DIOXINS IN BILE IN RELATION TO THOSE IN THE HUMAN LIVER AND BLOOD

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ABSTRACT The levels of 20 dioxin congeners, 7 polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs) and three coplanar polychlorinated biphenyls (Co-PCBs), in bile were examined in comparison with those in the blood and liver, in 27 autopsy cases. Total-TEQ values were the same in the bile (43.2 ± 30.9 pg TEQ/g lipid) and blood (43.1 ± 24.2 pg TEQ/g lipid), and three times higher in the liver (127.8 ± 57.4 pg TEQ/g lipid). Highly chlorinated PCDDs and PCDFs have a tendency to accumulate in the liver, and their levels in bile and blood were relatively low compared with those in the liver, with 1,2,3,4,6,7,8-heptachlorodibenzofuran having the highest tendency among the 20 congeners. Daily excretion in bile was calculated to be 54 pg TEQ at age 65, by assuming that daily bile secretion is 750 ml and is concentrated 7.5-fold in the gallbladder. The correlation between bile and blood total-TEQ was high, with a correlation coefficient of 0.89 among the 27 autopsy cases. Thus, the regression equation of $y = 1.14x - 6.02$ will provide us levels in bile (total TEQ per g lipid), knowing the blood total-TEQ level, where x is total-TEQ per g lipid of the blood. Furthermore, accumulation of dioxins was estimated to be 0.99, 0.70 and 1.91 pg TEQ/g lipid/year in bile, blood and liver, respectively.

KEY WORDS: PCDD, PCDF, Co-PCB, Bile, Blood, Liver

INTRODUCTION

At present in our environment, dioxins, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs), are mainly produced by heating waste in incinerators, and are then emitted in the exhaust gas and fly ash. Dioxins are chemically stable, lipophilic compounds, and they accumulate in fish, meat and dairy products (Schechter *et al.*, 1996; Toyoda *et al.*, 1999). Humans are exposed to dioxins mainly through food in daily life, and data of human exposure to dioxins based on blood levels are accumulating (International Agency for Research on Cancer, 1997; US Department of Health and Human Services, 1998).

However, studies of the distribution of dioxins in the human body and excretion from the body are very limited. We have recently clarified that gastrointestinal absorption of dioxins is not directly associated with the number of chlorine atoms of the congeners, and each compound has its own characteristic features of metabolism (Kitamura *et al.*, 2001). 1,2,3,7,8,9-hexachlorodibenzofuran (1,2,3,7,8,9-HxCDF), 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PeCDF), 1,2,3,4,7,8,9-heptachlorodibenzofuran (1,2,3,4,7,8,9-HpCDF), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), octachlorodibenzo-*p*-dioxin (OCDD) and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,4,7,8-HxCDD) were well absorbed from the gastrointestinal tract after ingestion, and OCDD, 2,3,7,8-TCDF and

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3,3',4,4'-tetrachlorobiphenyl (3,3',4,4'-TCB) were efficiently excreted from sebum but 1,2,3,4,7,8-HxCDD was scarcely excreted. In total-TEQ, about 22% of the ingested dioxins were excreted from feces and 29% from sebum, while the fate of the other 50% is not clear at present (Kitamura *et al.*, 2001). Since the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in humans has been estimated to be 5.8~9.5 years (Michalek *et al.*, 1996; Ott and Zober, 1996), and 33 years for 2,3,4,7,8-PeCDF (Rohde *et al.*, 1999), these dioxins might be subjected to enterohepatic circulation. Thus, prevention of absorption of dioxins from the intestines would be helpful for reduction of body burden of dioxins after accidental exposure. For this purpose, we first need to clarify levels of dioxins in bile. In this study, we clarified dioxin levels of seven major PCDDs, 10 PCDFs and three coplanar PCBs in the bile of 27 autopsy patients, in relation to blood levels, for estimation of dioxin in bile from blood levels.

MATERIALS AND METHODS

Chemicals

Authentic PCDDs, PCDFs and Co-PCBs, and $^{13}\text{C}_{12}$ -PCDDs, $^{13}\text{C}_{12}$ -PCDFs and $^{13}\text{C}_{12}$ -Co-PCBs were purchased from Wellington Laboratories (Ontario, Canada). All solvents used were of dioxin-analysis grade (Kanto Chemicals, Tokyo, Japan).

Sample preparation

Gallbladder bile, cardiac blood and liver tissue were obtained from 27 cadavers at autopsy with the permission of the bereaved families, and were stored at -80 °C until use. About 50 g of bile, more than 50 g of blood and about 5 g of liver tissue were stored from each cadaver. The ages and diagnoses of dead subjects are summarized in Table 1.

Preparation of samples for dioxin analysis

1. Bile

Forty g of bile was transferred to a 250 ml centrifuge-tube, 30 pg of $^{13}\text{C}_{12}$ -TCDD/F ~ HpCDD/F, 60 pg of $^{13}\text{C}_{12}$ -OCDD, 100 pg of $^{13}\text{C}_{12}$ -TCB~HxCB were added, and then a lipid fraction containing dioxins was obtained by extracting with 160 ml of acetone/*n*-hexane (2:1), and then *n*-hexane three times. The extracts were washed with ultra-pure water and the hexane fractions were pooled and dried by passing through a column containing 10 g Na_2SO_4 , concentrated to about 1 ml by a rotary evaporator, and then spontaneously dried by standing at room temperature. The lipid fraction was

then weighed. The lipid fraction was dissolved in 50 ml *n*-hexane, and treated with 20 ml conc H_2SO_4 to remove a large amount of lipid.

2. Blood

Fifty g of blood was transferred to a 250 ml centrifuge-tube, and to this $^{13}\text{C}_{12}$ -isomers were added as described above, and then dioxin extraction was performed within the same day. A lipid fraction that contained dioxins was extracted by the $(\text{NH}_4)_2\text{SO}_4$ -ethanol/hexane method (Miyata *et al.*, 1998).

3. Liver

The method reported by Iida *et al.* (1999) was applied with a minor modification. Briefly, 1.5 g of liver tissue was homogenized in a mortar in the presence of the same amount of Na_2SO_4 (Beck *et al.*, 1990) and transferred to a 100 ml tube. Then, $^{13}\text{C}_{12}$ -isomers were added. A lipid fraction was obtained by extracting with 50 ml acetone/*n*-hexane (2:1), and then *n*-hexane three times.

Clean-up

The lipid fraction and the *n*-hexane fraction treated with H_2SO_4 were dissolved in 2-3 ml *n*-hexane and subjected to a clean-up process. Clean-up was achieved by passing the fraction through a multi-layer column composed of Na_2SO_4 , silica gel, 10% (w/w) AgNO_3 -silica gel, silica gel, 22% (w/w) H_2SO_4 -silica gel, silica gel, 44% (w/w) H_2SO_4 -silica gel, silica gel, 2% (w/w) KOH-silica gel and silica gel (Miyata *et al.*, 1989). After elution with 150 ml of *n*-hexane the specimen was evaporated to a small volume. The concentrate was applied to an active carbon-impregnated silica gel column (Masuzaki *et al.*, 1999), washed with 200 ml of 25% (v/v) dichloromethane/ *n*-hexane, and then eluted with 200 ml of toluene. Five μl of *n*-nonane containing $^{13}\text{C}_{12}$ -1,2,3,4-TCDD and $^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDD spiking substances was added to this vessel (Safe, 1991). Samples dissolved in *n*-nonane were subjected to GC/MS.

GC/MS

PCDDs, PCDFs and Co-PCBs were analyzed by GC-MS, according to the method used by the Environmental Protection Agency, U.S. (EPA, USA, Office of Water, 1994). The analytical conditions were as follows: gas chromatography was performed with an HP 6890 series unit (Hewlett-Packard, Palo Alto, CA) equipped with a Finnigan MAT-95S (Finnigan MAT GmbH, Bremen, Germany). The column used was a

DB5MS fused silica capillary column, 0.25 mm i.d. $\times$ 60 m, with 0.25 μ m film thickness (J&W Scientific, Folsom, CA). The column temperature was maintained at 140 $^{\circ}$ C for 1 min, heated to 220 $^{\circ}$ C at a rate of 17 $^{\circ}$ C/min, heated to 310 $^{\circ}$ C at a rate of 3 $^{\circ}$ C/min, and maintained at 310 $^{\circ}$ C for 4 min. The injection temperature was 260 $^{\circ}$ C, ion source temperature was maintained at 250 $^{\circ}$ C and the carrier gas (helium) flow rate was 1.2 ml/min. The ionizing current, ionizing energy and accelerating voltage were 1 mA, 60 eV and 5 kV, respectively. The resolution of the mass spectrometer was maintained at about 10,000 throughout the work, and the analysis was carried out according to a selected ion monitoring (SIM) using 50 selected ions, M^{+} and M^{+2} for TCDD, TCDF and TCB, and M^{+2} and M^{+4} for other congeners. The ^{13}C standard of OCDF was not available. Deviations of the ion intensity ratio values of samples were less than 15% of the theoretical values.

A standard curve for quantification of each chemical was obtained using authentic congeners. Recovery of all congeners was more than 50%. Vehicle controls were lower than the detection limits for all dioxins examined in this study.

Statistical analysis

SPSS version 10.0J (1999) was used for the statistical analyses.

RESULTS

Levels of dioxins in the bile, blood and liver

Dioxin levels in amount and TEQ in the bile, blood and liver are summarized in Table 2. OCDD is the most abundant among 20 congeners in these organs, followed by three Co-PCBs on a pg/g lipid basis.

The total pg TEQ values per g lipid were almost the same in bile and blood at 43.2 ± 30.9 and 43.1 ± 24.2 , respectively (Table 2). On the other hand, it is three times higher in the liver, being 127.8 ± 57.4 pg TEQ. The lipid weights in bile, blood and liver were 1.24 ± 0.75 , 0.4 ± 0.04 and $5.09 \pm 2.50\%$, respectively, and total TEQ per unit tissue-weight was 3 times higher in bile than in blood, 38 times higher in liver than in blood, and 12 times higher in liver than in bile. Table 2 also shows the TEQ values of the congener groups. The contribution of each group to the total-TEQ was the same in blood and bile; however, the contribution of PCDDs was relatively small, and that of PCDFs was relatively large in the liver (PCDDs, 36%; PCDFs, 34%) compared with the bile and blood (PCDDs, 42%; PCDFs, 27%). Among 20 congeners, the contribution of 3,3',4,4',5-pentachlorobiphenyl (3,3',4,4',5-PeCB), 2,3,4,7,8-PeCDF and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD) were the most, accounting for more than 70% of the total-TEQ in all these three organs. 1,2,3,6,7,8-HxCDD and 2,3,7,8-TCDD fol-

Table 1. Diagnoses at death.

Female		Male	
Age	Disease	Age	Disease
29	Acute myelocytic leukemia	21	Cerebral palsy
33	Spinal cord tumor	50	Brain tumor
53	Polyarteritis	52	Dissecting aneurysm
58	Pancreatic carcinoma	63	Acute cardiac infarction
73	Rheumatoid arthritis	63	Lung cancer
74	Malignant lymphoma, Diabetes mellitus	65	Pulmonary fibrosis
75	Thalamic hemorrhage	66	Pancreatic carcinoma
76	Acute myelocytic leukemia	67	Hepatoma, Gastric cancer
77	Urinary bladder cancer	68	Primary biliary cirrhosis
83	Cor pulmonale	68	Urothelial cancer
84	Urothelial cancer	68	Malignant lymphoma, Colon cancer
85	Ovarian cancer	68	Prostate cancer
		73	Lung cancer
		77	Hepatoma, Malignant lymphoma
		77	Lung cancer

lowed and accounted for more than 10% of the total TEQ in the three organs.

The characteristics of organ distribution of each compound were examined (Table 3). Ratios of the values per g lipid in the bile to the blood were between 0.5 and 1.5, indicating that distribution in these body fluids of each compound was almost the same, except for OCDF, which was one-tenth the value in bile of that in blood. It was also revealed that highly chlorinated congeners of PCDDs and PCDFs were lower in bile than in blood, although the levels of all three PCB congeners were almost the same in the bile and blood.

The ratio of the amount in the liver to the blood on a g lipid basis revealed that 1,2,3,4,6,7,8-HpCDF has the highest tendency to accumulate in the liver; distribution to the blood was low, and to the bile was lower. The other highly chlorinated congeners of PCDDs and PCDFs showed a similar tendency, although the extent was lower in the other congeners. Among the three Co-

PCBs, the tendency was opposite to the number of chlorine atoms, and the ratio of the liver TEQ to the bile TEQ was the highest with 3,3',4,4'-TCB on a g lipid basis.

Daily secretion of dioxins into the bile

Daily secretion of bile from the liver is known to be about 500 ~1000 ml, and it is concentrated 5~10-fold in the gallbladder. Thus, the daily amount of gallbladder bile can be calculated as 100 ml, assuming that the daily bile secreted from the liver is 750 ml and concentrated 7.5-fold. Based on this value, the daily secretion into bile of the total-TEQ, and of each congener group is indicated in Table 4. Daily secretion was calculated to be 53.9 ± 52.5 pg TEQ at age 65, from an average of the 27 cases.

Relationship among bile, blood and liver TEQ

Correlation between blood TEQ and bile TEQ is

Table 2. PCDD / PCDF / Co-PCB levels in bile, blood and liver.

	Mean $\pm$ SD (pg / g lipid)						Mean $\pm$ SD (pg TEQ / g lipid)					
	Bile		Blood		Liver		Bile		Blood		Liver	
2,3,7,8-TCDD	3.6 $\pm$	3.5	2.6 $\pm$	2.0	8.0 $\pm$	6.5	3.6 $\pm$	3.5	2.6 $\pm$	2.0	8.0 $\pm$	6.5
1,2,3,7,8-PeCDD	10.5 $\pm$	8.3	9.9 $\pm$	6.4	24.4 $\pm$	16.8	10.5 $\pm$	8.3	9.9 $\pm$	6.4	24.4 $\pm$	16.8
1,2,3,4,7,8-HxCDD	2.9 $\pm$	2.9	4.1 $\pm$	3.1	10.8 $\pm$	6.1	0.3 $\pm$	0.3	0.4 $\pm$	0.3	1.1 $\pm$	0.6
1,2,3,6,7,8-HxCDD	29.7 $\pm$	24.7	41.1 $\pm$	24.9	101 $\pm$	52.7	3.0 $\pm$	2.5	4.1 $\pm$	2.5	10.1 $\pm$	5.3
1,2,3,7,8,9-HxCDD	4.2 $\pm$	3.2	6.1 $\pm$	4.8	12.7 $\pm$	6.3	0.4 $\pm$	0.3	0.6 $\pm$	0.5	1.3 $\pm$	0.6
1,2,3,4,6,7,8-HpCDD	20.0 $\pm$	47.4	43.0 $\pm$	112	144 $\pm$	170	0.2 $\pm$	0.5	0.4 $\pm$	1.1	1.4 $\pm$	1.7
OCDD	305 $\pm$	596	548 $\pm$	615	2650 $\pm$	3860	0.0 $\pm$	0.1	0.1 $\pm$	0.1	0.3 $\pm$	0.4
2,3,7,8-TCDF	1.1 $\pm$	1.2	0.9 $\pm$	1.0	2.4 $\pm$	1.8	0.1 $\pm$	0.1	0.1 $\pm$	0.1	0.2 $\pm$	0.2
1,2,3,7,8-PeCDF	0.6 $\pm$	0.6	0.9 $\pm$	0.7	3.0 $\pm$	2.3	0.0 $\pm$	0.0	0.0 $\pm$	0.0	0.2 $\pm$	0.1
2,3,4,7,8-PeCDF	19.7 $\pm$	16.4	17.7 $\pm$	13.0	61.3 $\pm$	40.1	9.9 $\pm$	8.2	8.8 $\pm$	6.5	30.6 $\pm$	20.1
1,2,3,4,7,8-HxCDF	5.6 $\pm$	4.2	8.4 $\pm$	6.7	32.2 $\pm$	19.3	0.6 $\pm$	0.4	0.8 $\pm$	0.7	3.2 $\pm$	1.9
1,2,3,6,7,8-HxCDF	6.4 $\pm$	6.4	10.5 $\pm$	9.3	53.9 $\pm$	38.2	0.6 $\pm$	0.6	1.1 $\pm$	0.9	5.4 $\pm$	3.8
2,3,4,6,7,8-HxCDF	2.8 $\pm$	2.4	5.9 $\pm$	7.0	26.8 $\pm$	19.2	0.3 $\pm$	0.2	0.6 $\pm$	0.7	2.7 $\pm$	1.9
1,2,3,7,8,9-HxCDF	0.3 $\pm$	0.5	0.7 $\pm$	0.8	2.7 $\pm$	2.4	0.0 $\pm$	0.1	0.1 $\pm$	0.1	0.3 $\pm$	0.2
1,2,3,4,6,7,8-HpCDF	2.2 $\pm$	3.1	5.3 $\pm$	6.2	53.0 $\pm$	61.6	ND		0.1 $\pm$	0.1	0.5 $\pm$	0.6
1,2,3,4,7,8,9-HpCDF	0.2 $\pm$	0.6	0.7 $\pm$	1.1	8.0 $\pm$	6.6	ND		ND		0.1 $\pm$	0.1
OCDF	0.3 $\pm$	0.6	0.7 $\pm$	1.1	8.2 $\pm$	10.7	ND		ND		ND	
3,3',4,4'-TCB	34.2 $\pm$	78.4	62.4 $\pm$	62.4	210 $\pm$	369	ND		ND		ND	
3,3',4,4',5-PeCB	125 $\pm$	105	122 $\pm$	71.3	353 $\pm$	180	12.5 $\pm$	10.5	12.2 $\pm$	7.1	35.3 $\pm$	18.0
3,3',4,4',5,5'-HxCB	127 $\pm$	244	121 $\pm$	87.5	274 $\pm$	162	1.3 $\pm$	2.4	1.2 $\pm$	0.9	2.7 $\pm$	1.6
PCDDs	376 $\pm$	649	655 $\pm$	724	2950 $\pm$	4020	18.0 $\pm$	13.8	18.1 $\pm$	10.9	46.6 $\pm$	25.7
PCDFs	39.1 $\pm$	30.6	51.6 $\pm$	39.2	251 $\pm$	129	11.5 $\pm$	9.3	11.6 $\pm$	8.4	43.2 $\pm$	24.5
Co-PCBs	286 $\pm$	309	306 $\pm$	181	837 $\pm$	491	13.7 $\pm$	11.1	13.5 $\pm$	7.6	38.0 $\pm$	19.0
Total	701 $\pm$	773	1010 $\pm$	826	4040 $\pm$	4110	43.2 $\pm$	30.9	43.1 $\pm$	24.2	128 $\pm$	57.4

demonstrated in Fig. 1. The correlation coefficient r was 0.89, and the regression equation was $y = 1.14x - 6.02$, where y indicates total-TEQ per g lipid of bile, and x is total-TEQ per g lipid of the blood. We also examined the relationship between blood total-TEQ and liver total-TEQ. As demonstrated in Fig. 1, this also showed good correlation, with a correlation coefficient of 0.73, and the relationship can be expressed as $y = 1.74x + 52.9$, where y is total-TEQ in terms of per g lipid of liver and x is total-TEQ per g lipid of blood.

Accumulation rate of dioxins in bile, blood and liver

Although the number of samples was not large, the ages of the subjects were distributed relatively well between 21 and 85. Therefore, we examined by age accumulation rates of dioxins in the bile, blood and liver, for total-TEQ and each of five congeners mostly contributing to the total-TEQ. Regression analysis between total-TEQ and age revealed that there is rela-

tively good correlation in these organs, with correlation coefficients of 0.52 ($p < 0.01$), 0.47 ($p < 0.05$) and 0.54 ($p < 0.01$) in bile, blood and liver, respectively (Table 5). Further it was estimated that dioxins appeared in bile after about age 20, in contrast to the blood in which dioxins appeared from age zero (Fig. 2.).

In bile, all of the major five congeners also showed a positive correlation between age and their levels, although there are some exceptional cases in blood and liver (Table 5). Regression lines indicated that all of the major five congeners appeared after about age 20 in bile, although appearance patterns are different by congener in blood and liver (Fig. 2).

Relationship between dioxin levels and disease at death

Eight of 12 cases of women and 10 of 15 cases of men were associated with malignant changes: three lung cancers, three malignant lymphomas, two urothe-

Table 3. Comparisons of PCDD / PCDF / Co-PCB levels among bile, blood and liver.

	Bile / Blood	Liver / Blood	Liver / Bile
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
pg /g lipid basis			
2,3,7,8-TCDD	1.5 $\pm$ 1.5	3.3 $\pm$ 2.7	3.7 $\pm$ 5.1
1,2,3,7,8-PeCDD	1.1 $\pm$ 0.6	3.2 $\pm$ 3.2	3.1 $\pm$ 2.4
1,2,3,4,7,8-HxCDD	0.7 $\pm$ 0.4	3.7 $\pm$ 2.9	7.0 $\pm$ 6.0
1,2,3,6,7,8-HxCDD	0.7 $\pm$ 0.5	3.1 $\pm$ 2.5	6.5 $\pm$ 7.1
1,2,3,7,8,9-HxCDD	0.8 $\pm$ 0.7	3.1 $\pm$ 2.6	4.4 $\pm$ 4.6
1,2,3,4,6,7,8-HpCDD	0.7 $\pm$ 0.6	9.3 $\pm$ 12.1	24.8 $\pm$ 41.0
OCDD	0.8 $\pm$ 1.1	7.2 $\pm$ 6.6	20.4 $\pm$ 33.2
2,3,7,8-TCDF	1.5 $\pm$ 1.8	2.1 $\pm$ 1.3	2.6 $\pm$ 2.3
1,2,3,7,8-PeCDF	0.8 $\pm$ 1.2	3.9 $\pm$ 3.7	6.4 $\pm$ 10.5
2,3,4,7,8-PeCDF	1.1 $\pm$ 0.6	4.2 $\pm$ 2.2	4.9 $\pm$ 4.8
1,2,3,4,7,8-HxCDF	0.8 $\pm$ 0.5	5.4 $\pm$ 3.6	7.9 $\pm$ 5.1
1,2,3,6,7,8-HxCDF	0.7 $\pm$ 0.5	7.6 $\pm$ 6.7	15.2 $\pm$ 16.2
2,3,4,6,7,8-HxCDF	0.7 $\pm$ 0.6	7.3 $\pm$ 5.8	16.3 $\pm$ 16.9
1,2,3,7,8,9-HxCDF	0.7 $\pm$ 1.7	4.0 $\pm$ 5.0	5.6 $\pm$ 8.7
1,2,3,4,6,7,8-HpCDF	0.5 $\pm$ 0.6	21.6 $\pm$ 34.3	39.6 $\pm$ 58.6
1,2,3,4,7,8,9-HpCDF	0.5 $\pm$ 1.1	8.5 $\pm$ 8.8	12.3 $\pm$ 13.3
OCDF	0.1 $\pm$ 0.2	6.1 $\pm$ 10.7	18.9 $\pm$ 33.9
3,3',4,4'-TCB	0.8 $\pm$ 1.2	8.1 $\pm$ 13.7	13.6 $\pm$ 27.0
3,3',4,4',5-PeCB	1.1 $\pm$ 0.7	3.8 $\pm$ 2.7	4.8 $\pm$ 4.0
3,3',4,4',5,5'-HxCB	0.9 $\pm$ 0.9	2.9 $\pm$ 1.8	6.8 $\pm$ 7.2
Congener group-pg TEQ/g lipid basis			
PCDDs	1.0 $\pm$ 2.1	1.9 $\pm$ 1.9	2.7 $\pm$ 4.5
PCDFs	0.9 $\pm$ 1.4	3.5 $\pm$ 4.3	3.7 $\pm$ 5.8
Co-PCBs	1.0 $\pm$ 1.5	2.0 $\pm$ 1.4	2.7 $\pm$ 3.3
Total	1.1 $\pm$ 2.8	2.6 $\pm$ 3.2	3.2 $\pm$ 7.3

lial cancers, two pancreatic cancers, two hepatomas, two acute myelocytic leukemias and one cancer in the spinal cord, ovary, brain, stomach, colon, bladder and prostate. Three cases in men were associated with double cancers. Total-TEQ ranged from 17.1 to 287.3 pg TEQ per g lipid in the liver. However, there was no correlation between cancer diagnosis and the level of dioxins.

DISCUSSION

This study first demonstrated that the dioxin level in bile is almost the same as the level in blood in terms of TEQ per g lipid. The same level as in blood was observed not only as the mean, but also in the 27 cases, with a correlation coefficient of 0.89. The bile and blood levels were one-third of that in the liver in terms

of TEQ per g lipid. Iida *et al.* (1999) also reported a similar trend in the ratio between blood and liver, although the ratio of liver to blood was 4.6 in their cases. It is noteworthy that the dioxin burden in liver is 38 times higher than that in blood in terms of per organ weight. These dioxins in liver would be distributed to various organs, including bile and blood. However, there was a tendency that congeners with higher numbers of chlorine atoms were less efficiently distributed to bile when compared with blood, and OCDF was hardly distributed to bile.

It has been reported that 2,3,7,8-TCDD is not detected in the bile of guinea pigs after intra-peritoneal administration, and it was suggested that the fecal excretion of 2,3,7,8-TCDD in this animal resulted from direct intestinal elimination (Olson, 1986). However, in the present study it was demonstrated that 2,3,7,8-TCDD is present in human bile at levels similar to blood, in terms of per g lipid.

Regression analysis of the 27 cases revealed that bile dioxin levels (total-TEQ) can be deduced based on blood total-TEQ levels with an equation of $y = 1.14x - 6.02$, where x = total-TEQ value of blood (on g lipid basis). Further, we can deduce liver dioxin levels, by the equation of $y = 1.74x + 52.9$ with a correlation coefficient of 0.73, with x = total-TEQ value of blood. Iida *et al.* (1999) also demonstrated the presence of a good correlation between the blood and liver in levels of several congeners of PCDDs, PCDFs and Co-PCBs, although they did not conduct studies on total-TEQ.

We recently clarified that about 50% of the total-TEQ of dioxins ingested is excreted, 22% in feces and 29% in sebum (Kitamura *et al.*, 2001). The fate of the remaining 50% is not known. One possibility is accumulation in the body. Therefore, we examined the effect of age on dioxin levels, and it was revealed that total-TEQ and the major five congeners mostly contributing to the total-TEQ increased with age in bile, blood and liver (Fig.2). The accumulation rate of dioxins was estimated to be 0.99, 0.70 and 1.91 pg total-TEQ/ g lipid / year in bile, blood and liver, respectively (Table 5). However, it is necessary to pay attention to the fact that these rates might be largely affected by the assize of historical exposure. The accumulation rate was about 2 times higher in the liver than in the bile or blood (Table 5). It is noteworthy that dioxins are estimated to be present at age naught in the blood and liver; however, they showed a tendency to appear in bile after about age 20. Since the number of samples of about age 20 was small, and furthermore, we did not examine samples from less than age 20, it is necessary

Table 4. Daily secretion of dioxins into bile.

	pg TEQ/day
PCDDs	22.8 ± 23.2
PCDFs	14.3 ± 14.3
Co-PCB	16.7 ± 17.7
Total	53.9 ± 52.5

Fig. 1. Dioxin TEQ: Relationship among blood, bile and liver. Open circles indicate bile and blood TEQ, and closed circles liver and blood TEQ of each autopsy sample.

Dioxins in bile of humans.

Fig. 2-1. Relationships between age and total-TEQ, and age and the major five dioxin congeners. a), total-TEQ in bile, blood and liver: b), 2,3,7,8-TCDD in bile, blood and liver: c), 1,2,3,7,8-PeCDD in bile, blood and liver.

Fig. 2-2. Relationships between age and total-TEQ, and age and the major five dioxin congeners. d), 1,2,3,6,7,8-HxCDD in bile, blood and liver: e), 2,3,4,7,8-PeCDF in bile, blood and liver: f), 3,3',4,4',5-PeCB in bile, blood and liver.

to confirm this point. However, by assuming that this is the case, two possible mechanisms for the delayed appearance in bile can be considered: secretion to bile might occur after accumulation of dioxins in liver has reached certain levels, or processes involved in the metabolism of dioxins might change after age about 20.

It was calculated that daily secretion of dioxins from bile is 54 pg TEQ, which corresponds to 30~40% of the total ingestion of dioxins of the Japanese, based on the data from a study by the Ministry of Health and Welfare (Toyoda *et al.*, 1999). Since our previous study (Kitamura *et al.*, 2001) indicated that 80% of dioxins to which intestines are exposed might be absorbed, prevention of absorption from intestines would be one important approach to reducing the body burden of dioxins. Aozasa *et al.* (2000) reported efficient elimination of dioxins by feeding chitosan-bound chlorophylline to rats. This line of study may be useful to reduce the body burden of dioxins in humans.

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Table 5. Relation between age and TEQ.

Dioxin congeners	Organ	Regression equation*		Correlation coefficient	p value
		a	b		
20 Congeners (total)	Bile	0.991	- 20.9	0.520	<0.01
	Blood	0.704	- 2.37	0.473	<0.05
	Liver	1.91	4.36	0.539	<0.01
2,3,7,8-TCDD	Bile	0.082	- 1.74	0.386	<0.05
	Blood	0.048	- 0.567	0.402	<0.05
	Liver	0.182	- 3.82	0.467	<0.05
1,2,3,7,8-PeCDD	Bile	0.203	- 2.76	0.407	<0.05
	Blood	0.185	- 2.09	0.479	<0.05
	Liver	0.238	8.86	0.236	>0.05
1,2,3,6,7,8-HxCDD	Bile	0.065	- 1.26	0.438	<0.05
	Blood	0.031	2.07	0.210	>0.05
	Liver	0.099	3.70	0.313	>0.05
2,3,4,7,8-PeCDF	Bile	0.206	- 3.55	0.421	<0.05
	Blood	0.199	- 4.10	0.511	<0.01
	Liver	0.494	- 1.53	0.411	<0.05
3,3',4,4',5-PeCB	Bile	0.280	- 5.73	0.446	<0.05
	Blood	0.089	6.46	0.208	>0.05
	Liver	0.474	4.37	0.440	<0.05

* Regression equation, $y = ax + b$ (pg TEQ /g lipid), $x = \text{age (year)}$

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