CONCENTRATION OF POLYCHLORINATED DIBENZO-p-DIOXINS AND THEIR RELATED COMPOUNDS IN THE HUMAN BILE IN RELATION TO THOSE IN THE LIVER AND BLOOD

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Introduction

PCDDs and related compounds are persistent environmental contaminants to cause adverse biological effects (1). It is important to find the way for excreting accumulated dioxins from the heavily exposed human body to prevent the future illness. We investigated PCDDs/PCDFs/coplanar PCBs levels in bile from the six autopsy cases and compared of these to those in the liver and blood. The concentrations between PCDDs, PCDFs and PCBs in bile, blood and liver was analyzed and the possibility of enhanced excretion from the body was discussed.

Subjects and Methods

Subjects: Six autopsy cases were provided (Table 1). All cases were autopsied within 2 hours after death. More than 50 ml cardiac blood, about 15 g liver tissues, and about 50 ml bile from the gallbladder were storered in a deep refrigerator until analysis. Permission for analyzing dioxins was obtained from bereaved family.

Table 1 Outline of autopsy

No	Age	Sex	Cause of death	Couse	Occupation
15640	33	Female	Spinal tumor	5 months	House wife
15643	75	Female	Cerebralpalsy	5 days	House wife
15671	53	Female			
15645	68	Male	Pneumonia	2 months	
15649	63	Male			
15678	50	Male			

Preparation of analytical samples from blood and organ specimens : The weights of liver, bile and blood from the normal subjects were approximately 3, 40 and 50g, respectively. $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$-PCBs were added as internal standard to liver homogenates and bile samples and extracted three times with 50 ml each of acetone/ hexane (2:1) (2,3). For blood samples, each sample was extracted three times with each of 50ml ethanol/ hexane (1:3) (4). These extracts were washed with distilled water. The n-hexane layers were dried over anhydrous sodium sulfate, evaporated to dryness, and the residual lipid were weighted. The residues were dissolved into 2-3ml of n-hexane and applied to an “multi-layer column” reported by Miyata et al (5). 150Ml of n-hexane was passed through the column and the effluent was evaporated. The concentrates were applied to an “AC column”, washed with 50ml of 10% (v/v) dichloromethane/ n-hexane,

then eluted with 150ml of Toluene. The elutes were evaporated at room temperature to almost empty. 5μl of n-nonane containing $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD spiking substances were added to this "empty " vessel.

Chemicals: Native polychlorinated dibenzodioxins (PCDDs), native polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs) and authentic standards for the above compounds were purchased from the Cambridge Isotope Laboratories (Massachusetts, USA). The internal standards, $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$-PCBs were also purchased from the above company. An active carbon (Ac) column was prepared followed by the method in literature (2). 10%Silver nitrate/ silica gel (AgSi), 22% sulfuric acid/ silica gel, 44% sulfuric acid/ silica gel and 2% potassium hydroxide/ silica gel were purchased from the Wako Pure Chemicals Ind. Co. Ltd. (Osaka, Japan). Ultra-pure water was supplied from a Milli-Q SP TOC system (the Japan Millipore Co. Ltd., Tokyo, Japan).

Analysis of PCDDs and their related compounds: A GC/MS, which consisted of a Finnigan MAT-95S mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) and a HP-6890A gas chromatograph (Hewllet-Packard, Palo Alto, California, U.S.A.) was used. A DB-5MS fused silica capillary column, 0.25 mm i.d. ×60m, df=0.25mm (J&W Scientific, Folsom, California, U.S.A.) was operated at the column temperatures of: 120 ℃ for 1 min, heated to 220 ℃ at the program rate of 15 ℃/min, to 300℃ at the rate of 3℃/min, and finally, maintained at 300 ℃ for 10min. The resolution of a mass spectrometer was maintained from 10,000 to 12,000 throughout the work. The analysis was carried out according to a SIM using 42 selected ions. The injection temperature and ion source temperature were maintained at 260℃, and the carrier gas (helium) pressure was 14 psi. Ionizing current, ionizing energy, accelerating voltage, and ion multiplier voltage were 1mA, 60eV, 5kV and 2kV, respectively.

Results and Discussion

There is a limited information on the occurrence of PCDDs and related compounds in human bile. Rappe et al. (6) reported that a direct assessment of exposure can be suggested from the analytical values of blood, adipose and other tissue samples and feces and bile.

Concentrations of PCDDs, PCDFs and Co-PCBs are shown in Table 2 (Table 2). Concentrations of TCDD and PeCDD in bile and blood were almost similar, but those of highly chlorinated dioxins yielded less in the bile. OCDD was the least in bile, and it was high in both blood and liver. This trend was similar in the concentrations of dibenzofurans, although highly chlorinated PCDFs were rare in normal human body. Total TEQ in the bile, blood and liver was shown below.

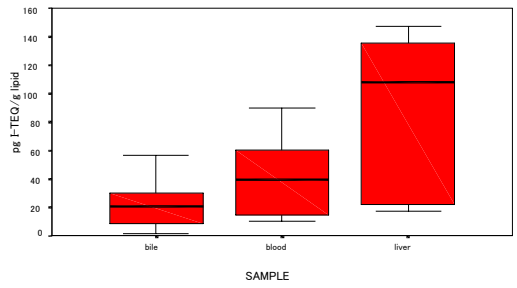

Table 2. Concentrations of PCDDs/PCDFs/Co-PCBs in samples

	bile median	blood median	liver median
D2378	0.47	1.07	2.29
D12378	3.89	3.82	15.73
D123478	1.55	3.14	4.25
D123678	22.31	30.86	90.6
D123789	2.47	4.4	9.81
D1234678	7.85	28.68	69
OCDD	211.34	1235.32	1837.08
F2378	0.57	1.24	0.92
F12378	0.51	0.66	1.52
F23478	8.46	22.7	31.77
F123478	3.56	8.08	26.29
F123678	6.11	11.18	61.13
F234678	1.63	3.59	13.13
F123789	0.45	0.75	1.87
F1234678	2.26	5.54	30.24
F1234789	0.54	1.18	8.04
OCDF	0.53	.	1.82
P77	19.03	42.85	10.75
P126	60.97	110.61	223.51
P169	55.22	66.37	123.53
TEQ	20.92	39.67	107.98

Table 3 shows the correlations of PCDDs and related compounds in bile and the liver to those in the blood. For bile, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF and 2,3,4,6,7,8-HxCDF had significant correlations to those in blood ($r > 0.832$) and 1,2,3,4,6,7,8-HpCDD, OCDD, 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. A 3,4,5,3',4'-PeCB had a good correlation to those in the liver ($r > 0.854$). For the liver, 1,2,3,4,6,7,8-HpCDD, OCDD and 2,3,4,6,7,8-HxCDF had good correlations to those in the blood ($r > 0.902$).

PCDDs, PCDFs and PCBs in the environment have been extensively studied in the past, but their influence on humans is still unclear, except for the high dose exposure by accident (7). We found the workers in the waste incinerator in Japan were chronically exposed to certain level of dioins. Excretion of accumulated dioxins in the body becomes urgently necessary. In this study, we found that the congener levels in the bile was quite similar to that of the congener levels in the blood, especially for low chlorinated dioxins. This suggested that the excretion of dioxins may be promoted by controling bile excretion and reabsorption from the intestine.

Table 3 Correlations between blood, bile and the liver by each congener

Congener	Blood	Bile	Liver	Congeners	Liver	Bile
2378-TCDD	1.000	0.149	0.526	12378-PeCDD	1.000	0.198
123678-HxCDD	1.000	0.138	0.047	123678-HxCDD	1.000	0.362
123789-HxCDD	1.000	0.899	0.362	123789-HxCDD	1.000	0.506
1234678-HpCDD	1.000	0.996	0.985	1234678-HpCDD	1.000	0.996
OCDD	1.000	0.859	0.902	OCDD	1.000	0.990
Total (PCDD)	1.000	0.907	0.934	Total (PCDD)	1.000	0.991
12378-PeCDF	1.000	0.386	0.237	12378-PeCDF	1.000	0.363
23478-PeCDF	1.000	0.628	0.115	23478-PeCDF	1.000	0.365
123478-HxCDF	1.000	0.924	0.765	123478-HxCDF	1.000	0.939
123678-HxCDF	1.000	0.832	0.587	123678-HxCDF	1.000	0.906
234678-HxCDF	1.000	0.834	0.918	234678-HxCDF	1.000	0.936
1234678-HpCDF	1.000	0.166	0.348	123789-HxCDF	1.000	0.869
Total(PCDF)	1.000	0.794	0.391	1234678-HpCDF	1.000	0.764
			1234789-HpCDF	1.000	0.176	
Total-TEQ(PD+DF)	1.000	0.912	0.935	OCDF	1.000	0.746
343'4'-TCB	1.000	-0.219	-0.099	Total(PCDF)	1.000	0.865
3453'4'-PeCB	1.000	0.455	0.219	Total-TEQ(PD+DF)	1.000	0.991
3453'4'5'-HxCB	1.000	-0.272	-0.428	343'4'-TCB	1.000	0.218
Total(PCB)	1.000	0.091	-0.198	3453'4'-PeCB	1.000	0.854
Total	1.000	0.866	0.881	3453'4'5'-HxCB	1.000	0.710
				Total (PCB)	1.000	0.78
				Total	1.000	0.991

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