TENTATIVE IDETIFICATION OF DIOXIN-LIKE COMPOUNDS IN HOUSE DUSTS COLECTED FROM JAPAN

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Abstract
Our previous study demonstrated dioxin-like activity level in indoor dusts collected from Japan was relatively higher than that in contaminated sediments. Exposure assessment indicated the average daily dose (ADD) of dioxin-like compounds via house dust exceeded ADDs of dioxins via food, indicating dust is a significant exposure pathway to children for dioxin-like compounds. This study has been conducted to identify dioxin-like compounds in sulfuric acid treatment extracts of indoor dusts by applying HPLC and Dioxin-Responsive Chemical-Activated LUciferase gene eXpression (DR-CALUX®) assay. A whole extract of mixed sample composed of 7 house dusts was fractionated using normal phase-HPLC using a nitrophenylpropylsilica column (NITRO-HPLC) and all the NITRO-HPLC fractions were assessed for dioxin-like activity using DR-CALUX assay. The first fraction by NITRO-HPLC indicated the highest dioxin-like activity. Furthermore, this fraction was fractionated using reverse phase-HPLC using an octadecylsilica column (ODS-HPLC). All 90 fractions were evaluated the dioxin-like activity by using the DR-CALUX assay. Based on a result of elution patterns on standard solutions, the dioxin-like compounds in ODS-HPLC fractions showing higher activity were estimated as a part of polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs). Finally, tentative identification by HRGC/HRMS for DR-CALUX inductive chemicals in ODS-HPLC fractions suggested that PentaCBs, HeptaCBs, TetraCNs, PentaCNs, HeptaCNs, and TeBDD seem to contribute to the whole activity in the extract of indoor dust.

Introduction

Exposure of children such as infants and toddlers to pollutants via indoor dust is suggested to be greater than by other pathways.[1] Actually, the analyses of brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) in house dust have been conducted all over the world, suggesting that the importance of house dust as the routes of children exposure to PBDEs.[2-4] Furthermore, our previous study also detected polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs) in indoor dust, and their concentrations are higher than those of contaminated sediment.[5] These results suggest children are exposed to not only indoor use chemicals but also their degradation products and/or impurities. It thus emphasizes the need to evaluate the chemical risks posed to children by indoor dust ingestion. It must pick up the related compounds with potential risk for children to propose their control strategy.

Therefore, our previous study have investigated dioxin-like and TTR-binding activities in sulfuric acid treatment extracts of house and office dust using DR-CALUX and TTR-binding assay (*in vitro* competitive human TTR-binding assay).[6] These activity levels in indoor dust samples were indicated to be higher than those in contaminated sediments. Exposure results demonstrated that house dust might be a significant dioxin-like compounds and TTR-binding compounds exposure pathway for children. After that, 2,4,6-Tribromophenol and 2,3,4,5,6-Pentachrolophenols as main TTR-binding compounds in indoor dusts were identified and quantified using HPLC and TTR-binding assay with GC/MSD, leading to their control strategy.[7] However, compounds contributing to dioxin-like activity were not identified until now.

Purpose of this study was to identify dioxin-like compounds in sulfuric acid treatment extracts of indoor dust samples tentatively. First, we prepared an acid-resistant extract of mixed sample composed of 7 house dusts in order to increase target dioxin-like compounds amount in final extract. Subsequently, we subjected the mixed house dust extract to NITRO-HPLC combined with the DR-CALUX assay. We applied ODS-HPLC to NITRO-HPLC fraction shown higher activity. Furthermore, we investigated elution times for PCBs, PCNs, polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), PBDDs, and PBDFs on ODS-HPLC fractionation. Based on a result of elution patterns on these compounds, we estimated dioxin-like compounds contained in ODS-HPLC fractions. Finally, tentative HRGC/HRMS-SIM analyses were conducted for ODS-HPLC fractions indicating higher activity.

Materials and Methods

Sampling: Indoor dust samples were collected from 19 households (n=19) as house dust in Japan in May to December, 2005. Indoor dusts were mainly collected by vacuum cleaners. Sampling procedures for house dusts have been described elsewhere.[6] In this study, 7 house dust samples (house dust 3, 4, 6, 9, 17, 18, and 19) indicating comparatively higher dioxin-like activity[6] were selected as target samples for identifying dioxin-like compounds.

Extraction and clean-up: Approximately 10 g of each sample was extracted using Soxhlet with toluene. Toluene fraction was concentrated and transferred to *n*-hexane by rotary evaporation. After removing elemental sulfur with activated copper, *n*-hexane fraction was subjected to H_2SO_4 treatment. The *n*-hexane fraction was washed with water and dehydrated. This fraction was applied to an H_2SO_4-silica gel column. After elution with *n*-hexane, the solution was evaporated. The residue was dissolved in 200 μl of nonane. Each nonane fraction was mixed into one fraction, and it stored at 4 °C for subsequent NITRO-HPLC fractionation.

DR-CALUX assay: Dioxin-like activity was measured by means of the DR-CALUX assay using the rat hepatoma H4IIE cell line with an AhR-regulated luciferase gene construct (H4IIE-luc).[8] The conditions for cell culture and the procedure for the DR-CALUX assay have been described in detail elsewhere.[9,10]

HPLC fractionation for estimating dioxin-like compounds in house dust: A whole extract of mixed house dust was injected and then fractionated using NITRO-HPLC, which separates compounds according to the size and charge density of their aromatic systems.[9] All the NITRO-HPLC fractions were evaporated, and the residue was taken up in DMSO and then assessed for dioxin-like activity using DR-CALUX assay. Then, NITRO-HPLC fraction showing high dioxin-like activity was injected and fractionated using ODS-HPLC, which separates compounds according to their hydrophobicities.[10] All fractions were evaporated. Then the residue was also dissolved in DMSO and assessed using the DR-CALUX assay for subsequent tentative HRGC/HRMS-SIM analyses.

The conditions and the procedure for NITRO-HPLC and ODS-HPLC fractionation column have been described in detail elsewhere.[9,10] Although the cut-off points for NITRO-HPLC were fixed referring to previous studie,[9] those of ODS-HPLC were decided according to HPLC fractionation results for test compounds. As tested compounds, 14 PCBs, 8 PCNs, 2 PBBs, 6 PBDEs, 4 PBDDs, and 4 PBDFs were used in this study.

Results and Discussion

The dioxin-like activity profile of the separated fractions derived from mixed housed dust was investigated by means of DR-CALUX assay combined with NITRO-HPLC fractionation. As a result, 1st fraction showed relatively higher activity, which accounts for about 80% of the arithmetical sum of activities of all the fractions (Fig. 1). It suggests that the contribution of halogenated 2- or 3-ring aromatic compounds inducing DR-CALUX activity, such as PBDD/Fs, Co-PCBs, and PCNs[11] to the overall activity was higher because our previous study indicated these compounds elute into 1st fraction on NITRO-HPLC fractionation.[11]

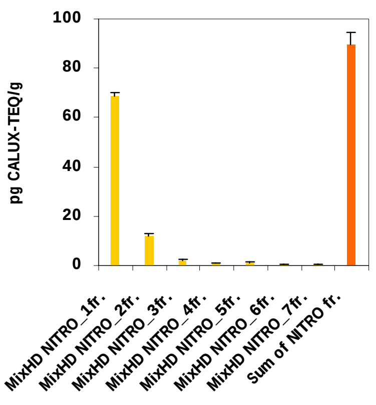

Fig. 1. The dioxin-like activity profile of the separated NITRO-HPLC fractions derived from mixed housed dust

NITRO-HPLC 1st fraction shown highest activity was separated into 90 fractions by ODS-HPLC fractionation, and then assessed using DR-CALUX assay. The dioxin-like activity profile derived from ODS-HPLC fractions was shown in Fig. 2. 35th to 60th ODS-HPLC fractions showed relatively higher activities. Especially, 35th fraction showed the highest activity. The 52nd, 40th, 44th, and 46th fractions also indicated relatively high activities. The dioxin-like activity profile of the separated ODS-HPLC fractions derived from mixed housed dust was similar to those of house dust 3, house dust 4 and office dust 5.[12] Although their potencies differed, ODS-HPLC fractions indicating higher activity were similar to these 3 dust samples.

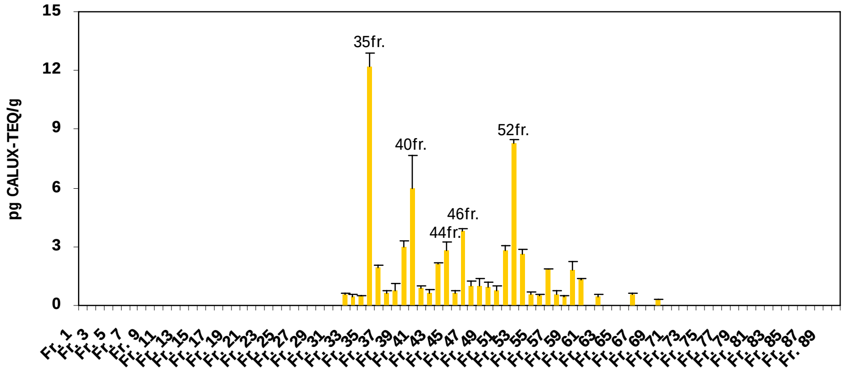

Fig. 2. The dioxin-like activity profile of the separated ODS-HPLC fractions derived from NITRO-HPLC 1st fraction of mixed housed dust

In order to estimate the active compounds contributed to the fractionated dioxin-like activity, we investigated the elution characteristics of PCBs, PCNs, PBBs, PBDEs, PBDDs, and PBDFs by ODS-HPLC. The elution times of these compounds were shown in Fig. 3. Considering the elution results for the standards and their relative potency on DR-CALUX,[11] we roughly estimated the dioxin-like compounds in ODS-HPLC fractions shown higher activity as follow: PentaCBs and HexaCBs for 35th fraction; HexaCNs, HeptaCNs, TetraBDDs, PentaBDDs, HexaBDDs, and PentaBDFs for 52nd fraction; HeptaCBs, PentaCNs and HexaCNs for 40th fraction; PentaCNs, HexaCNs, TetraBDDs, and PentaBDDs for 44th fraction; HexaCNs for 46th fraction.

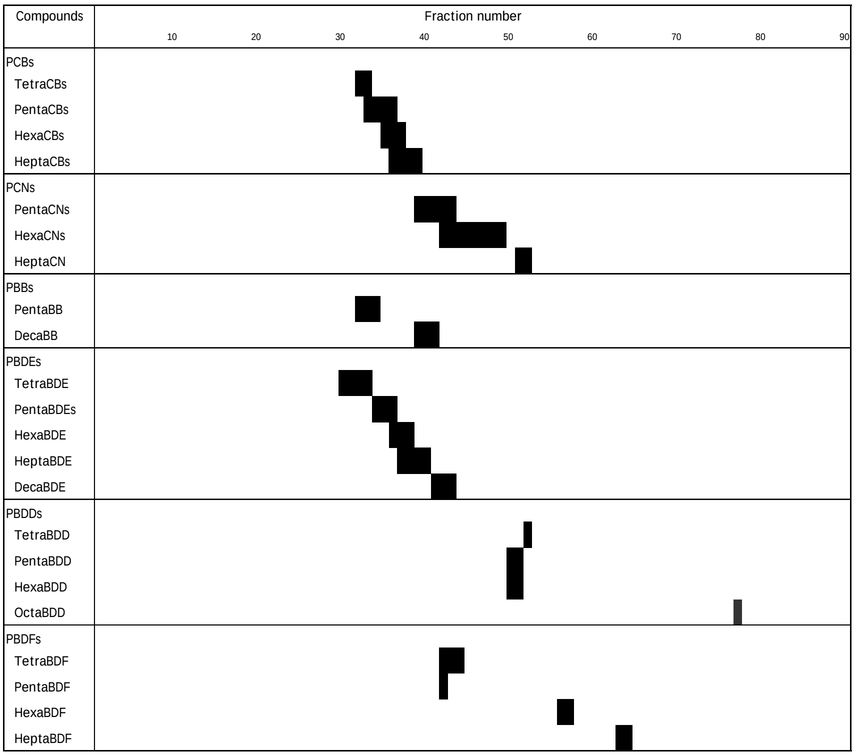

Fig. 3. Elution results of tested compounds on ODS-HPLC fractionation

And then, HRGC/HRMS-SIM analyses for ODS-HPLC fractions derived from house dust 4 were performed based on above mentioned information tentatively. As a result, halogenated 2- or 3-ring aromatic compounds including DR-CALUX-inducing compounds were assigned as shown in Fig. 4. Quantitative analysis for these detected compounds in the separated ODS-HPLC fractions derived from NITRO-HPLC 1st fraction of mixed house dust has been conducted in order to estimate their contribution to whole dioxin-like activity of indoor dust.

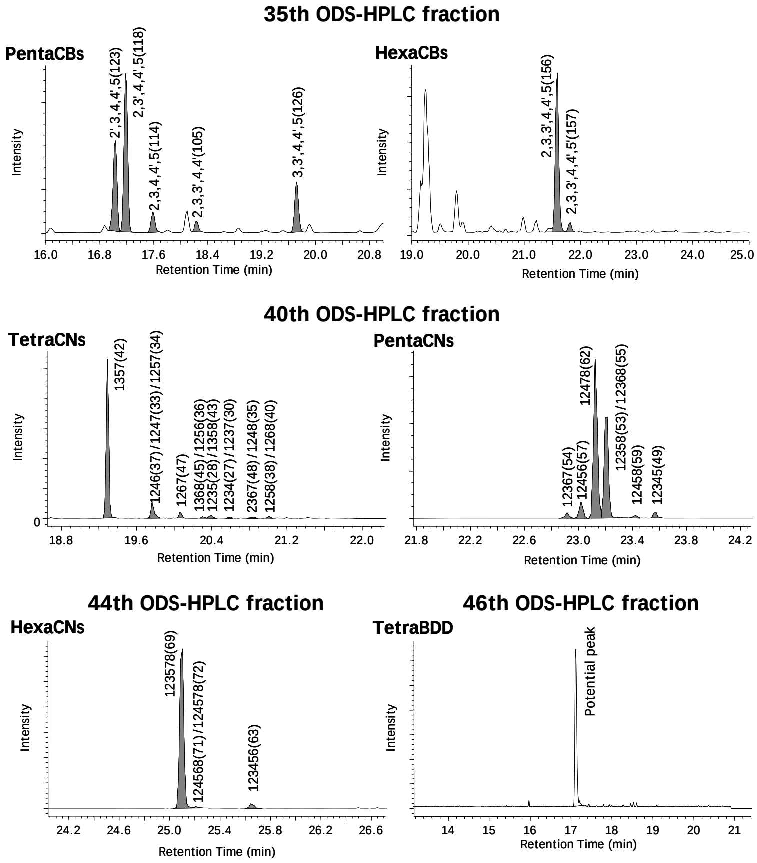

Fig. 4. Assigned halogenated 2- or 3-ring aromatic compounds including DR-CALUX-inducing compounds in each ODS-HPLC fraction shown higher activity derived from house dust 4

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References

1. Butte W, Heinzow B. *Rev Environ Contam Toxicol* 2002; 175:1.
2. Stapleton HM, Dodder NG, Offenberg JH, Schantz MM, Wise SA. *Environ Sci Technol* 2005; 39:925.
3. Jones-Otazo HA, Clarke JP, Diamond ML, Archbold JA, Ferguson G, Harner T, Richardson GM, Ryan JJ, Wilford B. *Environ Sci Technol* 2005; 39:5121.
4. Wilford BH, Shoeib M, Harner T, Zhu J, Jones KC. *Environ Sci Technol* 2005; 39:7027.
5. Suzuki G, Takigami H, Nose K, Takahashi S, Asari M, Sakai S. *Environ Sci Technol* 2007; 41:1487.
6. Suzuki G, Nose K, Takigami H, Takahashi S, Sakai S. *Organohalogen Comp* 2006; 68:1843.
7. Suzuki G, Takigami H, Nose K, Takahashi S, Asari M, Sakai S. *4th International Workshop on Brominated Flame Retardants (BFR2007).* P045. 2007.
8. Murk AJ, Legler J, Denison MS, Giesy JP, van den Guchte C, Brouwer A. *Fundam. Appl. Toxicol.* 1996; 33:149.
9. Suzuki G, Takigami H, Kushi Y, Sakai S. *Toxicol Lett* 2006; 161:174.
10. Suzuki G, Takigami H, Kushi Y, Sakai S. *Environ Int* 2004; 30:1055.
11. Behnisch PA, Hosoe K, Sakai S. *Environ Int* 2003; 29:861.
12. Suzuki G, Takigami H, Nose K, Takahashi S, Sakai S. *Organohalogen Comp* 2006; 68:177.