Chlorinated and brominated TTR binding compounds in house dust

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Introduction

Exposure of children such as infants and toddlers to pollutants via indoor dust is suggested to be greater than by other pathways (Butte & Heinzow 2002). Actually, the analyses of brominated flame retardants (BFRs) such as polybrominated duphenyl 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 (Stapleton et al. 2005, Suzuki et al. 2006). Their source is suggested to be household materials such as TV, PC, fabric products, and so on. These results suggest children are exposed to various indoor use chemicals but no investigations have addressed the endocrine-disrupting potencies in indoor dust. Thereupon, our previous study have investigated TTR (the thyroxin (T4)-transport protein in serum) binding activity, which means *in vitro* competition potency with T4 for binding to TTR, in the indoor dust using *in vitro* bioassay as an aspect of the endocrine-disrupting potency (Suzuki et al. submitted) because early brain development requires thyroid hormone, especially T4. We indicated TTR binding activities were higher than those in sediments and thyroid hormone homeostasis in children might be disrupted when TTR binding activities and ingestion rate for dust are high. Identification of TTR binding compounds in dusts is important for source identification and further exposure assessment, together with the proposal of their control strategy.

In this study, we tried to identify TTR binding compounds in sulfuric acid treatment extracts of indoor dust samples. First we separated TTR binding activity of indoor dust using HPLC fractionation and *in vitro* competitive human TTR binding assay (TTR binding assay), and then we applied silicagel column fractionation to HPLC fraction shown higher activity to separate neutral and phenolic compounds. Finally, we identified TTR binding compounds contained in indoor dusts using HRGC/LRMS.

Materials and Methods

Tested indoor dust extract: Investigated TTR binding potencies were 300–5000 pmol T4EQ (thyroxine (T4) equivalent)/g (median 1000 pmol T4EQ/g) as shown in Fig. 1 (Data cited from Suzuki

et al. submitted). Indoor dust extracts indicating higher TTR binding activities were selected in this study and subjected to HPLC fractionation for separating TTR binding compounds. The procedures of sampling, extraction and clean-up for indoor dusts have been described elsewhere (Suzuki et al. submitted). Briefly, dust sample was extracted using Soxhlet apparatus with toluene. Toluene fraction was transferred to *n*-hexane by rotary evaporation. After removing elemental sulfur with activated copper, *n*-hexane fraction was subjected to sulfuric acid treatment and applied to a sulfuric acid silicagel column. After elution with *n*-hexane, the solution was evaporated. The residue was dissolved in DMSO. The potency of dust sample to compete with thyroxin (T4) for binding to TTR was investigated using TTR binding assay according to our previous study (Suzuki et al. submitted).

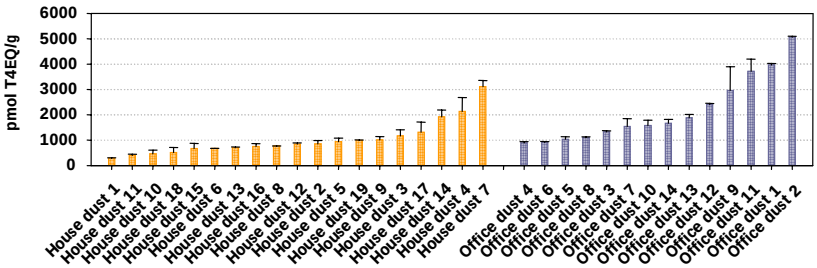

Figure 1 TTR-binding potencies in indoor dusts. Data cited from Suzuki et al. submitted.

Fractionation of TTR binding compounds with RP-HPLC: Selected indoor dust extracts were fractionated on reverse phase (RP)-HPLC using an octadecylsilica (ODS) column according to previous study (Suzuki et al. 2004) with modifications. A sample was injected and then fractionated over a period of 80.0 min: fractions were collected at 30-s intervals from 0 to 25.0 min, at 60-s intervals from 25.0 to 50.0 min and 120-s intervals from 50.0-80.0. All the fractions were evaporated, and the residue was taken up in DMSO and then assessed using the TTR binding assay.

Fractionation of TTR binding compounds with activated silicagel column: RP-HPLC fraction shown higher binding potency was fractionated using silicagel column. First, the HPLC fraction in DMSO was diluted with water and extracted three times with *n*-hexane. The extract was dehydrated and then evaporated to a small volume. The concentrate was then applied to a silicagel column. The first fraction was eluted with 1% (v/v) acetone/*n*-hexane, containing the neutral compounds. The second fraction, eluted with acetone, contained the phenolic compounds. Then the sample was evaporated, dissolved in DMSO and assessed using the TTR binding assay.

Identification of TTR binding compounds using HRGC/LRMS: TTR binding compound in silicagel fractionation sample was methylated by addition of ethereal diazomethane (24 hr at 4°C) before analysis using HRGC/LRMS. In this study, two different ionization techniques are used, which are electron ionization (EI) and electron-capture negative ionization (ECNI).

Results and Discussion

Based on results of our previous study, office dust 1 and 2, and house dust 4, 7 and 14 were selected for subsequent the TTR binding assay and RP-HPLC fractionation (Fig. 1). Indoor dusts indicating higher activity were injected and then separated into 90 fractions by ODS-HPLC. The TTR binding potency patterns in the 90 fractions derived from dusts were shown in Fig. 2. The some fractions during the early elution time indicated relatively higher binding potencies in five dust samples.

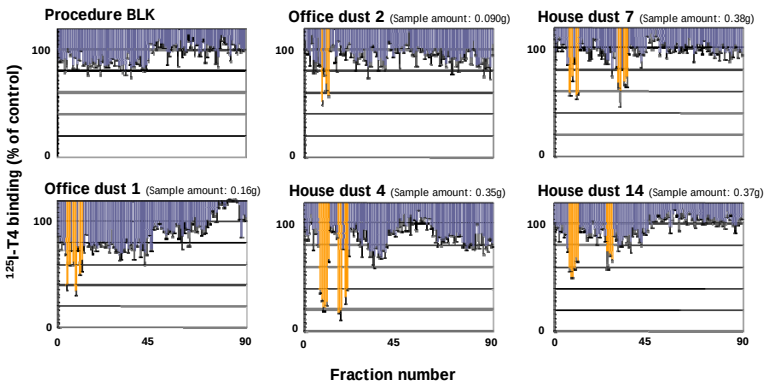

Figure 2 TTR-binding potency patterns in the 90 ODS-HPLC fractions derived from dust samples

Furthermore, several ODS-HPLC fractions, which indicated higher activity, were in common among dust samples. In order to obtain the further information for identifying the TTR binding compounds in dust samples, house dust extracts was subjected to the activated silicagel column. As a result, higher TTR binding potencies were indicated in the fractions containing the phenolic compounds and not shown in the fractions containing the neutral compounds (Table 1).

Table 1 Results of the silicagel column fractionation

Fraction number	Neutral fraction		Phenolic fraction	
	125I-T4 binding (% of control)			
	AVERAGE	STDEV	AVERAGE	STDEV
HD4_fr.8	108	14	55	2
HD4_fr.9	111	6	35	2
HD4_fr.10	96	2	28	2
HD4_fr.11	100	4	34	2
HD4_fr.12	98	3	71	1
HD4_fr.17	103	6	60	2
HD4_fr.18	102	3	66	2
HD4_fr.20	113	3	67	1
HD4_fr.21	115	5	82	3
HD7_fr.8	112	8	52	3
HD7_fr.9	110	1	55	5
HD7_fr.11	106	5	34	4
HD7_fr.12	111	3	35	0
HD7_fr.31	103	8	81	0
HD7_fr.32	103	6	64	3
HD7_fr.34	108	3	82	20
HD7_fr.35	110	4	85	3
HD14_fr.8	103	9	38	4
HD14_fr.9	113	12	27	5
HD14_fr.10	101	1	37	5
HD14_fr.11	91	6	50	5
HD14_fr.12	103	3	42	2
HD14_fr.26	104	3	64	3
HD14_fr.27	100	2	75	1
HD14_fr.28	98	2	70	5

After derivatizing compounds in the phenolic fractions of three house dusts possessing TTR binding activity (Table 1), analysis of methylated compounds was performed using EI and ECNI full-scan mode of HRGC/LRMS. As a result, the peaks, which didn't exist in the blank, were detected by HRGC /LRMS (EI and ECNI) in the phenolic fraction 8 to 12 of house dust 4, 7 and 14. On the other hand, there was no peak in other fractions of three house dusts, which might be due to the limit of detection. By comparison between the EI mass spectrums of these peaks and NIST (National Institute of Standards and Technology) reference spectrums, sample peaks in the phenolic fractions 8 to 12 of house dust 4, the phenolic fractions 8 and 9 of house dust 7, and the phenolic fractions 8 to 10 of house dust 14 were identified as pentachlorophenol (PeCP). Furthermore, although some peaks in the phenolic fractions 11 and 12 of these three house dust samples were identified as 2,4,6-tribromophenol (TriBP), sample peaks in the phenolic fractions 10 and 11 of house dust 4 and 11, and the phenolic fraction 11 of house dust 7 were identified as 2,3,4,6-tetrachlorophenol (TeCP). EI mass spectrum of

identified peaks in the phenolic fraction 11 of house dust 4 and NIST reference spectrum of MeO-PeCP, MeO-TriBP and MeO-TeCP were shown in Fig. 3 These compounds were also identified by comparing with the EI spectrums of these standard compounds.

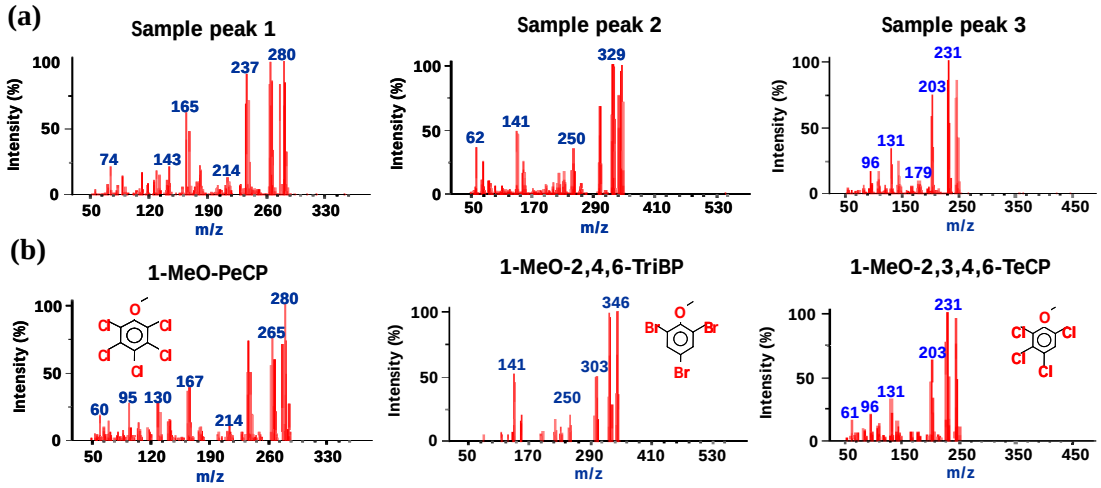

Figure 3 EI mass spectrums of three peaks detected in the phenolic fraction 11 of house dust 4 (a) and NIST reference spectrum of 1-MeO-PeCP, 1-MeO-2,4,6-TriBP and 1-MeO-2,3,4,6-TeCP (b).

Van den Berg (1990) has reported that PeCP was a competitor for the T4 binding site of TTR and the relative affinity of binding of PeCP to TTR was about twice that of T4. TriBP were also reported to be a very potent T4 competitor in the TTR binding assay with 10 times higher TTR binding potency than T4 (Hamers et al. 2006). On the other hand, although there are no data about TTR binding potency for TeCP, it might possess binding potency because its molecular structure is similar to PeCP and TriBP. Our results have shown that the above stated fractions contain PeCP, TriBP and TeCP as the TTR binding compounds. Presently we are trying to quantify concentrations of these compounds in a variety of dust samples collected.

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