Dioxin-like Compounds in Japanese Indoor Dusts: Brominated Dibenzofurans Strongly Contribute to Dioxin-Like Activity Evaluated by *In Vitro* Bioassay

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Introduction

Indoor dust is a sink for many kinds of pollutants (Butte & Heinzow 2002). Exposure via indoor dust of children, especially infants and toddlers, to pollutants is suggested to be greater than by other pathways, although it depends on their lifestyle and habit. In our previous study, not only polybrominated diphenylethers (PBDEs) but also polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) which are well-known as degradation products and/or impurities of PBDEs were detected in indoor dust collected from Japanese household and office (Suzuki et al. 2006). As well as PBDEs, PBDD/Fs concentration level in house and office dust is also comparably higher than those in contaminated sediment. Result thus emphasizes the need to evaluate their exposure risks to children by indoor dust ingestion because *in vitro* and *in vivo* studies suggest that PBDD/Fs have similar toxicity profiles to their chlorinated counterparts known as internationally controlled dioxins (Birnbaum et al. 2003).

Our previous study has investigated whole dioxin-like activities in sulfuric-acid-treated extracts of house and office dust using Dioxin-Responsive Chemical-Activated LUciferase gene eXpression assay (DR-CALUX®) (Suzuki et al. 2007a). Dioxin-like activities in indoor dust were 38–1400 pg CALUX-TEQ (2,3,7,8-TCDD equivalent)/g (median 160 pg CALUX-TEQ/g), which are three to five orders of magnitude higher than those in food samples. With our exposure model, we compared the average daily doses (ADDs) of dioxin-like compounds via house dust with the estimated ADDs of dioxins via air, soil, and food. Our exposure results suggest that house dust is an important medium of exposure to dioxin-like compounds, especially in children, in whom the ADD of dioxin-like compounds via house dust was greater than those of dioxins via food when the ingestion rate and CALUX-TEQ of house dust were high. Then, PBDD/Fs contributions to overall dioxin-like activity were estimated using the theoretical CALUX-TEQs calculated chemically using concentrations of 2,3,7,8-substituted PBDD/Fs (Suzuki et al. 2006) and their CALUX relative potencies to 2,3,7,8-TCDD (Behnisch et al. 2003) for house and office dusts. Median PBDD/F-derived CALUX-TEQ calculated was approximately 30% of the CALUX-TEQs detected in indoor dusts.

It is important to clarify existence and concentration of causative compounds related to *in vitro* dioxin-like activity in indoor dust because information for estimating exposure and risks via oral ingestion may be in demand for further improvement of the toxic equivalency factors (TEFs) concept for dioxin-like compounds (Van den Berg et al. 2006). Here, we used gas chromatography–high resolution mass spectrometry (GC-HRMS) to quantify polychlorinated dioxins and dibenzofurans (PCDD/Fs), coplanar-polychlorinated biphenyl (Co-PCBs) and polychlorinated naphthalene (PCNs), identified in our previous study (Suzuki et al. 2007b), in the sulfuric-acid-treated dust extracts. We further estimated

contribution of these well-known dioxin-like compounds to dioxin-like activity to determine the emerging dioxin-like compounds in indoor dust.

Materials and Methods

Sampling and Extraction for Tested Indoor Dusts. The procedures for the sampling and extraction of dusts have been described elsewhere (Suzuki et al. 2007a). Briefly, indoor dust samples were collected from households (n = 19) and office and laboratory from three institutions (n = 14) in Japan. A sieved dust (<1.0 mm) was extracted with a Soxhlet apparatus for 16 h with toluene.

Clean-up for Dioxin-like Compounds. A portion of crude toluene fraction (equal to about 1.5 g of dust sample) was transferred to *n*-hexane by rotary evaporation. After removal of the elemental sulfur with activated copper, the *n*-hexane fraction was subjected to sulfuric acid treatment and applied to a sulfuric acid silica gel column. After elution with *n*-hexane, this fraction was stored at 4°C for the evaluation with DR-CALUX assay (Suzuki et al. 2007a) and quantitative analysis. The stock solution was evaporated to a small volume after the addition of internal standards such as 13C-labeled PCDD/F (DF-LCS-C, Wellington Laboratories Inc, Canada), PCB (PCB-LCS-A, Wellington Laboratories Inc, Canada) and PCN (ECN-5102, Cambridge Isotope Laboratories, USA) mixture. Dust extracts were applied to a multi-layer silica gel column coupled with carbon reversible column (SUPLECO, USA). After elution with 100 ml of *n*-hexane, the multi-layer silica gel column and carbon reversible column were separated, and then carbon column was eluted with 40 ml of 25% (v/v) dichloromethane/*n*-hexane. Finally, carbon column was reversed and eluted with 100 ml of toluene in order to obtain PCDDs, PCDFs, Co-PCBs and PCNs. Eluted extract was evaporated to small amount, and then 13C-labeled PCDD/F (DF-IS-E, Wellington Laboratories Inc, Canada), PCB (PCB-IS-A, Wellington Laboratories Inc, Canada) and PCN (ECN-5261, Cambridge Isotope Laboratories, USA) were added to final extract as syringe spike.

Quantitative Analysis of Dioxin-like Compounds with GC-HRMS. The final solution was analyzed by GC-HRMS using a HP-6890 gas chromatography (Agilent, USA) equipped with an JMS-700 Mstation mass spectrometer (JEOL, Japan) employing the El (Electron Impact)-SIM (Selected Ion Monitoring) method at 11,000 resolutions. For the measurements of tetra- to hexa-CDD/Fs, hepta- to octa-CDD/F and dioxin-like PCBs, and PCNs, a SP-2331 (60 m × 0.32 mm i.d., 0.2 μm film thickness, SUPLECO, USA), a HT8-PCB (60 m × 0.25 mm i.d., not reported for film thickness, Kanto Chemical, Japan), and a DB-5MS (60 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, USA) capillary columns were used respectively. Target compounds were quantified by the isotope dilution method using 13C-labeled compounds. Recovery of 13C-labeled PCDD/Fs, PCBs and PCNs ranged between 64% and 108.4%.

Results and Discussion

In order to estimate contribution of well-known dioxin-like compounds to dioxin-like activity obtained by DR-CALUX, concentrations of PCDD/Fs, Co-PCBs and PCNs were measured in 33 stock sulfuric-acid-treated extracts of indoor dust samples collected from Japan.

Target dioxin-like compounds were detected in all stock extracts, indicating that PCDD/Fs, Co-PCBs and PCNs were ubiquitous in Japanese home and work environment. Concentrations of PCDDs, PCDFs, non-*ortho* Co-PCBs, mono-*ortho* Co-PCBs, and PCNs in Japanese indoor dusts were 440–15000 pg/g (median 1700 pg/g), 92–4400 pg/g (median 450 pg/g), 60–18000 pg/g (median 130 pg/g), 560–580000 pg/g (median 1400 pg/g), and 1600–49000 pg/g (median 4400 pg/g), respectively. The concentrations of

PBDDs and PBDFs reported by our previous study (Suzuki et al. 2006) were <2.5–2300 pg/g (median 54 pg/g) and 1100–29000 pg/g (median 4000 pg/g). Although the differences of concentration between PCNs and PBDFs were about one order of magnitude among indoor dust samples, those between PCDD/Fs and PBDFs was about two orders of magnitude. On the other hand, there exist differences of three orders of magnitude for Co-PCBs and PBDDs concentrations in Japanese indoor dusts, indicating their specific sources in indoor environment. The theoretical CALUX-TEQs calculated chemically using concentrations of analyzed dioxin-like compounds and CALUX relative potencies to 2,3,7,8-TCDD (Behnisch et al. 2003) for indoor dusts were shown in Figure 1. Theoretical CALUX-TEQs of PCDDs, PCDFs, non-*ortho* Co-PCBs, mono-*ortho* Co-PCBs, PCNs, and PBDFs in Japanese indoor dusts were 2.9–120 pg/g (median 14 pg/g), 2.0–170 pg/g (median 11 pg/g), 0.44–53 pg/g (median 1.4 pg/g), 0.014–10 pg/g (median 0.035 pg/g), 0.16–9.2 pg/g (median 0.46 pg/g), and 13–180 pg/g (median 47 pg/g), although the well-known dioxin-like PBDDs were not detected in our previous study (Suzuki et al. 2006). Based on median values, rank order in concentrations of well-known dioxin-like compounds were as follows: PBDFs > PCDDs > PCDFs > non-*ortho* Co-PCBs > PCNs > mono-*ortho* Co-PCBs. Contribution rate of the theoretical CALUX-TEQs (median) for PBDFs, PCDDs, PCDFs, non-*ortho* Co-PCBs, PCNs, and mono-*ortho* Co-PCBs to the experimental CALUX-TEQs were respectively 30%, 9%, 7%, 0.9%, 0.3%, and 0.02% of the CALUX-TEQs that we obtained experimentally for indoor dusts (Suzuki et al. 2007). These results indicate that PBDFs strongly contribute to dioxin-like activity in Japanese indoor dusts and sum of theoretical CALUX-TEQ for PBDFs, PCDD/Fs, Co-PCBs and PCNs accounted for 50% of the experimental CALUX-TEQ detected in indoor dusts based on median value.

Our results indicate that PBDFs, which are not the authorized dioxins such as PCDD/Fs and Co-PCBs (Van den Berg et al 2006), in Japanese indoor dust is the most important dioxin-like compounds based on the presented results for Japanese indoor dust. WHO-IPCS expert meeting requires data for non-authorized dioxin-like compounds such as PBDFs in human exposure pathway such as diet. Indoor dust is important as an exposure route for human, especially children (Butte & Heinzow 2002). In recent researches, PBDEs have been detected in indoor dust at relatively high concentrations (Harrad et al 2007, Stapleton et al 2005, Wilford et al 2005). Presumably, PBDFs would be detected in such dust samples because it is well-known as impurity and photo-degradation products of PBDEs (Kajiwara et al 2008). These results have pointed out PBDFs analysis for indoor dust is required for further improvement of TEFs concept for dioxin-like compounds. Furthermore, it is also suggested that the acid-resistant dioxin-like compounds other than analyzed dioxin-like compounds in this study also contribute strongly to the CALUX activity of indoor dust. We are searching the other dioxin-like compounds in indoor dust samples using effect-directed analysis composed of HPLC fractionation, DR-CALUX assay and GC-HRMS.

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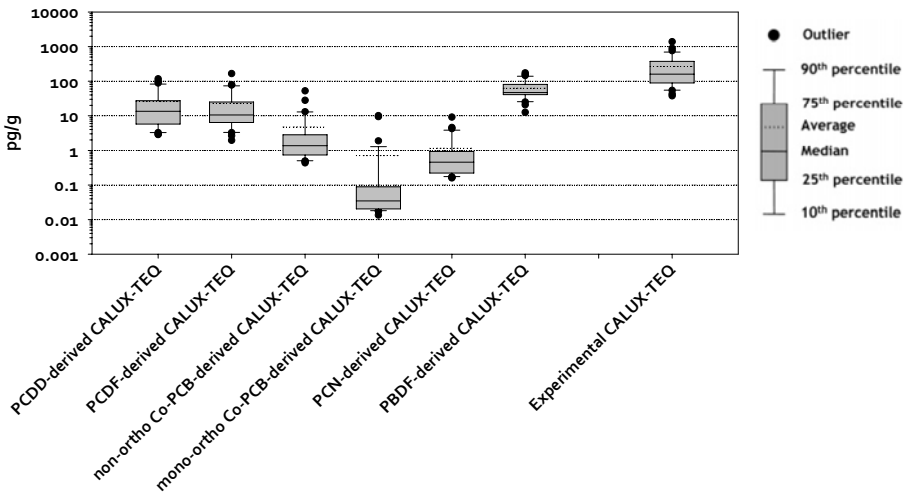

Fig. 1 The experimental CALUX-TEQ (Suzuki et al 2007a) and the theoretical CALUX-TEQs calculated chemically using PCDD/Fs, Co-PCBs, PCNs, and PBDFs concentrations and their CALUX relative potencies to 2,3,7,8-TCDD for indoor dusts

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