

図 - 11 Y軸断面の濃度分布結果

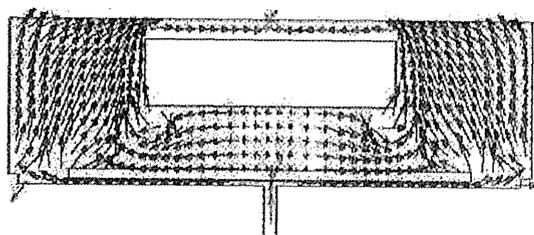


図 - 12 Y軸断面の風速ベクトル結果

表 - 6 DEHP 吸着量結果の比較

項目	計測値		比率
	チャンバ	プレート	
表面積[m ²]	0.27	0.011	2.5 : 1
DEHP 吸着量 実験値[ng]	1475	1126	1.3 : 1
DEHP 吸着量 数値計算値[ng/s]	4.46	2.64	1.7 : 1

2.4 各種プレートのDEHP吸着量評価

プレートの DEHP 吸着量の評価結果を図 - 13 に示す。図中の数値は相対変動係数を標記した。この結果より、プレートに吸着した DEHP 量は、石英プレートが一番多く、シラン処理プレートが一番少ない結果であった。シラン処理プレート測定時は、チャンバ壁面と治具に多く DEHP が吸着する傾向であり、プレートおよびチャンバ壁面と治具への DEHP 吸着量を表している合算値は、その他のプレートの値と同一レベルの結果であった。この理由は、シラン処理することによりプレート表面と DEHP の吸着力が低下したためと推定される。材料から放散する DEHP 量は一定であるため、プレートに吸着できなかった分の DEHP がチャンバ壁面などに吸着したものと考えられる。今後の試験では、石英プレートを用いることとした。

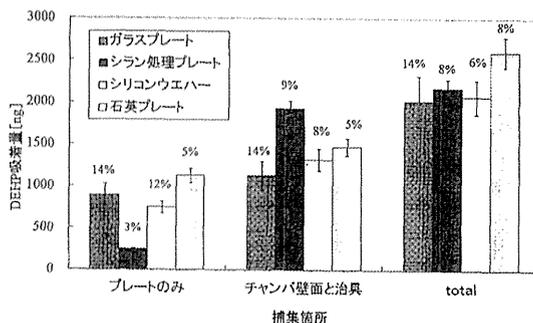


図 - 13 プレートのDEHP吸着量の評価結果 (n=3)

2.5 SUS保管容器による保管

DEHP を捕集したプレートを SUS の保管容器に保管した結果を図 - 14 に示す。この結果、保管することによりプレートに吸着した DEHP 量が低減することがわかった。チャンバ壁面に大きな差が無いのは、試験後すぐに定量評価しているからである。

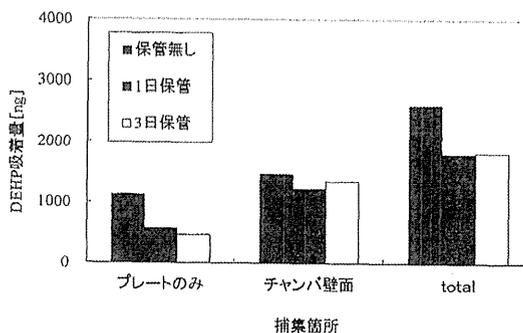


図 - 14 プレート保管における DEHP 吸着量

2.6 サンド容器による保管

捕集プレートと同一素材のプレートでサンド保管の評価結果を図 - 15 および図 - 16 に示す。これらの結果より、サンド保管により保管中における DEHP の減少は少なく、保管無しの捕集量と比較して 6% の誤差で捕捉できた。また、サンド保管における各プレートおよびチャンバへの DEHP の吸着量を図 - 17 に示す。この結果より、捕集プレートの上面に設置したプレートへの DEHP 移行が多いことがわかった。捕集プレートの下面に設置したプレートには DEHP の移行が殆ど無かった。これより、捕集したプレートの保管方法として、同一素材のプレートにより捕集面を遮蔽するようにプレートを設置すれば、吸着した DEHP が再放散によって減少することなく保管することが可能である。

プレート吸着による SVOCs 評価法の基礎検討

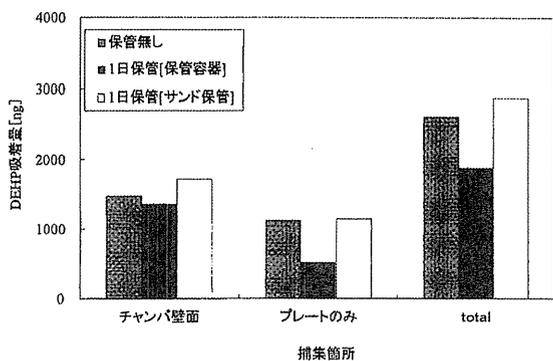


図 - 15 保管方法と DEHP 吸着量 (1 日保管 n=3)

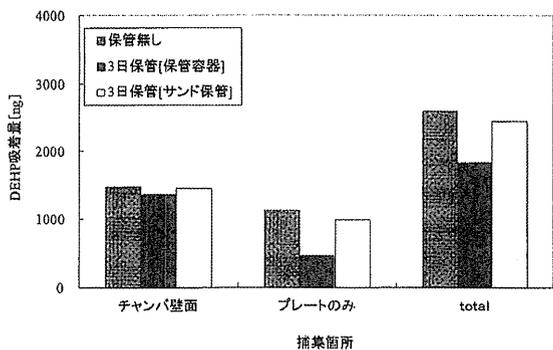


図 - 16 保管方法と DEHP 吸着量 (3 日保管 n=3)

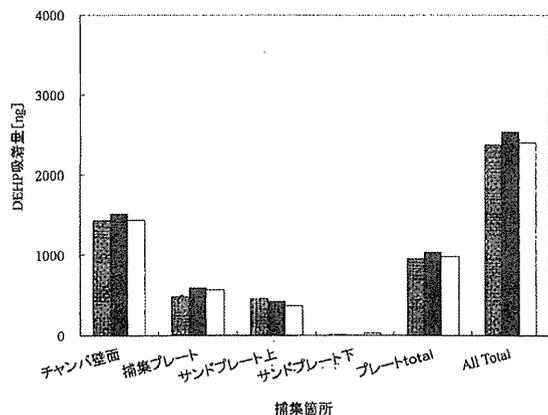


図 - 17 サンド保管における DEHP 吸着量 (3 日保管 n=3)

3. 考察

プレートに DEHP を吸着させて定量的に測定する際、捕集後の保管方法が重要になる。そこで、保管方法検討のために材料からの揮発する DEHP を利用した吸着方法を検討した。チャンバ内に揮発する DEHP はチャンバ内の気流の影響等を受けるために、材料とプレートの最適位置が存在することがわかった。この最適位置でプレートに DEHP を吸着させた後、保管方法について検討を実施したが、今までプレートに吸着した DEHP などの高沸点成分は脱離しな

いと考えていたが、実際は吸着した後に脱離することがわかった。このようにプレートに一旦吸着した DEHP などの SVOCs は吸着・脱離を繰り返していると推測される。この現象を同一素材のプレートによりサンドすることで、このプレート間の吸着・脱離現象に留まるため捕集プレートおよびサンドプレートを定量評価することにより一旦吸着させた DEHP の保管性を確保しながら定量評価することが可能となった。今回は DEHP について評価を行ったが、他の SVOCs についても同様の結果を得られるかは不明であるため、同様の評価が必要となる。

まとめ

本研究では、空気捕集では測定が困難とされていた SVOCs をプレートに吸着させることによって定量的に測定する手法を確立した。この際、捕集したプレートの保管方法が大きな課題であり、この保管方法について検討を実施した。

供試材料：フロアマット 5cm×5cm。

養生条件：25±1℃，40±5%RH，暗所条件，アルミ箔で包んで保管。

マイクロチャンバ測定条件：65℃，1時間加熱し，発生ガスを Tenax TA に捕集した後，GC/MS で定量評価した。更にその後，吸着ガスは 250℃まで昇温度させ Tenax TA に捕集して GC/MS で定量評価した。

- 1) フロアマットから放散する DEHP の測定を実施した結果、相対変動係数は約 8%であった。約 1 年経過しても放散量の減衰は無いことがわかった。
- 2) フロアマットから放散した DEHP をプレートに吸着させてプレートへの DEHP 吸着量の評価手法を検討した。この結果、フロアマットの下側にプレートを配置すると、マイクロチャンバ内でプレートに効率良く DEHP が吸着することがわかった。数値解析の結果より、マット下側にダウンフローが発生し、プレート表面に DEHP が拡散することにより効率的な DEHP 吸着ができる配置となっていることが明らかとなった。
- 3) プレート素材による DEHP 吸着量評価を行なった結果、本研究における試験条件では石英プレートが効率の良い吸着性能を有していることがわかった。一方、シラン処理プレートは吸着性能が低いことがわかった。
- 4) DEHP 捕集したプレートの保管方法を検討した結果、保管 1 日後には、約 38%まで減少した。しかし同一素材のプレートによりプレートの捕集面を遮蔽（サンドイッチ）することにより捕捉した DEHP 量の減少を防ぐことができた。この時は、捕集プレートとサンドイッチに用いたプレート 2 枚、合計 3 のプレートを定量

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評価した。これらの結果から2枚のプレートで実空間のDEHPを捕集した後、これらプレート捕集面を重ねて保管することによりDEHPの減少を防ぐことが可能となった。

謝辞

本研究を実施するにあたり、エスベック長尾祥大氏、カネカテクノロジーサーチ佐藤勝二氏からは部品測定および成分分析に関して懇切な指導・助言を受けた。また共同実験者の内藤敏幸氏、竹田哲也氏に対して深謝の意を表します。

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Basic Study on the SVOC Measuring Method Using Plate Adsorption

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Key Words: Analysis, Experiment, Measurement, SVOCs

Synopsis : To decrease the indoor VOC concentration in a vehicle the Japan Automobile Manufacturers Association, Inc. is tackling the self-imposed control of indoor VOC concentration in a vehicle.

Although high-boiling- point substances, such as phthalate ester, are part of the substances for self-imposed control, these high-boiling- point substances are difficult to quantity with sufficient accuracy in the collection using the conventional tube (Tenax-TA). Thus, we examined an evaluation technique that uses a glass plate as the STET medium of a high-boiling- point substances for quantitatively evaluating high-boiling-point

substances. This paper reports on the result of the method for selecting the plate for the quantitative evaluation technique of a high-boiling-point ingredient, and storage.

As a result, the adsorption of DEHP was excellent with a quartz plate.

The storage was able to maintain reduction the DEHP after a collection with 6% error by storing using a plate of the same material.

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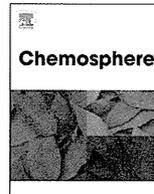
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Simple and rapid determination of PCDD/Fs in flue gases from various waste incinerators in China using DR-EcoScreen cells



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HIGHLIGHTS

- We construct an automated sample preparation device (SPD-600) for bioassay.
- We construct a new, sensitive and rapid reporter gene system for PCDD/Fs.
- Utilizing SPD-600 coupled cell to determine four different flue gases in China.
- Utilizing SPD-600 coupled cell can be a useful and prescreening method in China.

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ABSTRACT

In developing countries such as China, there is a strong need for simple and rapid bioassays for the determination of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in environmental samples; i.e., flue gas and fly ash from waste incinerators. In this study, we applied the DR-EcoScreen cell (DR-cell) assay to determination of PCDD/Fs in 78 flue gas samples obtained from various waste incinerators in China between 2009 and 2011. The flue gas samples were obtained from four kinds of incinerators, classified into hazardous, medical and municipal-solid waste, and iron ore sintering, and the flue gas extracts were cleaned up using an SPD-600 automated-sample preparation device for DR-cell assay. The PCDD/Fs values obtained from the DR-cell assay were compared with those obtained from conventional high resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS) analysis. The bioanalytical equivalent (BEQ) values obtained from the DR-cell assay were very closely correlated with the international toxicity equivalent (I-TEQ) values from HRGC–HRMS analysis ($r^2 = 0.98$, $n = 78$), while the BEQ values were 5.52-fold higher than the I-TEQ values, as the PCDFs, which account for 80% of the total I-TEQ value, were overestimated by DR cell-assay. Therefore, we multiplied the BEQ values from the DR-cell assay by a conversion coefficient (0.181, the reciprocal of 5.52), and could approximate the TEQ values from the HRGC–HRMS analysis. These results suggest that the DR-cell assay combined with SPD-600 cleanup provides a promising method for the simple and rapid screening of PCDD/Fs levels in flue gas samples, such as those from various waste incinerators in China.

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1. Introduction

China is facing growing environmental pressure due to the rapid economic development and urbanization occurring over the last three decades. Consequently, the quantity of various wastes has increased at a high rate, and their disposal has had a great impact on the environment and on public health. To dispose of

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huge amounts of solid waste at low-cost and in an environmentally friendly manner, many incinerators have been constructed in China. Although incineration offers many advantages, such as significant volume and mass reduction, some secondary pollution with the release of compounds, such as heavy metals (Jung et al., 2004; Yao et al., 2012), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Shibamoto et al., 2007; Ni et al., 2009).

These PCDD/Fs, so-called dioxins, are produced as unintentional by-products, and enter the environment via incineration, thermal processes and chemical manufacture (Zheng et al., 2008). These PCDD/Fs are widely distributed contaminants that are persistent and bio-accumulative, and can induce various toxic responses including immunotoxicity, carcinogenicity, as well as having adverse effects on reproduction, development, and endocrine functions via aryl hydrocarbon receptor (AhR) (Poland and Knutson, 1982; Safe, 1986; Fernandez-Salguero et al., 1996; Mimura et al., 1997). Therefore, the Chinese government has imposed standardized limits on PCDD/F emissions from flue gas of 1.0 ng I-TEQ N m⁻³ for municipal solid waste incinerators and 0.5 ng I-TEQ N m⁻³ for medical and hazardous waste incinerators. A better understanding of the levels and distribution of these compounds will allow more appropriate measures to be employed to reduce their emission.

The “gold standard” chromatographic technique based on high resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS) has been extensively used for the conventional determination of 17 PCDD/Fs (Firestone, 1991; Jong et al., 1993; Singh and Kulshrestha, 1997). The measured values of 17 PCDD/Fs are individually multiplied by a toxicity equivalency factor (TEF) and totaled to give I-TEQ values (Van den Berg et al., 2006). This method provides reliable data including the concentration of each of the 17 congeners in the test samples. However, it also requires expensive equipment and highly trained analysts, whilst the sample preparation procedures are time-consuming and costly. In particular, this method might be less than useful when rapid data on PCDD/Fs from a large set of test samples is required. For this reason, the development of a rapid and inexpensive screening method for PCDD/Fs remains a high priority, especially in developing countries with limited resources, such as China. Thus, there is a definite need to develop a faster and lower-cost bioassay methods for the determination of PCDD/Fs.

Reporter gene assays using hepatocarcinoma cells, which express the AhR gene and luciferase reporter gene containing the dioxin-responsive element (DRE), are applicable to the detection of dioxin-like compounds based on their activation of AhR (Garrison et al., 1996; Murk et al., 1996). Recently, Takeuchi have developed a new, sensitive and rapid reporter gene assay (DR-cell assay) using a genetically engineered stable cell line, designated DR-EcoScreen cells. The minimal detection limit (MDL) and 50% effective concentration (EC₅₀) of 2,3,7,8-TetraCDD (TCDD) in this DR-cell assay are 0.1 pM and 2.8 pM, respectively, with little variance observed in the data (within CV 10%), but other reporter gene assays, such as, Hepa1c1c7- and H4IIE-based CALUX assays, the MDL of 2,3,7,8-TCDD were reported to be 1 and 0.3 pM, and the EC₅₀ of 2,3,7,8-TCDD were reported to be 10 and 10 pM, respectively (Behnisch et al., 2002; Han et al., 2004). Besides high sensitivity, the DR-cell assay has unique advantages compared to other bioassays. As the DR-EcoScreen cells have very strong luminescence intensity and can be measured using a long-lived luciferase substrate, a bioassay using these cells does not require well-washing or medium changes during the procedure. Thus, the DR-cell assay is compatible with high-throughput automation and can reduce the overall workload in a laboratory. Most recently, based on a comparative study with HRGC–HRMS analysis, it has been reported that the DR-cell assay was helpful in determining low levels

of PCDD/Fs and dioxin-like polychlorinated biphenyls (PCBs) in ambient air samples (Anezaki et al., 2009) as well as in fish and seafood samples (Kojima et al., 2011).

In the present study, we investigated the applicability of the DR-cell assay to the determination of PCDD/Fs in 78 flue gas samples from four kinds of incinerators, including medical waste and municipal solid waste incinerators, in China as a prescreening step to the HRGC–HRMS method. In addition, we have now combined the DR-cell assay with a cleanup procedure utilizing an SPD-600 automated-sample preparation device. The bioanalytical equivalent (BEQ) values from the DR-cell assay were compared with the I-TEQ values from the HRGC–HRMS analysis, and we found that the values from both methods showed a very close correlation. Here, we provide evidence that the DR-cell assay coupled with SPD-600 cleanup might afford a promising method for the simple and rapid screening of PCDD/Fs in flue gas, such as that from various waste incinerators in China.

2. Materials and methods

2.1. Chemicals and cell culture materials

Acetone, *n*-hexane, toluene, and dichloromethane were obtained from J.T. Baker, Co., Ltd. (USA). Dimethyl sulfoxide (DMSO) and some kinds of silica gel for multi-layer column chromatography were obtained from Wako Pure Chemicals Inc., Ltd. (Osaka, Japan). The PCDD/F standards were obtained from Wellington Laboratories (Canada).

Fetal bovine serum (FBS), alpha-modified Eagle's minimum essential medium (α -MEM) and hygromycin were obtained from Invitrogen (San Diego, CA, USA). Glutamine and penicillin–streptomycin (antibiotics) solutions were obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). A 0.25% trypsin/0.02% ethylenediamine tetra-acetic acid (EDTA) disodium salt solution was obtained from Life Technologies (Paisley, UK). The luciferase substrate, Steady-Glo™ reagent, was purchased from Promega (Madison, WI, USA).

2.2. Collection, extraction, and cleanup of flue gas samples

We collected 19, 20, 21, and 18 flue gas samples from hazardous waste incinerators (i.e., chemical plants, and pesticide and paint factories), medical waste incinerators, municipal solid waste incinerators, and iron ore sintering furnaces, respectively, in China between 2009 and 2011. PCDD/Fs in flue gas were captured by a quartz filter cylinder, and XAD-2 resin with a vacuum pump (TCR TECORA, Italy) (gas volume approximately 3 m³, 0 °C and 1 atm). Each sample was then extracted with 300 mL of toluene by soxhlet for 24 h. The solvent was reduced to around 1 mL in a rotary evaporator, then 100 mL *n*-hexane was added. The *n*-hexane solution was treated with 20 mL of concentrated sulfuric acid until the *n*-hexane layer became colorless. After washing the extract twice with 50 mL of 2% NaCl solution, it was evaporated to a 1 mL in a rotary evaporator.

As shown in Fig. 1a, the cleanup of samples was conducted using two different methods: cleanup for the DR-cell assays employed an SPD-600 automated-sample preparation device (Kyoto Electronics Company, Ltd., Kyoto, Japan), whereas the cleanup for the HRGC–HRMS analysis used conventional manual chromatography columns, including a multi-silica gel column, an alumina column and a florisil column (JIS K0311, 1999). The SPD-600 device has a multilayer silica gel column (12.5 × 200 mm) and an alumina column (0.8 g). The multilayer column is composed, from bottom up, of silica gel (0.5 g), 10% AgNO₃ silica gel, 44% H₂SO₄ silica gel (10 g), and silica gel (0.5 g). The PCDD/Fs were adsorbed on the

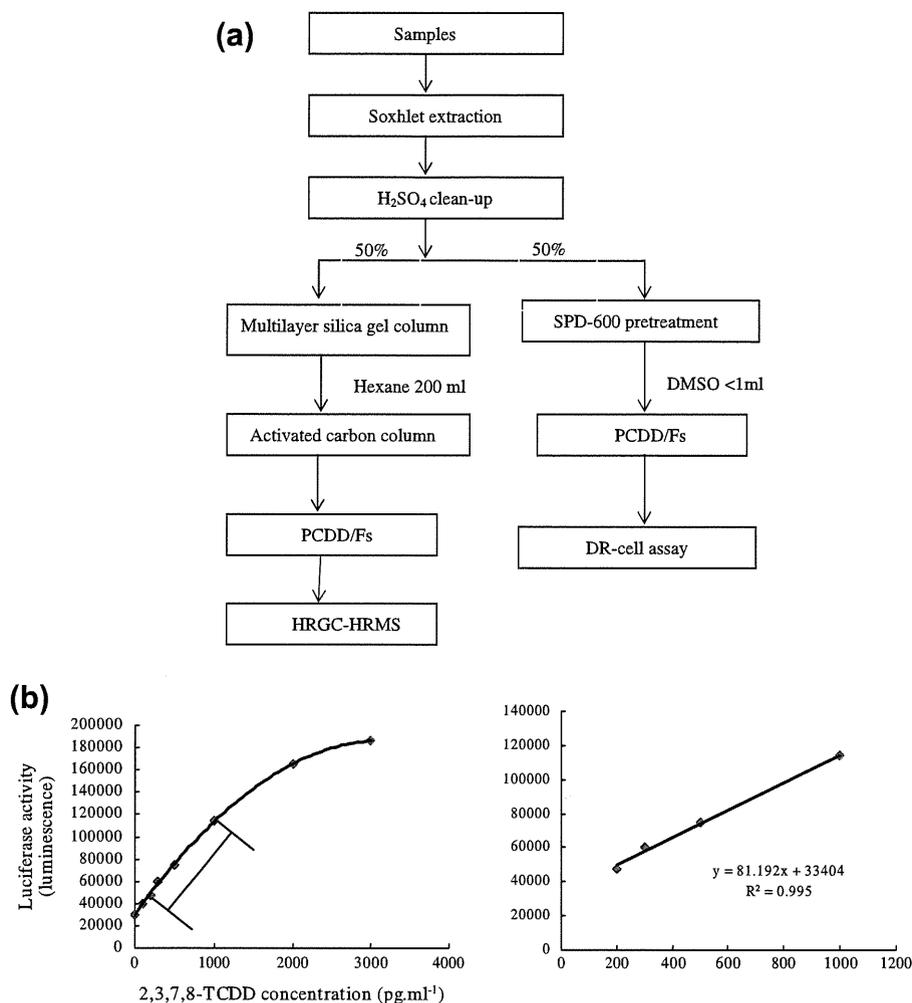


Fig. 1. Schema for the extraction and cleanup of flue gas samples for the DR-EcoScreen cell assay (a), and the standard curve and quantitative regression line for 2,3,7,8-TCDD as determined using this assay (b).

alumina column, and eluted with 0.7 mL DMSO. In the conventional manual cleanup for HRGC–HRMS, the extract was concentrated again then transferred to a vial. The solvent remaining in the vial was then reduced to about 20 μ L by a gentle stream of nitrogen. ¹³C-labeled PCDD/F recovery standard mixture was spiked prior to HRGC–HRMS analysis.

2.3. DR-EcoScreen cell bioassay

A highly sensitive AhR-mediated reporter cell line, DR-EcoScreen cells, developed from a mouse hepatoma Hepa1c1c7 cell line, was stably transfected with a reporter plasmid containing seven copies of DRE fused to a luciferase gene (Takeuchi et al., 2008). The DR-EcoScreen cells were maintained in α -MEM supplemented with 5% FBS, antibiotics, glutamine, and 150 μ g mL⁻¹ of hygromycin at 37 °C in an atmosphere of 5% CO₂ and 95% air under saturating humidity, and passaged twice every week by trypsinization with 0.25% trypsin/0.02% EDTA. For the screening assay, cells were trypsinized and suspended at a density of 1.0×10^5 cells mL⁻¹ in α -MEM containing 5% CD-FBS. Ninety μ L of the cell suspension was seeded in each well of a 96-well flat-bottomed plate (#136102 Nunclon™, Nalge Nunc, Denmark) at a final density of 9000 cells well⁻¹. After cultivation for 24 h at 37 °C, 10 μ L of each of the various flue gas samples dissolved in 10% DMSO was added to each well (final concentration of DMSO was 1%). Following cultivation for a further 24 h, 100 μ L of Steady-Glo™ reagent was

added to each well. The plate was then shaken at room temperature for five min, and the luminescence was measured with a microplate-reader (Wallac 1420 ARVO™ SX, Perkin-Elmer). The dioxin concentrations in the samples were calculated as cell-based BEQ values using the quantitative regression line of the standard curve for 2,3,7,8-TCDD (Fig. 1b).

The validation of extremely sensitive responses for PCDD/Fs measurement for this cell were done by repeatedly analyzing the calibration standards ($n \geq 5$). The lowest concentration of the coefficient of variation (CV) < 30% was defined as the limit of detection (LOD). The lowest concentration of CV < 20% was defined as the limit of quantification (LOQ). The LOD and LOQ values for the bioassay were 0.1 and 0.2 pg mL⁻¹ medium respectively. The LOD and LOQ could also be calculated to be 0.005 and 0.01 pg BEQ m⁻³ for about 1 L exhaust gas, respectively. And the quantitative regression line was set from 0.2 pg to 1.0 pg 2,3,7,8-TCDD mL⁻¹.

2.4. Quality control setting for DR-cell bioassay

Validation of this methodology requires strict quality control criteria, specifically designed for the DR-cell bioassay and quite different from the ones used in chemical analyses.

The first quality control is designed to assess the quality of the plates. For that purpose, a standard TCDD solution (50 pg mL⁻¹ DMSO) was analyzed for each plate. The concentration of this solution was chosen to be in the middle of the quantifiable. The

averages (μ) and RSDs (σ) of many test results provide an assessment of the measurement repeatability on the 96 wells plate. According to the Western Electric Handbook (Montgomery, 2001), the control limits were $\mu \pm 3\sigma$, and the warning limits were $\mu \pm 2\sigma$. When the standard solution was not within the warning limits, the DR-cell bioassay was re-checked. On the basis of these criteria, 85% of the plates were accepted.

Secondly, a quality control sample (Certified reference material) was assessed by six complete analytical runs including every step from the exposor and light detection. For the reproducibility, the same sample was analyzed in duplicate on 6 different days. The CV of the repeatability and reproducibility test were 5% and 8%, respectively. Those values were lower than 20%, and could be accepted.

Thirdly, Recoveries of PCDD/Fs by these methods have been previously validated (Kojima et al., 2011). Finally, for the results to be accepted, the RLU ratio between samples and DMSO blank must more than 1.5.

2.5. HRGC–HRMS analysis

The quantification of 17 PCDD/Fs was performed by HRGC–HRMS on a 6890N Series gas chromatograph (Agilent Technologies, USA) coupled to an AutoSpec Ultima NT high-resolution mass spectrometer (Waters, USA). The injector was operated in splitless mode and kept at 270 °C. A DB-5ms capillary column (60 m \times 0.25 mm i.d., 0.25 μ m film thickness; Supelco, USA) was used for separation of the PCDD/Fs congeners. The column oven temperature was programmed at 160 °C for 2 min, increased to 220 °C at a rate of 5 °C min⁻¹, maintained at that temperature for 16 min, then increased to 235 °C at a rate of 5 °C min⁻¹, again maintained at that temperature for 7 min, then finally increased to 330 °C at a rate of 5 °C min⁻¹. The ion source was operated at 220 °C, with an electron energy of 45 eV, and selective ion monitoring (SIM) mode was used at resolution of >10000. Instrument detection limits were determined as a signal-to-noise ratio of 3:1. The measurement values were multiplied by I-TEF (Van den Berg et al., 2006) and totaled to obtain the I-TEQ.

The limits of detection (LODs) of HRGC–HRMS were around 0.01 pg m⁻³ for Tetra- and Penta-CDDs/Fs, 0.001–0.003 pg m⁻³ for Hexa- and Hepta-CDDs/Fs, and 0.002–0.004 pg m⁻³ for Octa-CDD/F. When the value was lower than the LOQ, the actual concentration was calculated as one-half of the LOD.

3. Results and discussion

3.1. Recovery capability and repeatability of PCDD/F determination in combination with an SPD-600 automated-sample preparation device

The recovery capability and repeatability of the SPD-600 method were tested by replicate analysis (3 times), and compared with those from the manual method used in conventional HRGC–HRMS analysis. Reference samples spiked with an internal standard were purified and substituted with DMSO solutions using the SPD-600 device. Thereafter, the DMSO solutions were substituted with hexane by liquid–liquid extraction, then determined by HRGC–HRMS. Table 1 shows the recovery averages and relative standard deviations (RSDs) of 17 PCDD/F concentrations obtained using the SPD-600 method and manual method. The recoveries of the SPD-600 method ranged from 87% to 107% whereas those obtained using the manual method ranged from 78% to 122%, suggesting that scatter of the values from the SPD-600 method was smaller than that from the manual method. In addition, the RSDs obtained from SPD-600 method (2–8%) were also smaller than those from manual method (1–19%), indicating that the repeatability of

Table 1

Comparison of recoveries between manual packed column method and alumina column on SPD-600.

Congeners	Manual method (n = 3)		SPD-600(n = 3)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
2,3,7,8-TeCDD	79	12	93	2
1,2,3,7,8-PeCDD	91	12	100	4
1,2,3,4,7,8-HxCDD	99	14	99	4
1,2,3,6,7,8-HxCDD	104	13	92	3
1,2,3,7,8,9-HxCDD	97	17	106	5
1,2,3,4,6,7,8-HpCDD	122	5	105	4
OCDD	122	2	102	8
2,3,7,8-TeCDF	78	15	92	2
1,2,3,7,8-PeCDF	90	13	103	2
2,3,4,7,8-PeCDF	88	13	90	2
1,2,3,4,7,8-HxCDF	93	15	104	2
1,2,3,6,7,8-HxCDF	100	15	97	3
1,2,3,7,8,9-HxCDF	91	19	87	4
2,3,4,6,7,8-HxCDF	99	13	98	3
1,2,3,4,6,7,8-HpCDF	121	6	102	2
1,2,3,4,7,8,9-HpCDF	113	7	107	3
OCDF	118	1	101	5

SPD-600 method is superior to that of the manual method. These results indicate that the SPD-600 method may be a reliable and suitable for the PCDD/Fs cleanup procedure used in the DR-cell assay, and gives greatly improved efficiency.

The automated pre-treatment system, consisting of a multi-layer silica gel column and an alumina column, employs two special techniques (heating multi-layer silica gel and automatic solvent substitution) that could remove interference from contaminants such as aromatic hydrocarbons, and allow concentration directly to a small volume of DMSO solution, and potentially reducing human error.

3.2. Comparison between DR-cell assay and HRGC–HRMS analysis of PCDD/Fs in flue gas samples from four type incinerators

DR-cell assay and HRGC–HRMS analysis were used to estimate PCDD/Fs levels in flue gas samples obtained from hazardous waste incineration ($n = 18$), medical waste incineration ($n = 20$), iron ore sintering ($n = 21$) and municipal solid waste incineration ($n = 19$) in China (the gas volume is based on 0 °C and 1 atm).

The PCDD/F concentrations in 18 flue gas samples from hazardous waste incinerators were determined, the BEQ and I-TEQ values obtained from DR-cell assay and HRGC–HRMS analysis were compared. We found that the I-TEQ values from the HRGC–HRMS analysis ranged from 0.02 to 0.62 ng I-TEQ N m⁻³, whereas the BEQ values from the DR-cell assay ranged from 0.06 to 2.48 ng BEQ m⁻³. Fig. 2a shows a high correlation coefficient ($r^2 = 0.98$, $n = 18$, $p < 0.0001$) between the results of the DR-cell assay and HRGC–HRMS analysis, and the slope of the regression line was 4.10 for flue gas from hazardous waste incineration.

For medical waste incineration samples, the I-TEQ values ranged from 0.17 to 12 ng I-TEQ N m⁻³, and the BEQ values ranged from 0.71 to 93 ng BEQ m⁻³. Fig. 2b shows a high correlation coefficient ($r^2 = 0.95$, $n = 20$, $p < 0.0001$) between the two methods, and the slope of the regression line was 6.07 for flue gas from medical waste incinerators. The current emission limit for medical waste incinerators in China is 0.5 ng I-TEQ N m⁻³ (MEP, 2001), which is higher than the EU legal limit of 0.1 ng I-TEQ N m⁻³. This study reveals that 6 of 18 samples had emission levels above the current standard in China.

For iron ore sintering samples, the I-TEQ values ranged from 0.01 to 4.37 ng I-TEQ N m⁻³, and the BEQ values ranged from 0.05 to 23.52 ng BEQ m⁻³. Fig. 2c also shows a high correlation coefficient ($r^2 = 0.98$, $n = 21$, $p < 0.0001$) between the two

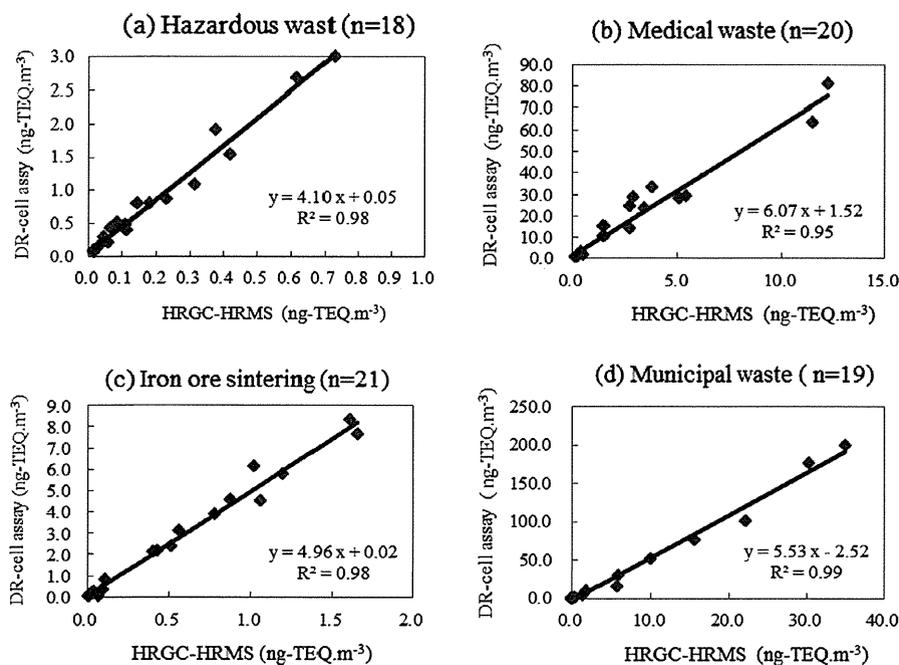


Fig. 2. Correlations between the values obtained from the DR-EcoScreen cell assay and those from the HRGC–HRMS analysis for the determination of PCDD/Fs in the flue gas samples from four kinds of incinerators (the gas volume is based on 0 °C and 1 atm).

methods, and the slope of the regression line was 4.96 for flue gases from iron ore sintering furnaces.

For the municipal solid waste incineration samples, the I-TEQ values ranged from 0.01 to 35 ng I-TEQ N m⁻³ and the BEQ values ranged from 0.09 to 270 ng BEQ m⁻³. Like other samples, a high correlation coefficient ($r^2 = 0.99$, $n = 19$, $p < 0.0001$) was obtained between the two methods (Fig. 2d), and the slope of the regression line was 5.53 for this type of samples.

We determined the concentrations of PCDD/Fs in the 78 flue gas samples collected from four kinds of incineration facilities in China, using both DR-cell assay and HRGC–HRMS analysis, and compared the respective BEQ and I-TEQ values obtained. Interestingly, although the BEQ values were 4.10 to 6.07-fold higher than the I-TEQ values, we found good linear relationships between the two methods for four types of samples. This tendency was similar to the results of our previous study using 80 ambient air samples obtained in Japan (Anezaki et al., 2009), for which the BEQ values were 3.66-fold higher than the I-TEQ values, although the two sets of data were closely correlated ($r^2 = 0.957$). In previous study, the discrepancy between the BEQ and I-TEQ values was thought to be due to differences between the WHO-TEF values and relative potency of the AhR agonistic activity estimated using the DR-cell assay (DR-REP) values (Anezaki et al., 2009). In this study, the reasons for discrepancy will be discussed in detail in the next paragraphs.

3.3. Contribution of 17 PCDD/Fs in flue gas samples from four kinds of incinerators

Fig. 3 shows the respective average contribution of 17 PCDD/Fs to the total I-TEQ values from the HRGC–HRMS analysis of the flue gas samples. The congeners in the four kinds of flue gas samples all similarly contributed to the total I-TEQ values. In both sets of samples, the contribution of PCDFs to the total I-TEQ was higher than those of PCDDs. 2,3,4,7,8-pentaCDF, in particular, was found to contribute approximately 40% to the total I-TEQ and to be main contributor, whose DR-REP value was 2.8-fold higher than its I-TEF value (Anezaki et al., 2009). This result was also supported

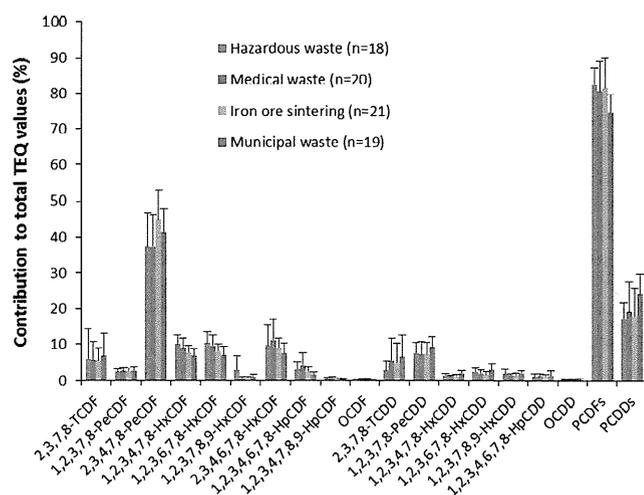


Fig. 3. Average contribution of 17 PCDD/F congeners to the total I-TEQ values of the flue gas samples obtained by the HRGC–HRMS analysis.

by other studies (Iino et al., 2003; Zhang et al., 2011). On the other hand, the DR-REP values for 7 PCDDs were lower than their I-TEF, except for 1,2,3,4,7,8-HxCDD, whereas the DR-REP values for the 10 PCDFs were higher than their I-TEF values, except for OCDF (Anezaki et al., 2009). This implies that the BEQ values obtained from the DR-cell assay could cause an underestimation and overestimation of PCDDs and PCDFs, respectively, compared to the I-TEQ values from the HRGC–HRMS using the I-TEF values. So, DR-TEQ were calculated by multiplying the concentration of each congener determined by HRGC–HRMS using its DR-REP value instead of I-TEF, and compared to DR-TEQ. Values from the DR-cell assay were found to be well correlated with those from the HRGC–HRMS analysis using the DR-REP values than I-TEF, and the R^2 decreased, and the fold of two methods decreased from 5.52 to 2.69 on average for all the samples. Thus, although the BEQ values are about 5-fold higher than the I-TEQ values, the discrepancy in BEQ and I-TEQ values

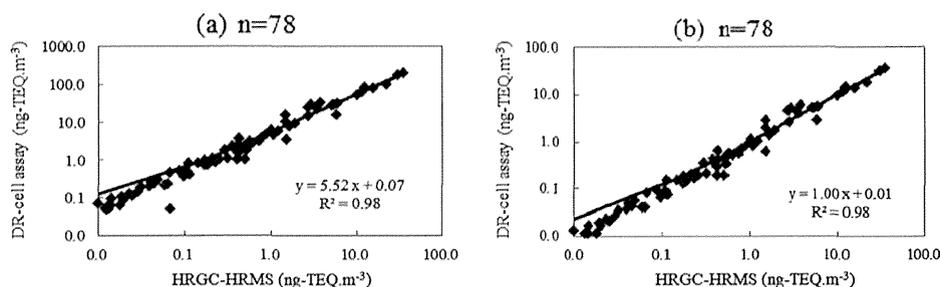


Fig. 4. Comparison between the values obtained from the DR-EcoScreen cell assay using the conversion coefficient 0.181 and the values from the HRGC–HRMS analysis for the determination of PCDD/Fs in the 78 flue gas samples (the gas volume is based on 0 °C and 1 atm).

was mainly due to differences between the WHO-TEF and DR-REP values. In addition, there is a possibility of contamination with AhR agonistic compounds, such as PAHs and brominated dioxin-like compounds, apart from the 17 PCDD/Fs in the purified samples.

3.4. Correction of values obtained from the DR-EcoScreen cell assay for approximation to values from the HRGC–HRMS analysis

Approximation of BEQ values from the DR-cell assay to I-TEQ values from the HRGC–HRMS analysis is required for the simple and rapid determination of PCDD/Fs in flue gas samples using the DR-cell assay as a prescreening tool for using the more costly HRGC–HRMS analysis. Therefore, we used the total results for the PCDD/F concentrations in the four kinds of flue gas samples obtained from the two methods. Fig. 4a shows a high correlation coefficient ($r^2 = 0.98$, $n = 78$) between the results of the two methods, and the slope of the regression line was 5.52 for flue gas samples in China. Following the Japanese Bio-manual (MEGJ, 2005), conversion scheme for the estimation of TEQ levels, we multiplied the values obtained from the DR-cell assay by the conversion coefficient 0.181, which is the reciprocal of the slope of the regression line (5.52). The converted values from the DR-cell assay were then compared with the values obtained using the HRGC–HRMS method. As shown as Fig. 4b, all of the converted values from the DR-cell assay of the 78 flue gas samples approximated (within 50–200%) the values obtained from the HRGC–HRMS analysis. These results suggest that the DR-cell assay, by applying the conversion coefficient 0.181, can be used to predict the values obtained using the HRGC–HRMS method in flue gas samples in China. This implies that the DR-cell assay combined with SPD-600 cleanup is a useful tool as a high-throughput prescreening method for large number of flue gas samples and other samples.

It is well known that HRGC–HRMS analysis is able to measure a wide range of pollutants quantitatively, and with high selectivity and sensitivity. However, the DR-cell bioassay has the following advantages: (1) rapid determination of the total potency of AhR agonists, (2) short procedure time, (3) low cost, (4) high sensitivity at a picogram level, and (5) the ability to predict the outcome of in vivo studies in terms of magnitude of effect. The use of the SPD-600 device for cleanup is thought to be very helpful in increasing these advantages. In 2005, Japan published the Bio-manual for determining the dioxin in flue gas from incinerators ($\leq 2 \text{ t h}^{-1}$), fly ash and screening in other environmental samples. In developing countries such as China, the quantity of various forms of waste is increasing, and the burning of waste is the most significant source of PCDD/Fs. The determination and survey of PCDD/Fs are needed on a wide-scale in support of economic development. Thus, the DR-cell assay is thought to be suitable for determining PCDD/Fs in various flue gas samples, and can be used as a rapid screening system prior to HRGC–HRMS analysis.

4. Conclusion

We applied the DR-cell assay to the determination of PCDD/Fs in flue gas from four types incinerators. The use of an SPD-600 automated-sample preparation device was very helpful for the rapid and simple determination of PCDD/Fs. Although the BEQ values from the DR-cell assay were higher than the I-TEQ values from HRGC–HRMS analysis, this discrepancy could be corrected by applying a conversion coefficient. This study demonstrated that the DR-cell assay can be used as a less expensive and less time-consuming alternative for screening purposes. Although it is currently unknown what specific chemicals in the environmental samples were responsible for the observed induction response, the bioassay system proposed herein was highly effective in screening a large number of samples. If used in coordination with HRGC–HRMS, it affords a powerful tool for risk assessment and the management of contaminated sites. The use of these two techniques in combination for the assessment of potential health hazards in contaminated sites would speed up the remediation processes and reduce costs.

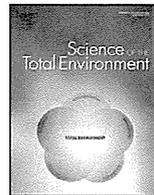
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Detection of 34 plasticizers and 25 flame retardants in indoor air from houses in Sapporo, Japan



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HIGHLIGHTS

- 34 semi-volatile organic compounds were detected in the indoor air samples.
- DEHT was detected in indoor air for the first time in Japan.
- Compounds with higher volatility tended to exist in a gaseous phase in indoor air.
- Compounds with lower volatility existed in a particulate phase in indoor air.
- TXIB (20.8 mg/m³) had the highest concentration among the 34 chemicals detected.

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ABSTRACT

Various plasticizers and flame retardants are contained in building materials and furniture produced for indoor environments. However, some of these material inclusions have been reported to cause endocrine-disrupting and mucosa-irritating effects. Because of the local climate, buildings in Sapporo are better insulated against cold weather than those in many other areas in Japan. In this study, we measured 59 compounds, including plasticizers (phthalates, adipates, and others) and flame retardants (organo-phosphates and brominated compounds), from indoor air samples from six houses in Sapporo. These compounds were measured separately in the gas phase and the particle phase using a two-stage cartridge equipped with a quartz fiber filter (1 μm mesh) and C₁₈ solid-phase extraction disk for sampling and analyzed by GC/MS and LC/MS/MS (for the detection of brominated flame retardants). Among the 59 compounds measured in this study, 34 compounds were detected from the indoor air of the six houses. The highest concentration among the 34 compounds found in a newly built house was 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (TXIB) at 20.8 μg/m³. Di(2-ethyl-1-hexyl)terephthalate (DEHT), which has been used in recent years as an alternative to di(2-ethyl-1-hexyl)phthalate (DEHP), was found in all six houses, although at low concentrations ranging from 0.005 to 0.027 μg/m³. To our knowledge, this is the first report of DEHT in indoor air in Japan. Among the compounds detected in this study, those with lower molecular weights tended to be captured in the C₁₈ solid-phase extraction disk rather than in the quartz fiber filter. These results suggest that compounds with higher volatility exist preferentially in the gas phase, whereas compounds with lower volatility exist preferentially in the particulate phase in indoor air.

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1. Introduction

The development of new building materials, furnishings, and consumer products in recent decades has resulted in a corresponding increase in new chemicals in indoor environments (Alaee et al., 2003; Rudel and Spengler, 2001; Schossler et al., 2011). Indoor chemical

concentrations in the 1990s were higher than those found 50 years prior because of the wider variety of chemicals used and suppression of air exchange rates in buildings to improve their energy efficiency when regulating residential thermal conditions. Health problems suspected to be induced by indoor air polluted with volatile organic chemicals (VOCs) have been observed in Japan, particularly in the 1990s (Seki et al., 2007). However, these problems have generally improved as a result of the Japanese Ministry of Health, Labor and Welfare setting guideline values for 13 compounds between 1997 and 2002. Nevertheless, “non-regulated” chemicals have been used in place of

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the 13 regulated compounds and they have caused sporadic health problems (Kamijima et al., 2002; Kobayashi et al., 2010).

Semi-volatile organic compounds (SVOCs) are a group of chemicals that have higher boiling points than VOCs. As a result of the lower volatility of SVOCs, their concentrations in indoor air are generally much lower than those of VOCs (Kanazawa et al., 2010); however, a number of SVOCs have been detected in indoor dust (Bornehag et al., 2005; Meeker and Stapleton, 2010). In addition, SVOCs, such as pesticides, plasticizers, and flame retardants, have been suggested to cause adverse health effects, including ocular and nasal symptoms and endocrine-disrupting effects (Kojima et al., 2004, 2009, 2013; Rudel and Perovich, 2009; Takeuchi et al., 2005a, 2005b; Wieslander and Norbäck, 2010).

Plasticizers and flame retardants are indispensable because they add strength, plasticity and safety to modern buildings; therefore, large amounts of these chemicals are used in a variety of building materials and furniture. As previously mentioned, these groups contain chemicals categorized as VOCs, SVOCs, and organo-halogen compounds. Although the level of consumption and diversity of these chemicals have been increasing, information on the concentrations of these chemicals in indoor air is limited to only a small number of compounds. Several organo-halogen compounds have been used as flame retardants because of their low cost and relative incombustibility. Brominated flame retardants are one of the main groups of organo-halogen flame retardants used for furniture and in the plastic parts of electrical products (Alaee et al., 2003). The concentrations of these compounds in the indoor air of houses and office buildings in Tokyo have been reported to be relatively low compared to those of organophosphorus flame retardants (Saito et al., 2007); however, they were detected in house dust in both Ottawa and Tokyo (Takigami et al., 2009; Wilford et al., 2005), and their persistence and bioaccumulation are thought to be hazardous to human health (Schechter et al., 2003).

Sapporo is the capital city of Hokkaido, the northernmost prefecture of Japan and a Winter Olympics city. It receives heavy snowfall over the long winter season; therefore, many buildings are well-insulated and people stay indoors for long periods of time. We investigated the concentrations of indoor air chemicals in several buildings in Sapporo, including the houses of patients with sick-building syndrome and/or chemical sensitivity (Takeuchi et al., 2004, 2005b, 2007). In this study, we examined methods for measuring compounds containing newly applied chemicals, such as 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) and Di-2-ethyl-1-hexyl-terephthalate (DEHT), in indoor air samples in both the gas phase and the particulate phase. These methods were applied to indoor air samples from the living rooms and bedrooms of six houses in Sapporo, and a total of 59 chemicals, 34 plasticizers and 25 flame retardants were measured.

2. Materials and methods

2.1. Chemicals

The test chemicals used in this study were of the highest available purity and purchased or provided as analytical standards from the companies listed in Table 1. The chemical names and their abbreviations for every category are also listed in Table 1. Anthracene- d_{10} was purchased from Wako Pure Chemical Industry (Osaka, Japan) and used as an internal control in the GC/MS analysis in this study.

2.2. Sampling and analysis of indoor air chemicals

Air samples were collected from six two-story houses, including one newly built house, in Sapporo. The ages of the houses were approximately two weeks (House 6) and five (House 1), fifteen (Houses 3 and 5), thirty (House 4), and fifty (House 2) years old. Five of the houses were conventional wooden houses, whereas the new house was a brick-built house. Carpets were found in the living rooms of all houses except

Table 1

The 59 compounds measured in this study.

No.	Chemical name	Abbreviation	Supplier
<i>19 phthalates</i>			
1	Dimethyl phthalate	DMP	Wako ^a
2	Diethyl phthalate	DEP	Wako
3	Diisopropyl phthalate	DiPP	TCI ^b
4	Dipropyl phthalate	DPP	Wako
5	Diisobutyl phthalate	DiBP	Wako
6	Dibutyl phthalate	DBP	Wako
7	Dipentyl phthalate	DPeP	Kanto ^c
8	Diisohexyl phthalate	DiHP	Kanto
9	Butyl phthalyl butyl glycolate	BPBG	Wako
10	Dihexyl phthalate	DHP	Wako
11	Butyl benzyl phthalate	BBzP	Kanto
12	Diisooheptyl phthalate	DiHPp	Aldrich ^d
13	Di (2-ethyl-1-hexyl) phthalate	DEHP	Wako
14	Diheptyl phthalate	DHPp	Wako
15	Dicyclohexyl phthalate	DcHP	Wako
16	Diphenyl phthalate	DPhP	Wako
17	Diocetyl phthalate	DOP	Wako
18	Diisononyl phthalate	DiNP	Wako
19	Diisodecyl phthalate	DiDP	Wako
<i>15 non-phthalate plasticizers</i>			
1	1,2-Cyclohexane dicarboxylic acid diisononyl ester	DINCH	Wako
2	Di (2-ethyl-1-hexyl) terephthalate	DEHT	Wako
3	Tris (2-ethyl-1-hexyl) trimellitate	TEHTm	Wako
4	2,2,4-Trimethyl-1,3-pentanediol monoisobutyrate	TXIB	Aldrich
5	1-Methyl-2-pyrrolidinone	1M2Pd	Wako
6	Dimethyl adipate	DMA	Wako
7	Diethyl adipate	DEA	Wako
8	Diisopropyl adipate	DiPA	Wako
9	Dibutyl adipate	DBA	Wako
10	Diisobutyl adipate	DiBA	Wako
11	Di (2-ethyl-1-hexyl) adipate	DEHA	Kanto
12	Dibutyl sebacate	DBSb	Wako
13	Di (2-ethyl-1-hexyl) azelate	BEHAz	Wako
14	Diocetyl azelate	DOAz	Wako
15	Bis (2-ethyl-1-hexyl) sebacate	BEHSb	Wako
<i>14 phosphorous flame retardants</i>			
1	Trimethyl phosphate	TMP	Wako
2	Triethyl phosphate	TEP	Wako
3	Tripropyl phosphate	TPrP	Aldrich
4	Tributyl phosphate	TBP	Wako
5	Tris (2-chloroethyl) phosphate	TCPEP	Wako
6	Tris (2-chloro-1-methylethyl) phosphate	TCMEP	Wako
7	Tris (1,3-dichloro-2-propyl) phosphate	TDCPP	Wako
8	Tris (2-butoxyethyl) phosphate	TBEP	Wako
9	Triphenyl phosphate	TPhP	Wako
10	Tri (2-ethyl-1-hexyl) phosphate	TEHP	Wako
11	2-Ethyl-1-hexyl diphenyl phosphate	EHDPhP	TCI
12	Cresyl diphenyl phosphate	CsDPhP	TCI
13	Tricresyl phosphate	TCsP	Wako
14	Trixylenyl phosphate	TXP	DH ^e
<i>11 brominated flame retardants</i>			
1	2,4,4'-Tribromodiphenyl ether	BDE-28	Well
2	2,2',4,4'-Tetrabromodiphenyl ether	BDE-47	Well
3	2,2',4,4',6'-Pentabromo diphenyl ether	BDE-100	Well
4	decabromodiphenyl ether	BDE-209	Well
5	2,4,6-Tribromophenol	TBPh	Wako
6	Pentabromophenol	PBPh	Wako
7	hexabromo benzene	HBBz	Wako
8	Hexabromocyclododecane	HBCD	Wako
9	Tetrabromobisphenol A	TBBPA	TCI
10	Tetrabromobisphenol A bis 2,3-Dibromopropylether	TBBPA-BPBE	TCI
11	1,2-Bis pentabromophenyl ethane	BPBPHE	TCI

^a Wako: Wako Pure Chemical Industry (Osaka, Japan).

^b TCI: Tokyo Chemical Industry (Tokyo, Japan).

^c Kanto: Kanto Chemical Co. Inc. (Tokyo, Japan).

^d Aldrich: Sigma-Aldrich Co. (St. Louis, MO, U.S.A.).

^e DH: Daihachi Chemical Industry (Osaka, Japan).

for House 4, which had wooden floors, and all living rooms were fitted with a television, sofa, and curtains. All houses except the oldest (House 2) were equipped with forced ventilation systems. The indoor air samples were collected from the living rooms and bedrooms. The air sampling rate was 10 L/min, and the sampling was performed for 12 h during the day in the living rooms and at night in the bedrooms. The sampling was performed during summer (July or August) in 2012 except for in the newly built house, which was sampled in November. An air sampling cartridge with a two-stage filter was used for the isolation of the indoor air chemicals as described elsewhere (Saito et al., 2007; Takeuchi et al., 2004, 2005b, 2007). A quartz fiber filter GMF 150 (1 μm of mesh size) (GL Sciences Inc., Tokyo, Japan) was used as the first stage to catch particulate chemicals larger than 1 μm in diameter, and a solid-phase extraction disk (Empore 2215 FF C₁₈ Disk) (3M Company, Saint Paul, MN, U.S.A.) was used as the second stage to catch gaseous chemicals that were not captured by the first-stage filter. The filters, cartridges and all of the glassware tools were rinsed with high-grade acetone (acetone for pesticide residue and polychlorinated biphenyl analysis produced by Wako Pure Chemical Industries Ltd., Osaka, Japan) to remove certain contaminants, such as phthalates and organophosphate flame retardants, prior to use. After indoor air sampling, each stage of the filters was rolled up and placed in 10-mL test tubes along with 10 μL of 100 $\mu\text{g}/\text{mL}$ anthracene-d₁₀ added as an internal control. Compounds were extracted from the filters by ultrasonication for 15 min with 10 mL of acetone and then concentrated to 1 mL using a rotary evaporator. The concentrated extracts were subjected to GC/MS or LC/MS/MS (for the analysis of brominated chemicals).

2.3. Instruments and analytical conditions

Except for the brominated flame retardants, the analysis of the test compounds was performed using a Shimadzu QP-2010 GC/MS system equipped with a DB-5MS column (30 m \times 0.25 mm i.d.). The following parameters were used for quantitative detection in this study: injection volume, 2 μL ; carrier gas, helium; and column head pressure, 72 kPa. The GC oven was initially maintained at 40 °C for 2 min, then increased by 25 °C/min to 200 °C and 40 °C/min to 280 °C, where it was maintained for 6 min, and finally increased by 10 °C/min to 320 °C, where it was maintained for 7 min. The analysis of the brominated test compounds was performed using an LC/MS/MS system (Waters ACQITY UPLC and AB SCIEX Triple Quad 5500) in negative atmospheric pressure ionization (APCI) mode with a BEH C₁₈ column (2.1 mm i.d. \times 50 mm). The injection volume was 10 μL , and the solvent program of the UPLC was initially maintained at 80% methanol in water for 4 min, then gradually changed to 100% methanol over the next 2 min, and finally maintained at 100% methanol for the last 9 min. The flow rate was 0.4 mL/min for the first 10 min and 0.5 mL/min thereafter. The following parameters were used for quantitative detection in this study: temperature, 400 °C; nebulizer gas, 30 psi; nebulizer current, –5A; curtain gas, 15 psi; and collision gas, 12 psi.

2.4. Quality assurance/quality control

The limit of detection (LOD) was defined as the amount of each test compound that yielded S/N = 3 in the analysis. The limits of quantification (LOQ) of the indoor chemicals were calculated from their LODs (except for DEP, DBP, DEHP, TXIB, and TBP, which is described later), the area of the peaks in the analysis of the test compounds extracted from the filters, and air sampling volume (7.2 m³). Breakthrough testing and recovery studies were performed as part of the setup of the air analysis method. A quartz fiber filter was spiked with 3 μg of each test chemical. The spiked filter was then installed as the first stage of the filter cartridge, and the second and the third stages were fitted with solid-phase extraction disks. Purified indoor air (14.4 m³) was passed through

the cartridge (n = 3). No breakthrough, which was defined by the detection of the spiked test chemicals from the extract of the third-stage filter, was found in this study. The chemicals were extracted from the first- and second-stage filters, and the percentage recoveries were calculated. Reproducibility was also evaluated by collecting duplicate air samples in our laboratory. To check for contamination and pollution by the chemicals during the transport of the sampling materials and air sampling, we used a “travel blank,” which was an additional cartridge equipped with the same type of filters used for the air sampling that traveled together with the sampling materials. The five compounds (DEP, DBP, DEHP, TXIB, and TBP) were detected at low concentrations even in the extracts of the rinsed filters, including those of the travel blanks. Therefore, the LOQs of those compounds were set at the three-fold value of the highest concentrations among the travel blanks in this study. The indoor air concentrations of these compounds were calculated by subtracting the travel blank values from the indoor air sample values.

3. Results and discussion

3.1. Phthalates

Table 2 shows the concentrations of the 12 phthalates detected in the indoor air from the six houses, and the compounds in Table 2 are listed by molecular weight in Table 3. DBP at 220 $\mu\text{g}/\text{m}^3$ and DEHP at 120 $\mu\text{g}/\text{m}^3$ are the guideline values that were set by the Japanese Ministry of Health, Labor and Welfare (Committee on Sick House Syndrome: Indoor Air Pollution, 2002); these compounds were detected in the indoor air samples at concentrations less than fifty-fold smaller than the guideline values (Table 2). However, these compounds and their metabolites have been frequently detected in human urine samples (Carlstedt et al., 2013; Bertelsen et al., 2013), which suggest the continuous exposure of humans to DBP and DEHP. Moreover, Carlstedt et al. (2013) reported that polyvinyl chloride flooring, which is known to contain phthalates, is related to phthalate uptake in humans. Although dietary exposure and exposure to indoor dust (presumably through unintentional dust ingestion) are the main exposure routes, phthalates in indoor air should not be ignored as a source of continuous exposure through inhalation and as an indicator of their source.

3.2. Non-phthalates

Table 2 shows the concentrations of the 10 non-phthalate plasticizers detected in the indoor air from the six houses. The concentration of TXIB (20.8 $\mu\text{g}/\text{m}^3$), which was detected in the newly built house, was the highest among the 34 chemicals detected in this study (Table 2). Seven compounds were found at concentrations higher than 1 $\mu\text{g}/\text{m}^3$, and the order of their maximum concentrations was as follows: TXIB > DMA > DiPA > DBP > DBA = 1M2Pd > DEHP. TXIB is used as a plasticizer and solvent and surface stabilizer for water-based paints (Wilke et al., 2004); we previously found TXIB and 1M2Pd at much higher concentrations (maximum concentrations were over 510 $\mu\text{g}/\text{m}^3$ and 1000 $\mu\text{g}/\text{m}^3$, respectively) in the indoor air of a newly built primary school in Hokkaido in which teachers and students had suffered from sick-building syndrome (Kobayashi et al., 2010). Bönisch et al. (2012) reported that exposure to TXIB (32 \pm 6 $\mu\text{g}/\text{m}^3$) and 1M2Pd (51 \pm 11 $\mu\text{g}/\text{m}^3$) for 5 h/day for 19 days increased the allergic immune response in ovalbumin-sensitized mice, reduced IL-12 production in maturing dendritic cells, and enhanced airway inflammation after adoptive dendritic cell transfer into Balb/c mice. Therefore, the concentrations of chemicals in the indoor air of newly built buildings should be taken into consideration even if water-based paints were used instead of conventional oil-based paints. Moreover, it is desirable to vacate buildings when the concentrations of chemicals are high and to provide adequate ventilation systems to prevent sick-building syndrome.

Table 2
Concentrations of the 34 compounds in the indoor air of the six houses ($\mu\text{g}/\text{m}^3$).

No.	Chemical	House 1		House 2		House 3		House 4		House 5		House 6		LOQ ^c
		LR ^a	BR ^b	LR	BR	LR	BR	LR	BR	LR	BR	LR	BR	
1	1M2Pd	2.8	1.8	– ^d	–	–	–	–	–	0.14	0.53	–	0.20	0.002
2	DMA	3.2	6.1	0.39	0.91	0.88	0.97	0.40	0.074	0.090	0.37	9.8	13.2	0.006
3	TEP	–	–	–	–	–	–	–	–	–	–	0.018	0.019	0.002
4	DMP	0.057	0.076	0.10	0.21	0.068	0.12	0.069	0.16	0.057	0.17	– ^e	–	0.004
5	TXIB	8.8	7.4	10.4	13.7	5.9	7.3	2.9	1.2	0.35	2.2	20.8	10.4	0.02
6	DEP	0.29	0.10	0.23	0.35	0.16	0.26	0.14	0.31	0.14	0.44	0.01	0.02	0.01
7	TPrP	0.054	–	–	–	–	–	–	–	–	–	0.32	0.15	0.001
8	DiPA	–	0.036	0.40	–	6.3	2.5	0.070	0.005	0.50	0.13	–	–	0.003
9	DiBA	0.44	0.41	0.92	0.80	0.46	0.68	0.38	0.027	0.064	0.18	0.34	0.028	0.005
10	DBA	0.73	0.002	1.2	1.2	1.5	2.8	0.80	0.097	0.19	0.15	0.007	0.003	0.001
11	TBP	0.06	0.07	0.08	0.11	0.09	0.08	0.06	0.09	0.04	0.08	0.05	0.04	0.01
12	DiBP	0.17	0.19	0.14	0.22	0.19	0.20	0.094	0.11	0.20	0.062	0.059	0.023	0.004
13	DBP	0.39	0.64	2.7	3.3	2.6	4.0	1.3	3.1	1.3	2.1	0.14	0.10	0.03
14	TCEP	0.037	0.028	0.041	0.060	0.065	0.024	0.025	0.032	–	–	0.013	0.022	0.002
15	BBzP	0.008	0.008	0.008	0.010	0.022	0.072	–	0.032	0.064	0.042	0.004	0.005	0.001
16	DBSb	–	0.079	0.061	0.098	0.092	0.081	0.065	0.061	0.036	0.017	–	0.004	0.002
17	TPhP	–	0.066	0.071	0.074	0.13	0.078	–	–	0.015	0.038	–	–	0.001
18	TCMEP	–	–	0.04	0.08	0.13	0.03	0.06	0.09	–	–	0.01	0.02	0.01
19	DcHP	–	0.004	–	–	0.014	–	–	–	–	–	–	–	0.006
20	TBPh	1.4	1.2	2.1	1.9	1.1	0.9	1.6	1.0	1.1	1.5	0.1	0.2	0.0001
21	DiHpP	–	–	0.021	–	–	–	–	0.012	–	–	–	–	0.002
22	DHpP	0.006	0.011	0.004	–	0.023	0.008	–	–	–	0.013	–	–	0.006
23	EHDPhP	–	–	–	–	–	–	–	–	–	–	–	–	0.03
24	TcSP	–	–	–	0.018	–	–	–	–	–	–	–	–	0.001
25	DEHA	0.04	0.03	0.11	0.42	0.35	0.05	0.04	0.003	0.46	0.59	–	0.009	0.02
26	DEHP	0.80	0.51	0.38	1.1	2.4	1.1	1.4	0.82	1.5	0.77	0.31	0.48	0.02
27	DOP	–	–	–	–	–	0.005	–	–	–	–	–	–	0.001
28	DEHT	0.007	0.006	0.006	0.009	0.006	0.007	0.006	0.006	0.005	0.027	0.005	0.005	0.002
29	TBEP	0.032	–	–	0.040	–	–	0.043	0.032	–	–	–	–	0.006
30	DiNP	0.050	0.13	0.052	0.070	–	0.008	0.29	0.23	0.031	0.36	0.012	0.021	0.006
31	DiDP	0.03	0.06	0.03	–	0.02	–	0.03	0.03	–	0.19	–	0.016	0.02
32	BEHSb	0.094	0.10	0.11	0.13	0.12	0.11	0.098	0.098	0.021	0.073	0.013	0.007	0.001
33	TDCPP	–	–	0.018	–	–	–	–	–	–	–	–	–	0.006
34	HBBz	–	–	–	–	–	–	–	–	–	–	0.3	–	0.0001

^a LR: living room.

^b BR: bed-room.

^c LOQ: limit of quantitation.

^d Not detected or lower than the LOQ.

^e Compounds not detected were as follows (LOQ): DiPP (0.001), DPP (0.001), DPeP (0.001), DiHP (0.001), BPBG (0.001), DHP (0.001), DPhP (0.001), DEA (0.002), BEHAz (0.002), DINCH (0.006), DOAz (0.002), TEHTm (0.001), TMP (0.002), TEHP (0.001), EHDPhP (0.03), CsDPhP (0.001), TXP (0.06), BDE-28 (0.0001), BDE-47 (0.0001), BDE-100 (0.0001), BDE-209 (0.0001), BPBPHE (0.0001), HBCD (0.0001), TBBPA (0.0001), TBBPA-BPBE PBPh (0.0001).

DEHT has been used in recent years as an alternative to DEHP (Schossler et al., 2011), and although there have been no previous reports on the indoor air concentrations of DEHT, its detection in house dust samples was reported by Nagorka et al. (2011) in Germany. They reported that DEHT in house dust increased steadily from 1997 to 2009 (Nagorka et al., 2011). In this study, we found DEHT in all six houses, although at low concentrations ranging from 0.005 to 0.027 $\mu\text{g}/\text{m}^3$ (Table 2). This is the first report of DEHT in indoor air samples in Japan. Abe et al. (2012) reported that DEHT was detected in 37% of 101 samples obtained from polyvinyl chloride (PVC) toys for sale on the Japanese market in 2009. Therefore, the use of DEHT may be spreading in Japan, including Sapporo and Germany (Nagorka et al., 2011).

3.3. Organo-phosphorus flame retardants

Table 2 shows the concentrations of the nine organo-phosphorus flame retardants detected in the indoor air from the six houses. Among the 14 organo-phosphorus flame retardants tested, nine compounds were detected in indoor air samples from the six houses (Table 2). Among these nine compounds, four (TPrP, TBP, TPhP, and TCMEP) were detected at concentrations higher than 0.1 $\mu\text{g}/\text{m}^3$. TPrP was detected at the highest concentration (0.32 $\mu\text{g}/\text{m}^3$) among the 14 organo-phosphorus flame retardants tested in this study. However, TPrP was only detected in two houses (Houses 1 and 6). In addition, although the concentration of TPrP in House 6 was greater than 0.1 $\mu\text{g}/\text{m}^3$, it was detected at a low concentration (0.054 $\mu\text{g}/\text{m}^3$) in the living room

in House 1 (Table 2). Bergh et al. (2011) measured six phthalates and 11 organophosphates in 30 indoor locations in Stockholm and reported that phthalate esters were more abundant than organophosphates and typically showed ten-fold higher total concentrations. These results suggest that the concentrations of organo-phosphorus flame retardants in indoor air tend to be lower than the concentrations of certain plasticizers, such as DBP and DEHP. However, Kanazawa et al. (2010) reported that TBEP, TCEP, and TCMEP were detected in the floor dust samples from 41 dwellings in Sapporo at high concentrations (5890, 308, and 291 $\mu\text{g}/\text{g}$ in the maximum concentrations, respectively). Saito et al. (2007) reported that the use of floor polish agents containing TBEP could affect the concentration of TBEP in indoor air. TBEP was detected in the highest concentration in the living room in House 4, which has wooden flooring (Table 2).

3.4. Brominated flame retardants

Table 2 shows the concentrations of the two brominated flame retardants detected in the indoor air from the six houses. TBPh was detected in the indoor air samples from the six houses at very low concentrations ranging from 0.1 to 2.1 ng/m^3 (Table 2). Saito et al. (2007) indicated that TBPh was vulnerable to oxidative degradation. Based on our previously described recovery test, the recovery rate of TBPh was 54% (TBBPA was 55%), whereas the recovery rates of most of the other compounds were approximately 100% (data not shown). Therefore, the concentrations of TBPh in this study (Table 2) might be underestimated by approximately

Table 3
Proportion of total captured in the quartz fiber disk for the 34 SVOCs detected in this study (%).

No.	Chemicals	MW (g/mol)	House 1		House 2		House 3		House 4		House 5		House 6	
			LR ^a	BR ^b	LR	BR	LR	BR	LR	BR	LR	BR	LR	BR
1	1M2Pd	99	0	0	0	0	- ^c	-	-	-	0	0	-	0
2	DMA	174	2	1	0	0	0	0	0	0	0	0	0	0
3	TEP	182	-	-	-	-	-	-	-	-	-	-	100	100
4	DMP	194	0	0	0	0	0	0	0	0	0	0	-	-
5	TXIB	216	0	0	0	0	1	0	1	0	0	0	0	0
6	DEP	222	0	0	0	0	1	0	2	0	0	0	0	0
7	TPrP	224	0	-	-	-	0	0	-	-	-	-	0	0
8	DiPA	230	0	0	3	-	2	8	0	0	16	11	0	26
9	DiBA	258	1	5	2	1	8	5	2	8	16	9	11	7
10	DBA	258	8	100	0	0	13	4	24	100	0	0	100	100
11	TBP	266	12	11	36	5	34	23	23	12	0	0	12	20
12	DiBP	278	1	3	3	1	11	8	4	0	14	100	15	18
13	DBP	278	3	10	10	1	23	16	8	2	9	8	63	68
14	TCEP	285	40	50	61	25	100	100	100	39	-	-	100	100
15	BBzP	312	53	55	100	100	100	94	-	55	90	90	100	100
16	DBSb	314	-	100	100	100	100	100	100	100	100	100	-	100
17	TPhP	326	-	100	100	100	100	100	-	-	100	100	-	-
18	TCMEP	327	0	0	100	11	100	100	100	71	100	100	100	100
19	DcHP	330	-	0	-	-	67	-	-	-	-	-	-	-
20	TBPh	330	0	0	0	0	0	0	0	0	0	0	0	0
21	DiHpP	362	-	-	100	-	-	-	-	100	-	-	-	-
22	DHpP	362	52	53	66	-	91	100	-	-	-	65	-	-
23	EHDPhP	362	-	-	-	-	-	100	100	100	-	-	-	-
24	TCsP	368	-	-	-	100	-	-	-	-	-	-	-	-
25	DEHA	370	97	93	100	89	100	96	100	-	100	100	-	-
26	DEHP	390	100	99	99	98	100	100	100	100	100	99	100	100
27	DOP	390	-	-	-	-	-	100	-	-	-	-	-	-
28	DEHTp	390	100	100	100	100	100	100	100	100	100	100	100	100
29	TBEP	397	100	-	-	100	-	-	100	100	-	-	-	-
30	DiNP	418	100	100	100	100	-	100	100	100	100	100	100	100
31	DiDP	446	100	100	100	-	100	-	100	100	-	100	100	100
32	BEHSb	426	100	100	100	100	100	100	100	100	100	83	100	100
33	TDCPP	430	-	-	100	-	-	-	-	-	-	-	-	-
34	HBBz	551	-	-	-	-	-	-	-	-	-	-	0	-

^a LR: living room.

^b BR: bed-room.

^c Not detected or lower than the LOQ (shown in Tables 2).

50% of the actual values, and we are currently conducting an additional study using quartz fiber filters treated with an antioxidant to prevent the oxidative degradation of TBPh. In addition, HBBz was detected at a very low concentration (0.3 ng/m³) from the living room of House 6 (Table 2). These results are similar to the results reported by Saito et al. (2007) in which they measured TBPh and HBBz in the indoor air of 18 houses in Tokyo. Although brominated flame retardants are widely used in the interiors of houses in Japan, they were only detected at low concentrations in this study, which may have resulted from the low volatility of brominated compounds. Moreover, TBPA and BDE-209, which are commonly used brominated flame retardants in Japan, were not detected in this study. Further study is required to clarify the trends in concentrations of brominated flame retardants in the indoor air of dwellings across Japan as a whole.

3.5. Phase of the 34 SVOCs detected in this study

The gas and particle phases of the 34 compounds were measured separately using a combination of a solid-phase extraction disk and quartz fiber filter as previously described. These compounds were caught by one or both of the two stages at different rates. Table 3 shows the detection ratios of the 34 compounds from the quartz fiber filter, which was used for the indoor air sampling in the six houses. Similar to Table 2, the compounds in Table 3 are listed by molecular weight, and the compounds with a smaller molecular weight tended to be captured in the C₁₈ solid-phase extraction disk. These results suggest that compounds with a higher volatility preferentially exist in the gas phase in indoor air, whereas compounds with lower volatility preferentially exist in the particulate phase. Kanazawa et al. (2010) and

Schossler et al. (2011) calculated the logarithm of the octanol-air distribution coefficient (K_{OA}) of 22 and 37 chemicals, respectively, according to the method of Weschler and Nazaroff (2008). The 27 compounds detected in the present study included 19 (seven phthalates, DEHA, and 11 organophosphates) calculated by Kanazawa et al. (2010) and eight (five phthalates, DIBA, DINCH, and TOTM) calculated by Schossler et al. (2011). Among the 27 compounds for which a log K_{OA} value was calculated, TMP had the lowest value (1.4), and TOTM had the highest value (16.24) (Kanazawa et al., 2010; Schossler et al., 2011). The log K_{OA} values of indoor air chemicals, which were captured equally by both stages in this study, were 5.0 (TBP), 8.41 (DiBP), 8.63 (DBP), 9.02 (BBzP), and 11.12 (DHPp) (Kanazawa et al., 2010; Schossler et al., 2011). These results suggest that the border between the two phases was within the range of these log K_{OA} values. A comparison of the values of log K_{OA} and the MWs in Table 3 shows a rough correlation. However, HBBz and TBPh were captured by the C₁₈ solid-phase extraction disk at 100%, and these results suggest that HBBz and TBPh exist in a gaseous phase in the indoor air, although other smaller compounds, such as DBSb, TPhP, and TCMEP, mainly existed in a gaseous phase. Bromine atoms in the structures of HBBz and TBPh might affect their phases. Because the indoor air samples in this study were obtained in the summer, the preference for the compounds to exist in a gas phase may have been higher than it would have been in winter.

However, many of these SVOCs have been reported to be endocrine-disrupting chemicals, and the molecular sizes of some of these SVOCs are an appropriate fit for the ligand-binding pockets of certain hormone receptors, such as those of estrogen and androgen. In our previous study, the transcriptional activities of SVOCs via those receptors were investigated using mammalian cell-based reporter gene assays, and

we found that several phthalates, organophosphorus flame retardants, and brominated flame retardants possess agonistic and/or antagonistic activities via these receptors (Kojima et al., 2009, 2013; Takeuchi et al., 2005a).

Chemicals in indoor air are important because of their continuous exposure through inhalation, which allows them to directly enter the bloodstream via the lungs and escape from digestive organs and metabolism by the liver; therefore, their exposure pathway differs from that of compounds ingested with food.

4. Conclusions

In this study, we measured 59 SVOCs in indoor air samples from 12 rooms in 6 houses in Sapporo, Japan, and we isolated 34 compounds, including DEHT, which was found for the first time in indoor air samples in Japan. The concentration of TXIB ($20.8 \mu\text{g}/\text{m}^3$) detected in a newly built house was the highest among the 34 chemicals detected in this study. Among the chemicals detected, seven compounds (TXIB, DMA, DiPA, DBP, DBA, 1M2Pd, DEHP) were found at concentrations higher than $1 \mu\text{g}/\text{m}^3$. These results suggest that a variety of plasticizers and flame retardants are present in the indoor environments of houses in Sapporo.

These chemicals contribute to increased quality and safety of furniture and building materials, and new compounds are continually being added to the list of such chemicals. Although the concentrations of the indoor air chemicals detected in this study were lower relative to cases related to sick-building syndrome, it is difficult to identify the concentration limits for indoor air chemicals with regard to human health because of a lack of knowledge regarding the pathogenesis of sick-building syndrome and/or chemical sensitivity. Further study of indoor chemicals in houses and office buildings is required to prevent health problems and improve indoor environments.

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