DIOXIN-LIKE COMPOUNDS IN HOUSE AND OFFICE DUSTS: ESTIMATION OF AVERAGE DAILY DOSE AND TOXICITY IDENTIFICATION EVALUATION

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Introduction

The rapid decrease in PBDE consumption after 1990 could be due to the industry's voluntary phasing out of PBDEs in Japan because of global concern regarding the potential adverse environmental and health effects of them and their thermal-breakdown products.[1] However, lots of products such as TV and PC have contained PBDEs used in the past, which may be source of them in the indoor air.[2] Recently, the analyses of PBDEs in house dust have been conducted all over the world, indicating that PBDE concentrations of house dust are relatively higher than other media such as sediment.[3-6] Although lots of researchers are taking an increasing interest in the importance of house dust as the routes of human exposure to PBDEs, there is no investigation about the chronic toxicities, such as endocrine disrupting potencies, of the house dust containing PBDEs.

In this study, we investigated the *in vitro* AhR-binding activity, as related toxicity for PBDEs and their thermal-breakdown products (e.g. PBDD/Fs), of house and office dust using the DR-CALUX assay (DR-CALUX®: Dioxin-Responsive Chemical-Activated Luciferase gene eXpression). First we compared the obtained data with the AhR-binding activity reported in foods and sediments, and then we tried to identify the indoor source of AhR-binding compounds (dioxin-like compounds) by evaluating the relevance of the AhR-binding activities in dusts and the investigated indoor information. Finally, we estimated the average daily dose (ADD) of dioxin-like compounds via house dust and tried to identify the dioxin-like compounds in dust samples.

Materials and Methods

Sampling: House dust (HD) and office dust (OD) samples were collected from 19 households (n=19) and 3 institutions (n=14) in Japan in May to December, 2005. HDs were collected by vacuum cleaners. While ODs 6, 7, and 9 to 14 were also collected from vacuum cleaner bags used in the office and laboratory, OD 1 to 5 and 8 were collected with a broom from the floor of office and laboratory manually Dust was transferred to an all stainless steel sieve (< 1.0 mm), covered with the steel lid, and shaken automatically using Sieve Shaker AS300 (Retsch Co., Ltd.). Sieved dust was collected in a glass bottle covered with aluminum foil. Samples were stored at room temperature until analysis. A questionnaire survey was performed for households and institutions where dusts were collected. To identify factors affecting chemical loadings, data investigated was included cleaning frequency, area, year of construction, floor type, ventilation condition, the number and use time of electric appliance such as TVs and computers, etc.

Extraction and Clean-up: Approximately 5.0 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. A portion of the 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 100 µl of DMSO and stored at 4 °C for subsequent DR-CALUX assay and HPLC fractionation.

DR-CALUX assay: AhR-binding 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).[7] The conditions for cell culture and the procedure for the DR-CALUX assay have been described in detail elsewhere.[8,9]

Estimation of average daily dose of dioxin-like compounds via house dust: The average daily dose (ADD) of dioxin-like compounds via HD was estimated and compared with the estimated ADD of dioxins via air, soil and food. Ingestion rate for HD, air, soil, and food,[3-6,10,11] and WHO-TEQs in air, soil and food[12-16] were quoted from previous studies. ADDs of dioxin-like compounds (1) and dioxins (2) were calculated for each media as:

$$ADD_{child/adult}=C_{dust}\times IR_{dust} \quad (1)$$

where $ADD_{child/adult}$ is average daily dose for child or adult (pg CALUX-TEQ/day), C_{dust} is concentration of CALUX-TEQ in dust (pg CALUX-TEQ/g), and IR_{dust} is ingestion rate of dust (g/day).

$$ADD_{child/adult}=C_{media}\times IR_{media} \quad (2)$$

where $ADD_{child/adult}$ is average daily dose for child or adult (pg WHO-TEQ/day), C_{media} is concentration of dioxins in air, soil and food (pg WHO-TEQ/m^3 or pg WHO-TEQ/g), and IR_{media} is ingestion rate of air, soil and food (m^3/day or g/day).

Toxicity Identification and Evaluation approach for dioxin-like compounds in house dust: The conditions and the procedure for HPLC fractionations using a nitrophenylpropylsilica (NITRO) and an octadecylsilica (ODS) column have been described in detail elsewhere.[8,9] First, a whole extract of dust shown high activity was injected and then fractionated using normal phase-HPLC on a NITRO column, which separates compounds according to the size and charge density of their aromatic systems.[9] Then, NITRO-HPLC fraction shown high activity was injected and fractionated using reverse phase-HPLC on an ODS column, which separates compounds according to their hydrophobicities.[8] All fractions were evaporated. Then the residue was taken up in DMSO and assessed using the DR-CALUX assay. A part of ODS-HPLC fraction was transferred to *n*-nonane, and analyzed using HRGC-HRMS.

Results and Discussion

CALUX-TEQ in house and office dust: The whole extract of dusts had significant dioxin-like activity. The CALUX-TEQs in HDs ranged from 38 to 900 pg/g (median 110 pg/g) while a concentration range of 67 to 1,400 pg/g (median 220 pg/g) was found in ODs. The difference of concentration was one order of magnitude at the maximum among HDs and two orders of magnitude at the maximum among ODs. The CALUX-TEQs in ODs tend to be higher than those in HDs. The CALUX-TEQs of dust samples obtained in this study were about three orders of magnitude higher than those of food samples such as meats, fishes and dairy products collected in Belgian market.[17] It suggests that the CALUX-TEQs of dust samples are extremely high level among the sources of human exposure to dioxin-like compounds. Furthermore, the CALUX-TEQs in dust samples are relatively high as compared to sediment samples obtained from various countries.[18,19]

Source identification of dioxin-like compounds: Although correlation between the CALUX-TEQs of dust

samples and data investigated in a questionnaire was examined, there was no significant association ($P < 0.05$). Then, further investigation for source identification of dioxin-like compounds was conducted on OD4. The indoor carpet was obtained from the office which OD4 was collected because dust matrix of OD4 may be mainly derived from a carpet material. The CALUX-TEQs in the surface and the inside of carpet were 94 and 73 pg/g respectively, and they were below 25% as compared with the CALUX-TEQ of OD4. Compared the dose–response curve on The DR-CALUX assay of OD4 with that of carpet materials, they differed clearly. Behnisch et al (2003) have indicated that the dose–response curves on the DR-CALUX assay are depending on the kind of compound. These results suggest that the composition of dioxin-like compounds contained in OD4 is different from that of carpet materials and the indoor carpet is not source of dioxin-like compounds in case of OD4.

Results of estimated average daily dose of dioxin-like compounds via house dust: ADD of dioxin-like compounds via HD was estimated and compared with the estimated ADD of dioxins via air, soil and food. The ADD of dioxin-like compounds via HD is higher than the ADD of dioxins via food when the ingestion rate and CALUX-TEQ for HD were relatively high (Fig. 1). It suggests that HD may be a significant dioxin-like compounds exposure pathway for human, particularly children.

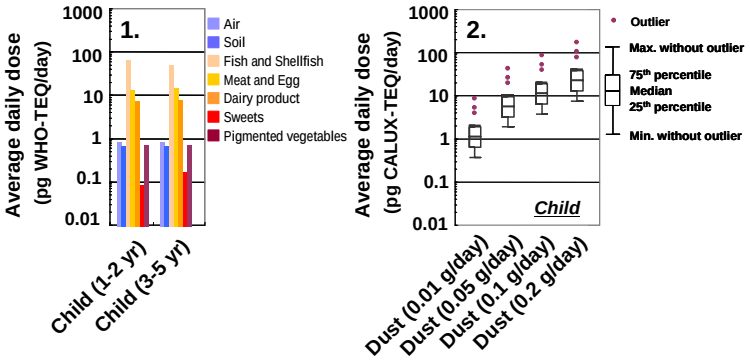

Fig. 1 The estimated ADD of dioxins via air, soil and food (1), and ADD of dioxin-like compounds via HD (2)

Fractionation of dioxin-like compounds in dust samples by HPLC methods: First, a whole extract of HD4 shown high activity was injected and then separated into seven fractions with NITRO-HPLC fractionation. The CALUX activity patterns in the seven fractions derived from HD4 after 24 h exposure are shown in Fig. 2-1. Fraction 1 showed relatively higher activity, which accounts for 90% or more of the arithmetical sum of the CALUX activities of all the fractions. Taking the elution results for the standards into consideration, we estimated that the contribution of the CYP1A-inducing HAHs, such as PCDDs, PCDFs, Co-PCBs, PCNs, PBDD/Fs, and PBDEs,[20,21] to the overall activity was higher. NITRO-HPLC first fraction shown highest activity was injected and then separated into 90 fractions by ODS-HPLC fractionation. The CALUX activity patterns in the 90 fractions derived from NITRO-HPLC first fraction after 24 h exposure are shown in Fig. 2-2. Fractions 30 to 70 showed relatively higher activities. The CALUX activity patterns of HD3 and OD5 were similar to that of HD4 although their potencies differed. The ODS-HPLC fractions, which indicated higher activity, were similar among dust samples.

Toxicity identification and evaluation of dioxin-like compounds in house dusts: Taking the clean-up methods and the elution results of NITRO-HPLC fractionation into consideration, we estimated that dioxin-like compounds contained in dust samples are 2-3 ring halogenated aromatic compounds. Higher activity fractions selected by using ODS-HPLC fractionation and the DR-CALUX assay were analyzed by HRGC-HRMS. As a result, there were lots of compounds including non-identifiable compounds in those fractions. Now, we are trying to separate the dioxin-like compounds individually with further fractionation and the DR-CALUX assay.

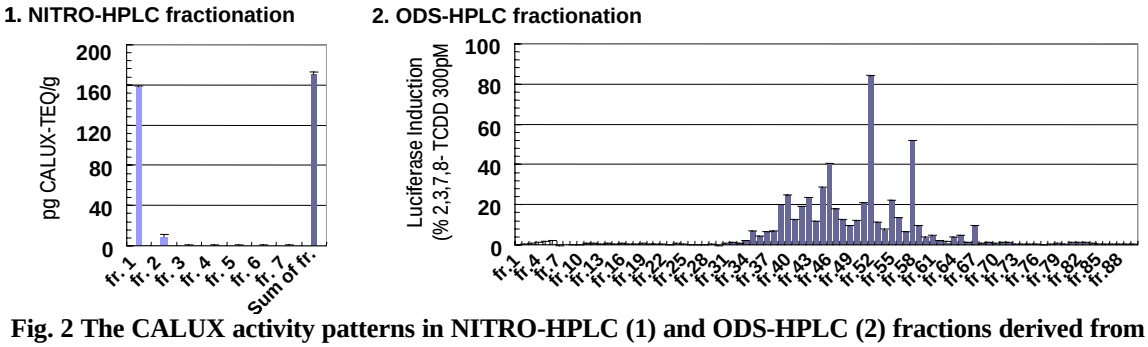

Fig. 2 The CALUX activity patterns in NITRO-HPLC (1) and ODS-HPLC (2) fractions derived from whole extract and NITRO-HPLC first fraction of HD4

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