MONO- TO OCTA- CHLORINATED DIBENZO-*p*-DIOXINS AND FURANS IN HUMAN SERUM: INDICATOR CONGENERS AND TEQS

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Abstract

The mono- to tri-chlorinated compounds have been less studied, however the less chlorinated PCDD/Fs may give valuable information for environmental and biological samples. Similarly, the isomer profiles can provide to estimate the origin of dioxin and related compounds. The mean human serum concentrations of $\sum Cl_{1\text{-}8}DD/F$ and 17-toxic congeners were 1890 and 398 pg/g lipid (11.9 I-TEQ pg/g lipid), respectively. The profile for PCDDs was dominated by most chlorinated congeners: OCDD (> 58%), while decreasing concentrations with increasing degree of chlorination were seen for PCDFs; MCDF(> 83%), DiCDF (> 6 %). $\sum Cl_{1\text{-}3}DD/Fs$ accounted for 77% of the serum concentrations of $\sum Cl_{1\text{-}8}DD/F$. These results suggest that we are exposed to a large amount of $\sum Cl_{1\text{-}3}DD/Fs$. Moreover, MCDF contributed more than 60% of $\sum Cl_{1\text{-}8}DD/Fs$ and highly correlated with $\sum Cl_{1\text{-}8}DD/Fs$. Above all, 2-MCDF could be a predictive indicator for $\sum Cl_{1\text{-}8}DD/Fs$ (r=0.94). In regard to TEQ concentrations, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD were predominant congeners and contributed more than 65 % of TEQ concentrations.

Introduction

Due to the high toxicity of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) substituted in 2,3,7,8-poistion, a lot of attention was paid to the congener specific analysis of tetra- to octachlorinated dibenzo-*p*-dioxins and dibenzofurans in the last two decades. Since the 17 toxic congeners exhibit the dioxin related toxicity, not a lot of attention was paid to less chlorinated congeners were not assigned with TEF values [1,2].

However, the analysis of less chlorinated congeners seems interesting from several point of view. For the investigation of the mechanisms of PCDD/F formation, the less chlorinated compounds offer valuable additional information. For on-line measurement of PCDD/Fs, some recent research projects focused on estimating TEQ values by measurement of less chlorinated PDDD/F as surrogates[3]. To establish this correlation, it seems important to measure not only the total amount of less chlorinated homologues but to calculate and correlate specific congeners. Furthermore, the less chlorinated PCDD/Fs may give valuable information for environmental and biological samples. There is no information, to our knowledge, about those compounds in human samples. The analysis of congener distributions in human serum samples is the key to estimating the origin of dioxins and related compounds.

Materials and Methods

Sampling & Storage: Seventy one human serum samples consisted of 32 males and 39 females were obtained in 2006 from volunteer MSWI workers (n=11), nearby residents (n=49), and referential inhabitants (n=11) in Korea. Information such as age, smoking, dietary habit, body weight and height etc. was obtained from a questionnaire survey. All samples were kept frozen at –20℃ until analysis.

Sample analysis: Approximately 30 g was analyzed for congener-specific PCDD/Fs as well as lipid content. Unfrozen serum samples were spiked with a mixture of $^{13}C_{12}$-labeled PCDD/Fs as internal standards. They were mixed with sodium oxalate saturated water. The solution was extracted 3 times using 200 mL of 2:1 acetone/hexane for each extraction. The resultant organic layer was filtered and evaporated to dryness in order to evaluate the lipid content in the samples. Lipid content was determined gravimetrically. Dried samples were resuspended in hexane and subjected to further cleanup via multilayer-silica, alumina column. Identification and quantification of each congener was performed on HP 6890N gas chromatograph coupled to a JEOL JMS-800D high-resolution mass spectrometer.

Data analysis: Descriptive statistics were used to characterize the level of the congeners and homologues in the

serum samples. One-way analysis of variance (ANOVA) was used for normally distributed variables and Kruskal-Wallis test, for the remaining variables. The correlation between the variables (age, proportional body fat, body burden, etc.) and serum concentrations of the congeners was examined using Pearson correlation coefficients. All statistical analyses were carried out using SPSS 12.0. A significance level of 0.05 was used for all tests.

Results and Discussion

PCDD/F Levels in Human Serum

The mean concentrations of $\sum Cl_{1\text{-}8}$DD/Fs and 17-toxic congeners were 1890 pg/g lipid and 398 pg/g lipid (11.9 WHO-TEQ pg/g lipid), respectively. The $\sum Cl_{1\text{-}8}$DD/F concentrations and those of the individual homologues in the sera from 3 groups were similar, and there was no significant difference among the 3 groups except for TEQs (Table 1). The profile for PCDDs was dominated by most chlorinated congeners: OCDD (> 58%), while decreasing concentrations with increasing degree of chlorination were seen for PCDFs; MCDF(> 83%), Cl_2DF (> 6 %) . $\sum Cl_{1\text{-}3}$DFs contributed about 94% of PCDFs detected in human blood samples. Due to lower octanol water partition coefficient for less chlorinated compounds, it is difficult to bioaccumulate[4]. Nevertheless, $\sum Cl_{1\text{-}3}$DD/Fs accounted for 77% the serum concentrations of $\sum Cl_{1\text{-}8}$DD/F. These results suggest that we are exposed to a large amount of $\sum Cl_{1\text{-}3}$DD/Fs. Especially, MCDF contributed more than 60% of $\sum Cl_{1\text{-}8}$DD/Fs and highly correlated with $\sum Cl_{1\text{-}8}$DD/Fs. In regard to TEQ concentrations, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD were predominant congeners and contributed more than 65 % of TEQ concentrations.

Table 1. Each homologue and total PCDD/F and TEQ concentrations (pg/g lipid) in human serum

	Total (n=71)		Resident (n=49)		Worker (n=11)		Reference (n=11)		*p*-value
	mean	median	mean	median	mean	median	mean	median	
MCDD*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
DiCDD	170	165	168	158	125	156	224	207	0.56
TrCDD	15.6	6.72	13.7	6.20	9.16	7.64	30.7	9.72	0.35
TeCDD	4.91	2.24	4.87	2.12	5.71	5.37	4.26	ND	0.71
PeCDD	6.38	4.49	6.41	3.74	3.46	3.30	9.14	7.50	0.06
HxCDD	10.5	10.2	11.0	10.3	6.13	6.40	12.6	12.8	0.23
HpCDD	15.3	12.2	17.0	12.8	8.93	8.28	14.0	13.2	0.05
OCDD	318	215	355	215	193	165	282	263	0.37
MCDF	1130	1000	1090	537	1140	927	1260	1380	0.79
DiCDF	83.5	82.3	85.1	85.1	71.1	77.5	88.9	82.3	0.77
TrCDF	72.3	67.2	72.3	67.2	58.0	58.0	86.2	77.8	0.36
TeCDF	9.59	8.52	9.52	7.49	8.06	7.25	11.5	8.77	0.63
PeCDF	16.3	12.6	17.7	13.1	10.7	10.9	15.5	16.1	0.16
HxCDF	27.1	23.7	29.7	23.9	17.7	17.0	25.3	25.1	0.17
HpCDF	14.3	9.94	15.1	9.81	11.8	9.24	13.4	11.5	0.93
OCDF	1.69	ND	1.20	ND	4.19	ND	1.40	ND	0.03
$\sum Cl_{1\text{-}8}$DD/Fs	1890	1600	1900	1340	1680	1450	2080	2120	0.78
$\sum Cl_{4\text{-}8}$DD/Fs	424	299	467	325	270	211	389	375	0.11

$\sum Cl_{1-3}DD/Fs$	1470	1320	1430	992	1410	1260	1690	1910	0.78
TEQs	11.9	10.1	12.9	10.3	6.6	7.0	12.6	11.4	0.00

*N/A; not available due to recovery rate (lower than 10%)

Due to no difference among 3 groups, we additionally compared between concentrations of the males and those of the females. There were also no remarkable differences for all homologues between gender groups except for HpCDD and OCDF. Moreover, $\sum Cl_{4-8}DD/Fs$ of the females were higher than those of the males. Although levels of the males were relatively lower than those of the females, distribution fraction of $\sum Cl_{1-3}DD/Fs$ for the males was significant higher than those for the females; 84% vs. 73%. That result may be come from differences of dietary habit, ventilation rate, and metabolic rate, etc.

Distribution of Monochlorinated Dibenzofuran Congeners

The overall serum MCDF concentration of all subjects was 1130 pg/g lipid. 2-MCDF was the most predominant congener which contributed 53.8% and 26.9% of total MCDF and total PCDD/F concentrations, respectively. 3-MCDF accounted for more than 24% of total MCDFs. Each 1-MCDF and 4-MCDF contributed about 10% of total MCDFs (Figure 1(a)). Ryu *et al.* found that the 2- and 3-MCDF isomer fractions were greater than the 1- and 4-MCDF isomer fractions in flue gas sample from stoker-type municipal waste incinerator[5]. Although no information is available for food and air about MCDF profiles, comparison between serum and stack MCDF profiles indicates that 2-MCDF is more resistant to degrade and easier to accumulate in human than other MCDF congers. A study about 2-MCDF toxicity showed that 2-MCDF tended to remain more in the adipose tissue and whole blood than 3-MCDF, even though results of the rats experiment[6].

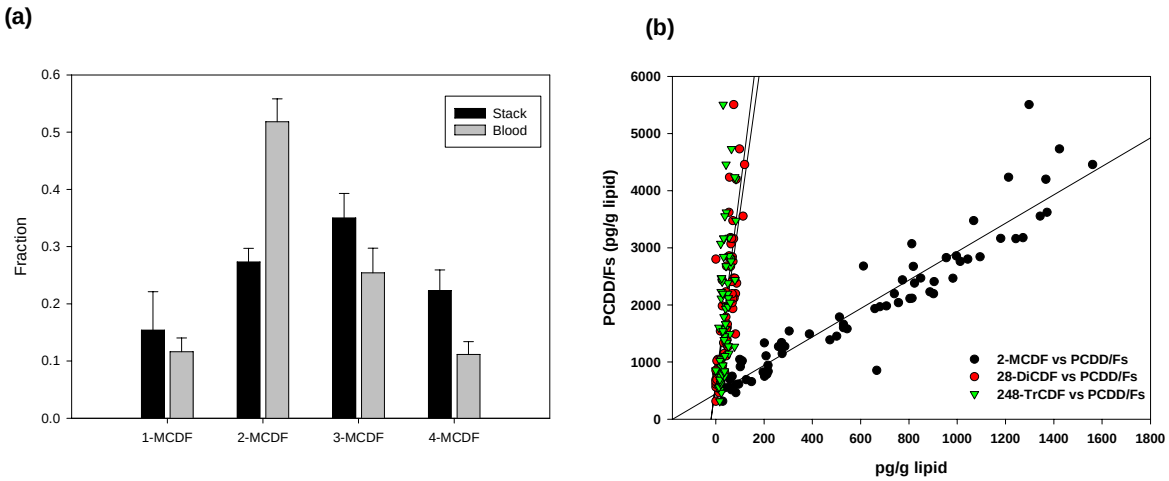

Figure 1. (a) MCDF distribution fraction of MWI stack gas (data from Ryu *et al.*) and human serum, (b) scatterplots of total PCDD/Fs versus 2-MCDF, 2,8-DiCDF, and 2,4,8-TrCDF congener

Indicator Congener

$\sum Cl_{1-8}DFs$ contributed more than 70% of $\sum Cl_{1-8}DD/Fs$, and moreover, $\sum Cl_{1-3}DFs$ accounted for 68% of $\sum Cl_{1-8}DD/Fs$; hence, we investigated the correlation between the serum concentrations of $\sum Cl_{1-8}DD/F$ and each of PCDF homologue. Thus, we found that MCDF showed the highest correlation coefficient value (r=0.96). 2-MCDF, 2,8-DiCDF, and 2,4,8-TrCDF were the predominant congeners in each homologue. These congeners also showed correlation to $\sum Cl_{1-8}DD/Fs$ (Figure 2(b)). 2-MCDF, 2,8-DiCDF, and 2,4,8-TrCDF had correlation coefficient values of 0.94, 0.77, 0.55, respectively. Therefore, 2-MCDF could be a predictive indicator for $\sum Cl_{1-8}DD/Fs$, although 2-MCDF was relatively rapidly metabolized and excreted from the body.

In summary, considering all PCDD/F congeners including 17 toxic-compounds, we are exposed to highly amount of PCDD/Fs and less chlorinated dibenzofurans accounted for more than 95% of total serum PCDF concentrations. The mean serum concentrations of $\sum Cl_{1\text{-}8}DD/F$ and TEQ were 1890 and 11.9 pg/g lipid. MCDF was the predominant homologue and contributed about 60% of $\sum Cl_{1\text{-}8}DD/Fs$, especially, 2-MCDF had strong correlation. Up to now, there is little information about less chlorinated dibenzo-*p*-dioxin/furans. Further environmental and food monitoring would be useful to get the valuable information by comparing full congener profiles of human serum samples.

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References

1. Buser H.-R. and Rappe C. *Anal. Chem.*1991; 56: 442
2. Ryan J.J., Conacher H.B.S., Panopio L.G., Lau B.P.-Y., Hardy J.A., and Masuda Y. *J. Chromatogr.* 1991; 541: 131
3. Gullet B.K. and Wikstroem E. *Organohalogen Compounds* 1998; 36: 179
4. Barron M.G. *Environ. Sci. Technol.* 1990; 24: 1612
5. Ryu J.-Y., Mulholland J.A., Dunn J.E., Iino F., and Gullet B.K. *Environ. Sci. Technol.* 2004; 38: 5112
6. Tohyama C., Hirano S., and Suzuki K.T. *Arch. Environ. Contam. Toxicol.* 1992; 22: 176