, and [$^{13}C_{12}$]-PCBs as internal standards were added to these samples, extracted twice with acetone/hexane (2:1) using Polytron® (KINEMATICA GmbH, Luzern, Switzerland). These extracts were then washed twice with distilled water. The *n*-hexane layers were treated with anhydrous sodium sulfate, evaporated at 40 °C, stand overnight at room temperature and the lipid contents were gravimetrically determined. The residues were dissolved in *n*-hexane, and treated three times with concentrated sulfuric acid, applied to a silver nitrate/silica gel column, eluted with 100 ml of *n*-hexane, and evaporated until there was a small volume of residue. The residues were fractionated in three fractions, 50 ml of 10% (v/v) dichloromethane/*n*-hexane, 50 ml of *n*-hexane, and 100 ml of toluene. The 10% (v/v) dichloromethane/*n*-hexane and toluene fractions were evaporated at 40 °C, and dissolved in *n*-nonane, respectively. The toluene fraction was used to determine PCDDs, PCDFs and non-*ortho*-substituted chlorinated biphenyls (Co-PCBs), and the 10% (v/v) dichloromethanel/*n*-hexane fraction was used to determine mono-*ortho* and di-*ortho*-chlorine substituted biphenyls (mono-*ortho*- and di-*ortho*-PCBs).

Table 1
Details of sample informations in the present study

No.	Age	Sex	Autopsy record	Clinical diagnosis
1	73	Male	May 2, 1998	Lung cancer
2	33	Female	May 6, 1998	Cervical cord tumour
3	29	Female	May 17, 1998	Acute myelocytic leukemia
4	68	Male	May 19, 1998	Pulmonitis
5	65	Male	May 28, 1998	Lung fibrosis
6	76	Female	June 9, 1998	Acute myelocytic leukemia
7	85	Female	June 22, 1998	Ovarian cancer
8	87	Male	August 2, 1998	Pulmonitis
9	42	Male	March 9, 1999	Haemorrhagia cerebri
10	74	Female	April 5, 1999	Lymphoma malignum, Hemolytic anemia
11	73	Female	April 10, 1999	Pneumocystis carinii
12	84	Female	April 16, 1999	Ovarian cancer
13	62	Male	April 16, 1999	Multiple organ failure
14	67	Male	April 27, 1999	Lung cancer
15	34	Male	May 7, 1999	Colon cancer
16	41	Male	May 10, 1999	Lymphoma malignum
17	68	Male	May 10, 1999	Heart infarction, esophageal cancer, prostate cancer
18	48	Female	May 25, 1999	Lung cancer
19	75	Female	June 8, 1999	Endometrial cancer
20	70	Female	June 9, 1999	Cardiac sarcoidosis
21	81	Male	June 21, 1999	Cerebrospinal meningitis, kidney failure
22	79	Male	June 26, 1999	Liver cancer
23	66	Male	July 3, 1999	Multiple organ failure
24	67	Male	July 14, 1999	Lung cancer, prostate cancer, renal cancer, thyroid cancer
25	87	Female	July 16, 1999	Brain infarction
26	17	Male	July 23, 1999	Acute lymphatic leukemia
27	49	Female	July 23, 1999	Breast carcinoma
28	57	Male	August 5, 1999	Lymphoma malignum

2.4. Analysis of PCDDs and related compounds

PCDDs and related compounds were analyzed by a gas chromatography–mass spectrometry (GC–MS). The analytical conditions were as follows: gas chromatography used an HP-5890 A series II (Hewlett-Packard, Palo Alto, CA), equipped with an Autospec Ultima E, (Micromass, Manchester, UK); the column used was an BPX-5 fused silica capillary column, 0.32 mm i.d. × 60 m, 0.25 μm film thickness (SGE International, Victoria, Australia); the column temperature was maintained at 150 °C for 1 min, heated to 220 °C at a rate of 20 °C/min, heated to 320 °C at a rate of 3 °C/min, then maintained at 320 °C for 4.2 min. The injection temperature and ion source temperature were, respectively, maintained at 280 and 270 °C, and the carrier gas (helium) flow rate (constant flow) was 1.3 ml/min. The ionizing current, ionizing energy, accelerating voltage, and trap current were 550 mA, 40 eV, 8 kV and 750 μA, respectively. PCDDs, PCDFs and coplanar PCBs (non-*ortho*-chlorine substituted biphenyls) were analyzed in a single ion recording mode. The resolution was maintained 8000–10 000 at 10%. Analysis of tetrachlorodibenzo-*p*-dioxins (TCDDs), pentachloro-*p*-dioxins (PeCDDs), hexachlorodibenzo-*p*-dioxins (HxCDDs), heptachlorodibenzo-*p*-dioxins (HpCDDs) and octachlorodibenzo-*p*-dioxin (OCDD) used [$^{13}C_{12}$]-2,3,7,8-TCDD, [$^{13}C_{12}$]-1,2,3,7,8-PeCDD, [$^{13}C_{12}$]-1,2,3,4,7,8-HxCDD, [$^{13}C_{12}$]-1,2,3,6,7,8-HxCDD, [$^{13}C_{12}$]-1,2,3,7,8,9-HxCDD, [$^{13}C_{12}$]-1,2,3,4,6,7,8-HpCDD and [$^{13}C_{12}$]-1,2,3,4,6,7,8,9-OCDD as internal standards, respectively. The analysis of tetrachlorodibenzofurans (TCDFs), pentachlorodibenzofurans (PeCDFs), hexachlorodibenzofurans (HxCDFs), heptachlorodibenzofurans (HpCDFs) and octachlorodibenzofuran (OCDF) used [$^{13}C_{12}$]-2,3,7,8-TCDF, [$^{13}C_{12}$]-1,2,3,7,8-PeCDF, [$^{13}C_{12}$]-2,3,4,7,8-PeCDF, [$^{13}C_{12}$]-1,2,3,4,7,8-HxCDF, [$^{13}C_{12}$]-1,2,3,6,7,8-HxCDF, [$^{13}C_{12}$]-1,2,3,7,8,9-HxCDF, [$^{13}C_{12}$]-2,3,4,6,7,8-HxCDF, [$^{13}C_{12}$]-1,2,3,4,6,7,8-HpCDF, [$^{13}C_{12}$]-1,2,3,4,7,8,9-HpCDF and [$^{13}C_{12}$]-1,2,3,4,6,7,8,9-OCDF as internal standards, respectively. The analysis of 3,3′,4,4′-tetrachlorobiphenyl (TCB), 3,4,4′,5-TCB, 3,3′,4,4′,5-pentachlorobiphenyl (PeCB) and 3,3′,4,4′,5,5′-hexachlorobiphenyl (HxCB) used [$^{13}C_{12}$]-3,3′,4,4′-TCB, [$^{13}C_{12}$]-3,4,4′,5-TCB, [$^{13}C_{12}$]-3,3′,4,4′,5-PeCB and [$^{13}C_{12}$]-3,3′,4,4′,5,5′-HxCB as internal standards, respectively. [$^{13}C_{12}$]-1,2,3,4-TCDD was used as a syringe spike. The mono-*ortho*-PCBs analysis used [$^{13}C_{12}$]-2,3,3′,4,4′-PeCB,

[$^{13}C_{12}$]-2,3,4,4',5-PeCB, [$^{13}C_{12}$]-2,3',4,4',5-PeCB, [$^{13}C_{12}$]-2',3,4,4',5-PeCB, [$^{13}C_{12}$]-2,3,3',4,4',5-HxCB, [$^{13}C_{12}$]-2,3,3',4,4',5'-HxCB, [$^{13}C_{12}$]-2,3',4,4',5,5'-HxCB and [$^{13}C_{12}$]-2,3,3',4,4',5,5'-HpCB as internal standards, respectively. The di-*ortho*-PCBs analysis used [$^{13}C_{12}$]-2,2',3,3',4,4',5-HpCB and [$^{13}C_{12}$]-2,2',3,4,4',5,5'-HpCB as internal standards, respectively.

2.5. Estimation of PCDDs and related compounds in livers and adipose tissues

The TEQ in the samples were calculated with the WHO-2,3,7,8-TCDD equivalency factor (TEF). Statistical significance was computed by linear regression analysis. A probability of 0.05 or less was considered significant. When a result was under the quantitation limit (0.5 pg/g on lipid basis), calculation of TEQ values was presented as zero. The determination limits for each congeners were indicated in Table 2.

3. Results and discussion

We previously reported concentrations of PCDDs/DFs in Japanese human tissues and blood in 1989 (Iida et al., 1999). Eleven years later, we analyzed those of Japanese livers and mesenteric adipose tissues. During the 11 years from 1989 to 1999, several regulations on PCDDs/DFs and related compounds discharged from facilities, as well as incinerator facilities, were enacted and enforced in Japan. As a result, the mean levels of PCDDs/DFs in livers and adipose tissues were 57 pg TEQ/g on a lipid basis and 49 pg TEQ/g on a lipid basis in 1998 or 1999, respectively. The mean non-*ortho*-chlorine substituted biphenyls levels showed 20 pg TEQ/g on a lipid basis and 17 pg TEQ/g on a lipid basis in livers and adipose tissues, respectively, and the mean total mono-*ortho*-chlorine substituted biphenyls level was 13.0 pg TEQ/g on a lipid basis in livers and 21.6 pg TEQ/g on a lipid basis in adipose tissues.

Basic statistical parameters with regard to age, lipid content of examined livers and adipose tissue samples, PCDDs/DFs and related compound levels on a lipid basis are summarized in Table 2. The mean levels of PCDDs/DFs in livers and adipose tissues were 238 pg TEQ/g on a lipid basis and 57 pg TEQ/g on a lipid basis in 1989, respectively (Iida et al., 1999). In 1999, these levels were 57 pg TEQ/g on a lipid basis and 49 pg TEQ/g on a lipid basis, respectively. A reduction to about a quarter was observed in the livers for most congeners,

Table 2
Concentrations of PCDDs, PCDFs and non-*ortho*-substituted PCBs in human livers and adipose tissues[a]

Liver, lipid basis (pg/g lipid)					Mesenteric adipose tissue, lipid basis (pg/g lipid)					Determination limits (pg/g lipid)
Isomers	Mean	Max	Min	SD	Isomers	Mean	Max	Min	SD	
2,3,7,8-TCDD	1.7	10	ND	3.1	2,3,7,8-TCDD	4.0	10	ND	2.6	0.5
1,2,3,7,8-PeCDD	18	80	ND	20	1,2,3,7,8-PeCDD	18	42	ND	11	0.5
1,2,3,4,7,8-HxCDD	6.7	38	ND	10	1,2,3,4,7,8-HxCDD	4.8	14	0.51	3.8	1
1,2,3,6,7,8-HxCDD	56	201	10	47	1,2,3,6,7,8-HxCDD	58	156	11	36	1
1,2,3,7,8,9-HxCDD	6.4	31	ND	8.4	1,2,3,7,8,9-HxCDD	8.7	19	ND	5.3	1
1,2,3,4,6,7,8-HpCDD	77	380	ND	99	1,2,3,4,6,7,8-HpCDD	26	119	ND	23	1
OCDD	1949	14916	116	3462	OCDD	621	3362	32	708	2
Total PCDDs	2115	15498	151	3607	Total PCDDs	741	3621	63	755	
2,3,7,8-TCDF	4.7	28	ND	6.4	2,3,7,8-TCDF	2.0	5.0	ND	1.3	0.5
1,2,3,7,8-PeCDF	7.9	34	ND	10	1,2,3,7,8-PeCDF	1.4	3.5	ND	0.97	0.5
2,3,4,7,8-PeCDF	43	157	12	36	2,3,4,7,8-PeCDF	33	75	11	19	0.5
1,2,3,4,7,8-HxCDF	22	102	ND	24	1,2,3,4,7,8-HxCDF	10	22	3.5	5.6	1
1,2,3,6,7,8-HxCDF	36	172	7.0	38	1,2,3,6,7,8-HxCDF	12	34	4.0	7.9	1
2,3,4,6,7,8-HxCDF	11	58	ND	13	2,3,4,6,7,8-HxCDF	4.4	15	ND	3.4	1
1,2,3,7,8,9-HxCDF	1.1	14	ND	2.8	1,2,3,7,8,9-HxCDF	0.62	2.1	ND	0.55	1
1,2,3,4,6,7,8-HpCDF	15	75	ND	20	1,2,3,4,6,7,8-HpCDF	4.5	8.2	ND	1.8	1
1,2,3,4,7,8,9-HpCDF	2.5	21	ND	5.5	1,2,3,4,7,8,9-HpCDF	2.1	7.2	ND	1.9	1
OCDF	3.9	23	ND	6.6	OCDF	7.1	21	ND	4.7	2
Total PCDFs	147	582	41	123	Total PCDFs	77	159	26	39	
3,4,4',5-TCB(#81)	14	72	ND	20	3,4,4',5-TCB(#81)	14	41	ND	11	5
3,3',4',4'-TCB(#77)	131	564	8.7	134	3,3',4',4'-TCB(#77)	77	332	8.3	68	5

Table 2 (*continued*)

Liver, lipid basis (pg/g lipid)					Mesenteric adipose tissue, lipid basis (pg/g lipid)					Determination limits (pg/g lipid)
Isomers	Mean	Max	Min	SD	Isomers	Mean	Max	Min	SD	
3,3′,4,4′,5-PeCB(#126)	188	882	27	175	3,3′,4,4′,5-PeCB(#126)	160	548	25	106	5
3,3′,4,4′,5,5′-HxCB(#169)	76	315	4.9	70	Total non-*ortho*-PCBs	326	1127	60	220	
Total non-*ortho*-PCBs	409	1817	58	380	3,3′,4,4′,5,5′-HxCB(#169)	74	209	14	54	5
Total dioxins	2670	16825	282	3977	Total dioxins	1144	4898	154	970	
Lipid content (%)	4.9	1.8	14	3.1	Lipid content (%)	70	36	87	13	
Age	63	87	17	19						
2,3,7,8-TCDD	1.7	10	ND	3.1	2,3,7,8-TCDD	4.0	10	ND	2.6	
1,2,3,7,8-PeCDD	18	80	ND	20	1,2,3,7,8-PeCDD	18	42	ND	11	
1,2,3,4,7,8-HxCDD	0.67	3.8	ND	1.0	1,2,3,4,7,8-HxCDD	0.48	1.4	0.05	0.38	
1,2,3,6,7,8-HxCDD	5.6	20	1.0	4.7	1,2,3,6,7,8-HxCDD	5.8	16	1.1	3.7	
1,2,3,7,8,9-HxCDD	0.64	3.1	ND	0.84	1,2,3,7,8,9-HxCDD	0.9	1.9	ND	0.53	
1,2,3,4,6,7,8-HpCDD	0.77	3.8	ND	1.0	1,2,3,4,6,7,8-HpCDD	0.26	1.2	ND	0.23	
OCDD	0.19	1.5	0.01	0.35	OCDD	0.06	0.34	0.003	0.07	
Total PCDDs	27	99	3.0	26	Total PCDDs	29	68	6.1	18	
2,3,7,8-TCDF	0.45	2.8	ND	0.63	2,3,7,8-TCDF	0.20	0.50	ND	0.13	
1,2,3,7,8-PeCDF	0.40	1.7	ND	0.48	1,2,3,7,8-PeCDF	0.07	0.17	ND	0.05	
2,3,4,7,8-PeCDF	22	79	6.2	18	2,3,4,7,8-PeCDF	17	38	5.7	10	
1,2,3,4,7,8-HxCDF	2.2	10	ND	2.4	1,2,3,4,7,8-HxCDF	1.0	2.2	0.35	0.56	
1,2,3,6,7,8-HxCDF	3.6	17	0.70	3.8	1,2,3,6,7,8-HxCDF	1.2	3.4	0.40	0.79	
2,3,4,6,7,8-HxCDF	1.1	5.8	ND	1.3	2,3,4,6,7,8-HxCDF	0.43	1.5	ND	0.34	
1,2,3,7,8,9-HxCDF	0.11	1.4	ND	0.28	1,2,3,7,8,9-HxCDF	0.06	0.21	ND	0.05	
1,2,3,4,6,7,8-HpCDF	0.14	0.75	ND	0.20	1,2,3,4,6,7,8-HpCDF	0.04	0.08	ND	0.02	
1,2,3,4,7,8,9-HpCDF	0.03	0.21	ND	0.05	1,2,3,4,7,8,9-HpCDF	0.02	0.07	ND	0.02	
OCDF	0.0004	0.002	ND	0.001	OCDF	0.001	0.002	ND	0.0005	
Total PCDFs	30	111	8.4	25	Total PCDFs	20	44	6.8	11	
3,4,4′,5-TCB(#81)	0.001	0.01	ND	0.002	3,4,4′,5-TCB(#81)	0.001	0.004	ND	0.001	
3,3′,4′,4′-TCB(#77)	0.01	0.06	0.001	0.01	3,3′,4′,4′-TCB(#77)	0.01	0.03	0.001	0.01	
3,3′,4,4′,5-PeCB(#126)	19	88	2.7	17	3,3′,4,4′,5-PeCB(#126)	16	55	2.5	11	
3,3′,4,4′,5,5′-HxCB(#169)	0.76	3.2	0.05	0.70	3,3′,4,4′,5,5′-HxCB(#169)	0.74	2.1	0.14	0.54	
Total non-*ortho*-PCBs	20	91	2.8	18	Total non-*ortho*-PCBs	17	57	2.7	11	
Total dioxins	76	290	19	65	Total dioxins	66	136	19	37	

ND: less than the determination limit.

[a] $N = 28$, 12 women and 16 men aged from 19 to 87.

but TEQ levels were similar for adipose tissues in both years. Although the sample group examined in a different set from 1989 to 1999 did not have the same size and age structure, it is obvious, that the PCDDs/DFs levels decreased over this time period in their livers (Fig. 1). This finding suggests that the distribution of PCDDs/DFs differs in human livers and adipose tissues. This tendency can be found for most detectable congeners with 2,3,7,8-substituted chlorinated DDs/DFs. 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF and 1,2,3,4,6,7,8-HpCDF concentrations in livers considerably decreased from those in 1989 ($p < 0.05$).

The mean non-*ortho*-chlorine substituted biphenyls levels were 20 pg TEQ/g on a lipid basis and 17 pg TEQ/g on a lipid basis in livers and adipose tissues, respectively. These TEQ values were similar to those in 1989 (Iida et al., 1999). However, concentrations of 3,3′,4,4′-TCB and 3,3′,4,4′,5,5′-HxCB, mainly originating from incineration, exhaust, fumes, etc., decreased as compared with those in 1989 (Iida et al., 1999). In livers, mean 3,3′,4,4′-TCB concentration was 131 pg/g on a lipid basis, and 7.7-fold higher than that (17 pg/g on a lipid basis) in 1989 ($p < 0.05$). The mean 3,3′,4,4′,5-PeCB concentration was 188 pg/g on a lipid basis, and 0.61-fold lower than that (310 pg/g on a lipid basis) in 1989 ($p < 0.05$). The mean 3,3′,4,4′,5,5′-HxCB concentration was stationary as

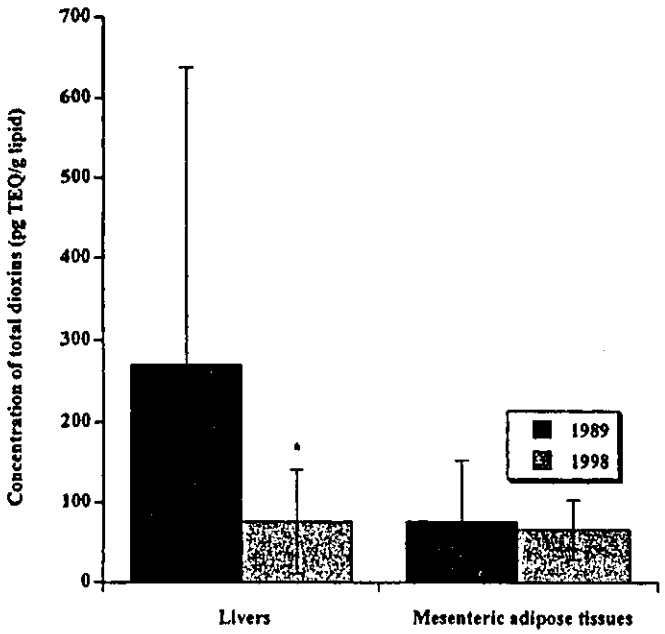

Fig. 1. Comparison of total dioxins concentrations in human liver and adipose tissues between in 1989 and in 1998. Each bar represents the mean ± SD of 8 samples in 1989 and of 28 samples in 1998. *Significantly different from total dioxins concentrations in 1989 ($p < 0.01$).

compared with that in 1989 ($p < 0.05$). In adipose tissues, the mean 3,3′,4,4′-TCB concentration (77 pg/g on a lipid basis) was also 12-fold higher than that (6.6 pg/g on a lipid basis) in 1989, that of 3,3′,4,4′,5-PeCB concentration was stationary as compared with that in 1989, and the 3,3′,4,4′,5,5′-HxCB concentration (74 pg/g on a lipid basis) was 0.57-fold lower than that (130 pg/g on a lipid basis) in 1989 ($p < 0.05$). We did not analyze mono-*ortho*-chlorine substituted biphenyls in the previous report. In the present study, we investigated mono-*ortho*-chlorine substituted biphenyls levels in human livers and adipose tissues. The mean total mono-*ortho*-chlorine substituted biphenyls level was 13.0 pg TEQ/g on a lipid basis in livers and 21.6 pg TEQ/g on a lipid basis in adipose tissues. These PCDDs/DFs levels in men ($N = 16$) were similar to those in women ($N = 12$) at $p < 0.05$.

The toxic contribution of mono- and non-*ortho*-chlorine substituted biphenyls was larger than that of PCDDs/DFs in a Japanese total diet study (Toyoda et al., 1999). Furthermore, fish and shellfish contributed to the high level of these congeners (Ministry of Health, Labor and Welfare, Government of Japan, 2000), and showed marked accumulation in aquatic wildlife. (Ministry of the Environment, Government of Japan, 1999a, September) In the atmospheric environment (air, soil, sediment, water and dust fall), the TEQ values of mono- and non-*ortho*-chlorine substituted biphenyls were under 10% against the total TEQ values. However, the toxic contribution of mono- and non-*ortho*-chlorine substituted biphenyls was about 70% in aquatic wildlife. This finding suggests that mono- and non-*ortho*-chlorine substituted biphenyls were stronger than PCDDs/DFs in terms of the biological magnification and/or biological accumulation. 3,3′,4,4′,5-PeCB and 3,3′,4,4′,5,5′-HxCB originated from combustible sources; both congeners levels decreased in human adipose tissues, and the 3,3′,4,4′,5-PeCB level only decreased in human livers. In Germany, the mean blood concentrations of PCDDs/DFs were 43.7 pg TEQ/g on a lipid basis in 1989, but the levels of PCDDs/DFs were 20.7 pg TEQ/g on a lipid basis between 1996 and 1998. The reduction to about half was found for most congeners (Wittsiepe et al., 2000). Several governmental policies on dioxin discharges were made, and improvement of incineration facilities has been made quickly in Japan. PCDDs, PCDFs, and mono- and non-*ortho*-chlorine substituted biphenyl levels may have decreased in human livers and adipose tissues because of these governmental policies on dioxin discharges over the last decade. Only the 3,3′,4,4′-TCB concentration in human liver and adipose tissue increased over the last decade (Ministry of the Environment, Government of Japan, 1999b), even though that in Japanese breast milk and blood decreased (Matsueda et al., 1993; Iida et al., 1999). We should investigate other sources of 3,3′,4,4′-TCB in the future.

Nakanishi et al. (2000) reported that the body burden of PCDDs and related compounds in humans should not be estimated with TEF, but with each particular bioavailavility of PCDDs/DFs and related compounds. Therefore, we estimated the correlations of PCDDs, PCDFs and related compounds levels between human livers and adipose tissues, summarized in Figs. 2–5, and tried to compare them with the same correlations in 1989. The correlation coefficient (r) between the concentrations on a lipid basis in human livers and in human adipose tissues were computed by linear regression and correlation analyses. No correlations with 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,7,8,9-HpCDF, 1,2,3,4,6,7,8-HpCDF, OCDF and 2′,3,4,4′,5-PeCB (IUPAC No. 123) were found. The other congeners correlated well between the concentrations on a lipid basis in human livers and in human adipose tissues. PCDDs correlations between human liver and adipose tissue were almost the same as those in 1989 ($p < 0.05$). In PCDFs, the correlation of 1,2,3,6,7,8-HxCDF in 1989 was significant ($p < 0.05$), but was not significant in the present report. The correlation of 3,3′,4,4′-TCB (IUPAC No. 77) in 1989 was not significant, but was significant in 1998 ($p < 0.05$). The other correlations were similar to the data in 1989. The dioxin congeners showing significant correlations between the concentrations in human livers and those in human adipose tissues may indicate similar behavior (accumulation, metabolism, etc.) in the human liver and adipose tissue. The correlative PCDDs isomers may have a

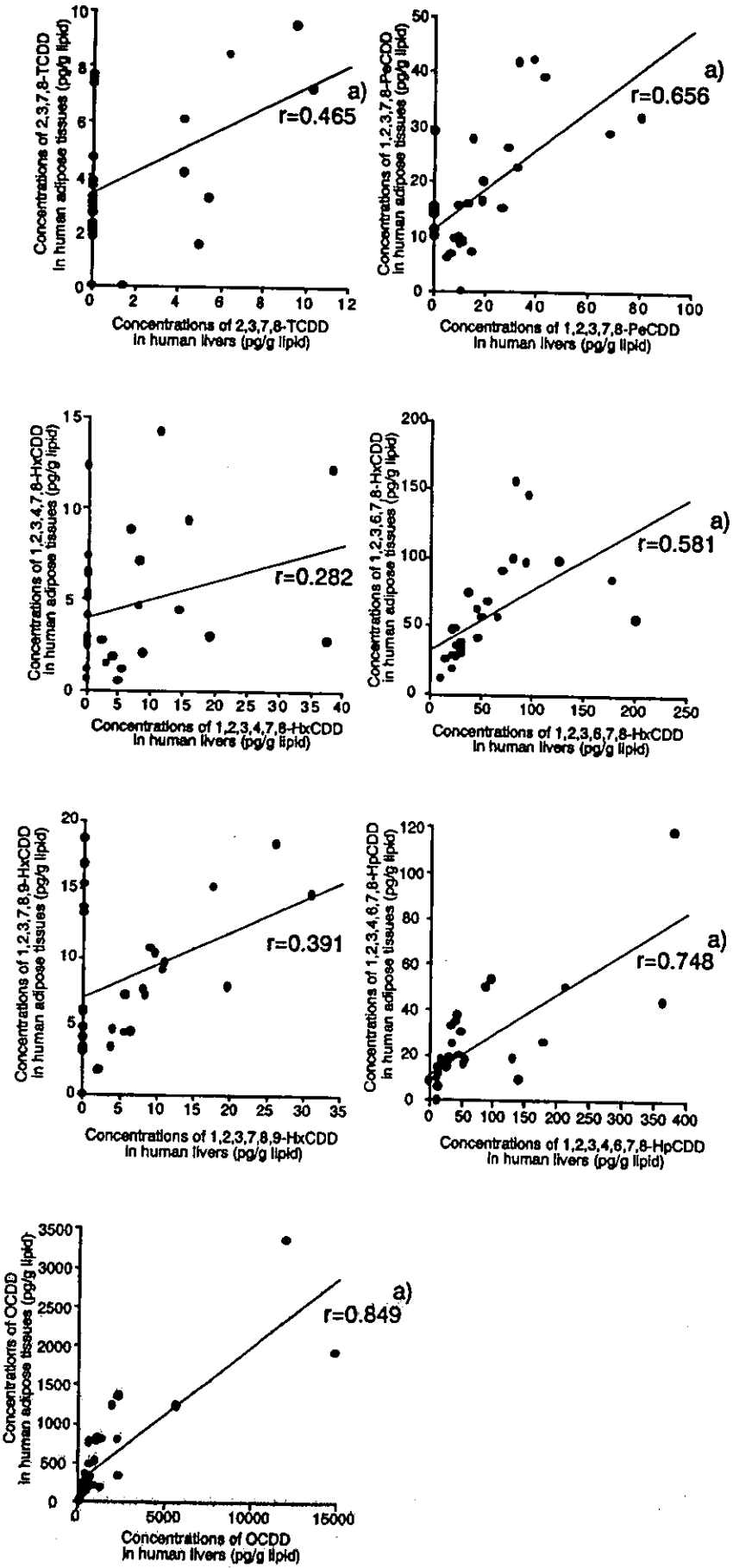

Fig. 2. Correlations between PCDDs concentration in human livers and in human adipose tissues: (a) significant at $p < 0.05$.

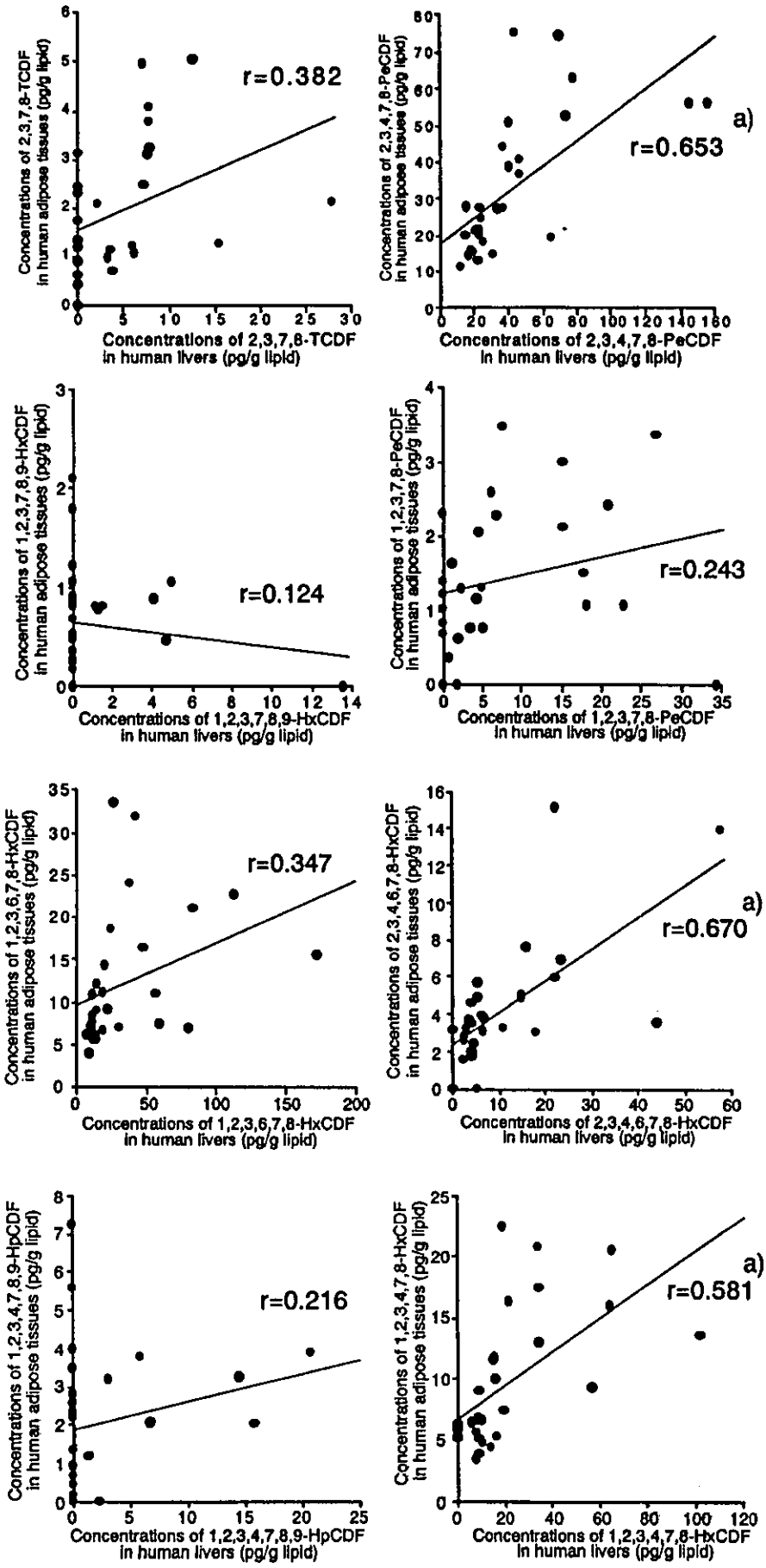

Fig. 3. Correlations between PCDFs concentration in human livers and in human adipose tissues: (a) significant at $p < 0.05$.

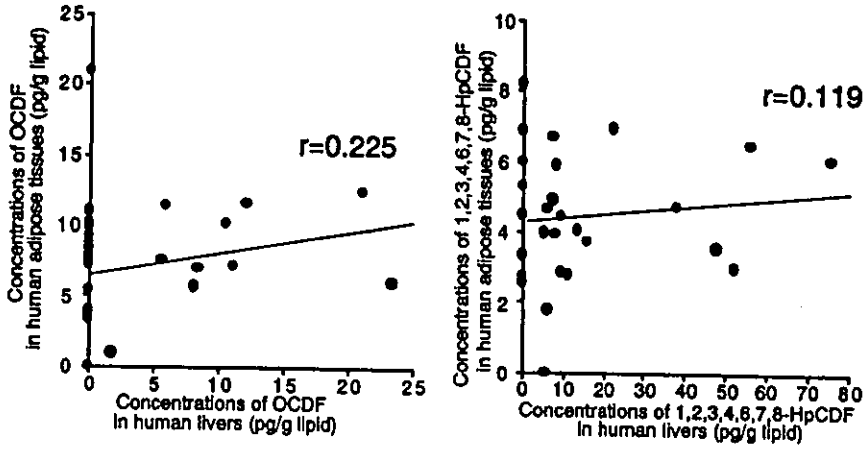

Fig. 3 (*continued*)

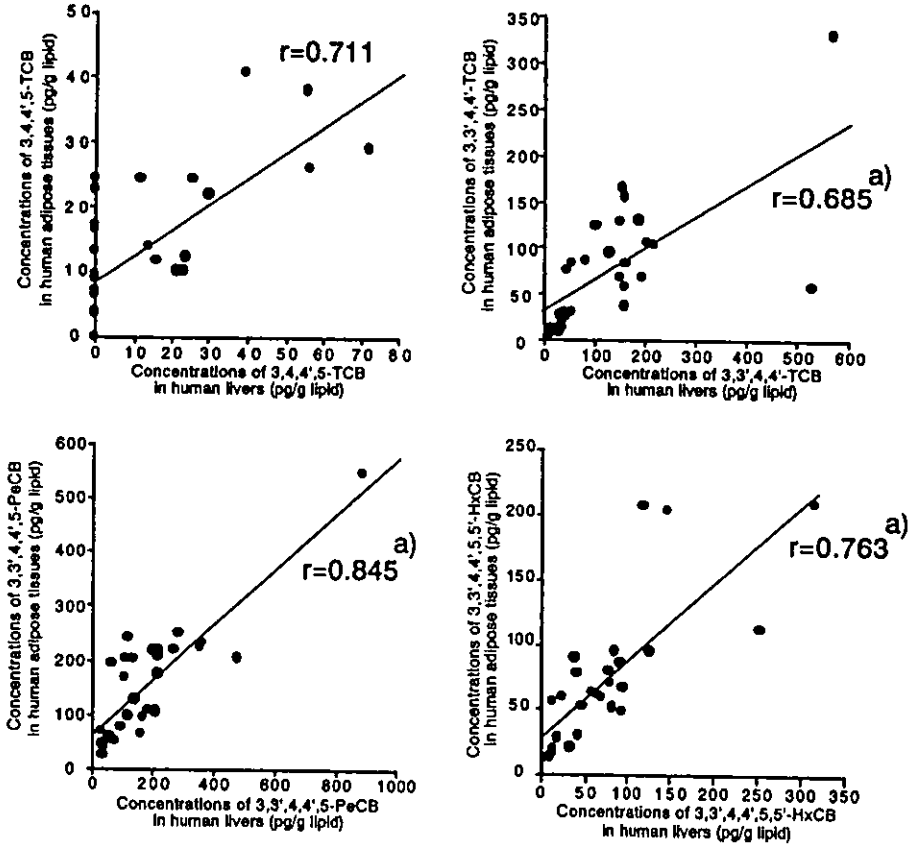

Fig. 4. Correlations between non-*ortho*-PCBs concentration in human livers and in human adipose tissues: (a) significant at $p < 0.05$.

similar behavior in human liver and adipose tissue. On the contrary, most PCDFs isomers may have different behaviors in human liver and adipose tissue, while 2′,3,4,4′,5-PeCB (IUPAC No. 123) may also have a different behavior in human liver and adipose tissue.

Generally, TEQ values of PCDDs/DFs and related compounds in the Japanese environment and dietary intake decreased from 1997 to 1998. Indeed, PCDDs/DFs and related compounds concentrations decreased in Japanese human liver and adipose tissue, but the non-*ortho*-PCBs concentrations were unchanged. The other PCBs congeners also had high concentrations in Japanese human liver and adipose tissue. Therefore, we conclude that non-, mono- and di-*ortho*-PCBs have a high bioavailability and highest accumulative effect than PCDDs/DFs, and the risks of PCBs as environmental endocrine disrupting chemicals for humans and wildlife are greater than those of PCDDs/DFs.

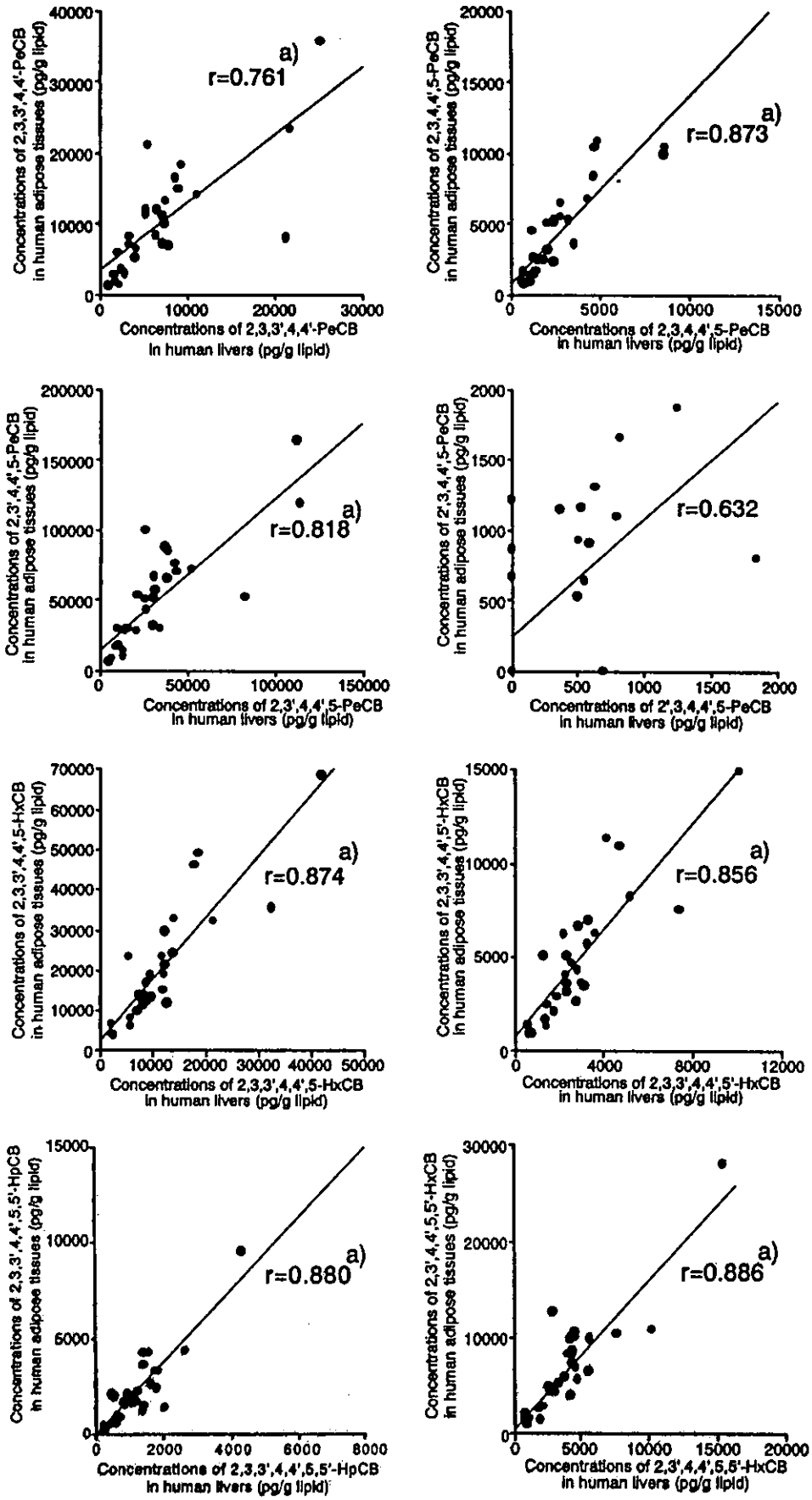

Fig. 5. Correlations between mono- and di-*ortho*-PCBs concentration in human livers and in human adipose tissues: (a) significant at $p < 0.05$.

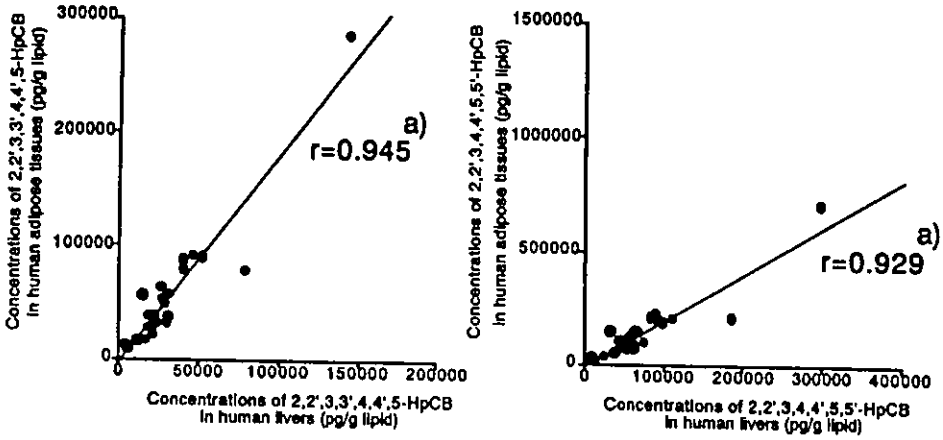

Fig. 5 (*continued*)

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