Environmental Pollution 252 (2019) 784–793

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Abundances and concentrations of brominated azo dyes detected in indoor dust

Birendra Dhungana [a, **], Hui Peng [b], Steven Kutarna [b], Gisela Umbuzeiro [c], Sujan Shrestha [a], Jing Liu [a], Paul D. Jones [d], Bikram Subedi [e], John P. Giesy [d], George P. Cobb [a, *]

[a] Department of Environmental Science, Baylor University, Waco, TX, United States
[b] Department of Chemistry, University of Toronto, Toronto, ON, Canada
[c] School of Technology, FT-UNICAMP, Sate University of Campinas, Limeira, Brazil
[d] Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[e] Department of Chemistry, Murray State University, Murray, KY, United States

ARTICLE INFO

Article history:
Received 20 March 2019
Received in revised form
20 May 2019
Accepted 30 May 2019
Available online 4 June 2019

Keywords:
House dust
Brominated compounds
Flame retardants
Mutagenic
High-resolution mass spectrometry

ABSTRACT

Dust samples were collected from four indoor environments, including childcare facilities, houses, hair salons, and a research facility from the USA and were analyzed for brominated compounds using full scan liquid chromatography high-resolution mass spectrometry. A total of 240 brominated compounds were detected in these dust samples, and elemental formulas were predicted for 120 more abundant ions. In addition to commonly detected brominated flame retardants (BFRs), nitrogen-containing brominated azo dyes (BADs) were among the most frequently detected and abundant. Specifically, greater abundances of BADs were detected in indoor dusts from daycares and salons compared to houses and the research facility. Using authentic standards, a quantitative method was established for two BADs (DB373: Disperse Blue 373 and DV93: Disperse Violet 93) and 2-bromo-4,6-dinitroaniline, a commonly used precursor in azo dye production, in indoor dust. Generally, greater concentrations of DB373 ($\leq$3850 ng/g) and DV93 ($\leq$1190 ng/g) were observed in indoor dust from daycares highlighting children as a susceptible population to potential health risk from exposure to BADs. These data are important because, to date, targeted analysis of brominated compounds in indoor environments has focused mainly on BFRs and appears to underestimate the total amount of brominated compounds.

1. Introduction

Halogenated compounds, especially brominated flame retardants (BFRs), are known for their persistence in the environment, bioaccumulation and toxic potencies (Birnbaum and Staskal, 2004; Saunders et al., 2013; Zhang et al., 2009). BFRs encompass wide classes of compounds, such as, polybrominated benzene, polybrominated diphenyls and polybrominated diphenyl ethers (PBDEs). Other than BFRs, hundreds to thousands of naturally produced brominated compounds in the marine environment have also been reported (Peng et al., 2016a). Several studies have shown that BFRs, such as PBDEs, hexabromocyclododecanes (HBCDs), 2-ethylhexyl-2,3,4,5-tetrabromo-benzoate (TBB), are widely present in various environmental matrices including indoor dusts (Brits et al., 2016; De Oliveira et al., 2006; Stapleton et al., 2008; Guigueno and Fernie, 2017) as well as in animal and human tissues and in serum (Guigueno and Fernie, 2017; Alaee et al., 2003; Sugeng et al., 2017). Traditionally, BFRs are analyzed using gas chromatography mass spectrometry, and various strategies employed in such analysis have been reviewed previously (Brits et al., 2016). However, it is important to note that previous targeted studies emphasized quantification of brominated compounds previously known to be in the environment, which may not represent all brominated compounds of concern and present in the environment. For example, chlorinated or brominated disperse dyes are commonly used as textile dyes, specifically for synthetic fibres (de Aragão Umbuzeiro et al., 2005). Although production and consumption statistics of brominated azo dyes (BADs) are not

* Corresponding author.
** Corresponding author.
E-mail address: george_cobb@baylor.edu (G.P. Cobb).

https://doi.org/10.1016/j.envpol.2019.05.153
0269-7491/

available, azo dyes being one of the most important class of dyes used (Disperse Dye - an overvie, 2019), application of BADs could be significant. X-ray fluorescence analysis revealed significant bromine (Br) concentrations, in the range of hundreds of mg/kg to g/kg in common household objects, such as curtains, television casings, roll screens and stuffed toys. These concentrations, are several orders of magnitude greater than could be attributed to known BFRs (~*ng*/g). (Takigami et al., 2009).

Full scan data acquisition and subsequent suspect screening (hereafter we will simply use the terms "suspect screening") using high-resolution mass spectrometry (HRMS) provides a promising way to identify previously unknown halogenated compounds in indoor dust. Data-independent acquisition (DIA) allows unbiased MS and MS/MS data acquisition across the whole mass range while data-dependent acquisition (DDA) captures MS/MS data of only more abundant ions (Koopmans et al., 2018). Thus, in combination with chromatographic peak deconvolution, DIA is expected to outperform DDA regarding sensitivity and coverage. Using a DIA approach, a previous study determined over 1000 brominated compounds in house dusts, and previously undetermined brominated azo dyes (BADs) were determined to be predominant compounds (Peng et al., 2016b). However, concentrations of BADs could not be determined due to the lack of authentic standards. In addition, the potential occurrence of BADs in different types of indoor environments remains unclear.

As one of the major reservoirs for pollutants, indoor dust has been suggested as a significant exposure pathway for humans (Moschet et al., 2018; Guo and Kannan, 2011), and BADs in indoor dust could pose risks to human health. For example, 2-bromo-4,6-dinitroaniline (BNA), an important raw material used to synthesize azo dyes, was detected in several house dusts (Peng et al., 2016b). Ames *Salmonella assay* of BNA exhibited significant mutagenicity at environmentally relevant concentrations (Peng et al., 2016b). A more recent study suggested multigenerational reproductive toxicities of BNA to zebrafish at concentrations as little as 0.5 μg/L (Ma et al., 2018). Several other toxicological studies, including in vitro micronucleus assays in cell cultures, and in vivo studies of rodents, indicated genotoxic and mutagenic effects of different azo dyes and/or their degradation products (de Aragão Umbuzeiro et al., 2005; Vacchi et al., 2017; Oliveira et al., 2006; Chequer et al., 2011; Fernandes et al., 2019; Fernandes et al., 2018). Genotoxic and mutagenic pollutants in childcare facilities' dust could be of a greater concern for children due to their developmental stage and increased exposure resulting from their greater hand-to-mouth activities (US EPA National Center for Environmental Assessment, 2018).

In this study, suspect screening of brominated compounds in indoor environments was conducted using a data-independent precursor isolation and characteristic fragment (DIPIC-Frag) HRMS approach (Peng et al., 2015), with a particular focus on BADs. Thirty-seven samples collected from diverse indoor environments, including daycares (facilities that provide childcare during the day while parents work), hair salons, houses and a research facility from the USA, were analyzed. Occurrence, distribution and relative abundance of hundreds of brominated compounds detected in these indoor dusts were compared, and concentrations of five of the most frequently detected brominated compounds including BNA and two BADs, Disperse Blue (DB373) and Disperse Violet (DV93), were quantified by use of authentic standards. Daily intake of BADs through dust ingestion was also estimated for different age groups in daycares and houses. This is the first study to report concentrations of potentially mutagenic BADs in indoor dust. In addition, samples of cloth and carpet were analyzed as possible sources of brominated compounds in indoor environments.

2. Materials and methods

2.1. Chemicals and materials

Authentic standards of 8 PBDE congeners, tetrabromobisphenol A (TBBPA), γ-hexabromocyclododecane (γ -HBCD), 6-fluoro-2-2′,4,4′-tetrabromodiphenyl ether (F-BDE47) and 2-ethylhexyl-tetrabromobenzene (TBB) were purchased from AccuStandard (New Haven, CT, USA). 2-Bromo-4,6-dinitroaniline (BNA) was purchased from Sigma-Aldrich (St. Louis, MO). Isotopically labelled bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (d_{34}, $^{13}C_6$-TBPH) was purchased from Wellington Laboratories Inc. (Guelph, ON, Canada, Cat. No. MBEHTBP). Two brominated azo dyes, DB373 (CAS No.: 51,868-46-3) and DV93 (CAS No.: 52,697-38-8), were purified from technical products using flash column chromatography. Purities of these compounds were >95% as assessed using HPLC-UV/Vis (Umbuzeiro et al., 2017). Florisil (6 mL, 500 mg, 30 μm) solid-phase extraction (SPE) cartridges were purchased from Waters (Milford, MA, USA), and polypropylene centrifuge tubes were purchased from Fisher Scientific (Hempton, NH, USA). Dichloromethane (DCM), hexane, methanol, and acetone were all of analytical grades or higher and were purchased from Fisher Scientific. Ultrapure water (produced by Genpure water system, Thermo Fisher Scientific) was used for chromatographic separation and rinsing equipment.

2.2. Collection of samples

A total of 37 samples of indoor dusts from children daycare facilities, hair salons, houses, and a research facility were collected from 8 states across the USA. In addition, samples from potential sources of brominated compounds including, clothes, carpets, toys (plastic toys and stuffed animals), and TV casing were collected from a daycare and a house from which dust samples were also collected and analyzed. Details of collection methods and criteria of indoor dusts and potential sources are provided in supplementary material (SM). Abbreviations used for sample names are as follows, AP: apartment/house, DC: daycare, SL: salon, RB: research building.

3. Sample extraction and cleanup

Dust samples were extracted and cleaned up using previously described methods (Peng et al., 2016b). Briefly, isotopically labelled d_{34}, $^{13}C_6$-TBPH was added to approximately 0.1 g of each dust type 1 h prior to extraction. Each sample was extracted sequentially with 5 mL methanol and 5 mL dichloromethane (DCM). Methanol and DCM extracts were combined and evaporated. The extract was dissolved in 500 μL of DCM, loaded onto Florisil cartridge, and eluted with 6 mL DCM. Each final extract was evaporated to dryness, reconstituted in 200 μL of acetone and kept frozen at −20 °C until further analysis. Thirty microliters of 2 mg/L F-BDE47 were added to the reconstituted solution before analysis. Detailed experimental procedure of sample extraction and cleanup is provided in SM.

3.1. LC-MS/MS data acquisition and analysis

Aliquots of extracts (5 μL) were analyzed using a Q Exactive Focus HRMS coupled to a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA HPLC). Chromatographic separation was achieved with a Hypersil GOLD™ C18 column (3 μm, 2.1 mm × 50 mm; Thermo Fisher Scientific) using ultrapure water and methanol as mobile phases. To maximize ionization of analytes, data were acquired using atmospheric pressure photo- and chemical ionization (APPI/APCI) sources

operated in dual APPI/APCI mode (Ion Max, Thermo Scientific).

Data were acquired in full scan and parallel reaction monitoring (PRM) mode separately. Full scan (MS) data were acquired using 300 Da-*m/z*-wide windows at resolution R = 70,000 (at *m/z* 200) with a maximum of 1×10^6 ions collected within 200 ms. PRM (MS/MS) scans were recorded, using collision induced dissociation energies, 15 and 25 eV, at a resolution R = 35,000 (at *m/z* 200) with a maximum of 1×10^5 ions collected in 60 ms using 5-*m/z*-wide precursor ion isolation windows per scan. Detailed experimental procedure of LC-MS/MS data acquisition and analysis is provided in SM.

3.2. Quality assurance/quality control

Abundances (chromatographic peak areas of the greatest intensity isotopic peaks) were obtained for all detected brominated compounds with detection limit arbitrarily set at 1000 counts. This set limit represented less than three times signal counts generally observed for background level and below detection limit as observed from calibration standards (Table S2). Internal standard F-BDE47 was used for correcting run-to-run instrumental variations and utilized to normalize area under all other chromatographic peaks of interest. Peak abundances of brominated compounds were corrected by a single surrogate standard d_{34}, $^{13}C_6$-TBPH in corresponding samples, where the mean recovery of d_{34} $^{13}C_6$-TBPH was 87.0 ± 21.4% in all the samples. The use of single surrogate standard for multiple compounds was mainly due to the lack of commercially available surrogate standards for novel compounds. To avoid sample contamination, all equipment and consumables (e.g., centrifuge tubes, glass Pasteur pipets, syringes, storage vials and test tubes) used in this analysis were rinsed with acetone (3 times) followed by hexane (3 times) before use. At least one procedural blank was incorporated for every batch of samples. In a procedural blank, BDE209, penta-, and hexa-BDEs and TBB were detected; therefore, the analytical responses of these compounds were blank corrected. Compounds with abundances less than 3 times the background abundance were considered not detected.

3.3. Chemometric data analysis

An in-house computation R program was used to analyze the DIPIC-Frag data to annotate formulas, which has been described in our previous studies (Peng et al., 2015). Briefly, the bromine peaks (*m/z* = 78.9188) were extracted from each PRM windows for screening brominated compounds. Precursor ions of bromine fragment peaks were identified from corresponding PRM windows (5 *m/z*), and were aligned by correlating chromatographic peak shapes. Possible confounding candidates were further excluded by matching to characteristic isotopic peaks of brominated compounds. Formulas of detected features were predicted by integrating exact *m/z* and isotopic peak information as described in our previous studies. MS/MS spectra were manually checked to confirm the formulas predicted by automated data analysis workflow. The features with predicted formulas were matched across indoor dusts according to their retention times (<0.1 min) and exact *m/z* (3 ppm). Chemical formulas were set to contain up to 80 C, 160 H, 10 N, 10 O, 10 Br, 2 Cl, 2I and 2 S per molecule. Numbers of Br or Cl atoms were constrained based on information from distinguishable isotopic patterns of Br and Cl.

3.4. Quantitative analysis

For quantitative analysis of indoor dust, data were collected in both full scan and PRM scan modes. Although linear calibration curves ($R^2 > 0.99$) were observed using molecular $[M]^{\cdot -}$ ion in full scan or fragment $[Br]^-$ ion in PRM scan of BADs, to minimize probable interference in dust analysis, the fragment $[Br]^-$ ion was used for quantification of BADs as well as for BNA, HBCD, and BD209. Instrument detection limits (IDLs) and procedural detection limits (PDLs) were determined as 3*s/m (where, s is the standard deviation of peak area for four-replicate analyses and m slope of calibration curve) (Shrivastava and Gupta, 2011; Broekaert and Harris, 2015). While IDLs were determined by analyses of diluted standard solutions, PDLs were determined based on replicate analyses of spiked procedure blank, both at a concentration of approximately six times the corresponding IDLs. Recoveries of five brominated compounds were determined from procedural spiked blanks where analytes at 200 μg/L ($n = 4$) were spiked to the solvent and carried through the same analytical process as samples. Recoveries for these compounds ranged from 73 ± 11% (BNA) to 86 ± 14% (BDE 209) (Table S2).

Estimated concentrations of BADs, for which authentic standards were not available, were obtained by using of response factors (RFs) of DB373 and DV93. RFs of DB373 and DV93 in individual sample were calculated by dividing chromatographic peak area of $[M]^{\cdot -}$ ion in full scan acquisition by determined concentrations of the compound.

3.5. Data treatment and statistical analyses

Heat map and hierarchical cluster analyses were performed in MATLAB. Only brominated compounds with detection frequencies of >50% were used for correlation, regression, and cluster analyses. Statistical significance was defined as $p < 0.05$.

Exposure to brominated compounds through dust ingestion was calculated using previously reported parameters as (Guo and Kannan, 2011; US EPA National Center for Environmental Assessment, 2018):

$$\text{Daily intake (DI) through dust ingestion} = \frac{C_{dust} \cdot IEF \cdot DIR}{M}$$

where, C_{dust} is the azo dye concentration (geometric mean, GM) in dust, IEF is the indoor exposure fraction, DIR is the rate of ingestion of dust (6 months–1 year: 0.04, 1 < 2 years: 0.05, 2–6 years: 0.03, and adults: 0.03 g/day), (US EPA National Center for Environmental Assessment, 2018) and M is the average body weight (6 months to <1 year: 7.5 kg, 1 < 2 years: 11.5 kg, 2–6 years: 18 kg, and adults: 75 kg) (Fryar et al., 2016). IEFs, averaged over a week, were calculated to be 0.52 and 0.48 for a child, respectively, in daycare and home, and 0.36, and 0.64 for an adult daycare worker, respectively, in a daycare and home (SM).

4. Results and discussion

4.1. Suspect screening of brominated compounds in indoor dust

The primary focus of the study was to screen brominated compounds in dust collected from diverse indoor environments. A total of 240 peaks brominated compounds were detected in at least one indoor dust sample by extracting the bromine fragment peak (*m/z* = 78.9188) from each of the 120 PRM windows, and then aligning precursor ions using the computational algorithm (DIPIC-Frag) as documented previously (Peng et al., 2015). Among these, formulas of 120 compounds were determined by chemometric strategies that incorporated retention time, exact *m/z* analysis of precursor and fragment ions and relative isotopic abundances (see SM). Variation in retention times (4.6–13.1 min), *m/z* ratios (229–700) and chemical compositions in the determined formulas (for example, N = 1–10, Br = 1–10) of these compounds suggest

diverse physical-chemical characteristics. Fewer brominated compounds were detected in the current study than in our previous similar study (549). Because of greater uncertainty when predicting formulas with increased *m/z*, the current study emphasized analysis of smaller compounds. Fewer compounds detected in the current study is, to some extent, due to data collection over a smaller mass range (100–700) compared to the mass range (100–1000) in the previous study. However, other possible reasons for observed differences in the number of detected compounds are: i) indoor dusts were collected from different locations, which may have different compounds profiles; and ii) PRM was adopted in the current study, while data independent acquisition (DIA) mode was used in the previous study, and the uneven isolation PRM window may sacrifice sensitivity due to less efficient isolation of analytes with *m/z* at edges of the isolation windows. Nevertheless, it is important to note that the majority of abundant compounds detected in the current study and previous study are similar.

Well-known BFRs, such as PBDEs (tetra-, penta-, hexa- and deca-BDE), HBCD, and TBBPA, were detected with greater abundances. Although ions of the type $[M - Br + O]^-$ were the most abundant for PBDEs and such ions were also detected for deca-BDE (BDE209), the fragment ion corresponding to C_6OBr_5 (exact *m/z* = 486.5830, rt = 11.54 min) had the greatest spectral intensity. The greater spectral intensity of C_6OBr_5 fragment compared to commonly observed most intense $[M - Br + O]^-$ type ion for PBDE in APCI/APPI is well documented (Debrauwer et al., 2005). This is consistent with results of the previous study that C_6OBr_5 was also detected as a top 10 signal. Complicated fragmentation pathways, as well as adducts, posed significant challenges to match formulas to public databases. Similar to the previous study, to avoid any potential bias, formulas of the detected ions, rather than predicted neutral compounds, were reported in the study.

Consistent with the previous study, nitrogen-containing (N-containing) brominated compounds were the predominant class of compounds detected in this study (Table 1). As noted earlier (Peng et al., 2016b), this group of compounds contained only one or two bromine atoms but large number of nitrogen (1–9) and oxygen (1–10) atoms. Most of the detected BADs contained a BNA ($C_6H_5BrN_3O_2$) substructure, as evidenced by MS/MS spectra. By searching predicted formulas of abundant N-containing brominated compounds against databases with different probable adducts ($[M - H]^-$, $[M]^-$, $([M - Br]^-$, $M - Br + O]^-$), these compounds were putatively identified as BADs. In addition, DB373, DV93 and BNA were successfully confirmed by retention time congruence, exact *m/z*, and MS/MS spectra (Fig. 1). It is important to note that the data analysis technique utilized in the current study, DIPIC-Frag, correctly identified formulas of these three compounds. Consistent with previous studies (Peng et al., 2016b; Ferguson and Stapleton, 2017), BADs (for example, DB373 and DV93) produced radical anions resulting from the electron capture as the most dominant ions in the negative-ion APCI/PI. Resonance stabilized large core structures (Ph-N_2-Ph) with multiple electron withdrawing groups (*e.g.*, NO_2 and X) in halogenated, azo compounds could favor simple electron capture over typically observed ionization by the loss of a hydrogen or other groups.

4.2. Prediction of structures of previously unknown brominated compounds

In the absence of authentic standards, structures of other N-containing compounds could not be confirmed. Nevertheless, MS/MS spectra were evaluated with the aim of gaining structural insight of these compounds. Fragment ions of several N-containing compounds were consistent with the fragment ions generally observed for brominated azo compounds. For example, fragment ions of *m/z* 229.9330 and 244.9200 that were observed in the MS/MS spectra of both authentic azo standards, DB373 and DB 93, were commonly observed in N-containing brominated compounds, especially in mono-brominated compounds (Fig. S1). The *m/z* 244.9200 ion in the MS/MS spectra is possibly due to the breakage of azo nitrogen and aromatic linkage as highlighted by red coloration in the structures of DB373 and DV93 (Fig. 1). MS/MS spectra of DB373 were reported previously (Peng et al., 2016b). Considering the fact that authentic azo compounds primary ionized by electron capture, predicted formulas of the precursor ions, $C_{21}H_{23}O_9N_6Br$, $C_{21}H_{21}O_{10}N_6Br$ and $C_{23}H_{25}O_{10}N_6Br$ (Fig. S1), are likely to be radical anions of corresponding molecules. A formula search for $C_{21}H_{21}O_{10}N_6Br$ in online database Chemspider suggested a compound Disperse Blue 79:1 (CAS No.: 88,938-51-6) as the only possible candidate. Search for formulas $C_{21}H_{23}O_9N_6Br$ and $C_{23}H_{25}O_{10}N_6Br$ resulted in three and six compounds, respectively, all with azo structures. Similarly, the predicted formula ($C_{17}H_{15}O_2N_5Br_2$) and MS/MS spectra of ion with *m/z* = 478.9597, suggested that this compound was most likely to be the Disperse

Table 1
Mass to charge ratios (*m/z*), retention times (rt), and predicted formulas of the top 20 most abundant brominated compounds identified in the indoor dust.

m/z	rt (min)	Formula	Abundance	Detection frequency (%)
482.5873	11.54	C_6OBr_5 (BDE209)	9.20E+08	100
478.9598	6.25	$C_{17}H_{15}O2N_5Br_2$	1.59E+08	100
532.0720	7.51	$C_{21}H_{21}O_6N_6Br$ (DB373)	7.54E+07	95
624.0817	6.15	$C_{23}H_{25}O_{10}N_6Br$	7.19E+07	73
478.0611	6.85	$C_{18}H_{19}O_5N_6Br$ (DV93)	4.26E+07	92
596.0514	5.76	$C_{21}H_{21}O_{10}N_6Br$	1.66E+07	78
482.8809	10.19	$C_{15}H_{18}O_3Br_3$ (TBB)	1.65E+07	100
458.0706	7.31	$C_{19}H_{19}O_3N_6Br$	1.38E+07	62
346.9200	6.05	$C_{11}H_7O_5NBrCl$	1.35E+07	97
526.0257	6.51	$C_{20}H_{20}O_6N_4BrCl$	1.26E+07	70
500.6987	8.65	$C_{12}H_5O_2Br_4$ (peta-BDE)	1.19E+07	100
638.9956	12.08	$C_{24}H_{34}O_5Br_3$ (TBPH)	1.06E+07	100
424.9252	6.25	$C_{14}H_{11}O_2N_4Br_2$	1.04E+07	92
492.0399	6.60	$C_{18}H_{17}O_6N_6Br$	5.48E+06	62
259.9313	4.50	$C_6H_3O_4N_3Br$ (BNA)	3.44E+06	81
636.6410	7.41	$C_{16}HO_8Br_4$	3.35E+06	57
582.0721	5.86	$C_{21}H_{23}O_9N_6Br$	3.30E+06	95
634.6438	7.96	$C_{12}H_{17}Br_6$ (HBCD)	3.04E+06	86
501.0892	7.54	$C_{21}H_{22}O_4N_6Br$	2.97E+06	62
482.5874	10.90	C_6OBr_5	2.90E+06	62

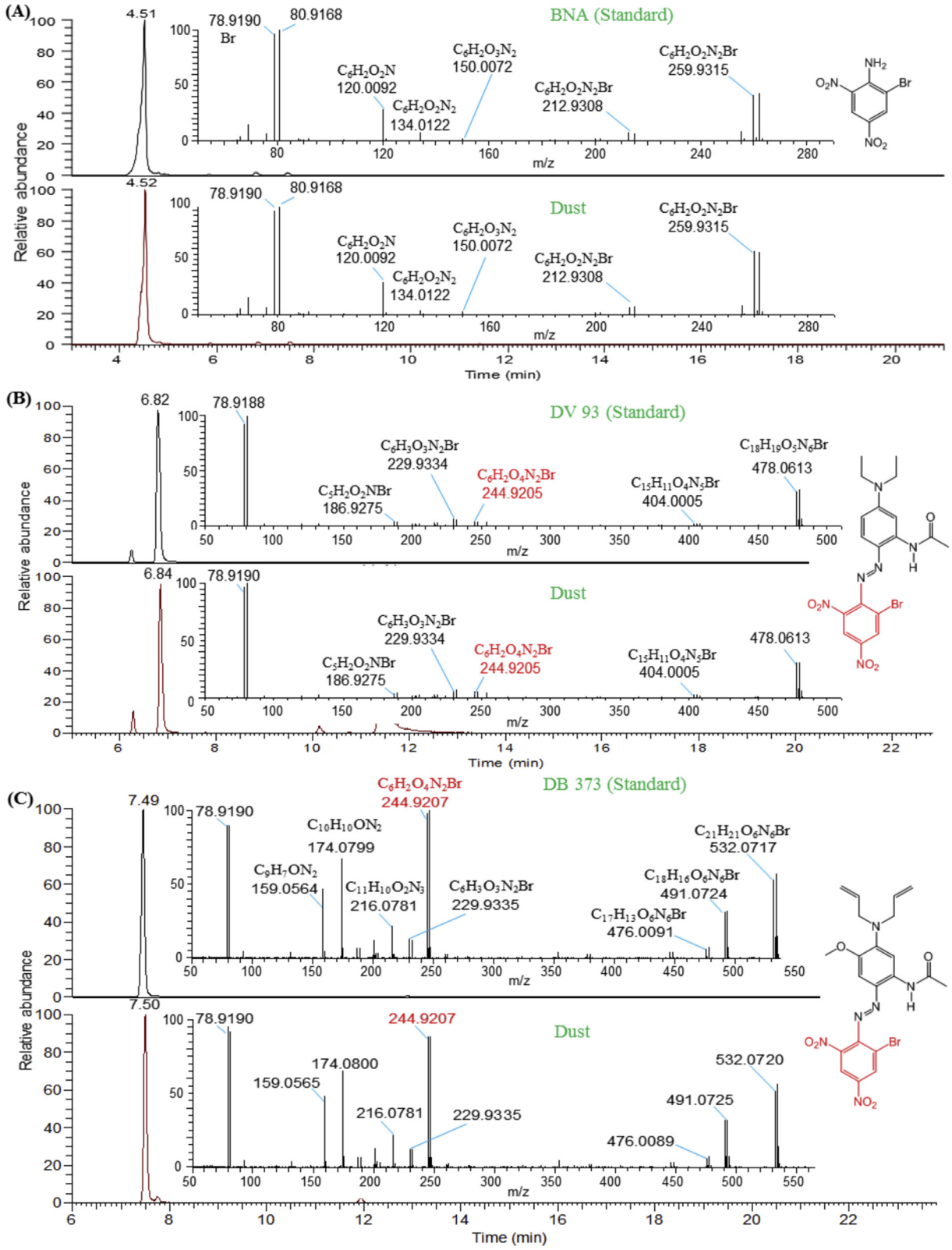


Fig. 1. Identification of (A) 2-bromo-4,6-dinitroaniline (BNA), (B) Disperse Violet 93 (DV93), and (C) Disperse Blue 373 (DB373) in a dust sample by comparing chromatographic congruence and fragmentation with synthetic standards. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Orange 61 (Fig. S2). These observations suggest that N-containing compounds detected in the current study are likely to be BADs or their derivatives. Based on elemental composition and/or some similarity of fragmentation pattern with identified BADs, more than 20 other compounds detected in house dust appear to be BADs (Table S4), but in the absence of authentic standards, these compounds could not be identified. Future studies for identification and quantification of these BADs in indoor environment could be important.

4.3. Distribution of brominated compounds across different indoor dusts

Interestingly, abundances of brominated compounds varied more substantially among samples from various indoor environments compared to dust collected from different geographic locations. Based on compound profiles, a hierarchical correlation plot (Fig. 2) segregated samples into two broad groups, A and B. Dust samples from houses and research facilities are clustered in group B, while group A mainly contained samples from daycares and hair salons with one sample from a house (AP12) in TX. The average abundance of 60 brominated compounds in the group A was 3.4 times greater than in the group B. Apparent clustering of sample types according to indoor environments, despite being collected from different states, and having different brominated compound distribution profiles among various samples suggest differences in emission/accumulation pattern of brominated compounds in different indoor environments.

Similarly, brominated compounds were clustered into three hierarchical groups (Fig. 2, Tables S3 and S4). Compounds in group I, BDE209, TBB, TBPH, and, penta-BDE, are the well-known BFRs. These compounds were detected in all dust samples, and together, compounds in group I comprised 67.0% of the total abundance of the 60 compounds. The average abundance of BDE209 (i.e., C_6OBr_5 ion) was greatest among all brominated compounds with abundances in the range 2.4×10^6 to 1.9×10^{10}. Other common FRs, such as, tetra-, hexa-BDEs, and HBCD, exhibited greater detection frequency (80–100%) and are clustered into group III. The total abundance of group III compounds was 2.4%.

In addition to common BFRs, BADs were among the most abundant analytes detected in these samples and were clustered into group II (Table 1 and Fig. 2). Compared to the ubiquitous BFRs, BADs showed heterogeneous distribution across indoor environment, leading to distinct compound profiles in daycare and salon indoor dusts. Among the 60 most abundant brominated compounds, 35 were predicted to be N-containing (Fig. 2). The majority of frequently detected and abundant azo compounds, including identified BADs (i.e. DB373, and DV93) and BNA, and other predicted to be BADs, were clustered in group II. DB373 and DV93 and BNA were among 20 frequently detected and abundant compounds in indoor dust (Fig. 3), and were detected in 35, 34, and 30, respectively, of the 37 samples. The ion whose formula was predicted to be $C_{17}H_{15}O_2N_5Br_2$ was likely Disperse Orange 61 (Ferguson and Stapleton, 2017). This compound was present in all dust samples and had the second greatest abundance among all brominated compounds and the greatest abundance among N-containing compounds. Higher abundance of Disperse Orange 61 in indoor dusts may be due to greater extent of its use in dye blends to produce different color shades. Abundances of group II compounds was 30.5% of the total abundance of 60 compounds where the five most abundant azo compounds together represented 80% of the total abundance of N-containing compounds.

Results of the previous study had suggested greater total abundances of azo compounds compared to common BFRs in indoor dust. Contrary to that previous study, the current dataset showed the total abundance of common brominated compounds was greater than azo compounds by more than 2-fold (Tables S3 and S4). It appears that significantly greater abundance of

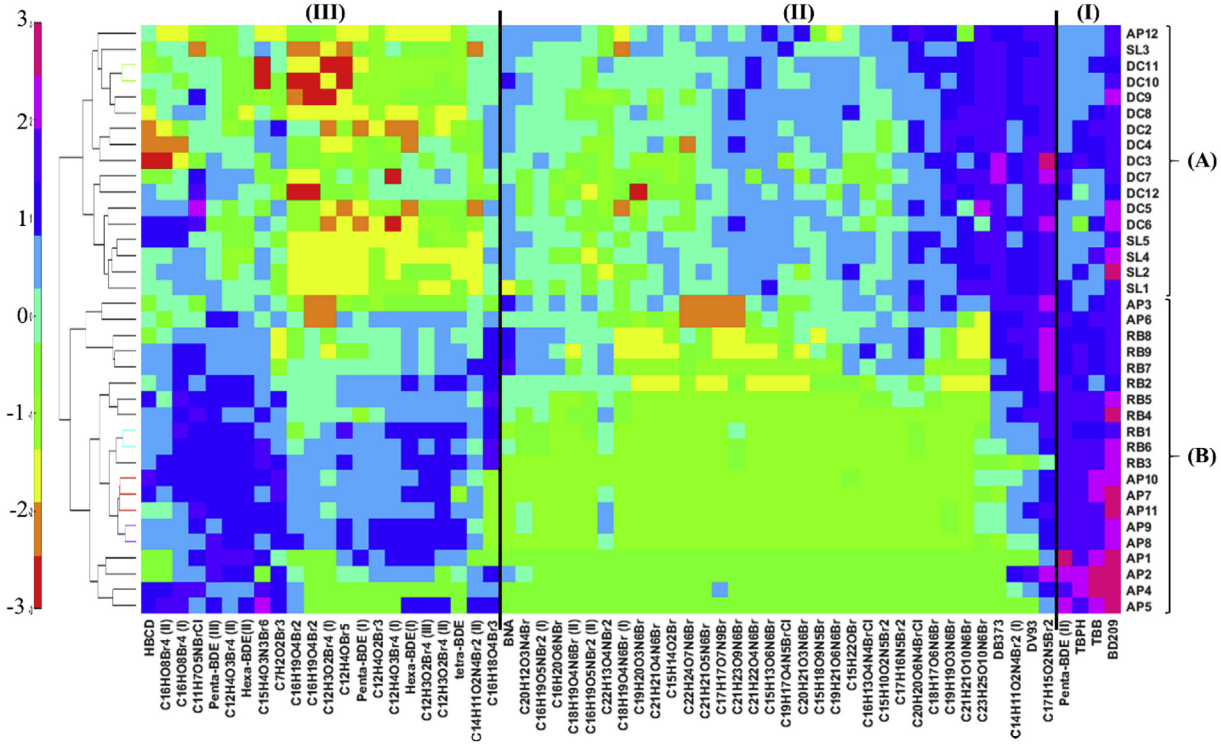


Fig. 2. Heat map and hierarchical clustering of 60 more abundant brominated compounds in indoor dust samples. Overlaid black lines separate three major compound clusters. AP: apartment/house, DC: daycare, SL: salon, RB: research building.

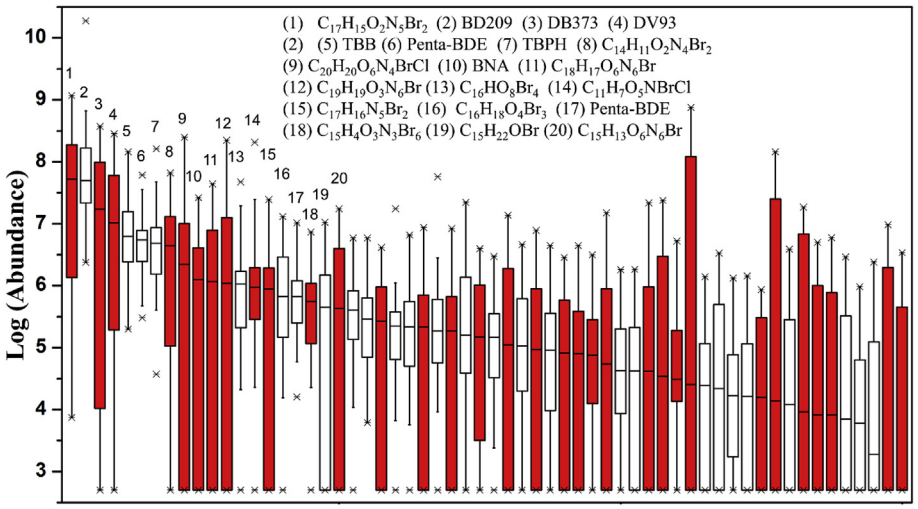


Fig. 3. Boxplot of the abundances of brominated compounds in indoor dusts. Brominated azo dyes (BADs) are shown in red. Box plots are in the order of decreasing (left to right) median abundance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

BDE209 observed in two samples, SL2 ($=1.9 \times 10^{10}$) and AP7 ($=1.1 \times 10^{10}$), skewed the overall calculation of total abundance of common BFRs in the current study resulting in greater abundance of common BFRs as compared to azo compounds. However, in the majority of samples (57% of all samples), azo compounds had greater total abundance than common BFRs. In addition, in all 10 daycare dusts, brominated azo dyes had greater total abundances compared to common BFRs. When samples SL2 and AP7 were excluded from calculations, abundances of groups I, II and III compounds were determined to be 24.6%, 69.9%, and 5.5%, respectively, of the 60 compounds. Consistent with our previous study, these results suggest over two-fold greater total abundance of BADs compared to common BFRs.

A greater abundance of BADs was observed in daycares' dust than in other indoor dusts (Fig. 2). The average abundance of BADs in daycares was 3.6-fold greater than the average abundance in other indoor dusts. The majority of BADs had relatively high abundances in dust from two daycares in Texas (DC10 and DC11). Dusts from other daycare facilities (DC3 – California, DC4 and DC7 – Ohio, DC9 – South Dakota, DC2 and DC6 – Kentucky); salons (SL3 and SL5 – Kentucky) and a house (AP12 – Texas) exhibited greater abundances of BADs compared others. Since children are more susceptible to indoor dust exposure due to their hand-to-mouth activities, greater abundances of azo compounds in daycares determined in the current study suggest quantification of potentially toxic BADs in such facilities is crucial.

4.4. Concentration of brominated compounds in indoor dust

To quantify abundant brominated compounds in indoor dust, a targeted method was developed for compounds, DB373, DV93, BNA, BD209 and γ-HBCD. All three BADs showed 10-fold greater sensitivities than BDE209. The PDLs were BNA: 0.3, DV93: 0.2, DB373: 0.5, γ-HBCD: 0.3, and BDE209: 7 μg/g). Partly due to the robustness of APCI-APPI to matrix effects as documented in previous studies, sufficient recoveries (73–81%) were achieved for all three BADs.

With the quantitative targeted method, concentrations of three BADs, DB373, DV93 and BNA, and two common FRs, γ-HBCD and BDE209, were determined in 13 dusts from four apartments, four salons, and five daycares (Fig. 4 and Table S5). While the geometric mean (GM) of BDE209 (929 *ng*/g of dust) was greatest among five

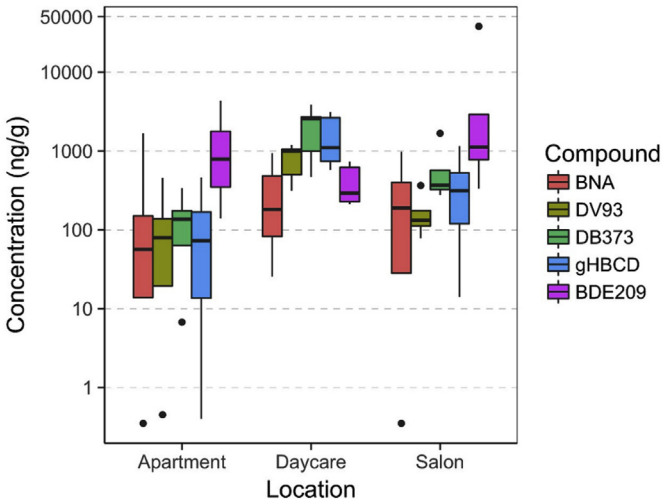


Fig. 4. Box plots representing concentrations of five brominated compounds in indoor dust. Data for indoor dusts from four apartments, five daycares and four salons are shown. In each box plot, the lower and upper boundary of the box represent the 25th and 75th centile, respectively; the mid-line represents the median and the extreme lines show the greatest and lowest value excluding outliers which are shown as black circles.

brominated compounds quantified in this study, BADs were detected with particularly greater concentrations in daycare dusts, which was consistent with untargeted screening results. For instance, DB373 was detected in all 13 samples, and concentrations were in the range of 9–3800 *ng*/g of dust where the greatest concentration was observed in DC3, a sample from daycare. Concentrations of BNA and DV93 were in the range BQL-1780 and BQL-1155 *ng*/g of dust, respectively. Concentrations of two DV93 and DB373 in some indoor dust (e.g., DC10 and DC11) were greater than concentrations of BDE209. Based on results of previous studies (Peng et al., 2016b; Ma et al., 2018) brominated azo compounds present in indoor dust at concentration determined in this study, specifically BNA in dust samples AP3, SL5 and DC5 could have toxic potency.

While potential sources of common brominated compounds in indoor dust are well documented (Flame Retardants; Johnson et al.,

2013), the presence of BADs in indoor dust were reported only recently, and sources of these compounds in indoor dust are largely unknown. We analyzed clothes, carpets, toys (plastic toys and stuffed animals), and television casings as potential sources of these compounds. While BFRs were detected with great concentrations in electronic devices such as TVs, some of BADs were identified in cloth and/or carpet samples (Figs. S3–S5), albeit with variations in abundances. Specifically, a pooled cloth sample showed substantially higher level of Disperse Orange 61 and DB373. A list of 20 more abundant brominated compounds that were identified in cloth sample, and concentrations of BADs whose standards were available is provided in Table S6. With this information, textiles may be a significant source for BADs in indoor environments, but this cannot explain the greater concentrations of BADs in daycares compared to the house. Future studies to more accurately apportion sources of BADs in daycare are warranted.

4.5. Estimation of brominated azo dye daily intake by children

Lack of authentic standards for other BADs prevented quantification of these compounds in indoor dust. However, assuming that BADs have similar instrumental sensitivities as DB373 and DV93 because of their similar structures, the concentrations of four other BADs were estimated by using average response factors (RFs) of DB373 and BV93, separately (Table 2). Indeed, the sensitivity of DV93 and DB373 were quite similar (0.2–0.5 μg/L for IDLs). Because of the lower average RF of DB373 compared to DV93, estimated concentrations from average RF of DB373 are slightly greater than DV93. However, the majority of concentrations calculated both ways differed by less than 25%. Generally, greater concentrations of BADs were observed in daycares and salons than in other samples.

Daily intake (DI) of BADs through the dust ingestion were estimated for different age groups in daycares and houses that we sampled (Table 3 and Table S7). Incorporating only detectable data for mean concentrations provided a conservative estimate of these exposures. Although daily intake through dermal uptake was not estimated, based on previous studies of common environmental contaminants, dermal uptake of BADs could be orders of magnitude less than through dust ingestion. The estimated daily intake of BADs through dust ingestion were greater for infants (6 months to <1 year) than other age groups. The difference between infants and adults was an order of magnitude. Although daily intake of BADs through dust appear to be orders of magnitude less than reported for other common classes of contaminants such as phthalates (Subedi et al., 2017), with limited toxicity data for BADs, it is difficult to determine risks of these compounds to humans through dust ingestion. Semi-quantitative determination of unknown azo compounds in indoor dust and their uptake through dust ingestion in the current study are meant to estimate concentrations of BADs in indoor dust. Future quantification of these compounds in indoor dust and their daily intake by humans coupled with a better understanding of toxicological responses to these azo compounds would allow more comprehensive risk assessments.

5. Conclusion

Dusts collected from three types of indoor environments from the USA were analyzed by use of a full scan, liquid chromatography

Table 2
Concentration (*ng*/g) of brominated azo dyes (BADs) in indoor dust. Concentration calculated using response factor of DB373 (upper panel) and DV93 (lower panel) are shown.

Compound	Group A*						All samples			
	Daycare (N = 11, n = 11)			Salon (N = 5, n = 5)			N = 37			
	Min	Max	GM	Min	Max	GM	Min	Max	GM	n
DB373	163	3480	1230	274	3150	1160	BQL	3480	361	35
DV93	66.1	2040	637	97.1	614	310	BQL	2670	98.2	34
$C_{21}H_{23}O_9N_6Br$	15.0	174	61.3	13.2	101	44.3	BQL	174	41.2	23
$C_{21}H_{21}O_{10}N_6Br$	7.12	1350	219	111	417	279	7.4	1350	190	29
$C_{23}H_{25}O_{10}N_6Br$	26.3	2410	731	265	2180	848	8.4	7060	609	27
$C_{17}H_{15}O_2N_5Br_2$	345	9610	1770	423	5100	1600	4.5	11,000	344	37
DB373	143	3060	1080	241	2770	1020	BQL	3060	318	35
DV93	58.0	1790	560	85.1	540	273	BQL	2340	87.2	34
$C_{21}H_{23}O_9N_6Br$	13.2	153	54.1	11.2	89.4	39.2	BQL	153	36.1	23
$C_{21}H_{21}O_{10}N_6Br$	7.02	1190	192	97	367	245	6.5	1190	167	29
$C_{23}H_{25}O_{10}N_6Br$	23.3	2120	643	233	1910	745	7.4	6200	536	27
$C_{17}H_{15}O_2N_5Br_2$	303	8440	1560	372	4480	1410	4.0	9680	302	37

N: Total number of samples in each group.
n: number of samples with quantifiable analyte concentrations, which were included in determining Minimum, Maximum and Geometric Mean One house sample, that was in Group A, is not included.
BQL: below quantification limit.

Table 3
Daily intake (DI) of brominated azo dyes (*ng*/kg-body mass/day)[a] through dust ingestion for various age groups in daycares and houses.

Age group	DB373	DV93	$C_{21}H_{23}O_9N_6Br$	$C_{21}H_{21}O_{10}N_6Br$	$C_{23}H_{25}O_{10}N_6Br$	$C_{17}H_{15}O_2N_5Br_2$	$\sum BAD_D$[b]	$\sum BAD_{(D+H)}$[c]
6 months to < 1 year	3.38	1.75	0.17	0.60	2.01	4.86	12.76	14.42
1 to <2 years	2.95	1.53	0.15	0.53	1.75	4.25	11.16	12.62
2 to <6 years	0.98	0.51	0.05	0.18	0.58	1.42	3.72	4.21
Adults[d]	0.18	0.09	0.01	0.03	0.11	0.26	0.67	0.81

[a] DI through dust ingestion were calculated assuming weekly averaged time spent by a child at home and daycare to be 52 and 48%, respectively, and by an adult 64 and 32%, respectively.
[b] $\sum BAD_D$: total DI of brominated azo dyes (BADs) in daycare.
[c] $\sum BAD_{(D+H)}$: total DI of BADs in daycare and house.
[d] Daycare employees.

high-resolution mass spectrometry method with a focus on screening of brominated compounds. In addition to commonly monitored flame retardants (BFRs), brominated azo dyes (BADs) were detected at relatively great abundances. Most samples (57% of all samples) exhibited greater abundances BADs than other BFRs. Using authentic standards, three BADs (Disperse Blue 373, Disperse Violet 93 and 2-bromo-4,6-dinitroaniline) were, for the first time, quantified in indoor dusts. Generally, higher concentrations of BADs were observed in indoor dusts from childcare facilities than from houses. The estimated daily intake of BADs through dust ingestion were as much as 10-fold greater for infants than other age groups. Overall, results suggest BAD prevalence in indoor dusts represents a potential exposure routes for humans.

Acknowledgment

This study was supported by funds from the Faculty Research Investment Program (FRIP) and the Vice Provost for Research at Baylor University. We gratefully acknowledge the Baylor University Mass Spectrometry Center (BU-MSC) for continued instrumental support. The Baylor University Postdoctoral Fellowship Program provided salary to support the primary author. Prof. Giesy was supported by a visiting professorship from the Department of Environmental Sciences, Baylor University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.05.153.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

References

Alaee, M., Arias, P., Sjödin, A., Bergman, Å., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ. Int. 29 (6), 683–689. https://doi.org/10.1016/S0160-4120(03)00121-1.

Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: cause for concern? Environ. Health Perspect. 112 (1), 9–17.

Brits, M., de Vos, J., Weiss, J.M., Rohwer, E.R., de Boer, J., 2016. Critical review of the analysis of brominated flame retardants and their environmental levels in africa. Chemosphere 164, 174–189. https://doi.org/10.1016/j.chemosphere.2016.08.097.

Broekaert, J.A., Harris, C. Daniel C., 2015. Quantitative chemical analysis, 9th ed. Anal. Bioanal. Chem. Heidelb. 407 (30), 8943–8944. https://doi.org/10.1007/s00216-015-9059-6.

Chequer, F.M.D., Lizier, T.M., de Felício, R., Zanoni, M.V.B., Debonsi, H.M., Lopes, N.P., Marcos, R., de Oliveira, D.P., 2011. Analyses of the genotoxic and mutagenic potential of the products formed after the biotransformation of the azo dye disperse red 1. Toxicol. Vitro 25 (8), 2054–2063. https://doi.org/10.1016/j.tiv.2011.05.033.

de Aragão Umbuzeiro, G., Freeman, H.S., Warren, S.H., de Oliveira, D.P., Terao, Y., Watanabe, T., Claxton, L.D., 2005. The contribution of azo dyes to the mutagenic activity of the cristais river. Chemosphere 60 (1), 55–64. https://doi.org/10.1016/j.chemosphere.2004.11.100.

De Oliveira, D.P., Kuhlmann, M.L., De Aragão Umbuzeiro, G., 2006. Evaluation of the presence of mutagenic dyes in sediments from cristais river. Soil Sediment Contam. 15 (5), 455–462. https://doi.org/10.1080/15320380600846882.

Debrauwer, L., Riu, A., Jouahri, M., Rathahao, E., Jouanin, I., Antignac, J.-P., Cariou, R., Le Bizec, B., Zalko, D., 2005. Probing new approaches using atmospheric pressure photo ionization for the analysis of brominated flame retardants and their related degradation products by liquid chromatography–mass spectrometry. J. Chromatogr. A 1082 (1), 98–109. https://doi.org/10.1016/j.chroma.2005.04.060.

Disperse dye - an overview | ScienceDirect topics. https://www.sciencedirect.com/topics/engineering/disperse-dye. (Accessed 4 May 2019).

Ferguson, P.L., Stapleton, H.M., 2017. Comment on "mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust. Environ. Sci. Technol. 51 (6), 3588–3590. https://doi.org/10.1021/acs.est.7b00372.

Fernandes, F.H., Botasso-Nasciutti, M.O., Svio, A.L.V., Souza, L., da, C.M., Fernandes-Cal, J.R., Cardoso, F.F., Fontes, M. R. de M., Albuquerque, A.F., Munari, C.C., Kummrow, F., et al., 2018. In vivo genotoxicity of a commercial C.I. Disperse red 1 dye. Environ. Mol. Mutagen. 59 (9), 822–828. https://doi.org/10.1002/em.22226.

Fernandes, F.H., Umbuzeiro, G. de A., Salvadori, D.M.F., 2019. Genotoxicity of textile dye C.I. Disperse Blue 291 in mouse bone. Marrow. Mutat. Res. Toxicol. Environ. Mutagen. 837, 48–51. https://doi.org/10.1016/j.mrgentox.2018.10.003.

Flame retardants. https://www.niehs.nih.gov/health/topics/agents/flame_retardants/index.cfm. (Accessed 4 May 2019).

Fryar, C.D., Gu, Q., Ogden, C.L., Flegal, K.M., 2016. Anthropometric reference data for children and adults: United States, 2011-2014. Vital Health Stat 3 (39), 1–46.

Guigueno, M.F., Fernie, K.J., 2017. Birds and flame retardants: a review of the toxic effects on birds of historical and novel flame retardants. Environ. Res. 154, 398–424. https://doi.org/10.1016/j.envres.2016.12.033.

Guo, Y., Kannan, K., 2011. Comparative assessment of human exposure to phthalate esters from house dust in China and the United States. Environ. Sci. Technol. 45 (8), 3788–3794. https://doi.org/10.1021/es2002106.

Johnson, P.I., Stapleton, H.M., Mukherjee, B., Hauser, R., Meeker, J.D., 2013. Associations between brominated flame retardants in house dust and hormone levels in men. Sci. Total Environ. 445–446, 177–184. https://doi.org/10.1016/j.scitotenv.2012.12.017.

Koopmans, F., Ho, J.T.C., Smit, A.B., Li, K.W., 2018. Comparative analyses of data independent acquisition mass spectrometric approaches: DIA, wisim-DIA, and untargeted DIA. Proteomics 18 (1). https://doi.org/10.1002/pmic.201700304.

Ma, Z., Peng, H., Jin, Y., Zhang, X., Xie, X., Jian, K., Liu, H., Su, G., Tang, S., Yu, H., 2018. Multigenerational effects and demographic responses of zebrafish (Danio rerio) exposed to organo-bromine compounds. Environ. Sci. Technol. 52 (15), 8764–8773. https://doi.org/10.1021/acs.est.8b00569.

Moschet, C., Anumol, T., Lew, B.M., Bennett, D.H., Young, T.M., 2018. Household dust as a repository of chemical accumulation: new insights from a comprehensive high-resolution mass spectrometric study. Environ. Sci. Technol. 52 (5), 2878–2887. https://doi.org/10.1021/acs.est.7b05767.

Oliveira, D.P., Carneiro, P.A., Rech, C.M., Zanoni, M.V.B., Claxton, L.D., Umbuzeiro, G.A., 2006. Mutagenic compounds generated from the chlorination of disperse azo-dyes and their presence in drinking water. Environ. Sci. Technol. 40 (21), 6682–6689. https://doi.org/10.1021/es061020p.

Peng, H., Chen, C., Saunders, D.M.V., Sun, J., Tang, S., Codling, G., Hecker, M., Wiseman, S., Jones, P.D., Li, A., et al., 2015. Untargeted identification of organo-bromine compounds in lake sediments by ultrahigh-resolution mass spectrometry with the data-independent precursor isolation and characteristic fragment method. Anal. Chem. 87 (20), 10237–10246. https://doi.org/10.1021/acs.analchem.5b01435.

Peng, H., Chen, C., Cantin, J., Saunders, D.M.V., Sun, J., Tang, S., Codling, G., Hecker, M., Wiseman, S., Jones, P.D., et al., 2016. Untargeted screening and distribution of organo-bromine compounds in sediments of lake Michigan. Environ. Sci. Technol. 50 (1), 321–330. https://doi.org/10.1021/acs.est.5b04709.

Peng, H., Saunders, D.M.V., Sun, J., Jones, P.D., Wong, C.K.C., Liu, H., Giesy, J.P., 2016. Mutagenic azo dyes, rather than flame retardants, are the predominant brominated compounds in house dust. Environ. Sci. Technol. 50 (23), 12669–12677. https://doi.org/10.1021/acs.est.6b03954.

Saunders, D.M.V., Higley, E.B., Hecker, M., Mankidy, R., Giesy, J.P., 2013. In vitro endocrine disruption and TCDD-like effects of three novel brominated flame retardants: TBPH, TBB, & TBCO. Toxicol. Lett. 223 (2), 252–259. https://doi.org/10.1016/j.toxlet.2013.09.009.

Shrivastava, A., Gupta, V.B., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles Young Sci. 2 (1), 21. https://doi.org/10.4103/2229-5186.79345.

Stapleton, H.M., Allen, J.G., Kelly, S.M., Konstantinov, A., Klosterhaus, S., Watkins, D., McClean, M.D., Webster, T.F., 2008. Alternate and new brominated flame retardants detected in U.S. House dust. Environ. Sci. Technol. 42 (18), 6910–6916. https://doi.org/10.1021/es801070p.

Subedi, B., Sullivan, K.D., Dhungana, B., 2017. Phthalate and non-phthalate plasticizers in indoor dust from childcare facilities, salons, and homes across the USA. Environ. Pollut. 230, 701–708. https://doi.org/10.1016/j.envpol.2017.07.028.

Sugeng, E.J., de Cock, M., Schoonmade, L.J., van de Bor, M., 2017. Toddler exposure to flame retardant chemicals: magnitude, health concern and potential risk- or protective factors of exposure: observational studies summarized in a systematic review. Chemosphere 184, 820–831. https://doi.org/10.1016/j.chemosphere.2017.06.041.

Takigami, H., Suzuki, G., Hirai, Y., Sakai, S., 2009. Brominated flame retardants and other polyhalogenated compounds in indoor air and dust from two houses in Japan. Chemosphere 76 (2), 270–277. https://doi.org/10.1016/j.chemosphere.2009.03.006.

Umbuzeiro, G.A., Szymczyk, M., Li, M., Chen, Y., Vendemiatti, J.A.S., de Albuquerque, A.F., dos Santos, A., Maselli, B. de S., Kummrow, F., Vinueza, N.R., et al., 2017. Purification and characterization of three commercial phenylazoaniline disperse dyes. Color. Technol. 133 (6), 513–518. https://doi.org/10.1111/cote.12307.

US EPA National Center for Environmental Assessment. W. D.; phillips, L. Exposure factors handbook chapter 5 (update): soil and dust ingestion. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=337521. (Accessed 25 October 2018).

Vacchi, F.I., Vendemiatti, J. A. de S., da Silva, B.F., Zanoni, M.V.B., Umbuzeiro, G. de A.,

2017. Quantifying the contribution of dyes to the mutagenicity of waters under the influence of textile activities. Sci. Total Environ. 601–602, 230–236. https://doi.org/10.1016/j.scitotenv.2017.05.103.

Zhang, S., Bursian, S.J., Martin, P.A., Chan, H.M., Tomy, G., Palace, V.P., Mayne, G.J., Martin, J.W., 2009. Reproductive and developmental toxicity of a pentabrominated diphenyl ether mixture, DE-71, to ranch mink (Mustela vison) and hazard assessment for wild mink in the great lakes region. Toxicol. Sci. 110 (1), 107–116. https://doi.org/10.1093/toxsci/kfp095.

Abundances and Concentrations of Brominated Azo Dyes Detected in Indoor Dust

Birendra Dhungana,[1] Hui Peng,[2] Steven Kutarna[2], Gisela Umbuzeiro[3], Sujan Shrestha,[1] Jing Liu,[1] Paul D. Jones,[4] Bikram Subedi,[5] John P. Giesy,[4] George P. Cobb[1]

[1]Department of Environmental Science, Baylor University, Waco, TX, United States

[2]Department of Chemistry, University of Toronto, Toronto, ON, Canada

[3]School of Technology, FT-UNICAMP, Sate University of Campinas, Limeira, Brazil

[4]Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada

[5]Department of Chemistry, Murray State University, Murray, KY, United States

Supplementary Material

Collection of samples. A total of 37 samples of indoor dusts from daycares, hair salons, houses, and a research facility were collected from 8 states across the USA (**Table S1**). Samples of dust from the research facility and one house (AP12) were collected using a Eureka Mighty-Mite vacuum cleaner (model 3670) into a cellulose extraction thimble (Whatman International) as described previously. [1] Other samples came from standard vacuum cleaner bags and were transferred directly into aluminum foil before transport to laboratories at Murray State University and subsequent shipment to Baylor University. Dust samples were sieved using 1.4 mm USA Standard Testing Sieve #4 and stored at -20 °C until further sample treatments as described previously.[2] In addition, samples from potential sources of brominated compounds including, clothes, carpets, toys (plastic toys and stuffed animals), and TV casing were collected from a daycare and a house from which dust samples were also collected and analyzed. The following criteria were used while selecting potential source materials of brominated azo dye in house dusts: 1) household objects that were previously reported to contain brominated compounds (e.g., TV casing, plastic toys),[3] 2) colored fabrics (e.g., clothes, window curtains), and 3) materials that are in direct contact with floors (e.g., carpet). Samples were cut into small pieces (~2 mm), and extracted using the same method that was used for dusts.

Samples from daycare facilities, salons and houses were collected in September/October 2016. While samples from a research facility was collected in the month October of 2017.

Sample Extraction and Cleanup

Dust samples were extracted and cleaned up using previously described methods. [1] Briefly, isotopically labelled d_{34}, $^{13}C_6$-TBPH was added to approximately 0.1 g of each dust type one hour prior to extraction. Each sample was extracted sequentially with 5 mL methanol and 5 mL

dichloromethane (DCM). The extraction process involved vigorous shaking for 30 min, sonication for 30 min (Aquasonic, Model, VWR, Part No. 97044-010), followed by centrifugation at 2,000 g for 10 min (Sorvall ST 16R, Thermo Scientific). Methanol and DCM extracts were combined and evaporated to dryness under a gentle stream of nitrogen at 35 ^{o}C. The extract was dissolved in 500 µL of DCM, loaded onto pre-conditioned Florisil cartridge, preconditioned with 6 mL of acetone and then 6 mL of DCM and, eluted with 6 mL DCM. Each final extract was evaporated to dryness under a gentle stream of nitrogen, reconstituted in 200 µL of acetone and kept frozen at −20 ^{o}C until further analysis. Thirty microliter of 2 mg/L F-BDE47 was added to the reconstituted solution before analysis.

LC-MS/MS Data Acquisition and Analysis. Aliquots of extracts (5 µL) were analyzed using a Q Exactive Focus HRMS coupled to a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA HPLC). Chromatographic separation was achieved with a Hypersil GOLD^{TM} C18 column (3 µm, 2.1 mm × 50 mm; Thermo Fisher Scientific) using ultrapure water (A) and methanol (B) as mobile phases. Initially 20% of B was increased to 80% in 2 min, then increased to 100% at 10 min and held static for 13 min, followed by a decrease to initial conditions of 20% B, and held for 2 min to allow equilibration to initial conditions. The flow rate was 0.35 mL/min. Column and sample compartment temperatures were maintained at 30 °C and 8 °C, respectively. To maximize ionization of analytes, data were acquired using atmospheric pressure photo- and chemical ionization (APPI/APCI) sources operated in dual APPI/APCI mode (Ion Max, Thermo Scientific). APCI/APPI source parameters were optimized to enhance overall signal intensities and to minimize insource fragmentation. Optimized APCI

parameters were: discharge (or corona) current, 6 μA; capillary temperature, 180 °C; sheath gas, 20 L/h; auxiliary gas, 5 L/h; and probe heater temperature, 320 °C.

Data were acquired in full scan and parallel reaction monitoring (PRM) mode separately. Full scan (MS) data were acquired using 300 Da-*m/z*-wide windows at resolution R = 70,000 (at *m/z* 200) with a maximum of 1×10^6 ions collected within 200 ms. PRM (MS/MS) scans were recorded, using collision induced dissociation energies, 15 and 25 eV, at a resolution R = 35,000 (at *m/z* 200) with maximum of 1×10^5 ions collected in 60 ms using 5-*m/z*-wide precursor ion isolation windows per scan. In these experiments, 120 5-*m/z*-wide windows between 100 and 700 *m/z*, were grouped into six separate methods, each of which contained 20 windows.

Data Treatment and Statistical Analyses. Heat map and hierarchical cluster analyses were performed in MATLAB. For abundances less than the detection limit, half of the detection limit (peak abundance of 500) was assigned to avoid missing values in the statistical analysis.[1] Only brominated compounds with detection frequencies of >50% were used for correlation, regression, and cluster analyses. Statistical significance was defined as $p < 0.05$.

Exposure to brominated compounds through dust ingestion was calculated using previously reported parameters as: [4,5]

$$\text{Daily intake (DI) through dust ingestion} = \frac{C_{dust} \,.\, IEF \,.\, DIR}{M}$$

where, C_{dust} is the azo dye concentration (geometric mean, GM) in dust, IEF is the indoor exposure fraction, DIR is the rate of ingestion of dust (6 months to 1 year: 0.04, 1 <2 years: 0.05, 2 to 6 years: 0.03, and adults: 0.03 g/day),[5] and M is the average body weight (6 months to <1 year: 7.5 kg, 1 <2 years: 11.5 kg, 2 to 6 years: 18 kg, and adults: 75 kg).[6] IEFs were obtained by assuming that a child does not ingest dust during his/her seep (10 hours/per day), and a daycare-

attending child spends 2/3 of his/her active hours/day and 5 days/week in a daycare (*i.e.*, IEF = 2/3*5/7). Similarly, an adult childcare worker spends 8 hours/day (i.e., 1/2 of his/her active hours/day) and 5 days/week in a daycare (*i.e.*, IEF = 1/2*5/7). IEFs, averaged over a week, were calculated to be 0.52 and 0.48 for a child, respectively, in daycare and home, and 0.36, and 0.64 for an adult daycare worker, respectively, in a daycare and home.

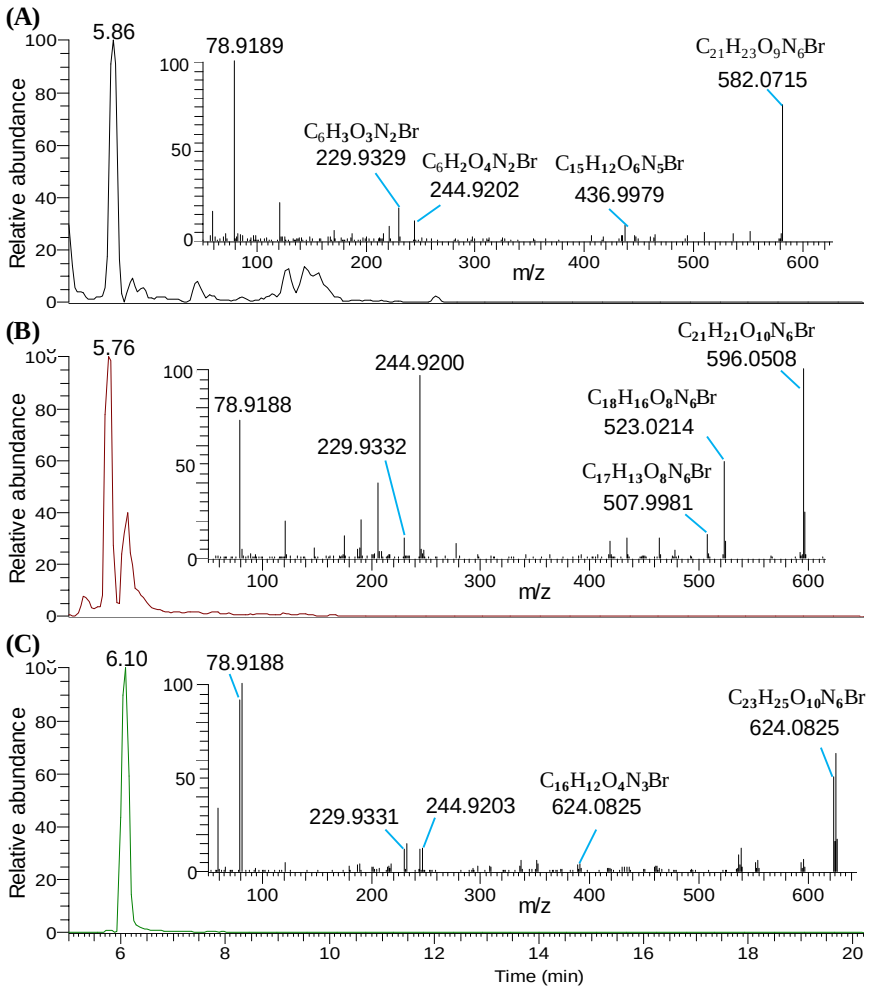


Figure S1. Chromatographic peaks and MS/MS spectra (insets) of compounds predicted to have formulas (A) $C_{21}H_{23}O_9N_6Br$, (B) $C_{21}H_{21}O_{10}N_6Br$, and (A) $C_{23}H_{25}O_{10}N_6Br$ showing characteristic fragmentation of mono-brominated azo dyes, especially fragment ions of m/z 244.9200 and 229.9328.

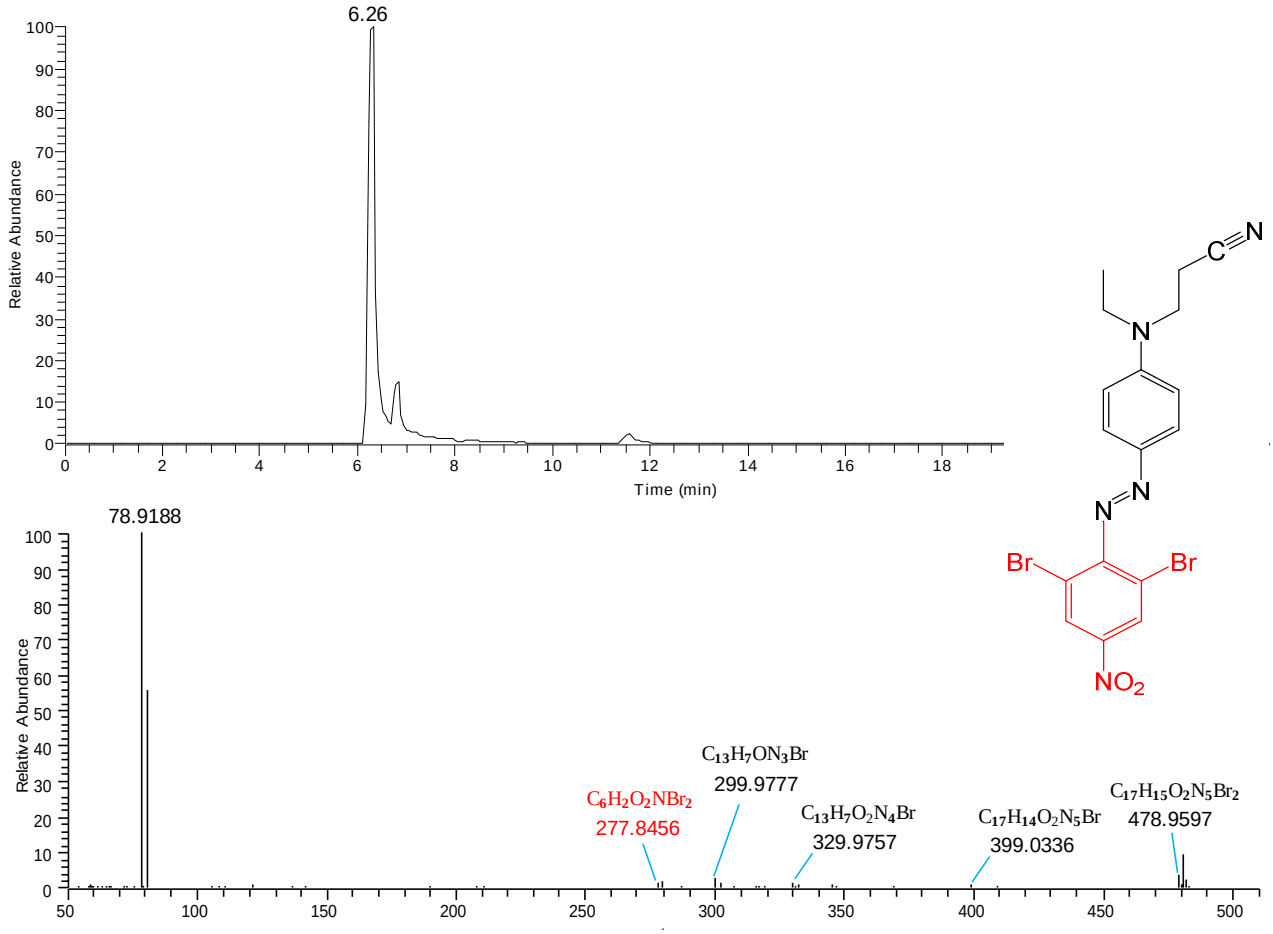


Figure S2. Chromatographic profile and MS/MS spectrum of a compound whose formula was predicted to be $C_{17}H_{15}O_2N_5Br_2$ (*m/z* = 478.9597). Database search suggested Disperse Orange 61 (structure shown in the figure) as a probable candidate. Fragment ions commonly observed for a brominated azo dye, especially the fragment ion resulting from the breakage of bond between azo nitrogen and aromatic ring (shown in red) indicate the compound likely to be a Disperse orange 61.

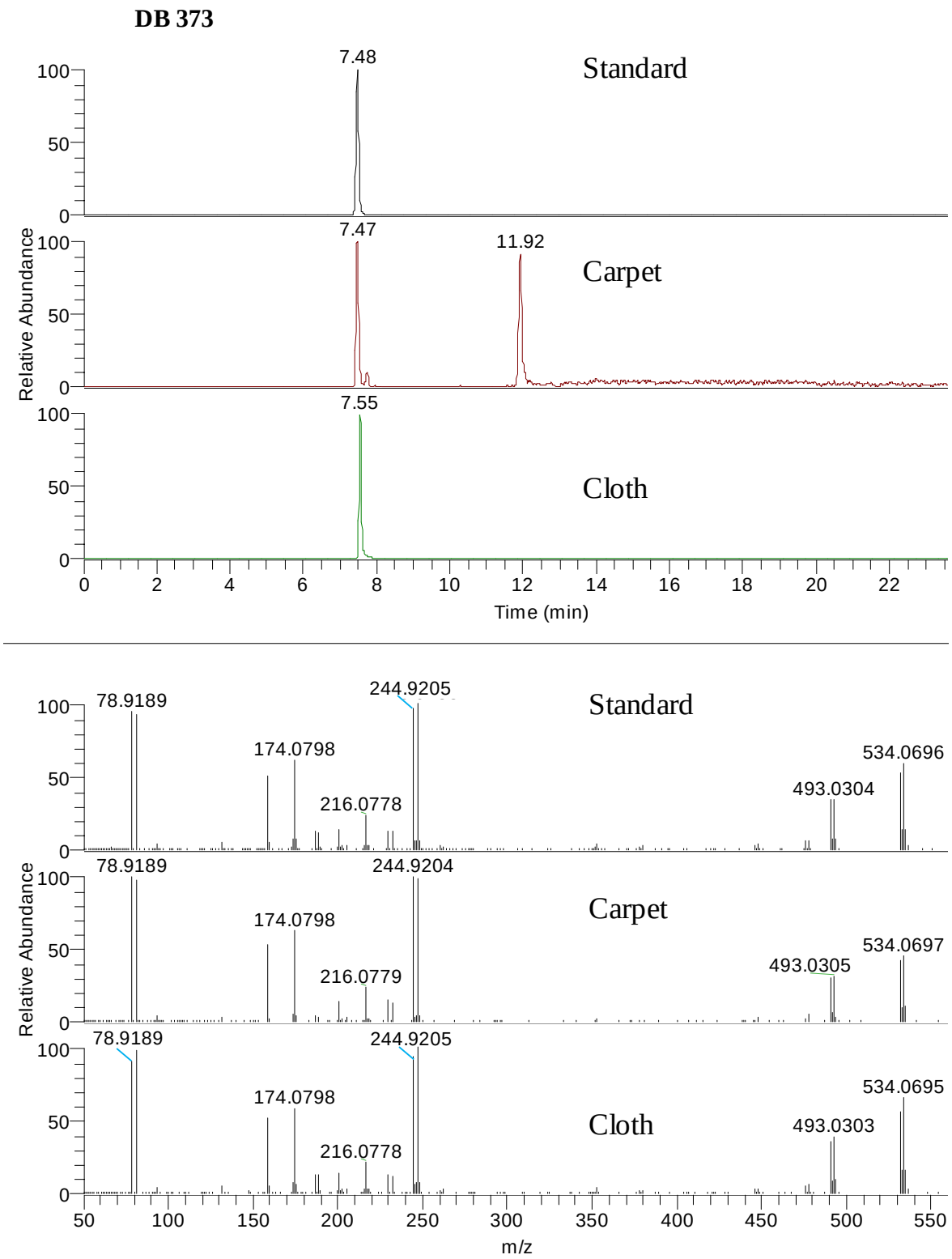


Figure S3. Identification of DB373 in carpet and samples of cloth by chromatographic (upper panels) and MS/MS (lower panels) spectra congruence.

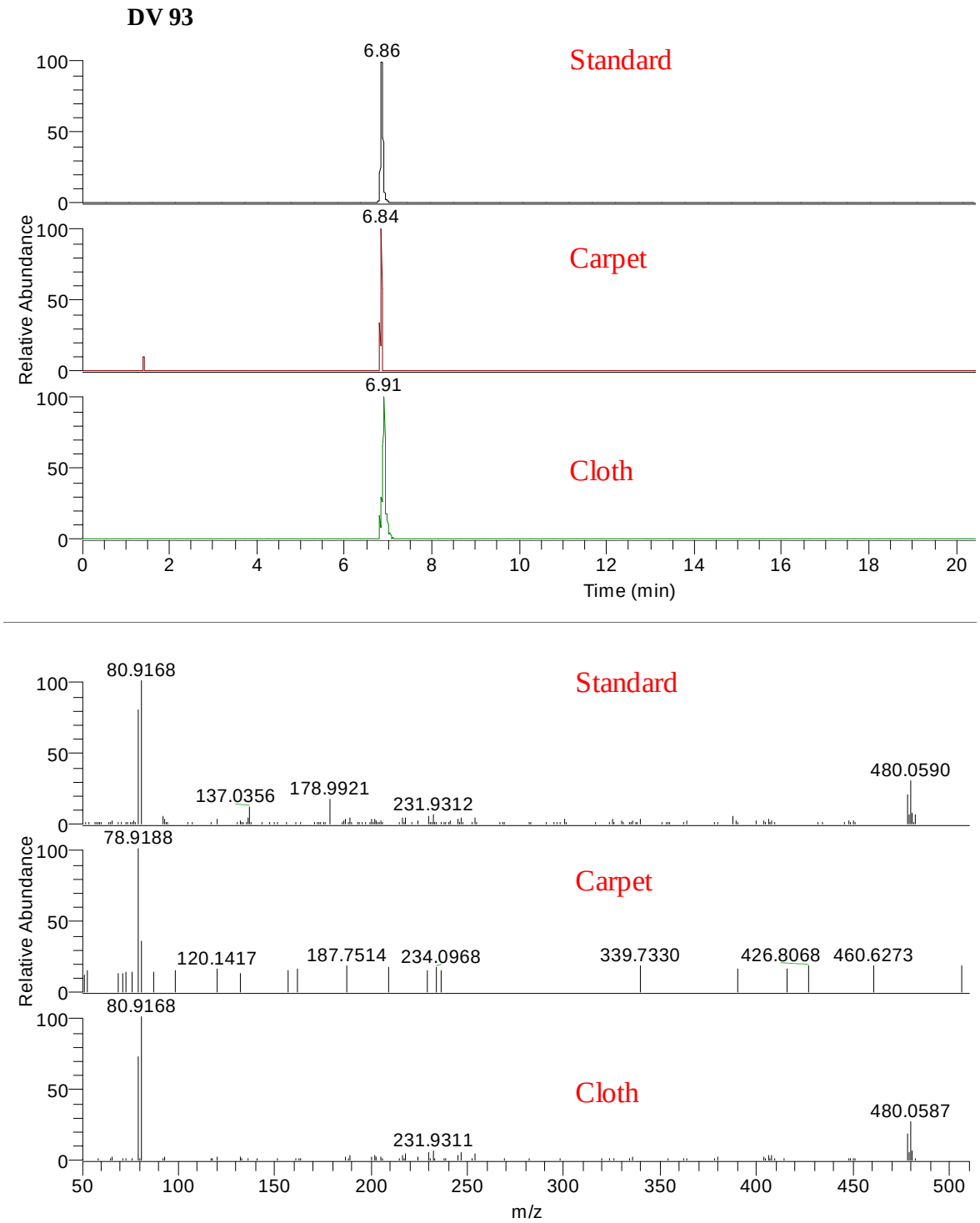
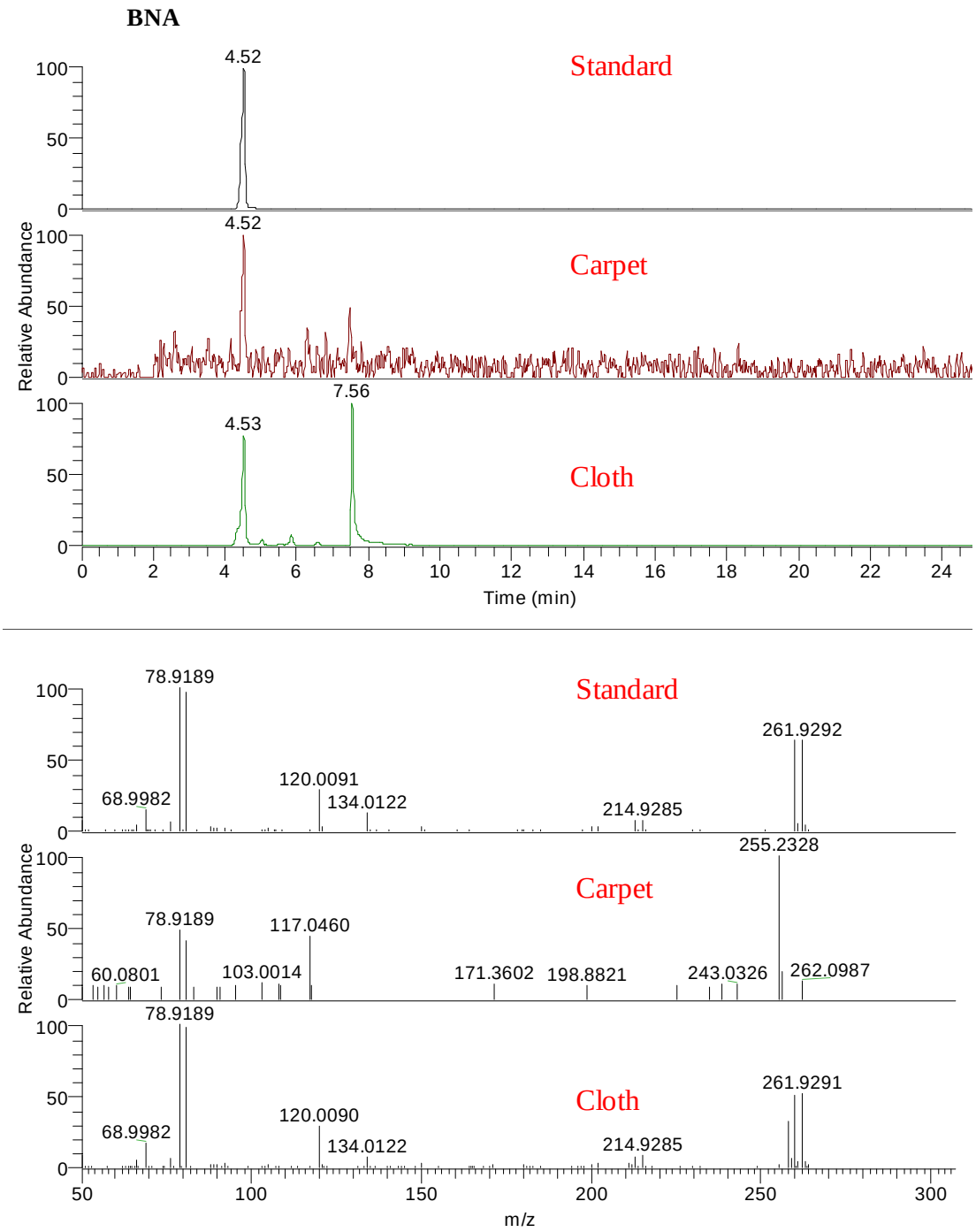


Figure S4. Identification of DB373 in carpet and samples of cloth by chromatographic (upper panels) and MS/MS spectra (lower panels) congruence.



126

127 **Figure S5.** Identification of DB373 in carpet and samples of cloth by chromatographic (upper
128 panels) and MS/MS spectra (lower panels) congruence.
129

130

131

Table S1. List of samples analyzed in the current study.*

Symbol	Sampling loacations
Childcare facilities	
DC2, DC5, DC6	Murray, KY
DC3	El Cerrito, CA
DC4, DC7	Hubbard, OH
DC8	West Lafayette, IN
DC9	Brooking, SD
DC10, DC11, DC12	Waco, TX
House/Apartments	
AP1	Medway, MA
AP2	Silver Springs MD
AP3, AP6	El Cerrito, CA
AP4	San Diego, CA
AP5, AP7, AP8, AP11	Murray, KY
AP9, AP10, AP12	Waco, TX
Hair salon	
SL1, SL3, SL5	Murray, KY
SL2	Lafayette, IN
SL4	Waco, TX
Research facility	
RB1-RB9	Waco, TX

*Different symbols represent samples collected from different facilities, except for research facility where different symbols represent samples from different locations in the same facility. Although 38 samples were collected initially, a sample from daycare, DC1, was compromised during analysis so data for this sample was not included. However, to facilitate possible comparison with previous study that used a portion of these samples for the targeted analysis phthalate and non-phthalate plasticizers, sample IDs in the current studies remained the same as the previous study.

Table S2. Recovery, instrumental detection limits (IDL) and method detection limits (PDL) of five brominated compounds.

Compound	% Recovery		Detection limit	
	Average (n = 4)	st dv	IDL, µg/L	PDL, *n*g/g
BNA	73.3	10.5	0.31	0.77
DV93	77.6	8.7	0.22	0.65
DB373	80.6	12.7	0.46	1.13
γ-HBCD	75.2	8.1	0.33	0.93
BDE-209	85.7	14.3	6.57	13.9

Table S3. Mass to charge ratios (*m/z)*, retention times (rt), and average abundances of Group I and II compounds represented in Figure 2.

rt, min	m/z	Formula	Total abundance
Group I			
8.65	500.6987	Penta-BDE (II)	4.4.E+08
10.19	482.8809	TBB	6.1.E+08
11.54	482.5873	BDE209	3.4.E+10
12.08	638.9956	TBPH	3.9.E+08
Group III			
7.23	297.0857	$C_{15}H_{22}OBr$	4.1.E+07
7.41	636.6407	$C_{16}HO_8Br_4$ (I)	1.2.E+08
7.68	480.7077	tetra-BDE	2.4.E+07
7.78	636.6406	$C_{16}HO_8Br_4$ (II)	4.3.E+07
7.96	634.6438	HBCD	1.1.E+08
8.23	496.7029	Penta-BDE (I)	5.2.E+06
8.40	511.6898	$C_{12}H_4O_3Br_4$ (I)	1.0.E+07
8.40	496.7029	Penta-BDE (III)	6.3.E+07
8.61	511.6895	$C_{12}H_4O_3Br_4$ (II)	3.5.E+07
8.63	558.6184	$C_{12}H_4OBr_5$	1.6.E+07
8.65	416.7771	$C_{12}H_4O_2Br_3$ (I)	1.0.E+07
8.98	494.6878	$C_{12}H_3O_2Br_4$ (I)	2.8.E+06
9.21	574.6132	Hexa-BDE(I)	2.3.E+07
9.22	494.6878	$C_{12}H_3O_2Br_4$ (II)	2.9.E+07
9.58	574.6134	Hexa-BDE(II)	3.5.E+07
9.59	494.6875	$C_{12}H_3O_2Br_4$ (III)	1.7.E+07
10.15	354.76077	$C_7H_2O_2Br_3$	6.2.E+07
10.52	753.5295	$C_{15}H_4O_3N_3Br_6/C_{17}H_6O_4Br_6$	4.4.E+07
11.23	432.9656	$C_{16}H_{19}O_4Br_2$ (I)	4.8.E+06
11.75	432.9651	$C_{16}H_{19}O_4Br_2$ (II)	4.6.E+06
12.07	510.8768	$C_{16}H_{18}O_4Br_3$	7.3.E+07
Sum			3.6.E+10

Table S4. Mass to charge ratios (*m/z)*, retention times (rt), and average abundances of Group II compounds represented in Figure 2.

rt, min	m/z	formula	Average abundance
Group II			
4.50	259.9313	BNA	1.3.E+08
5.09	305.0182	$C_{15}H_{14}O_2Br$	1.4.E+07
5.33	459.0781	$C_{19}H_{20}O_3N_6Br$	1.3.E+07
5.76	596.0514	$C_{21}H_{21}O_{10}N_6Br$	6.2.E+08
5.79	449.9209	$C_{15}H_{10}O_2N_5Br_2$	3.3.E+07
5.86	582.0721	$C_{21}H_{23}O_9N_6Br$	1.2.E+08
5.92	493.0155	$C_{19}H_{17}O_4N_5BrCl$	6.1.E+07
6.05	452.0086	$C_{15}H_{13}O_6N_6Br$	9.9.E+07
6.05	346.92	$C_{11}H_7O_5NBrCl$	5.0.E+08
6.20	435.0101	$C_{20}H_{12}O_3N_4Br$	2.6.E+07
6.21	538.0445	$C_{17}H_{17}O_7N_9Br$	5.1.E+07
6.25	424.9252	$C_{14}H_{11}O_2N_4Br_2$ (I)	3.8.E+08
6.25	478.9598	$C_{17}H_{15}O_2N_5Br_2$	5.9.E+09
6.25	447.9779	$C_{17}H_{16}N_5Br_2$	9.1.E+07
6.27	462.9639	$C_{16}H_{19}O_5NBr_2$ (I)	3.1.E+07
6.34	401.0484	$C_{16}H_{20}O_6NBr$	1.7.E+07
6.49	462.9639	$C_{16}H_{19}O_5NBr_2$ (II)	6.5.E+06
6.51	526.0257	$C_{20}H_{20}O6N_4BrCl$	4.7.E+08
6.52	438.9812	$C_{16}H_{13}O_4N_4BrCl$	2.6.E+07
6.57	424.9253	$C_{14}H_{11}O_2N_4Br_2$ (II)	1.0.E+07
6.60	492.0399	$C_{18}H_{17}O_6N_6Br$	2.0.E+08
6.69	500.0814	$C_{21}H_{21}O_4N_6Br$	2.2.E+07
6.77	462.0661	$C_{18}H_{19}O_4N_6Br$ (I)	8.8.E+06
6.79	478.0609	$C_{18}H_{19}O_5N_6Br$	1.6.E+09
6.85	478.0611	DV93	2.7.E+09
6.87	462.0661	$C_{18}H_{19}O_4N_6Br$ (II)	1.4.E+07
6.98	512.9218	$C_{22}H_{13}O_4NBr_2$	1.1.E+07
7.29	508.0716	$C_{19}H_{21}O_6N_6Br$	6.4.E+07
7.31	458.0706	$C_{19}H_{19}O_3N_6Br$	5.1.E+08
7.45	516.0757	$C_{21}H_{21}O_5N_6Br$	2.9.E+07
7.48	563.0896	$C_{22}H_{24}O_7N_6Br$	1.6.E+07
7.49	491.0297	$C_{15}H_{18}O_9N_5Br$	4.6.E+07
7.51	532.072	DB373	2.8.E+09
7.54	501.0892	$C_{21}H_{22}O_4N_6Br$	1.1.E+08
7.79	472.0865	$C_{20}H_{21}O_3N_6Br$	4.4.E+07
Sum			1.7.E+10

Table S5. Concentration (ng/g) of azo dyes and flame retardants and geometric mean (GM) in indoor dust. BQL: Less than limit of quantification.

Sample ID	BNA	DV 93	DB 373	g-HBCD	deca-BDE
AP2	BQL	67.6	133	BQL	139
AP3	1670	453	338	459	1310
AP5	47.0	BQL	139	44.2	472
AP11	67.2	92.2	6.72	127	4340
SL1	BQL	78.1	275	13.7	1020
SL2	294	137	341	403	38000
SL4	121	126	395	243	1230
SL5	977	364	1670	1150	333
DC3	25.4	993	3850	1100	291
DC5	935	312	468	571	731
DC9	82.0	499	995	736	2120
DC10	482	1190	2550	3120	618
DC11	181	1050	2700	2630	227
GM	208	283	451	397	927

Table S6. Mass to charge ratios (*m/z)*, retention times (rt), and predicted formulas of the top 20 most abundant brominated compounds as observed in the in a pooled cloth sample of different colors. Abundance of these compounds in other materials and two dust samples (AP9 and DC9) are also provided. Measured concentration of DB373, DV93 and BNA for which standard were available are provided in parenthesis.

			Abundance					
m/z	**rt (min)**	**Formula/Compound**	**Pooled cloth**	**TV casing**	**Carpet**	**Plastic toys**	**AP9[a]**	**DC9[b]**
478.9598	6.28	$C_{17}H_{15}O_2N_5Br_2$	3.24E+09	2.13E+05	1.85E+06	3.01E+05	1.89E+06	1.99E+08
532.0721	7.51	DB373	2.48 E+09 (23200)[c]	BDL	3.65 E+05 (34.0)	BDL	1.05E+05	1058 E+08 (995)
491.0301	7.58	$C_{15}H_{18}O_9N_5Br$	8.78E+07	BDL	BDL	BDL	BDL	BDL
492.0399	6.60	$C_{18}H_{17}O_6N_6Br$	8.05E+07	BDL	BDL	BDL	BDL	7.90E+06
401.0491	6.34	$C_{17}H_{16}O_2N_5Br$	4.13E+07	BDL	BDL	BDL	BDL	1.16E+06
478.0611	6.85	DV 93	1.83E+07 (151)	BDL	2.65E+05 (2.28)	1.34E+04	1.98E+05	6.22 E+07 (499)
624.0817	6.15	$C_{23}H_{25}O_{10}N_6Br$	1.70E+07	BDL	BDL	BDL	1.32E+04	1.57E+08
464.9627	6.27	$C_{16}H_{19}O_5NBr_2$	1.40E+07	BDL	BDL	BDL	BDL	1.14E+06
424.9251	6.25	$C_{14}H_{11}O_2N_4Br_2$	1.09E+07	1.19E+04	BDL	1.42E+04	1.06E+05	1.30E+06
459.0781	5.33	$C_{19}H_{20}O_3N_6Br$	1.05E+07	BDL	BDL	BDL	BDL	6.54E+05
449.9211	5.79	$C_{15}H_{10}O_2N_5Br_2$	6.82E+06	BDL	BDL	BDL	BDL	1.26E+06
259.9313	4.50	BNA	4.36E+06 (912)	BDL	1.63E+03 (BDL)	BDL	BDL	3.92 E+06 (82)
452.0086	6.05	$C_{15}H_{13}O_6N_6Br$	4.15E+06	BDL	BDL	BDL	BDL	8.52E+06
259.9309	7.56	$C_6H_3O_4N_3Br$	4.01E+06	BDL	BDL	BDL	BDL	BDL
424.9251	6.57	$C_{14}H_{11}O_2N_4Br_2$	2.45E+06	3.92E+04	BDL	3.27E+04	5.16E+04	4.60E+05
435.0101	6.20	$C_{20}H_{12}O_3N_4Br$	6.88E+05	BDL	BDL	BDL	1.17E+04	2.46E+06
416.7771	8.65	$C_{12}H_4O_2Br_3$	4.19E+05	2.97E+04	BDL	BDL	7.95E+05	4.24E+04
563.0898	7.42	$C_{22}H_{24}O_7N_6Br$	4.03E+05	BDL	BDL	BDL	BDL	3.24E+05
210.9617	7.46	$C_5H_8O_4Br$	2.77E+05	BDL	BDL	BDL	BDL	BDL

[a]Pooled clothes and TV casing samples were collected from residential apartment, AP9.

[b]Carpet and plastic toys samples were collected from daycare, DC9

[c]DB373 concertation in pooled cloth sample represents estimated concentration as it was calculated using instrumental response outside of calibration range.

Table S7. Daily intake (DI) of brominated azo dyes (*n*g/kg-bw/day) through dust ingestion for various age groups in house.

Age group	**DB373** (n = 3)	**DV93** (n = 6)	**$C_{21}H_{23}O_9N_6Br$** (BQL)	**$C_{21}H_{21}O_{10}N_6Br$** (n = 1)	**$C_{23}H_{25}O_{10}N_6Br$** (n = 2)	**$C_{17}H_{15}O_2N_5Br_2$** (n = 9)	**$\sum BAD_H$**
6 months to < 1 year	0.82	0.08	-	0.02	0.59	0.15	1.67
1 to <2 years	0.71	0.07	-	0.02	0.52	0.14	1.46
2 to <6 years	0.24	0.02	-	0.01	0.17	0.05	0.49
Adults	0.07	0.01	-	0.00	0.05	0.01	0.14

n: number of samples with concentration higher than detection limits, which were used to calculate DI.

REFERENCES

(1) Peng, H.; Saunders, D. M. V.; Sun, J.; Jones, P. D.; Wong, C. K. C.; Liu, H.; Giesy, J. P. Mutagenic Azo Dyes, Rather Than Flame Retardants, Are the Predominant Brominated Compounds in House Dust. *Environ. Sci. Technol.* **2016**, *50* (23), 12669–12677. https://doi.org/10.1021/acs.est.6b03954.

(2) Subedi, B.; Sullivan, K. D.; Dhungana, B. Phthalate and Non-Phthalate Plasticizers in Indoor Dust from Childcare Facilities, Salons, and Homes across the USA. *Environ. Pollut.* **2017**, *230*, 701–708. https://doi.org/10.1016/j.envpol.2017.07.028.

(3) Flame Retardants https://www.niehs.nih.gov/health/topics/agents/flame_retardants/index.cfm (accessed May 4, 2019).

(4) Guo, Y.; Kannan, K. Comparative Assessment of Human Exposure to Phthalate Esters from House Dust in China and the United States. *Environ. Sci. Technol.* **2011**, *45* (8), 3788–3794. https://doi.org/10.1021/es2002106.

(5) US EPA National Center for Environmental Assessment, W. D.; Phillips, L. Exposure Factors Handbook Chapter 5 (Update): Soil and Dust Ingestion https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=337521 (accessed Oct 25, 2018).

(6) Fryar, C. D.; Gu, Q.; Ogden, C. L.; Flegal, K. M. Anthropometric Reference Data for Children and Adults: United States, 2011-2014. *Vital Health Stat. 3.* **2016**, No. 39, 1–46.