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# COMPREHENSIVE ANALYSIS OF HOUSE DUST USING GC×GC-HRTOFMS AND IN-HOUSE SOFTWARE

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## Introduction

Over the last decade, comprehensive two-dimensional gas chromatography (GC×GC) coupled with high-resolution time-of-flight mass spectrometry (HRTOFMS) has been applied to global analysis of environmental samples[1,2,3]. GC×GC/HRTOFMS allowed exhaustive detection of compounds with wide and accurate mass in only one measurement. Therefore, information available from GC×GC/HRTOFMS has dramatically increased compare with traditional methods. However, features of software in the market have been not adequate to analyze the data of GC×GC/HRTOFMS yet. In the past five years, therefore we have developed several original software for GC×GC/HRTOFMS, such as "T-SEN" (automatic peak sentinel tool) [4] , "CBEx" (selective extraction tool for halogenated compounds) [5] and "NMFwithDBcreator" (global spectral deconvolution tool based on non-negative matrix factorization) [6] . They have been applied to environmental samples, such as lake sediment (T-SEN), indoor air (CBEx), fly ash (CBEx), human urine (CBEx) and sea sediment (NMFwithDBcreator), respectively and they showed thier effectivity to help process comprehensive data from GC×GC/HRTOFMS.

Recently, number of children with a developmental disability has been increasing. Some environmental factors (e.g. exposure to chemicals) are suspected of leading to the emergence of the disease[7] . House dust is known as a sink of many kinds of pollutants, such as polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and polychlorinated dioxins/furans (PCDD/Fs) [8,9] . Moreover, house dust is considered as one of the major routes of chemical exposure to children, and suggested to be greater than the other pathways [8,9,10] . However actual levels and composition of pollutants in house dust widely differs in each case according to those reports. Therefore, elucidation of global and each levels and composition of hazardous compounds in house dust is needed.

In this study, comprehensive analysis using GC×GC/HRTOFMS and in-house software was applied for a house dust sample. Firstly, detection capability of compounds was evaluated by using a standard solution. Secondly, persistent organic pollutants (POPs), such as PCBs whose concentrations were certified by NIST, in extract of certified house dust sample (NIST SRM 2585) were automatically quantified by using "T-SEN". Finally, mass spectral deconvolution tool "NMFwithDBcreator" was used for non-target analysis of pollutants in a house dust sample. The deconvoluted exact mass spectra were searched in NIST library and in-house library which had been provided by measurement of several standard compounds and an elemental composition was estimated. As a result, several compounds not included in the certified compound list were tentatively estimated in the house dust sample.

## Materials and methods

Samples

Standard solution containing 17 PCDD/Fs, 27 POPs, 27 PCBs, 28 PBDEs and 16 PAHs (ES-5521, Cambridge Isotope Laboratories (CIL), Inc.), and ^{13}C-PAHs (ES-4087, CIL Inc.) were used for the evaluation of in-house developed software. House dust sample (NIST SRM 2585) was analyzed for characterization of existing contaminants. Approximately 1 g of house dust sample was Soxhlet extracted for 18 hours with 200 mL of 50 % hexane and 50 % acetone (volume fraction). ^{13}C-PCBs (EC-4977, CIL Inc.) was added to the house dust sample before Soxhlet extraction. The extract was concentrated up to 1 mL and 1 μL of the extract was used for the analysis without any clean-up. All solvents used were high-purity pesticides grade.

GC×GC-HRTOFMS

Analyses were performed with a Zoex KT2006 loop type modulator (Zoex Corporation, Houston, TX, USA) installed on an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a JEOL AccuTOF GCV 4G. InertCap 5MS/Sil (45 m length, 0.25 mm i.d., 0.1 μm film thickness, GL Sciences Inc.) was used for the first column and BPX-50 (0.9 m length, 0.10 mm i.d., 0.10 μm thickness, SGE) was used for the second column. An 1 μL-sample was injected into an inlet in the splitless mode with a 2 min splitless time at 280 ºC. During the injection, the GC was held at the initial temperature of 50 ºC. The GC was programmed from 50 ºC (held for 2 min) to 350 ºC (held for 0 min) at 3 ºC /min for both the first column and the second column. Helium was used as a carrier gas supplied at 1.8 mL/min. The modulation period was 7 sec. A HRTOFMS was operated at a multi-channel plate voltage of 2000 V and a mass range of m/z 35-850 using electron ionization (EI). The data acquisition speed was 33 Hz.

Data analysis software
GC×GC-HRTOFMS data were analyzed using two in-house software, "T-SEN" (automatic peak sentinel tool) and "NMFwithDBcreator" (global spectral deconvolution tool based on non-negative matrix factorization), and one commercial software "GC Image"(GC Image, LLC)).

**Results and discussion**
Effectivity of detection and quantification of known compounds by using software "T-SEN" was evaluated with the standard solutions (ES-5521) containing 17 PCDD/Fs, 27 POPs, 27 PCBs, 28 PBDEs and 16 PAHs. Firstly, a standard solution (200 pg/μL, 1μL) was measured by GC×GC/HRTOFMS in order to provide database for a T-SEN program which requires retention times on 1st and 2nd columns and several set of exact mass numbers and intensity ratios of those mass for quantification of compounds. In this study, 5 data set were initially provided for each compound. After preparation of the database, each 1 μL of the standard solution (5, 25, 50, 100 and 200 pg/μL) was measured and compounds registered in the database were automatically quantified by T-SEN at criterion of mass error: ±0.05. All compounds over 5 pg were faithfully detected and quantified when 3 dataset for each compound was employed even though PCB #15, 37, 126 and 169 were not detected in the 5 pg/μL of solution if 5 dataset had been demanded. It suggests that adequate intensity for all mass spectra was required to quantify by using T-SEN. Linearity of calibration curves of all compounds registered in the T-SEN database was also confirmed in the range from 5 to 200 pg ($R^2$ >0.98).

Fig. 1 shows a 2D total ion chromatogram (TIC) and 3D view of the house dust sample (NIST SRM 2585) measured by GC×GC/HRTOFMS. The several thousands of peaks were detected on 2D TIC even though no sample clean-up was applied. In consequence of the NIST library search, PCBs, PBDEs, polycyclic aromatic hydrocarbons (PAHs) and legacy POPs were identified in the house dust sample. PAHs and methyl PAHs were clearly separated from the unresolved complex mixture (UCM), consisting mainly of aliphatic hydrocarbons on the 2D TIC.

Concentrations of PCBs, PBDEs, PAHs and chlorinated pesticides in the house dust were automatically quantified by T-SEN and some were compared with certified values by NIST if the concentration was available in the publication. As a result, the concentrations obtained in this study were matched well to the certified values. For example, concentrations of PCB #118, #105 and #206 were 23.3, 10.8 and 2.9 pg/g (this study) against 26.3, 13.2, and 3.8 pg/g (NIST), respectively. Some compounds not listed in the NIST certification were also quantified by T-SEN regardless of determination by authentic method.

In this study, crude extract of the sample was directly measured by GC×GC/HRTOFMS to avoid loss of compounds included in the sample. Therefore it was supposed that insufficient separation of compounds despite of GC×GC application. For further quest of compounds and to obtain reliable result from mass library search to the house dust sample, global spectral deconvolution tool "NMFwithDBcreator" was used for non-target and detailed qualitative analysis. Original data was deconvoluted into 4 data set composed of 4 layers by the software, the number of layer had been set as a parameter before execution of the program. Fig.2 shows an example for the result of deconvolution. On the original 2D TIC (top of the figure), a peak was tentatively identified as trans-Chlordane having reverse match factor: 766. In top mass spectra, unique spectra of trans-Chlordane were observed in spectra of UCM. On the other hand, unique spectra of trans-Chlordane were clearly separated as shown in the layer 2 (bottom) from the spectra of UCM in layer 1 (middle), and the reverse match factor of NIST library search to the deconvoluted spectra was increased to 869. Incidentally mass error between measured and theoretical molecular mass of trans-Chlordane (m/z 405.7983) was calculated as -1.3 ppm.

Table 1 shows some results of NIST library searching to peaks from the house dust sample after the deconvolution. In the house dust sample, many kinds of compounds were detected. For example, methylparaben, propylparaben, triclosan and miconazole have been used for pharmaceutical and personal care products (PPCP), diazinone and chlorpyrifos are pesticide. Furthermore, several kinds of phosphorus-containing flame retardants and plasticizers, such as triethyl phosphate, triphenyl phosphate and tris-(methylphenyl) phosphate, were also detected. Isomers of tris-(methylphenyl) phosphate eluted linearly on the 2D map and group type analysis wouldbe possible. Phosphorus-containing flame retardants have recently been detected in the house dust sample because of the restrictions on the use of POPs and the manufacture of PBDEs[9] . Although some compounds were found from the original data almost all of their match factors were lower than that obtained after deconvolution. And some compounds could not be specified because of several reasons such as low match factor (< 700) of library search, and lack of intensity and/or separation even after deconvolution.

In conclusion, the combination of GC×GC/HRTOFMS and in-house software was very powerful for the comprehensive qualification and semi-quantification of wide range of compounds. Application of the methods to the house dust showed effective and useful to identify many kinds of compounds such as PCBs, PBDEs, PAHs, pesticides, phosphorus-containing flame retardants, plasticizers and PPCPs even if no sample clean-up was needed. However, exact mass spectra database for environmental pollutants is essential for further effective non-target analysis by GC(×GC)/HRTOFMS.

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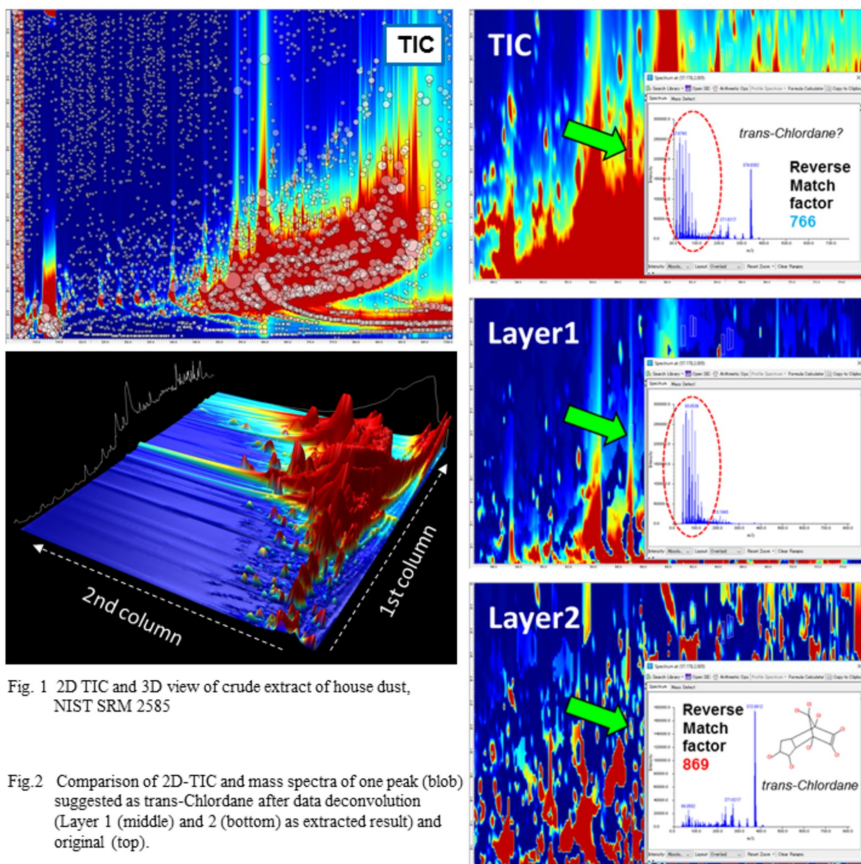
Fig. 1 2D TIC and 3D view of crude extract of house dust, NIST SRM 2585

Fig.2 Comparison of 2D-TIC and mass spectra of one peak (blob) suggested as trans-Chlordane after data deconvolution (Layer 1 (middle) and 2 (bottom) as extracted result) and original (top).

Table 1 The results of identification of some compounds in house dust sample after mass spectra deconvolution by an in-house software "NMFwithDBcreator"

| Compound Name | Library Formula | RT I (min) | RT II (sec) | MF | RMF | Exact mass | Detect mass | Mass error (ppm) |
|---|---|---|---|---|---|---|---|---|
| Methylparaben | $C_8H_8O_3$ | 36.06 | 1.91 | 741 | 850 | 152.0473 | 152.0463 | 6.93 |
| Propylparaben | $C_{10}H_{12}O_3$ | 42.48 | 1.81 | 683 | 813 | 180.0786 | 180.0780 | 3.85 |
| Diazinone | $C_{12}H_{21}N_2O_3PS$ | 47.73 | 1.62 | 804 | 818 | 304.1011 | 304.1003 | 2.33 |
| Chlorpyrifos | $C_9H_{11}Cl_3NO_3PS$ | 53.56 | 1.94 | 823 | 905 | 348.9263 | 348.9236 | 7.84 |
| Triclosan | $C_{12}H_7Cl_3O_2$ | 57.76 | 2.13 | 784 | 884 | 287.9512 | 287.9515 | -1.07 |
| Miconazole | $C_{18}H_{14}Cl_4N_2O$ | 79.69 | 3.40 | 721 | 898 | 413.9860 | 413.9849 | 2.71 |
| Triethyl phosphate | $C_6H_{15}O_4P$ | 21.94 | 1.21 | 731 | 833 | 182.0708 | 182.0714 | -3.26 |
| Triphenyl phosphate | $C_{18}H_{15}O_4P$ | 65.58 | 3.05 | 759 | 809 | 326.0708 | 326.0709 | -0.17 |
| Propylated triphenyl phosphate | $C_{21}H_{21}O_4P$ | 68.73 | 2.80 | 743 | 816 | 368.1177 | 368.1173 | 1.21 |
| Tris(methylphenyl) phosphate | $C_{21}H_{21}O_4P$ | 71.53 | 3.02 | 810 | 845 | 368.1177 | 368.1171 | 1.67 |
| Tris(methylphenyl) phosphate | $C_{21}H_{21}O_4P$ | 72.23 | 2.99 | 861 | 877 | 368.1177 | 368.1177 | 0.04 |
| Tris(methylphenyl) phosphate | $C_{21}H_{21}O_4P$ | 72.58 | 2.96 | 848 | 853 | 368.1177 | 368.1171 | 1.73 |
| Tris(isopropylphenyl) phosphate | $C_{27}H_{33}O_4P$ | 73.86 | 2.39 | 697 | 822 | 452.2116 | 452.2123 | -1.44 |
| Di-(t-butylphenyl) phenyl phosphate | $C_{26}H_{31}O_4P$ | 80.39 | 2.86 | 717 | 833 | 438.1960 | 438.1961 | -0.24 |