# Mutagenic Azo Dyes, Rather Than Flame Retardants, Are the Predominant Brominated Compounds in House Dust

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### **Supporting Information**

**ABSTRACT:** Characterization of toxicological profiles by use of traditional targeted strategies might underestimate the risk of environmental mixtures. Unbiased identification of prioritized compounds provides a promising strategy for meeting regulatory needs. In this study, untargeted screening of brominated compounds in house dust was conducted using a data-independent precursor isolation and characteristic fragment (DIPIC-Frag) approach, which used data-independent acquisition (DIA) and a chemometric strategy to detect peaks and align precursor ions. A total of 1008 brominated compound peaks were identified in 23 house dust samples. Precursor ions and formulas were identified for 738 (73%) of the brominated compounds; three large groups were found for the 140 high-abundance brominated compounds, and



only 24 (17%) of these compounds were previously known flame retardants. The predominant class of unknown brominated compounds was predicted to consist of nitrogen-containing compounds. Following further validation by authentic standards, these compounds (56%) were determined to be novel brominated azo dyes. The mutagenicity of one major component was investigated, and mutagenicity was observed at environmentally relevant concentrations. Results of this study demonstrated the existence of numerous unknown brominated compounds in house dust, with mutagenic azo dyes unexpectedly being identified as the predominant compounds.

## **INTRODUCTION**

A growing number of chemicals are being introduced into the commercial realm, yet information regarding environmental fates and toxic potencies of these chemicals is rarely available. Thus, assessing risks of these chemicals has posed a challenge for traditional, targeted testing strategies to meet evolving regulatory needs.<sup>1–3</sup> To address these issues, the U.S. Environmental Protection Agency (EPA) ToxCast Program has developed approaches for screening and prioritization to facilitate rapid hazard assessments of chemicals.<sup>4,5</sup> However, the universe of chemicals covered by the Toxic Substances Control Act has been estimated to number more than 75000.<sup>6</sup> This makes the characterization of toxicological profiles of all chemicals in use exceedingly difficult. This task is further complicated by the presence of unknown substances in

environmental matrices, such as natural products or byproducts that were not purposefully synthesized.<sup>7</sup> Indeed, toxic effects such as those mediated via nuclear receptors,<sup>8</sup> acute toxicities,<sup>9</sup> and mutagenicity<sup>10</sup> are mainly driven by unknown substances. Thus, establishment of a prioritized list of chemicals, including known and unknown compounds, is critical to reduce the time and expense of programs such as ToxCast and allow assessments of environmental mixtures to which humans and wildlife are exposed.

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Because of their persistence in the environment, bioaccumulation, and toxic potencies, halogenated compounds are a class of chemicals of special concern. Specifically, the brominated flame retardants (BFRs), such as hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), bis(2ethylhexyl)-tetrabromophthalate (TBPH), and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), are the most studied halogenated compounds.<sup>11–14</sup> Targeted monitoring of these BFRs has revealed their wide occurrence in human tissues<sup>15</sup> and association with multiple adverse effects,<sup>12</sup> but mass balance analysis by quantification of total organic halogens has revealed the existence of numerous unidentified organohalogens in the environment.<sup>16–18</sup> Thus, unbiased identification of unknown brominated compounds in the human environment is critical for improving our understanding of exposure scenarios and potential adverse effects on humans.

House dust has been widely used as an important environmental matrix in monitoring pollutants, especially BFRs.<sup>19–21</sup> Previous studies have found that ingestion of house dust is an important exposure pathway for humans, especially for children,<sup>22</sup> and significant associations between concentrations of pollutants in children and those in house dust have been reported.<sup>21</sup> Results of X-ray fluorescence analysis have revealed relatively high concentrations of total bromine in house dust and furniture in the range of approximately milligrams per gram, which was ~100-fold greater than that described for known BFRs (range of micrograms per gram),<sup>18</sup> indicating that most brominated compounds in house dust remain unidentified. Results of these studies indicated potential contributions of other classes, in addition to BFRs, to brominated compounds in dust.

In a previous study, it was found that high concentrations of TBPH and its byproducts occurred in house dusts collected from Saskatoon, Saskatchewan.<sup>20</sup> In this study, the dataindependent precursor isolation and characteristic fragment (DIPIC-Frag) method<sup>23</sup> was used for untargeted screening of brominated compounds in 23 samples of house dust collected from Saskatoon. Experimental evidence that hundreds of unknown brominated compounds existed in house dust was provided for the first time, and a novel class of mutagenic brominated azo dyes were identified as the predominant brominated compounds.

#### MATERIALS AND METHODS

**Chemicals and Materials.** Authentic standards of 10 congeners of PBDEs, tetrabromobisphenol A (TBBPA), polybrominated biphenyl (PBB), bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), and 2-ethylhexyl-tetrabromobenzene (TBB), were purchased from Wellington Laboratories Inc. (Guelph, ON). 2-Bromo-4,6-dinitroaniline (BNA) was purchased from Sigma-Aldrich (St. Louis, MO). Florisil (6 cm<sup>3</sup>, 500 mg, 30  $\mu$ m) solid-phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Dichloromethane (DCM), hexane, methanol, and acetone were all of omni-Solv grade and purchased from EMD Chemicals (Gibbstown, NJ).

**Collection of Dust and Sample Pretreatment.** Twentythree samples of dust were collected. A total of two or three samples were collected from different rooms per house, in Saskatoon, from May to August 2013 as described previously.<sup>20</sup> All house dust samples were collected from daycare houses, and children's toys and foam furniture were contained in these houses. There are zero to three furnishings or zero to three electronics in studied rooms. Floors of these rooms were covered by carpet or laminate. Dust was collected using a Eureka Mighty-Mite vacuum cleaner (model 3670) into a cellulose extraction thimble (Whatman International, Pittsburgh, PA), which was inserted between the vacuum tube extender and suction tube and was secured by a metal hose clamp.<sup>11,24</sup> Extraction thimbles were Soxhlet-extracted with DCM for 2 h and dried prior to use. The equivalent of the entire floor surface area was sampled in each room. All sampling components upstream of the extraction thimble were cleaned after each sampling event. Prior to the sample pretreatment, nondust particles, such as hair, were removed.

Dust samples were extracted by use of previously described methods, by use of two-step organic solvent extraction followed by Florisil cartridge cleanup,<sup>20</sup> which is described in the Supporting Information; 0.1 g of clothes was cut into small pieces and then extracted using the same method that was used for dust samples.

Mass Spectrometry and Chemometric Data Processing. Aliquots of extracts were analyzed using a Q Exactive UHRMS instrument (Thermo Fisher Scientific, San Jose, CA) equipped with a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific),<sup>23</sup> as described in the Supporting Information. Data were acquired using atmospheric-pressure photoionization in negative ion mode (APPI), and the total mass range for the nine methods was m/z 100–1000.

A novel chemometric strategy was developed to expand the number of detected brominated compounds and reduce the false positive rate of predicted precursor ions and compound formulas. Because the precursor ions and formulas for some compounds could not be identified, and to include all peaks for subsequent data analysis, a strategy similar to selected reaction monitoring (SRM) was used. In this strategy, rather than precursor ion peaks, the bromine fragment peaks from each data-independent acquisition (DIA) window were used for semiquantification, as described in previous studies.<sup>25,26</sup>

Quality Control and Assurance. To avoid contamination of samples, all equipment was rinsed regularly with acetone. One procedural blank (without house dust) was incorporated in the analytical procedure for every batch of samples. Twentyone brominated compound peaks were detected in the blank. Background contamination from blanks was subtracted from samples for subsequent data analysis, and those brominated compounds with abundances less than 3 times the background abundance in blanks were considered nondetects.

Because most identified brominated compounds were novel compounds for which no authentic standards were available, peak intensities were used to semiquantify their abundances in house dust, which has been done previously.<sup>23,25</sup> Such a semiquantitative strategy has been widely used for previous comparative proteomics and metabolomics studies,<sup>27</sup> and also untargeted chemical analysis studies.<sup>25</sup> Method detection limits (MDLs) could not be calculated, but a peak intensity cutoff of 1000 was incorporated into the DIPIC-Frag method as described previously,<sup>25</sup> and used as the MDL for the identified brominated compounds. For the 21 brominated compound peaks detected in blanks, values 3 times greater than the peak abundance were used as MDLs.

**Ames II Tests.** The Ames II test was conducted according to the manufacturer's protocol (Xenometrix, Basel, Switzerland).<sup>28</sup> 2-Nitrofluorene (2-NF) and 4-nitroquinoline N-oxide (4-NQO) were used as the positive controls for TA98 and TA100 strains, respectively. In brief, extracts of dust were diluted 2-fold with DMSO to obtain a series of concentrations.

The Ames test strains were exposed to extracts of house dusts in culture medium. Samples were tested in triplicate (plates), as well as a triplicate negative control (DMSO), and a triplicate positive control. Extracts of house dust were tested with both strains (TA98 and TA100), without the S9 liver enzyme extract. Cytotoxicity was investigated by measuring the OD<sub>600</sub> of the cultured strains according to the manufacturer's protocol, and no significant cytotoxicity was observed in the selected dose range. The number of yellow wells per 48 wells of one sample were counted visually as a measure of genotoxicity. The Ames test response was assumed to follow a binomial distribution, and a sample was considered genotoxic if the response of the sample was different from the response of the negative control with a certainty of 99%, as proposed by Heringa et al.<sup>28</sup>

**Data Treatment and Statistical Analyses.** All data analysis, including Pearson correlation, and cluster analysis were performed with an in-house R program. For those results that were smaller than the MDLs, half of the MDL (peak abundance of 500) was assigned to avoid missing values in the statistical analysis. Only brominated compounds with detection frequencies of >50% were used for correlation, regression, and cluster analysis. Statistical significance was defined as p < 0.05.

#### RESULTS

Library of Unknown Brominated Compounds in House Dust. A total of 1008 brominated compound peaks were detected by use of the DIPIC-Frag method. Compounds were distributed across 104 of the 160 DIA windows at m/zvalues of >200. By use of a chemometric strategy, which incorporated exact mass, homologue model, isotopic distribution, and chromatographic elution profiles,<sup>25</sup> precursor ions and molecular formulas were calculated for 738 (73.2%) of the 1008 brominated compound peaks. After isotopic peaks had been excluded, a final nonredundant library with 549 unique brominated compounds was established. By searching against the public database Chemspider, we found only 78 probable formulas of brominated compounds. These results indicated that most of the identified brominated compounds were "novel" and thus had not been included in the Chemspider database. This provided the first direct experimental evidence that hundreds of unknown brominated compounds exist in house dust.

The diversity of these brominated compounds was indicated by variation among their retention times (7.3-23.3 min), m/zvalues (229.9558-1000.868), and numbers of bromine atoms (1-9) (Figure 1A). Although large variations in abundances of brominated compounds were observed from  $\sim 10^3$  to  $2.0 \times 10^7$ arbitrary units (Figure 1B), the 10 most abundant brominated compounds contributed 66.1% of the total abundance. Such results provided an opportunity to establish a short list of prioritized brominated compounds by focusing on the most abundant compounds, although the conclusion might be limited by the different instrumental responses between chemicals.

Many well-known brominated compounds, such as TBBPA, PBDEs, and TBB/TBPH, were detected by use of the DIPIC-Frag method. One of the larger classes of brominated compound peaks with relatively great retention times and numbers of bromine atoms was identified as the PBDEs (compounds labeled as groups I and II in Figure 1A). In particular, the debrominated ion of BDE 209 ( $[M - Br + O]^-$ ,  $C_{12}O_2Br_9$ , m/z 886.2549)<sup>23,29</sup> was detected in 21 of the 23 samples of dust with relatively great abundances (e.g., the



Figure 1. Distributions of identified brominated compounds in house dust. (A) Distributions of brominated compounds by retention time (minutes) and m/z value. Sizes of the dots are proportional to abundances. Colors of dots represent numbers of bromine atoms. Brominated compounds from groups I and II (black) showed distribution patterns different from those of members of group III (blue). (B) Distributions of abundances (red dots) and cumulative contributions (blue dots) of brominated compounds and comparison to those of several identified brominated compounds. The cumulative contribution was calculated by the summed abundances divided by the total abundance.

greatest abundance was  $2.0 \times 10^5$  in dust 16). TBB and TBPH were also detected in 22 of the 23 samples, with the greatest abundance detected in dust 2 ( $5.3 \times 10^5$  and  $2.2 \times 10^5$  for TBB and TBPH, respectively). TBBPA was detected in only 13 of the 23 dust samples, with the greatest abundance in dust 8 (4.6  $\times 10^4$ ). Such results demonstrated the robustness of the DIPIC-Frag method for untargeted identification of known and unknown brominated compounds in house dust samples.

A heat map of the abundances of brominated compounds showed the heterogeneity of identified brominated compounds. Most of the compounds exhibited distinct patterns among the 23 dust samples (Figure S1). Only 140 of the 549 brominated compounds were detected in more than 13 (57%) of the 23 dust samples. Such results demonstrated the variability of profiles among the 23 dusts, which was consistent with the results mentioned above that the known compounds, PBDEs, TBB/TBPH, and TBBPA, exhibited the greatest abundances in different dust samples.

**Source Apportionment of Identified Brominated Compounds.** The 140 most abundant brominated compounds were further investigated to determine their potential sources (a library of these compounds is provided in the Supplementary Data). Heterogeneity was also observed for these compounds, and 23 dust samples were grouped into four clusters according to profiles of the relative abundances of brominated compounds (Figure 2A). These results highlighted the differences in emission patterns of brominated compounds



Figure 2. (A) Heat map and hierarchical clustering of 140 high-abundance brominated compounds in 23 dust samples. Color indicates logtransformed peak abundances of brominated compounds. Only brominated compounds with detection frequencies of >50% (13 samples) are shown. (B) Similarity heat map and hierarchical clustering of brominated compounds. Similarity between brominated compounds is defined as Pearson correlation coefficients among 23 dust samples.

in different rooms or houses, which provided an opportunity to use the correlation among brominated compounds to allocate their sources.

A correlation heat map was developed for the 140 most abundant brominated compounds by calculating a paired correlation matrix. Despite the heterogeneity of brominated compounds, it is surprising that only three groups were observed (groups I–III) (Figure 2B). These results indicated that most brominated compounds in house dust might originate from three common sources. Such results are quite different from those for brominated compounds detected in Lake Michigan sediments that showed poor correlations,<sup>25</sup> indicating the more heterogenic emission sources of natural brominated compounds in sediment.

A total of 17 compounds were clustered into group I (Figure 2B). Well-known PBDEs, such as BDE 209, BDE 183, BDE 99, and BDE 47, were all clustered into this group. Some other compounds, whose formulas were predicted to be  $C_{12}OBr_9$ ,  $C_{12}HOBr_8$ , and  $C_{12}H_2OBr_7$ , were also clustered into group I. Formulas of these compounds were different from that of the O<sub>2</sub> adduct ([M - Br + O]) of PBDEs, and these compounds were identified as polybrominated biphenyls (PBBs). Similar to PBDEs, PBBs are also important BFRs, and previous studies have reported their correlations of abundances with PBDEs in the environment.<sup>30</sup> Group I brominated compounds were thus categorized as "legacy BFRs".

Two compounds in group II were identified as TBB and TBPH, which are major constituents of Firemaster 550 (FM-550) and Firemaster BZ-54 (BZ-54) and are mainly used as replacements for PBDEs.<sup>31</sup> Thus, compounds in group II represent "newer" BFRs with sources common to TBB and TBPH. The moderate correlations of abundances of brominated compounds between group I and group II supported the role of group II compounds as replacements for PBDEs (Figure 2B). In addition to TBB and TBPH, five other brominated compounds were also clustered into this group. Formulas of these compounds were identified as those of polyoxygenated compounds. For example, a compound with m/z 510.8755 was

identified as  $C_{16}H_{18}O_4Br_3$ . On the basis of fragments in  $MS^2$  spectra, the compound was determined to be mono-(2ethyhexyl) tribromophthalate (BMEHP) (Figure S2). Considering the similar structure of BMEHP and TBPH, the compound might be an industrial byproduct or environmental degradation product of TBPH. It has been previously reported that TBPH could be biotransformed to a mono TBPH ester metabolite that exhibited peroxisome proliferator-activated receptor (PPAR) activity.<sup>32</sup> This study demonstrated that these bioactive compounds might also exist in human environments.

Thus, the unbiased strategy for identifying brominated compounds, in combination with a correlation matrix, was efficient for reducing the dimensions of the data set and identifying sources of known (PBDEs, PBBs, TBB, and TBPH) and unknown brominated compounds (e.g., BMEHP). In total, 24 BFRs (17 legacy and 7 new BFRs) were grouped together, indicating significant contributions of BFRs (17%) to the total brominated compounds in house dust.

Brominated Azo Dyes Are the Predominant Brominated Compounds. The largest group of chemicals observed (group III) consisted of 78 brominated compounds (Figure 1B), which contributed 56% of the 140 compounds, even more numerous than the BFRs (17%). These compounds were also detected with relatively great abundances, and 9 of the 10 most abundant brominated compounds were clustered into this group (Table 1). Total abundances of compounds in this group contributed 85% of the total abundance of the 140 brominated compounds, but commercially available standards should be tested in future studies to assess the exact contribution of each brominated compound.

Almost all compounds clustered in group III were determined to be nitrogen-containing compounds (Table 1). These compounds exhibited an elemental composition different from that of BFRs in groups I and II. In group III, most of the brominated compounds contained only one or two bromine atoms, but large numbers of nitrogen and oxygen atoms (Figure 3A). Thus, the N/C and O/C ratios of group III compounds

Table 1. m/z Values, Retention Times, and Predicted Formulas of the Top 10 Most Abundant Brominated Compounds Identified by the DIPIC-Frag Method in House Dust

m/z	rt <sup>a</sup> (min)	abundance	formula	group <sup>b</sup>
533.0724	11.7	$2.0 \times 10^{7}$	$C_{23}H_{23}O_7N_3Br$	III
478.9585	9.94	$1.2 \times 10^{7}$	$C_{17}H_{15}O_2N_5Br_2$	III
478.0603	10.6	$1.1 \times 10^{7}$	$\mathrm{C_{18}H_{19}O_5N_6Br}$	III
488.1203	10.9	$8.6 \times 10^{6}$	$C_{25}H_{29}O_5N_3Br$	III
482.9542	10.0	$2.5 \times 10^{6}$	$\mathrm{C_{16}H_{19}O_6NBr_2}$	III
488.5799	13.5	$2.4 \times 10^{6}$	C <sub>6</sub> OBr <sub>5</sub>	Ι
532.0694	11.7	$2.0 \times 10^{6}$	$\mathrm{C_{21}H_{21}O_6N_6Br}$	III
715.2162	11.9	$1.3 \times 10^{6}$	$C_{28}H_{42}O_{10}N_7Br$	III
715.2162	12.9	$1.3 \times 10^{6}$	$C_{28}H_{42}O_{10}N_7Br$	III
458.0694	11.9	$8.5 \times 10^{5}$	$C_{18}H_{23}O_7N_2Br$	III
<sup><i>a</i></sup> rt indicates	retention ti	me. <sup>b</sup> Group I ii	ndicates the group	of PBDEs

and PBBs, and group III indicates the largest group of azo dyes.

were typically high, but the Br/C ratio was low compared to those of BFRs (Figure 3B). The two most abundant compounds in group III, whose formulas were predicted to be  $C_{23}H_{23}O_7N_3Br$  [retention time (rt) = 11.7 min] and

 $C_{18}H_{19}O_5N_6Br$  (rt = 10.6 min), were complex molecules with large numbers of nitrogen and oxygen atoms (Figure 3C–F). Extensive queries of known chemical databases (Chemspider) resulted in no further information regarding the two compounds, which indicated that brominated compounds in group III might be a class of novel, complex, and abundant brominated compounds.

Further evaluation of the  $MS^2$  spectra of the compounds in group III showed that common fragments with an m/z value of 244.9198 or 231.9305 were detected; their formulas were predicted to be  $C_6H_2N_2O_4Br$  or  $C_6H_3N_2O_3Br$  (typical spectra shown in panels D and F of Figure 3). Such results indicated that  $C_6H_2N_2O_4Br/C_6H_3N_2O_3Br$  might be the chemical backbone of the brominated compounds in group III (Figure 3C,E). This is also consistent with the predicted formulas of brominated compounds in group III, with small numbers of bromine atoms but larger numbers of nitrogen and oxygen atoms.

Because the most abundant compounds in group III could not be identified from public databases because of the complexity of the chemical structure, brominated compounds with smaller masses in group III, whose structure may be easier



Figure 3. Elemental composition, chromatogram, and mass spectra of group III compounds (brominated azo dyes). (A) Nitrogen, oxygen, and nitrogen atom number of identified BFRs and group III compounds. Red dots indicate BFRs. Blue dots indicate group III compounds. (B) Element/ carbon ratios of identified BFRs and group III compounds. (C) Chromatogram of a highly abundant group III compound whose formula was predicted to be  $C_{23}H_{23}O_7N_3Br$ . (D) MS<sup>2</sup> spectra of a highly abundant group III compound whose formula was predicted to be  $C_{23}H_{23}O_7N_3Br$ . (E) Chromatogram of a highly abundant group III compound whose formula was predicted to be  $C_{18}H_{19}O_5N_6Br$ . (F) MS<sup>2</sup> spectra of a highly abundant group III compound whose formula was predicted to be  $C_{18}H_{19}O_5N_6Br$ . (F) MS<sup>2</sup> spectra of a highly abundant group III compound whose formula was predicted to be  $C_{18}H_{19}O_5N_6Br$ . (F) MS<sup>2</sup> spectra of a highly abundant group III compound whose formula was predicted to be  $C_{18}H_{19}O_5N_6Br$ .



**Figure 4.** Chromatogram and fragment pattern of a brominated compound whose formula was predicted to be  $C_6H_4N_3O_4Br$ . (A) Chromatogram of the compound from dust. (B) MS<sup>2</sup> spectra of the compound from dust. (C) Chromatogram of the compound from a commercial standard. (D) MS<sup>2</sup> spectra of the compound from a commercial standard.



Figure 5. Results of Ames tests (TA98) with azo dye and extracts of house dusts: (A) azo dye, (B) dust 16, (C) dust 2, and (D) dust 9. Concentrations in panels B–D indicate concentrations of house dust. Revertants indicated the number of individual mutated cells in 48 tested wells. Asterisks denote significant mutagenicity tested by a binomial distribution.

to elucidate, were investigated. One of the compounds with the smallest mass at m/z 261.9291 was evaluated (Figure 4A) and had a predicted formula of C<sub>6</sub>H<sub>3</sub>N<sub>3</sub>O<sub>4</sub>Br. The common C<sub>6</sub>H<sub>3</sub>N<sub>2</sub>O<sub>3</sub>Br fragment from group III compounds was also detected in the MS<sup>2</sup> spectrum of this compound (Figure 4B). Neutral loss of NO, NO<sub>2</sub>, and HBr was also observed in the MS<sup>2</sup> spectrum. The neutral loss of NO and NO<sub>2</sub> indicated that the compound contained an aromatic nitrogen group,<sup>33</sup> and the loss of double NO groups indicated two aromatic nitrogen groups. Because of the simple structure of the compound, on the basis of this fragment pattern, this compound was identified as bromo-dinitroaniline (BNA) (Figure 4B). In addition, this

compound was listed in the public database Chemspider. An authentic standard was obtained and used for further validation. The MS<sup>1</sup> spectra, retention time, and MS<sup>2</sup> spectra were all consistent between the authentic standard and putative compound in dust samples (Figure 4). When the standard was used for external calibration, the concentration of BNA was determined to be  $502 \pm 430$  ng/g in dust, which was similar to the concentration of TBPH (734  $\pm$  0.87 ng/g) in house dust collected from the same region.<sup>20</sup>

BNA is an important raw material for synthesis of azo dyes, the largest class of organic dyes used in clothing, leather, food, and toys.<sup>34</sup> The correlation of group III compounds with BNA,

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in addition to their large nitrogen atom number and common fragment of BNA, indicated these compounds were brominated azo dyes. Thus, samples of clothing were collected and extracted. Relatively high concentrations of BNA and other brominated dyes were detected in these materials (Figure S3). Microscopic analysis also found large proportions of clothing fibers in samples of house dust (data not shown). This information suggested that brominated azo dyes were the source of the largest group of brominated compounds in house dust.

**Mutagenicity of BNA and House Dust.** Considering the aromatic amine structure of BNA, the potential mutagenicity of BNA was tested by use of the Ames *Salmonella* assay. A strong positive response was observed with the frameshift mutation strain (TA98). Revertants were detected in 100% of wells in the 48-well plate, at the maximal concentration (20  $\mu$ g/mL) (Figure 5A). Significant mutagenicity was also detected at 1.25  $\mu$ g/mL, which was comparable to BNA concentrations in dust samples (e.g., 1.7  $\mu$ g/g in dust 16). Much weaker induction of revertants was detected in the base pair mutation strain TA100 at a concentration of 10  $\mu$ g of BNA/mL (Figure S4). The detection of mutagenicity of BNA is consistent with the results of a previous study.<sup>35</sup>

Three extracts of dust (dust 16, dust 2, and dust 9) were also tested for potential mutagenicity. All three samples of dust caused significant positive responses with the TA98 strain (Figure 5B–D), but not with the TA100 strain (Figure S4). These results were consistent with the concentrations of BNA in dust samples (1.7, 0.78, and 0.13  $\mu$ g/g for dust 16, dust 2, and dust 9, respectively). Dust 16, with the highest concentration of azo dyes and BNA, also resulted in the strongest positive response in the Ames assay.

#### DISCUSSION

Mounting data from the use of bioassays to assess mixtures for specific measurement end points have revealed that most biological activities in human environments are driven by unknown chemicals. As such, targeted monitoring and assessment of risks posed by single chemicals of known pollutants might underestimate risks to human health. Thus, the establishment of a prioritized list of chemicals stemming from the analysis of complex environmental mixtures and identification of unknown compounds is promising. In this study, an untargeted chemical screening strategy, in combination with statistical analysis and toxicity testing, efficiently deconvoluted a complex mixture of chemicals in house dusts and highlighted a class of prioritized chemicals.

Because the major source of compounds in house dust is human products, house dust is an ideal matrix for unbiased identification of unknown human synthetic compounds. Untargeted chemical screening revealed that numerous brominated compounds (1008 peaks) existed in house dust samples. Thus, the largest mass spectrometric library of brominated compounds [549 unique compounds (see Supplementary Data)], to date, in house dust was established. Unexpectedly, BFRs (PBDEs, PBBs, and TBB/TBPH) were found to contribute only a minor amount to the total abundance of brominated compounds, although historically, BFRs have been the most studied brominated compounds. These results were also consistent with results of previous studies that showed that concentrations of known BFRs were several orders of magnitude lower than those of total bromine in house dust.<sup>17,18</sup> The study presented here indicates that the

focus on BFRs in previous studies might underestimate potential exposures of humans to brominated compounds; however, only dust samples from Saskatoon were investigated in this study, and the application of the untargeted strategy for identification of unknown brominated compounds in house dust from other countries and regions is of great interest.

Brominated azo dyes were identified as the predominant group of brominated compounds (56% by compound number and 85% by peak abundance). The lack of information regarding these compounds in public databases might be due to the complexity of dye mixtures. These mixtures are generally synthesized by unspecific reaction routes, and as such, the chemical components of the mixtures have not been fully resolved. Azo dyes have been widely used in clothing, leather, food, toys, waxes, and plastics.<sup>37</sup> Currently, more than 3000 azo dyes have been developed in a broad spectrum of colors and represent more than 65% of the global dye market.<sup>34</sup> Because of their carcinogenic potential, azo dyes have been regulated by some governments. The European Union promulgated a limit of 30 mg/kg in consumer goods for 24 listed carcinogenic azo dyes.<sup>38</sup> Despite extensive study of azo dyes, limited information about the environmental existence of brominated azo dyes is available.<sup>39</sup> Here we unexpectedly identified numerous brominated azo dyes in house dust, which represented the largest group of unknown brominated compounds. Because the addition of a bromine atom to the aromatic ring might increase the persistence of compounds in the environment<sup>40</sup> and metabolic stability in humans,<sup>41</sup> these brominated azo dyes might exhibit toxicity greater than those of previously known nonbrominated azo dyes. The exact sources of the brominated components in azo dyes are unclear, but previous studies have reported the presence of chlorinated aromatic amines in azo dye mixtures.<sup>42</sup> Thus, brominated azo dyes might be synthesized via routes similar to those of chlorinated compounds. In support of this hypothesis, chlorinated analogues of several of the brominated compounds identified in this study were also detected, with similar abundances (Figure S5).

The azo dye (BNA) produced mutagenic effects at concentrations comparable to those in dust samples, which highlighted the potential health risk of these brominated azo dyes. Previous studies have suggested that mutagenicity detected in dust samples could not be explained by known chemicals,<sup>10</sup> and the detection of frameshift mutations in that study was also consistent with the toxic potency of BNA. Such results indicated that brominated azo dyes might contribute significantly to mutagenicity observed in these samples of dust. Although a correlation between concentrations of brominated azo dyes and mutagenicity of house dust was observed, the exact contributions of azo dyes to the mutagenicity of house dust could not be accurately determined, because BNA is the only azo dye for which an authentic standard is available commercially. On the basis of peak abundance, BNA is a relatively low-abundance compound compared to other identified brominated azo dyes. As exemplified by C<sub>23</sub>H<sub>23</sub>O<sub>7</sub>N<sub>3</sub>Br and C<sub>18</sub>H<sub>19</sub>O<sub>5</sub>N<sub>6</sub>Br, their concentrations were calculated to be as high as 6.6  $\pm$  8.7 and 27  $\pm$  32  $\mu$ g/g in house dust, respectively, based on the BNA standard. Such results highlight the necessity of synthesizing authentic standards of other highly abundant brominated azo dyes to more accurately investigate their potential toxicities and health risks. Alternatively, investigation of the potential presence of brominated azo dyes in various commercial azo dyes would be of interest

for clarifying the sources and mutagenicity of brominated azo dyes present in house dusts.

Deconvolution of the components and toxicities of environmental mixtures, in addition to traditional single-chemical risk assessment, is critical to more completely understand risks to human health. Numerous efforts have been undertaken to characterize toxicological profiles of chemicals since the release of the National Research Council (NRC) Report "Toxicity Testing in the 21<sup>st</sup> Century". Such database-based strategies will undoubtedly fill knowledge gaps regarding known chemicals. However, considering the large number of known and especially unknown substances in the environment, establishment of a list of prioritized chemicals based on potential exposures of humans and wildlife is critical to maximizing the efficiency of our efforts. Here, we demonstrate an alternative platform for efficiently and directly deconvoluting the components of a complex environmental mixture and identifying and prioritizing chemicals by a combination of untargeted chemical analyses, statistical analyses, and toxicity tests. The prioritized brominated compounds (azo dyes) identified in this study could be easily incorporated in future environmental monitoring and regulation.

#### ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03954.

Additional description of some methods and a heat map of all detected brominated compounds in house dust (Figure S1), the MS<sup>2</sup> spectrum of mono-(2-ethyhexyl) tribromophthalate (Figure S2), chromatograms of two brominated azo dyes from samples of clothing (Figure S3), results of Ames tests (TA100) with azo dye and house dust extracts (Figure S4), and detection of brominated and chlorinated analogues of organic azo dyes in house dust (Figure S5) (PDF) Somehengeth Date (XLSX)

Supplemental Data (XLSX)

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#### Notes

The authors declare no competing financial interest.

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#### REFERENCES

Richardson, S. D. Environmental mass spectrometry: emerging contaminants and current issues. *Anal. Chem.* 2012, 84 (2), 747–778.
 Judson, R.; Richard, A.; Dix, D. J.; Houck, K.; Martin, M.; Kavlock, R.; Dellarco, V.; Henry, T.; Holderman, T.; Sayre, P.; Tan, S.; Carpenter, T.; Smith, E. The toxicity data landscape for environmental chemicals. *Environ. Health Perspect.* 2009, 117 (5), 685–695.

(3) Collins, F. S.; Gray, G. M.; Bucher, J. R. Toxicology - transforming environmental health protection. *Science* **2008**, *319* (5865), 906–907.

(4) Judson, R. S.; Houck, K. A.; Kavlock, R. J.; Knudsen, T. B.; Martin, M. T.; Mortensen, H. M.; Reif, D. M.; Rotroff, D. M.; Shah, I.; Richard, A. M.; Dix, D. J. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. *Environ. Health Perspect.* **2009**, *118* (4), 485–492.

(5) Dix, D. J.; Houck, K. A.; Martin, M. T.; Richard, A. M.; Setzer, R. W.; Kavlock, R. J. The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol. Sci.* **2006**, *95* (1), 5–12.

(6) Toxic Substances Control Act of 1976; USC §2601 et seq. Public Law 94-469. 1976.

(7) Chen, D.; Hale, R. C.; Letcher, R. J. Photochemical and microbial transformation of emerging flame retardants: Cause for concern? *Environ. Toxicol. Chem.* **2015**, *34* (4), 687–99.

(8) Suzuki, G.; Tue, N. M.; Malarvannan, G.; Sudaryanto, A.; Takahashi, S.; Tanabe, S.; Sakai, S.; Brouwer, A.; Uramaru, N.; Kitamura, S.; Takigami, H. Similarities in the endocrine-disrupting potencies of indoor dust and flame retardants by using human osteosarcoma (U2OS) cell-based reporter gene assays. *Environ. Sci. Technol.* 2013, 47 (6), 2898–2908.

(9) Jeong, C. H.; Wagner, E. D.; Siebert, V. R.; Anduri, S.; Richardson, S. D.; Daiber, E. J.; McKague, A. B.; Kogevinas, M.; Villanueva, C. M.; Goslan, E. H.; Luo, W. T.; Isabelle, L. M.; Pankow, J. F.; Grazuleviciene, R.; Cordier, S.; Edwards, S. C.; Righi, E.; Nieuwenhuijsen, M. J.; Plewa, M. J. Occurrence and toxicity of disinfection byproducts in European drinking waters in relation with the HIWATE epidemiology study. *Environ. Sci. Technol.* **2012**, *46* (21), 12120–12128.

(10) Maertens, R. M.; Gagne, R. W.; Douglas, G. R.; Zhu, J. P.; White, P. A. Mutagenic and carcinogenic hazards of settled house dust II: Salmonella Mutagenicity. *Environ. Sci. Technol.* **2008**, *42* (5), 1754– 1760.

(11) Stapleton, H. M.; Allen, J. G.; Kelly, S. M.; Konstantinov, A.; Klosterhaus, S.; Watkins, D.; McClean, M. D.; Webster, T. F. Alternate and new brominated flame retardants detected in US house dust. *Environ. Sci. Technol.* **2008**, *42* (18), 6910–6916.

(12) Birnbaum, L. S.; Staskal, D. F. Brominated flame retardants: Cause for concern? *Environ. Health Perspect.* 2003, 112 (1), 9–17.

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

(14) Zhang, S.; Bursian, S. J.; Martin, P. A.; Chan, H. M.; Tomy, G.; Palace, V. P.; Mayne, G. J.; Martin, J. W. 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.* **2009**, *110* (1), 107–116. (15) Alaee, M.; Arias, P.; Sjodin, A.; Bergman, A. An overview of commercially used brominated flame retardants, their applications,

their use patterns in different countries/regions and possible modes of release. *Environ. Int.* 2003, 29 (6), 683–689.

(16) Wan, Y.; Jones, P. D.; Wiseman, S.; Chang, H.; Chorney, D.; Kannan, K.; Zhang, K.; Hu, J. Y.; Khim, J. S.; Tanabe, S.; Lam, M. H. W.; Giesy, J. P. Contribution of synthetic and naturally occurring organobromine compounds to bromine mass in marine organisms. *Environ. Sci. Technol.* **2010**, *44* (16), 6068–6073.

(17) Imm, P.; Knobeloch, L.; Buelow, C.; Anderson, H. A. Household exposures to polybrominated diphenyl ethers (PBDEs) in a Wisconsin cohort. *Environ. Health Perspect.* **2009**, *117* (12), 1890–1895.

(18) Takigami, H.; Suzuki, G.; Hirai, Y.; Sakai, S. Brominated flame retardants and other polyhalogenated compounds in indoor air and dust from two houses in Japan. *Chemosphere* **2009**, *76* (2), 270–277.

(19) Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Webster, T. F. Linking PBDEs in house dust to consumer products using X-ray fluorescence. *Environ. Sci. Technol.* **2008**, *42* (11), 4222–4228.

(20) Peng, H.; Saunders, D. M. V.; Sun, J. X.; Codling, G.; Wiseman, S.; Jones, P. D.; Giesy, J. P. Detection, identification, and quantification of hydroxylated bis(2-ethylhexyl)-tetrabromophthalate isomers in house dust. *Environ. Sci. Technol.* **2015**, *49*, 2999–3006.

(21) Stapleton, H. M.; Misenheimer, J.; Hoffman, K.; Webster, T. F. Flame retardant associations between children's handwipes and house dust. *Chemosphere* **2014**, *116*, 54–60.

(22) Harrad, S.; de Wit, C. A.; Abdallah, M. A. E.; Bergh, C.; Bjorklund, J. A.; Covaci, A.; Darnerud, P. O.; de Boer, J.; Diamond, M.; Huber, S.; Leonards, P.; Mandalakis, M.; Östman, C.; Haug, L. S.; Thomsen, C.; Webster, T. F. Indoor contamination with hexabromocyclododecanes, polybrominated diphenyl ethers, and perfluoroalkyl compounds: an important exposure pathway for people? *Environ. Sci. Technol.* **2010**, *44* (9), 3221–3231.

(23) Peng, H.; Chen, C. L.; Saunders, D. M. V.; Sun, J. X.; Tang, S.; Codling, G.; Hecker, M.; Wiseman, S.; Jones, P. D.; Li, A.; Rockne, K. J.; Giesy, J. P. 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.* **2015**, *87* (20), 10237–10246.

(24) Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Webster, T. F. Critical factors in assessing exposure to PBDEs via house dust. *Environ. Int.* **2008**, *34* (8), 1085–1091.

(25) Peng, H.; Chen, C. L.; Cantin, J.; Saunders, D. M. V.; Sun, J. X.; Tang, S.; Codling, G.; Hecker, M.; Wiseman, S.; Jones, P. D.; Li, A.; Rockne, K. J.; Sturchio, N. C.; Giesy, J. P. Untargeted screening and distribution of organo-bromine compounds in sediments of Lake Michigan. *Environ. Sci. Technol.* **2016**, *50* (1), 321–330.

(26) Peng, H.; Chen, C. L.; Cantin, J.; Saunders, D. M. V.; Sun, J. X.; Tang, S.; Codling, G.; Hecker, M.; Wiseman, S.; Jones, P. D.; Li, A.; Rockne, K. J.; Sturchio, N. C.; Cai, M. H.; Giesy, J. P. Untargeted screening and distribution of organo-iodine compounds in sediments from Lake Michigan and the Arctic Ocean. *Environ. Sci. Technol.* **2016**, *50* (18), 10097–10105.

(27) Sun, J. X.; Tang, S.; Peng, H.; Saunders, D. M. V.; Doering, J. A.; Hecker, M.; Jones, P. D.; Giesy, J. P.; Wiseman, S. A combined transcriptomic and proteomic approach to identify toxicity pathways in early-life stages of Japanese medaka (Oryzias latipes) exposed to 1, 2, 5, 6-tetrabromocyclooctane (TBCO). *Environ. Sci. Technol.* **2016**, 50 (14), 7781–7790.

(28) Heringa, M. B.; Harmsen, D. J. H.; Beerendonk, E. F.; Reus, A. A.; Krul, C. A. M.; Metz, D. H.; Ijpelaar, G. F. Formation and removal of genotoxic activity during UV/H2O2-GAC treatment of drinking water. *Water Res.* **2011**, *45* (1), 366–374.

(29) Zhou, S. N.; Reiner, E. J.; Marvin, C.; Helm, P.; Riddell, N.; Dorman, F.; Misselwitz, M.; Shen, L.; Crozier, P.; MacPherson, K.; Brindle, I. D. Development of liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry for analysis of halogenated flame retardants in wastewater. *Anal. Bioanal. Chem.* **2010**, 396 (3), 1311–1320.

(30) Sjodin, A.; Wong, L. Y.; Jones, R. S.; Park, A.; Zhang, Y.; Hodge, C.; Dipietro, E.; McClure, C.; Turner, W.; Needham, L. L.; Patterson, D. G. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polyhrominated biphenyl (PBB) in the united states population: 2003–2004. *Environ. Sci. Technol.* **2008**, *42* (4), 1377–1384.

(31) Ma, Y. N.; Venier, M.; Hites, R. A. 2-Ethylhexyl tetrabromobenzoate and bis(2-ethylhexyl) tetrabromophthalate flame retardants in the Great Lakes atmosphere. *Environ. Sci. Technol.* **2012**, 46 (1), 204–208.

(32) Springer, C.; Dere, E.; Hall, S. J.; McDonnell, E. V.; Roberts, S. C.; Butt, C. M.; Stapleton, H. M.; Watkins, D. J.; McClean, M. D.; Webster, T. F.; Schlezinger, J. J.; Boekelheide, K. Rodent thyroid, liver,

and fetal testis toxicity of the monoester metabolite of bis-(2-ethylhexyl) tetrabromophthalate (TBPH), a novel brominated flame retardant present in indoor dust. *Environ. Health Perspect.* **2012**, *120* (12), 1711–1719.

(33) Ma, Y.; Kind, T.; Yang, D. W.; Leon, C.; Fiehn, O. MS2Analyzer: A software for small molecule substructure annotations from accurate tandem mass spectra. *Anal. Chem.* **2014**, *86* (21), 10724–10731.

(34) Garcia-Lavandeira, J.; Salgado-Petinal, C.; Blanco, E.; Cela, R. A sensitive and efficient procedure for the high throughput determination of banned aromatic amines in textiles and leather products aided by advanced sample composition. *Anal. Bioanal. Chem.* **2010**, 397 (2), 751–763.

(35) Ackerman, J.; Sharma, R.; Hitchcock, J.; Hayashi, T.; Nagai, Y.; Li, S. L.; Lu, S.; Miret, J.; Tang, K.; Spence, F.; Aubrecht, J. Interlaboratory evaluation of the bioluminescent Salmonella reverse mutation assay using 10 model chemicals. *Mutagenesis* **2009**, *24* (5), 433–438.

(36) Hakk, H.; Letcher, R. J. Metabolism in the toxicokinetics and fate of brominated flame retardants—a review. *Environ. Int.* **2003**, 29 (6), 801–828.

(37) Yamjala, K.; Nainar, M. S.; Ramisetti, N. R. Methods for the analysis of azo dyes employed in food industry - A review. *Food Chem.* **2016**, *192*, 813–824.

(38) European ban on certain azo dyes. Quality and Environment. *Leather International;* Progressive Media Group Ltd.: London (http://www.tflchina.com/aboutus/Environment/eubanazodyes.pdf).

(39) Zocolo, G. J.; Pilon dos Santos, G.; Vendemiatti, J.; Vacchi, F. I.; Umbuzeiro, G. D.; Zanoni, M. V. B. Using SPE-LC-ESI-MS/MS analysis to assess disperse dyes in environmental water samples. *J. Chromatogr. Sci.* **2015**, *53* (8), 1257–1264.

(40) Stiborova, H.; Vrkoslavova, J.; Lovecka, P.; Pulkrabova, J.; Hradkova, P.; Hajslova, J.; Demnerova, K. Aerobic biodegradation of selected polybrominated diphenyl ethers (PBDEs) in wastewater sewage sludge. *Chemosphere* **2015**, *118*, 315–321.

(41) Gao, S. X.; Wan, Y.; Zheng, G. M.; Luo, K.; Kannan, K.; Giesy, J. P.; Lam, M. H. W.; Hu, J. Y. Organobromine compound profiling in human adipose: Assessment of sources of bromophenol. *Environ. Pollut.* **2015**, *204*, 81–89.

(42) Mortensen, S. K.; Trier, X. T.; Foverskov, A.; Petersen, J. H. Specific determination of 20 primary aromatic amines in aqueous food simulants by liquid chromatography-electrospray ionization-tandem mass spectrometry. J. Chromatogr. A 2005, 1091 (1–2), 40–50.

1	Supporting Information
2	Mutagenic Azo Dyes, Rather than Flame Retardants, are Predominant Brominated
3	Compounds in House Dust
4	
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13	Pages 9
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17	This supporting information provides figures describing (1) Heatmap of all detected brominated
18	compounds in house dust; (2) $MS^2$ spectrum mono-(2-ethyhexyl) tribromophthalate; (3)
19	Chromatograms of two brominated azo dyes in the samples of clothing; (4) Results of Ames tests
20	(TA 100) with azo dye and house dust extracts; (5) Detection of brominated and chlorinated
21	analogues of organic azo dyes in house dust.
22	

**Sample Pretreatment.** Dust samples were extracted by use of previously described methods.<sup>1</sup> 24 by use of two-step organic solvent extraction followed by Florisil cartridge cleanup. In brief, 25 approximately 0.1 g, dry mass (dm) of dust was transferred to a 15 mL centrifuge tube. Twenty 26 microliters of 1 mg/L mass-labeled internal standard  $d_{34}$ , <sup>13</sup>C<sub>6</sub>-TBPH and 5 mL of methanol were 27 added for extraction of house dust. Samples were vigorously shaken for 30 min followed by 28 sonication for an additional 30 min. The methanol extract was separated by centrifugation at 29 1,669 g for 10 min and transferred to a new tube. The extraction was repeated using 5 mL of 30 DCM. The methanol and DCM extracts were combined and blown to dryness under a gentle 31 stream of nitrogen. Extracts were dissolved in 500 µL of DCM and loaded onto Florisil 32 cartridges, which had been sequentially conditioned with 6 mL of acetone and then 6 mL of 33 DCM. Brominated compounds were eluted from Florisil cartridges using 5 mL of DCM, and 34 35 then 5 mL of acetone and 5 mL of methanol. Because a limited number of brominated compounds were detected in other fractions, only the DCM fraction from the cartridges was used 36 in this study for the screening of brominated compounds, as described previously.<sup>2, 3</sup> Final 37 extracts were blown to dryness under a gentle stream of nitrogen and reconstituted with 200 µL 38 of acetone for analysis. 39

40 **Mass Spectrometry.** Aliquots of extracts were analyzed using a Q Exactive<sup>TM</sup> UHRMS (Thermo 41 Fisher Scientific, San Jose, CA, USA) equipped with a Dionex<sup>TM</sup> UltiMate<sup>TM</sup> 3000 UHPLC 42 system (Thermo Fisher Scientific). Due to its previously determined ability<sup>2</sup> to separate 43 compounds and the sensitivity achieved with its use a Hypersil GOLD<sup>TM</sup> C18 column (3  $\mu$ m; 2.1 44 mm × 50 mm; Thermo Fisher Scientific) was selected for the present method. The injection 45 volume was 5  $\mu$ L. Ultrapure water (A) and methanol (B) were used as mobile phases. Initially 20% 46 of B was increased to 80% in 3 min, then increased to 100% at 8 min and held static for 19.5 min, followed by a decrease to initial conditions of 20% of B, and held for 2 min to allow for
equilibration. The flow rate was 0.25 mL/min. Temperatures of the column and sample
compartment were maintained at 30 °C and 10 °C, respectively.

Data were acquired in data-independent acquisition (DIA) mode.<sup>3</sup> Parameters for DIA 50 were one full MS<sup>1</sup> scan (100-1,000 m/z) recorded at resolution R=70,000 (at m/z 200) with a 51 maximum of  $3 \times 10^6$  ions collected within 100 ms, followed by six DIA MS/MS scans recorded at 52 a resolution R=35,000 (at m/z 200) with maximum of  $1 \times 10^5$  ions collected within 60 ms. DIA 53 data were collected using 5-m/z-wide isolation windows per MS/MS scan. Each DIA MS/MS 54 scan was selected for analysis from a list of all 5 m/z isolation windows. In these experiments, 55 180 5-m/z-wide windows between 100 and 1,000 m/z, were grouped into nine separate methods, 56 each of which contained 20 windows. The maximal mass range was set to 1,000 m/z because 57 initial experiments showed that few NSOICs detected in dusts had >1000 m/z. Small overlaps 58 59 with neighboring windows were used to reduce the likelihood of placing window edges on critical target peaks. Mass spectrometric settings for atmospheric pressure photoionization (APPI) 60 (-) mode were: discharge current, 10 µA; capillary temperature, 225 °C; sheath gas, 20 L/h; 61 auxiliary gas, 5 L/h; and probe heater temperature, 350 °C. 62



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Figure S1. Heatmap and hierarchical clustering of all brominated compounds identified in 23
 dust samples. Color indicates log-transformed peak abundances of brominated compounds.

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**Figure S2.** High-resolution  $MS^2$  spectrum of a novel brominated compound mono-(2-ethyhexyl) tribromophthalate, from DIA window at 515±2.5 *m/z*. 



73 74

Figure S3. Chromatograms of bromo-dinitroaniline and another brominated azo dye 75 76  $(C_{23}H_{23}O_7N_3Br)$  in the samples of clothing.





**Figure S4.** Results of Ames tests (TA 100) with azo dye and extracts of house dusts. (A) Azo

79 dye. (B) dust-16; (C) dust-2; (D) dust-9. Revertants indicated the number of individual mutated

80 cells in 48 tested wells. Asterisks denote significant mutagenicity tested by binomial distribution.





**Figure S5.** Detection of brominated and chlorinated analogues of organic azo dyes in house dust.

84 Chromatograms were only shown for two most abundant dyes.

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# 87 **REFERENCES**

- 88 (1) Peng, H.; Saunders, D. M. V.; Sun, J. X.; Garry, C.; Wiseman, S.; Jones, P. D.; Giesy, J.
- 89 P., Detection, identification, and quantification of hydroxylated bis(2-ethylhexyl)-
- tetrabromophthalate isomers in house dust. *Environ. Sci. Technol.* **2015**, *49*, *2999-3006*.
- 91 (2) Peng, H.; Chen, C. L.; Cantin, J.; Saunders, D. M. V.; Sun, J. X.; Tang, S.; Codling, G.;
- Hecker, M.; Wiseman, S.; Jones, P. D.; Li, A.; Rockne, K. J.; Sturchio, N. C.; Giesy, J. P.,
- 93 Untargeted screening and distribution of organo-bromine compounds in sediments of Lake
- 94 Michigan. Environ. Sci. Technol. 2016, 50 (1), 321-330.
- 95 (3) Peng, H.; Chen, C. L.; Saunders, D. M. V.; Sun, J. X.; Tang, S.; Codling, G.; Hecker, M.;
- 96 Wiseman, S.; Jones, P. D.; Li, A.; Rockne, K. J.; Giesy, J. P., Untargeted identification of organo-
- bromine compounds in lake sediments by ultrahigh-resolution mass spectrometry with the data-
- 98 independent precursor isolation and characteristic fragment method. Anal. Chem. 2015, 87 (20),
- 99 10237-10246.
- 100
- 101