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Prevalence of low chlorinated dibenzo-*p*-dioxin/dibenzofurans in human serum

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HIGHLIGHTS

- All PCDD/F congeners were first measured in human serum samples.
- $\sum Cl_{1-3}$DFs accounted for more than 95% of $\sum Cl_{1-8}$DFs.
- 2-MoCDF showed the maximum contribution and had a strong correlation in human serum.
- The combination of 2-MoCDF and OCDD could explain the 95.9% variation in human serum.

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ABSTRACT

Mono- to tri-chlorinated dibenzo-*p*-dioxin/dibenzofurans (DD/Fs) have not been studied as extensively as the 17 toxic 2,3,7,8-substituted congeners. In this study for the first time, mono- to octa-chlorinated DD/Fs were analyzed for seventy one human serum samples collected from incinerator workers as well as residents living near and far from the facility. The mean concentrations of $\sum Cl_{1-8}$DD/Fs and 17-toxic congeners were 1890 and 398 pg g^{-1} lipid (11.9 TEQ pg g^{-1} lipid), respectively. 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDD, and 1,2,3,6,7,8-HxCDD were predominant congeners that accounted for more than 78% of the TEQ concentrations. The profile for polychlorinated dibenzo-*p*-dioxins (PCDDs) was dominated by the most chlorinated congener, OCDD (>58%), while decreasing concentrations with increasing degree of chlorination were seen for polychlorinated dibenzofurans (PCDFs); MoCDFs (>83%) and DiCDFs (>6%). $\sum Cl_{1-3}$DD/Fs accounted for 77% of the serum concentrations of $\sum Cl_{1-8}$DD/Fs. These findings confirm that human beings are exposed to a large amount of $\sum Cl_{1-3}$DD/Fs. Moreover, MoCDFs contributed more than 60% of the $\sum Cl_{1-8}$DD/Fs and was highly correlated with $\sum Cl_{1-8}$DD/Fs. Thus, 2-MoCDF could be a predictive indicator for $\sum Cl_{1-8}$DD/Fs (r_s = 0.96), and the combination of 2-MoCDF and OCDD could explain the 95.9% variation in the serum of $\sum Cl_{1-8}$DD/Fs. These results suggest that low chlorinated DD/Fs should be studied extensively until these low chlorinated congeners will have been elucidated for their toxicities.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are ubiquitous contaminants from anthropogenic sources that are almost exclusively produced by thermal and chemical-industrial processes (Fiedler, 2007; Kulkarni et al., 2008), including incineration of municipal solid wastes (Chang and Lin, 2001; Abad et al., 2002), chlorine bleaching of pulp and paper mills (Rappe et al., 1987; Zheng et al., 2001), and manufacture of chlorinated compounds such as pesticides, herbicides, and fungicides (Sidhu and Edwards, 2002; Chen, 2004). PCDD/Fs are persistent and accumulate in the environment and organisms. Further, some congeners such as 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD) are highly toxic to humans (Kogevinas, 2001; Steenland et al., 2004; Schecter et al., 2006). These chemicals bind to the aryl hydrocarbon (Ah) receptor and can induce gene expression, disrupt normal hormone signaling pathways, and cause reproductive and developmental defects (Mandal, 2005). Moreover, the seven dioxin and ten furan congeners of the 210 PCDD/F congeners have been included in the internationally agreed toxic equivalency factor (TEF) system, which estimates the overall dioxin-like toxicity of compounds relative to 2,3,7,8-TCDD (Van den Berg et al., 1998, 2006). A great number of studies for PCDD/Fs have concentrated on 2,3,7,8-substituted toxic congeners; however, little research has been carried out on low chlorinated congeners (mono- to tri-chlorinated dibenzo-*p*-dioxin/dibenzofurans) which have not been assigned with TEF values.

Low chlorinated compounds offer valuable information on the formation mechanism of PCDD/Fs. Some researchers focused on estimating toxic equivalent (TEQ) values by measurement of low chlorinated DD/Fs as surrogate compounds (Gullett and Wikström,

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2000) and monitored the chlorination pathway in municipal solid waste incinerators (MSWI) by analyzing low chlorinated congeners of PCDD/Fs (Ryu et al., 2004, 2005, 2006). In addition, they obtained information on mono- to octa-chlorinated congeners of PCDD/Fs in fly ash samples from a full-scale MSWI (Lundin and Marklund, 2008). Lee et al. (2005) measured emission factors for house coal and seasoned hardwood burning in an open fire situation for mono- to octa-chlorinated DD/Fs. Accordingly, the low chlorinated DD/Fs can provide valuable information for environmental samples, and the analysis of congener distribution is the key to estimating of dioxins and related compounds. From a toxic point of view, 2-MoCDF caused embryotoxicities by independent mechanisms of the Ah receptor (Usami et al., 1993), and 3-MoCDFs showed significant mutagenecity (Michi et al., 1988). Furthermore, these compounds may induce neurological and immunological defects in human beings similar to non-dioxin-like PCBs, even though no such findings have been reported to date. However, to the best of our knowledge, there have been no previous analyses of low chlorinated PCDD/Fs in human samples.

In Korea, there are currently 32 MSWIs and about 1000 various incinerators (small-scale: <200 kg h^{-1} and medium-scale: >200 kg h^{-1}) in service, indicating that incineration has become an important method of treating wastes. We previously conducted a study to evaluate the effects of exposure to incineration emission on PCDD/F concentrations in serum samples (Park et al., 2009). In this study, we evaluated mono- to octa-chlorinated DD/Fs and the TEQs from individuals working in an MSWI, residents living near the facility, and the general population to map the concentrations and distribution of human serum samples and compare the results with our previous study. Additionally, we identified the plausible congeners that describe $\sum Cl_{1-8}$DD/Fs in human serum samples and evaluated the potential exposure of MSWI workers and residents living near the MSWI to dioxin compounds.

2. Materials and methods

2.1. Blood sampling

Sixty blood samples were obtained from worker volunteers at the MSWI (n: number of subjects = 11, denoted as W) and from the nearby residents living within 0.3 km of the MSWI (n = 49, denoted as R) in 2006. Additionally, 11 samples were obtained from individuals (denoted as B) living >10 km away from the MSWI. Information regarding the age, smoking habits, diet, occupational history, and medical history was obtained by conducting a survey, and the body weight and height of each subject was also measured during the survey. The valuable information about subjects was summarized in Appendices Table A.1. Approximately 100 mL of blood (without anticoagulant) was collected from each of the volunteers who had been instructed not to eat breakfast on the day of sample collection. Each sample was separated into serum and cruor and placed manually into 50 mL contamination-free (sterile) Pyrex bottles with Teflon-coated tops. All samples were kept frozen at −20 °C until analysis.

2.2. Chemical analysis

Approximately 30 g of serum was added to a 500 mL separating funnel and spiked with a set of ^{13}C-labeled PCDD/Fs (2-MoCDD, 2-MoCDF, 2,3-DiCDD, 2,8-DiCDF, 2,3,7-TrCDD, 2,4,8-TrCDF, and EPA method 1613 labeled compound stock solution; Cambridge Isotope Laboratories, Andover, MA, USA) as the surrogate internal standard. The samples were then mixed with sodium oxalate saturated water after which the solution was extracted three times using 200 mL of 2:1 acetone/hexane for each extraction. The resultant organic layer was filtered and evaporated to dryness to evaluate the lipid content in the samples. The lipid content was determined gravimetrically. Briefly, the dried samples were resuspended in hexane and then subjected to further cleanup via multilayer-silica, alumina column (Park et al., 2010). The cleaned extract was then analyzed by high-resolution gas chromatography/high-resolution mass spectrometry using an HP 6890 N gas chromatograph coupled with a JEOL 800D mass spectrometer in the electron impact mode after addition of a ^{13}C-labeled recovery standard. A DB5-MS column (140 °C (4 min) → 220 °C (15 °C min^{-1}) → 240 °C (1.5 °C min^{-1}, 2 min) → 310 °C (4 °C min^{-1}, 6 min), 60 m × 0.25 mm × 0.25 μm film thickness; J&W Scientific, CA, USA) was used for tetra- to octa-chlorinated DD/Fs, and a DB-Dioxin column (50 °C (1 min) → 150 °C (20 °C min^{-1}) → 270 °C (3 °C min^{-1}, 14 min), 60 m × 0.25 mm × 0.25 μm film thickness; J&W Scientific, CA, USA) was used in a separate GC run for mono- to tri-chlorinated DD/Fs measurement.

2.3. Quality assurance/quality control

Procedural blanks were analyzed with every nine samples to check for interference and/or contamination from solvents and glasswares. The limit of detection (LOD) for each PCDD/F congener was estimated to be 2.5 times the S/N value found for procedural blanks and this LOD was in the range of 2–20 pg g^{-1} lipid for human serum samples. Peaks were identified based on the corresponding retention times observed upon analysis of the standards. Quality control standards for PCDD/Fs were analyzed after every ten samples to monitor for instrument stability. The recoveries of $^{13}C_{12}$-labeled PCDD/Fs spiked into each sample were 52 ± 11% for 2,3-DiCDD, 76 ± 9% for 2,3,7-TrCDD, 52 ± 12% for 2-MoCDF, 71 ± 10% for 2,8-DiCDF, 78 ± 11% for 2,4,8-TrCDF, and in the range of 80–120% for the EPA 1613 labeled compounds. Samples below the LOD were assigned a value of zero in the subsequent data analysis, and the congeners of MoCDDs were not considered in our study due to the recovery of $^{13}C_{12}$ 2-MoCDD (under 20%). The vapor pressure of 2-MoCDD was 1.8 times higher than 2-MoCDF, i.e. 0.017 vs. 0.0096 Pa, thus 2-MoCDD could be easily evaporated in evaporation and dryness steps.

2.4. Data analysis

About 40 PCDD/F congeners were detected in human serum samples and descriptive statistics were used to characterize the levels of congeners. One-way analysis of variance (ANOVA) was used for normally distributed variables and the Kruskal–Wallis test was used for the remaining variables. The correlation between the variables (age, proportional body fat, body weight, etc.) and the serum concentrations of each congener was examined using the Spearman correlation coefficients. We chose the MSWI stack gas data provided by Ryu et al. (2004), as well as the ambient air and stack gas data from the National Institute of Environmental Research, Korea (NIER, 2006) as representative environmental samples. Multivariate analysis of the results was conducted to elucidate the differences in PCDD/F patterns between the environmental samples and those detected in human serum after normalization on the basis of total PCDD/F concentrations. Data matrices were evaluated by principal component analysis (PCA). Hierarchical cluster analysis (HCA) was used according to the average linkage between the groups on a square Euclidian distance matrix derived from the PCA scores in order to identify the homogeneous groups. All statistical analyses were carried out using SPSS 12.0 and a significance level of 0.05 was used for all tests.