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Test chamber and forensic microscopy investigation of the transfer of brominated flame retardants into indoor dust via abrasion of source materials

Rauert, Cassandra; Harrad, Stuart; Suzuki, G.; Takigami, H.; Uchida, N.; Takata, K.

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Corresponding Author: Ms. Cassandra Rauert,

Corresponding Author's Institution: University of Birmingham

First Author: Cassandra Rauert

Order of Authors: Cassandra Rauert; Stuart Harrad; Go Suzuki; Hidetaka Takigami; Natsuyo Uchida; Kyouko Takata

Abstract: Brominated flame retardants (BFRs) have been detected in indoor dust in many studies, at concentrations spanning several orders of magnitude. Limited information is available on the pathways via which BFRs migrate from treated products into dust, yet the different mechanisms hypothesised to date may provide an explanation for the wide range of reported concentrations. In particular, direct transfer of BFRs to dust via abrasion of particles or fibres from treated products may explain elevated concentrations (up to 210 mg g-1) of low volatility BFRs like decabromodiphenyl ether. In this study, an indoor dust sample containing a low concentration of hexabromocyclododecane, or HBCD, (110 ng g-1 ΣHBCDs) was placed on the floor of an in-house test chamber. A fabric curtain treated with HBCDs was placed on a metal mesh shelf 3 cm above the chamber floor and abrasion induced using a stirrer bar. This induced abrasion generated fibres of the curtain, which contaminated the dust on the chamber floor, and ΣHBCD concentrations in the dust increased to between 4 020 and 52 500 ng g-1 for four different abrasion experiment times. The highly contaminated dust (ΣHBCD at 52 500 ng g-1) together with three archived dust samples from various UK microenvironments, also known to contain high concentrations of BFRs, were investigated with forensic microscopy techniques. Fibres or particles abraded from a product treated with BFRs were identified in all dust samples, thereby accounting for the elevated concentrations detected in the original dust (3 500 to 88 800 ng g-1 ΣHBCD and 24 000 to 1 438 000 ng g-1 for BDE-209). This study shows how test chamber experiments alongside forensic microscopy techniques, can provide valuable insights into the pathways via which BFRs contaminate indoor dust.

Suggested Reviewers: Tom Webster
Boston University
twebster@bu.edu

Jeff Wagner
California Department of Public Health
Jeff.Wagner@cdph.ca.gov

Charles Weschler

University of Medicine and Dentistry of New Jersey
weschlch@umdnj.edu

Tunga Salthammer
Fraunhofer Institute for Wood Research
tunga.salthammer@wki.fraunhofer.de

Sutapa Ghosal
California Department of Public Health
Sutapa.Ghosal@cdph.ca.gov

Opposed Reviewers:

Cassandra Rauert,
Division of Environmental Health & Risk Management,
School of Geography Earth and Environmental Sciences,
University of Birmingham,
Birmingham,
B15 2TT,
UK
Tel. +44 121 414 5431
Fax +44 121 414 3078
Email c.b.rauert@bham.ac.uk

The Editor,
Science of the Total Environment

March 17, 2014

SUBMISSION OF ARTICLE "Test Chamber and Forensic Microscopy Investigation of the Transfer of Brominated Flame Retardants into Indoor Dust via Abrasion of Source Materials"

Dear Sir

I hereby submit the above paper for consideration by *Science of the Total Environment*. I believe it is ideal for publication in this journal as – amongst other things - it describes to our knowledge the first experimental evidence of BFR migration to dust via abrasion of treated products. The paper also discusses the applicability of a suit of forensic microscopy techniques for the analysis of dust contamination with BFRs.

I trust that I have submitted all the necessary information at the website, but if you require any further information in order to expedite the review process, please don't hesitate to get in touch. I look forward to hearing from you.

Best Regards

Cassandra Rauert

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TITLE

Test Chamber and Forensic Microscopy Investigation of the Transfer of Brominated Flame Retardants into Indoor Dust via Abrasion of Source Materials

Authors

C. Rauert[a], S. Harrad[a], G. Suzuki[b], H. Takigami[b], N. Uchida[b], K. Takata[b]

Affiliation

[a] School of Geography Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

[b] Center for Material Cycles and Waste Management Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

Corresponding Author

Cassandra Rauert

Division of Environmental Health & Risk Management, Public Health Building,

School of Geography, Earth & Environmental Sciences,

University of Birmingham,

Birmingham, B15 2TT, UK

Tel +44 (0)121 414 5431

Fax +44 (0)121 414 3078

c.b.rauert@bham.ac.uk

Abstract

Brominated flame retardants (BFRs) have been detected in indoor dust in many studies, at concentrations spanning several orders of magnitude. Limited information is available on the pathways via which BFRs migrate from treated products into dust, yet the different mechanisms hypothesised to date may provide an explanation for the wide range of reported concentrations. In particular, direct transfer of BFRs to dust *via* abrasion of particles or fibres from treated products may explain elevated concentrations (up to 210 mg g^{-1}) of low volatility BFRs like decabromodiphenyl ether. In this study, an indoor dust sample containing a low concentration of hexabromocyclododecane, or HBCD, (110 ng g^{-1} ΣHBCDs) was placed on the floor of an in-house test chamber. A fabric curtain treated with HBCDs was placed on a metal mesh shelf 3 cm above the chamber floor and abrasion induced using a stirrer bar. This induced abrasion generated fibres of the curtain, which contaminated the dust on the chamber floor, and ΣHBCD concentrations in the dust increased to between 4 020 and 52 500 ng g^{-1} for four different abrasion experiment times. The highly contaminated dust (ΣHBCD at 52 500 ng g^{-1}) together with three archived dust samples from various UK microenvironments, also known to contain high concentrations of BFRs, were investigated with forensic microscopy techniques. These techniques included Micro X-Ray Fluorescent Spectroscopy, Scanning Emission Microscopy coupled with an Energy Dispersive X-ray Spectrometer, Fourier Transform Infrared spectroscopy with further BFR analysis on LC-MS/MS. Using these techniques, fibres or particles abraded from a product treated with BFRs were identified in all dust samples, thereby accounting for the elevated concentrations detected in the original dust (3 500 to 88 800 ng g^{-1} ΣHBCD and 24 000 to 1 438 000 ng g^{-1} for BDE-209). This study shows how test chamber experiments alongside forensic microscopy techniques, can provide valuable insights into the pathways via which BFRs contaminate indoor dust.

Keywords

Brominated flame retardants, HBCDs, PBDEs, Migration pathways to dust, Product abrasion, Forensic microscopy, Test chambers

Research Highlights

- HBCD contamination of dust via source material abrasion reproduced in test chamber
- Fragments of plastic with elevated BDE-209 content identified in dust samples
- Results suggest high BFR concentrations in dust due to source material abrasion

1. Introduction

Brominated flame retardants (BFRs) are incorporated in numerous textile, plastic and foam products with extensive indoor applications. They are incorporated into consumer products in two main ways: (a) via an "additive" process where the BFR is physically mixed with the molten polymer, and (b) via a "reactive" process where the BFR is covalently bound to the polymer. BFRs incorporated via the additive process are considered loosely bound to the product and more available for release into the environment than those incorporated into materials in a "reactive" manner. Owing to their extensive indoor application and their low vapour pressures that favour air-to-dust partitioning, BFRs such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are ubiquitous and substantial contaminants of indoor dust (Besis and Samara, 2012; Covaci et al., 2006; Harrad et al., 2010a). Contact with indoor dust has thus been identified as an important human exposure pathway, particularly for young children who spend extended time periods crawling over surfaces and display extensive hand-to-mouth contact (Jones-Otazo et al., 2005). Consequently, improved understanding of the pathways via which BFRs migrate to dust from treated products can inform strategies to reduce exposure. To date however, limited experimental evidence is available about such migration pathways. Currently hypothesised pathways include: (1) volatilisation of BFRs from the treated product with subsequent partitioning to dust; (2) abrasion via physical wear and tear of the treated product (likely enhanced by UV degradation of the polymer), resulting in the transfer of particles or fibres of the treated product directly to dust; and (3) transfer via direct contact between the treated product and dust. Pathway (1) appears particularly relevant for more volatile BFRs incorporated additively into the product, and is expected to result in a homogeneous bromine distribution within the dust. In contrast, pathway (2) appears more applicable to the product-to-dust migration of less volatile BFRs, and/or those BFRs incorporated reactively into products. A non-uniform, or heterogeneous, bromine distribution is anticipated to result from this migration pathway. Finally, pathway (3) also appears more relevant for additive BFRs for which migration from the product matrix to the surface and uptake by surface dust occurs via sorption or other physical processes such as capillary forces. A homogeneous bromine distribution is hypothesised for this pathway.

Emission chamber studies to date have focused largely on measurement of emissions to air of BFRs and related semivolatile organic compounds (SVOCs) from treated products (Rauert et al., 2014). In contrast, very few studies have investigated the migration of SVOCs from products to dust, with – to the authors' knowledge - no such studies existing for BFRs. Specifically, the migration to dust of phthalates (a class of SVOCs) has been simulated in test chamber experiments that investigated migration pathways (1) and (3) (Clausen et al., 2004; Schripp et al., 2010). Moreover, while we reported recently (Rauert et al., submitted) on test chamber experiments examining volatilisation with subsequent partitioning to dust of HBCDs (pathway (1)); controlled test chamber experimental studies of the migration of BFRs from products to dust via pathway (2), or abrasion, have yet to be reported in the literature for any SVOC.

Forensic microscopy techniques such as energy dispersive Micro X-ray fluorescence spectroscopy (Micro XRFS) and scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS) have been utilised previously to provide information on the origins of dust contamination with BFRs. Suzuki et al, (2009) used Micro XRFS to map dust samples, identifying particles of high bromine content, with isolated particles analysed by GC-HRMS to determine their PBDE content. In a parallel study, Webster et al, (2009) utilised Micro XRFS for identification of areas of high bromine content in dust samples containing high concentrations of decabromodiphenyl ether (BDE-209), followed by SEM/EDS to provide compositional and morphological information. However, the XRFS and SEM/EDS techniques used in these preliminary studies can only confirm the existence of bromine, so additional confirmation is required of the presence of BFRs. Using GC-HRMS, Suzuki et al (2009) were able to identify and quantify the content of PBDEs (pg per bromine rich fragment) contained within individual bromine (Br)-rich particles isolated from a dust sample, with BDE-209 quantified in each isolated fragment. However, due to the uncertainty associated with the gravimetric determination, the mass of the particles themselves could not be measured. Subsequent studies by Ghosal and Wagner, (2013) and Wagner et al, (2013) reported the use of Raman micro-spectroscopy to study Br-rich particles, after identification with SEM/EDS, for non-destructive confirmation of the presence of PBDEs. Collectively, studies to date have all identified in dust samples the presence of particles or fibres originating from a product treated with BFRs, suspected to

migrate via abrasion or pathway (2). This pathway may provide an explanation for the high concentrations, up to 210 mg g^{-1} (Batterman et al., 2009), reported of the relatively non-volatile BDE-209 in some dust samples.

This study for the first time mimics experimentally the abrasion of a BFR source material and the subsequent migration of the abraded material into dust. The dust sample generated by this experiment was analysed with a combination of forensic microscopy techniques to identify the abraded material. To further show the applicability of these selected microscopy techniques to providing information on BFR migration pathways to dust, three 'real' dust samples, previously sampled from indoor microenvironments in the UK and containing high levels of BFRs, were analysed with the same sequence of techniques. This augmented further, the existing evidence that such highly contaminated dusts are due to the presence of a small proportion of fibres and/or particles abraded from BFR-treated materials.

2. Materials and Methods

2.1. Test Chamber Experiments

2.1.2. Experimental design for investigating the abrasion migration pathway.

A in-house designed and built test chamber was utilised to investigate migration pathway (2). The experimental design of the chamber is illustrated in Figure 1. Briefly, the chamber design consisted of a cylindrical stainless steel chamber (20 cm height, 10 cm diameter), with a removable aluminium mesh shelf. In this experiment, the shelf was placed 3 cm above the chamber floor and a magnetic stirrer bar, 40 mm x 8 mm, (Fisher Scientific, Leicestershire, UK) placed on the shelf to mimic abrasion. A piece of product treated with BFRs (the BFR source) was placed on the shelf and a known mass of dust placed on a glass fibre filter (GFF), situated on the chamber floor. The dust contained low concentrations of HBCDs and PBDEs (ΣHBCDs = 110 ng g^{-1} and ΣPBDEs = 280 ng g^{-1}). The chamber was sealed and placed on a magnetic stirrer plate, operated at 200 rotations per minute. In this way, abrasion was mimicked via direct contact between the rotating stirrer bar and the treated product, with the fibres and particles thus generated, falling through the mesh shelf and incorporated into the dust sample below. This process was conducted at room temperature, and repeated for four durations of 2, 3, 21, and 48 hours.

2.1.3. BFR source

Fabric curtains treated with technical HBCD were obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan. Concentrations of HBCDs in these curtains were: 18,000 mg/kg for α-HBCD, 7,500 mg/kg for β-HBCD and 17,000 mg/kg for γ-HBCD (Kajiwara et al., 2013).

2.1.4. Archived dust samples

Three UK dust samples were chosen for detailed analysis via forensic microscopy. These samples were previously identified as containing highly elevated concentrations of BDE-209 and HBCDs (Harrad et al., 2010b; Harrad et al., 2008) with the concentrations listed in Table 1. Each dust originated from a different microenvironment category (a residential living room, office and primary school for Dusts 1, 2, and 3 respectively) however little other information was available on microenvironment characteristics or putative sources for these samples.

2.2. Forensic Microscopy

2.2.2. Sample Preparation for Forensic Microscopy

The bulk dust sample to be examined was mixed thoroughly before use. A small quantity of dust (1 mg) was evenly distributed in a monolayer, with tweezers, onto a 25 x 25 mm square area of double sided carbon tab attached to a glass sample plate for analysis with Micro XRFS.

2.2.3. Forensic Microscopy Analysis

Archived and test chamber experiment-generated dust samples were mapped to locate areas of high bromine content with a Micro XRFS ('μRay' μEDX-1200, Shimadzu Co.) equipped with a Rhodium X-ray tube and Nickel filter as the X-ray filter. The instrument was operated with a tube voltage of 50 keV, tube current of 1000 μA, and beam diameter of 50 μm as described previously, (Suzuki et al., 2009). High speed bromine mapping was performed using a 0.5 second dwell time with step sizes of 50 μm in the x and y-directions. Initial total sample mapping was conducted continuously over the 25 x 25 mm sample area for 76 hours. To achieve more accurate characterisation of specific areas and possible fragments of high bromine content, areas identified as 'of interest' were remapped over smaller regions of 4 x 3 mm and 2 x 1.5 mm for 4.5 hours and 11 minutes respectively. Regions identified as

Br-rich were separated from the bulk sample and positioned on an aluminium stub for further analysis. Areas and fragments were then imaged with a LEXT OLS 4100 3D laser microscope (Olympus, Japan) using a 20 x magnification. SEM/EDS was performed on the sample areas with a JSM-7600F Field emission SEM (JEOL, Japan) equipped with a retractable backscattering electron detector and energy dispersive X-ray spectrometer (EDS) analyser with silicon drift X-ray detector, with analysis performed at an accelerating voltage of 20 kV. Due to the close proximity of the bromine Lα (1.480 keV) and aluminium Kα (1.486 keV) lines, bromine was confirmed by the presence of the bromine Kα line at 11.907 keV. Because the Lα peak counts were at least 1000 times higher than the background aluminium peak count (from the aluminium stub), interference was considered negligible. Particles identified as having high bromine content with SEM/EDS were then removed with a pair of tweezers and analysed with a Nicolet Continuμm Microscope connected to a Nicolet 6700 Fourier transform infrared (FTIR) spectrometer (Thermo Scientific, Waltham, USA) using transmission infrared microscopy, and an MCT/A detector. Samples were placed in a diamond compression cell and resolution was 4 cm^{-1} over a determination range of 4000-650 cm^{-1} with a cumulative number of 128 (77 sec). Sample spectra searches were conducted with the spectral library database provided with the software package (OMNIC Software, Thermo Scientific). The spectra searches were conducted on the entire sample spectrum as well as separate peaks of interest to identify the closest library matches (represented as a % match). Particles identified as containing a close spectral match to BDE-209 were removed from the ATR objective under a microscope, with a pair of tweezers and collected. All particles from each dust (10 for dust 1 and 15 for dust 2) were combined prior to extraction and LC-MS/MS analysis to quantify BFR content.

2.3. Determination of concentrations of HBCDs and PBDEs

2.3.1 Chemicals

All solvents used for extraction and analysis were of HPLC grade quality (Fisher Scientific, Loughborough, UK). Standards of HBCDs (α-HBCD, β-HBCD, γ-HBCD), BDE-209, labelled ^{13}C HBCDs (α-, β-, γ-), d_{18} γ-HBCD and labelled ^{13}C BDE-209 and ^{13}C BDE-100 were acquired from Wellington Laboratories (Guelph, ON, Canada). Florisil (60-100 mesh) and silica gel (60Å, 60-100 mesh) were provided from Sigma Aldrich (Dorset, UK) with concentrated sulfuric acid obtained from

Merck (Darmstadt, Germany). Glass fibre filters (GFF, 12.5 cm diameter, 1 μm pore size, Whatman, UK) were purchased from Agilent (UK).

2.3.2 Sample analyses

Dust samples generated by test chamber experiments and particles identified by FTIR as containing BFRs were extracted and analysed using modified in-house methods (Abdallah et al, 2008, 2009). A detailed description is provided as supplementary data. Briefly, samples were spiked with ^{13}C-HBCD and PBDE analogues as internal (surrogate) standards prior to pressurised liquid extraction (ASE, Dionex Europe, UK, ASE 350) with hexane:dichloromethane (1:1 v/v). After clean-up of the crude extracts via elution through sulfuric acid-impregnated silica (44% w/w), the eluates were evaporated and made up to 100 μL using d_{18} γ-HBCD and ^{13}C BDE-100 in methanol, as recovery determination (or syringe) standards. Analysis was conducted with a dual pump Shimadzu LC-20AB Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a SIL-20A autosampler, and a DGU-20A3 vacuum degasser. Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an APPI (PBDEs) or ESI (HBCDs) ion source, operated in negative ion mode.

3. Results

3.1. Test chamber abrasion experiments

Stirrer bar-induced abrasion in the test chamber was successful, with loosened fibres observed post-experiment on both the tested curtain and visible fibres in the dust on the chamber floor. The entire dust sample including all abraded fibres was extracted and analysed to determine concentrations of HBCDs. Figure 2 shows the pre- and post-experimental concentrations of HBCD diastereomers in dust for four replicates of this experiment. All four experiments were run for different time periods (2, 3, 21 and 48 hours for experiments 1, 2, 3 and 4 respectively). However, although there is a two orders of magnitude increase in concentrations of HBCDs post-experiment in all cases; there is no clear relationship between the concentration in the dust and the duration of the abrasion experiment. The large concentration increase is consistent with the hypothesis that curtain fibres (of high HBCD concentration) have been incorporated into the dust. Other experiments in the same test chamber examining transfer of HBCDs via volatilisation from the same HBCD-treated curtains with

subsequent deposition to the same dust (Rauert et al., submitted) reported much lower concentrations in chamber dust post experiment (average 610 ng ΣHBCDs/g), adding further weight to the hypothesis that different migration pathways may result in varying BFR concentrations in dust. The variable HBCD concentrations in these abrasion experiments is consistent with the abrasion of treated products in indoor microenvironments as 'wear and tear' of a product will not be uniform and will depend on factors such as: the product material (e.g. plastic or fabric), how and how often the product is used, as well as its age and extent to which it is exposed directly to UV light and consequent weathering. The abrasion induced in these test chamber experiments is highly intensive (forced), so does not represent realistic abrasion from e.g. 48 hour use of a curtain. However, the results may be interpreted as an acceleration test to mimic long-term abrasion. For example, if we assume 10 seconds daily movement/wear and tear of the fabric from opening and closing curtains, then the 2 hour chamber abrasion experiment may represent house dust concentration increment from abrasion over 720 days. Abrasive contact with other fabrics such as sofa covers, will likely be far more frequent, and our range of experimental durations may be viewed as reflecting abrasion of a variety of domestic and commercial fabrics. The ease with which abrasion can be replicated in these chamber experiments, suggests this is a feasible migration pathway. The highest concentration (48 hour abrasion) dust sample was analysed further with forensic microscopy techniques.

3.2. Microscopy analysis of the chamber generated dust sample

The Micro XRFS identified fibres of high bromine content in the dust sample, and these were analysed in closer detail using the SEM for elemental confirmation. Figure 3 presents the SEM backscattering image of a series of intertwined fibres and the EDS elemental profile, confirming the presence of bromine via the presence of both the Kα and Lα bromine spectral lines. The fibre was isolated and analysed on the FTIR for compositional information, with an 88% match returned for the polyester spectrum when searching the entire sample spectrum through the library. Peaks of interest in the sample spectrum were searched separately to increase the confidence of the match with the reference spectrum (in particular the strong stretch at ~1700 cm^{-1}, representative of a C=O double bond stretch, and weak stretches around 3000 cm^{-1}, representative of alkyl group stretches) and a 97% match for polyester was returned,

strongly suggesting the base polymer of these curtain fibres is a polyester. The HBCD spectrum was not distinguishable however, as the HBCD concentration in the curtain was below the LOD of the FTIR (5% HBCD content). Figure 4 shows the FTIR spectra of the fibre, alongside reference spectra of the polyester match, and the technical HBCD formulation for comparison. The limits of detection of the FTIR in particular, did not allow confirmation of HBCDs in identified fibres in this sample. However, the presence of Br-rich fibres were confirmed, suggesting the fibres originated from the HBCD treated curtain and demonstrating the applicability of these methods for identifying particles/fibres of high Br and high BFR content. To investigate this applicability further, three 'real' indoor dust samples were investigated with the same combination of methods, to determine if BFR-containing particles/fibres could be identified in high concentration dust samples.

3.3. Archived dust samples

3.3.2. Forensic Microscopy investigation

All three archived dusts were analysed with the Micro XRFS in triplicate. Areas containing high bromine content and bromine rich particles were identified in all samples with 2 to 10 bromine rich fragments per mg dust. Similarly, the study by Ghosal and Wagner, (2013) also reported ≤ 10 fragments per mg of analysed dust sample. As the incident X-ray excitation beam is a 50 μm square area, the mapping image provides an average of the bromine content in the sample rather than identification of individual bromine rich fragments. Moreover, particles smaller than 50 μm may be missed, creating a selection bias with this method, and requiring further SEM/EDS analysis for bromine confirmation. The bromine rich particles identified in these samples ranged in size from 30 to 260 μm in length; however, it is possible larger fragments may have fractured during dust collection preparation techniques (vacuuming, sieving etc) or during application of the dust to the double sided carbon tab. Figure 5 presents the Micro XRFS optical images and bromine mapping images of typical sample areas containing bromine rich fragments. Again, identified areas of interest (1 x 1 mm) were removed and placed on an aluminium stub for further analysis. The areas and suspected Br-rich fragments were examined with a laser microscope to provide detailed optical and 3D laser images. All fragments were visually different from the surrounding dust particles having a white or slightly yellow colouring and sharp edges, suggesting they may be pieces of a fractured polymer

rather than typical dust organic matter. Following the imaging, SEM analysis performed in backscattering mode and followed by EDS was conducted on the suspect Br-rich fragments, confirming the presence of bromine in all identified fragments as well as the presence of antimony. The particles were not coated before SEM/EDS to prevent interference with the subsequent FTIR analysis, and as a result there was a charging effect on the images. However, in backscattering mode, clear regions of bromine and antimony (located in the imaged bright areas) were observed over the particle surface and confirmed with EDS. Due to the charging effect and the uneven topography of the sample surface, quantitative elemental analysis was not possible; rather the SEM qualitatively identified the presence of these elements. Bromine and antimony were observed in distinct pockets on each particle surface in a heterogeneous fashion, similar to the study by Wagner et al, (2013) who observed clear pockets of bromine on particle surfaces. Figure 6 shows the backscattering electron images of particles from all three dusts and the related EDS profiles, confirming the presence of bromine and antimony. The high bromine content areas originally identified with the Micro XRFS, were all shown to be associated with distinct individual particles and a homogeneous bromine distribution over dust particles was not seen, a result consistent with migration via pathway (2), rather than pathway (1). After SEM/EDS analysis, these identified individual particles were removed from the sample area with a pair of tweezers for FTIR analysis. All particles identified in dust 3 were < 50 μm long and too small for removal, thereby preventing further FTIR analysis. Hence in dust 3, it was only possible to confirm the presence of bromine in the particles.

Eight particles from dust 1 and nine particles from dust 2 were analysed and all fragments from the same dust sample had very similar spectra, suggesting a common source. Library database searches of the spectra obtained from particles in dust 1 were obtained to identify closest component matches from the database. Firstly the entire spectrum was analysed for the top 3 matches in the database that combined, most closely matched the spectrum. An 88% match was found for the combination of BDE-209, antimony trioxide and an acrylic based industrial coating. To improve the accuracy of the spectral matches, individual peaks and areas of interest in sample spectra were run separately through the software to find the top match in the database for each peak/area. The absorption spectrum in the range of 900 to 1400 cm^{-1} (often

due to C-O bond stretches, particularly from ethers) returned a 95% confidence match with BDE-209, while the strong absorption at ~700 cm^{-1} (possibly from a Sb-O bond stretch) combined with the broad absorption at 3100-3500 cm^{-1} (from a Sb=O stretch) returned a 91% match for antimony trioxide. To identify the product polymer, the strong absorbance at ~1750 cm^{-1} (often from a C=O, or C=N stretch) combined with the absorbance at 2800-3000 cm^{-1} (often from various C-H bond stretches) returned a 98% match for a styrene acrylic. The same method was applied for spectra from particles in dust 2 with a 73% match obtained for the combination of BDE-209, antimony trioxide, and again an acrylic based industrial coating. Once more, comparison of individual peaks/areas resulted in higher confidence level matches with a 92% match for BDE-209, a 90% match for antimony trioxide, and a 94% match for an acrylic copolymer from the strong absorbance at ~1750 cm^{-1} and absorbance at 2800-3000 cm^{-1}. By investigating the spectral peaks/areas separately, the confidence of spectral matches with database spectra was improved greatly. The lower accuracy for the matched total spectrum from dust 2, is largely due to the presence of the extra broad peak at 1500 cm^{-1}, reducing confidence in the accuracy of the matches. This peak was similar to the broad peak seen in the reference calcium carbonate spectra, suggesting calcium carbonate may have been used as a resin filler in the polymer fragments isolated from dust 2.

The reference spectra for the acrylic copolymer, styrene acrylic and for an acrylonitrile-butadiene-styrene (ABS) copolymer are all similar, and hence are all possible matches for the polymer in the isolated fragments. ABS plastic was compared, as it is commonly flame-retarded with both BDE-209 and antimony trioxide, and it is thus plausible that the fragments may have originated from a source containing BDE-209 treated ABS plastic. Figure 7 presents the spectra, and software library database matches, for particles from dusts 1 and 2.

The limitations with these methods, primarily the high LODs of the instruments, constrain these analyses to dust with very high concentrations of BFRs. The Micro XRFS detects bromine concentrations $\geq 0.1\%$ in high speed mapping mode (0.5 sec dwell time) (Suzuki et al., 2009), and will identify with a high degree of confidence, high Br concentrations from fragments > 50 μm in length. Smaller fragments may not be identified, introducing a selection bias to this method, which is thus only suitable

for identifying Br-rich particles of Br content ≥ 0.1% and particle length > 50 μm (for this particular instrument). The LOD of the FTIR introduces further limitations to these methods, as the FTIR will only distinguish a BFR spectrum from the particle/fibre spectrum if present at > 5% BFR content. As seen with the analysis of the dust generated from abrasion of the HBCD curtain, where HBCDs were not identified with the FTIR in the isolated fibres, this is a restriction on successfully identifying BFRs in contaminated dust samples. Particle size is also a consideration with FTIR analysis, as particles need to be separated from the sample matrix for individual analysis with the diamond ATR objective. In this analysis, particles > 65 μm were successfully removed and analysed on the FTIR, with smaller particles (e.g. from dust 3) unable to be isolated using the present methods. This again created a selection bias in particles that could be analysed for the presence of BFRs, the resin material and other additives in the particle. The SEM/EDS, although providing a more specific elemental analysis than the Micro XRFS, is only a qualitative measure of bromine (and other element) content. The uneven topography of the dust sample creates difficulties for the detector to receive an accurate signal and the charging effect (caused by an excess of electrons, normally minimised by carbon or platinum coating of the sample) also reduces accuracy of any quantitative measurement, hence this method can only be used to identify the presence of certain elements. Furthermore, the SEM spectral lines for bromine and aluminium interfere, so a high bromine content and the presence of the Kα bromine line is needed for confirmation. Similarly, the antimony Lα and calcium Kα lines interfere, providing difficulties in identifying these elements unless one (antimony) is present at a much higher concentration.

3.3.3. Analysis of BFR concentration in isolated particles

As many BFR containing particles as possible (10 and 15 from dust 1 and 2 respectively), were collected and combined for determination of BDE-209 content. Table 1 lists the mass of BDE-209 (ng) quantified in the combined particles removed from each dust sample. On average, the particles removed in dust sample 1 were much smaller than in dust 2, and more particles were successfully removed from dust 2. The combination of these factors means that the total particle mass analysed in the particles isolated from dust 2 was much greater, explaining the higher BDE-209 content quantified in the isolated particles from this dust. As with previous studies

highlighted earlier, an accurate mass measurement could not be determined for the isolated particles, and hence the BDE-209 masses given can only confirm its very strong presence in these particles.

4. Conclusions

Abrasion of a HBCD treated curtain was successfully induced in a test chamber with forensic microscopy techniques identifying fibres of high bromine content throughout dust samples impacted by such induced abrasion. Application of these microscopic techniques to 'real' indoor dust samples displaying highly elevated concentrations of BDE-209, identified 2 to 10 bromine rich fragments per mg dust. These fragments were also identified as polymeric in origin and to contain elevated masses of BDE-209. Combined, this evidence suggests strongly that the highly elevated concentrations in these dust samples is due to the presence of such fragments that presumably arise via abrasion of friable polymeric material. Although these techniques are limited to the study of dust samples containing very high concentrations of BFRs; in this study they have shown that the abrasion migration pathway is a likely source of the elevated concentrations of BFRs detected in such indoor dust samples. This study raises questions about dust sampling and preparation techniques. Bromine rich particles, confirmed to contain BDE-209, of up to 260 μm in size were detected in this study, hence sample preparation techniques that sieve bulk dust samples to a particle size < 250 μm may potentially underestimate BFR concentrations in that dust sample. Moreover, the heterogeneity of the distribution of BFR-rich particles in the dust samples studied here, implies obtaining a representative subsample of such dusts for analysis is problematic. Consequently, analysis of replicate subsamples may be required to obtain an accurate picture of the BFR concentration in dust from such microenvironments.

5. Acknowledgements

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Figures and Tables

Figure 1: Schematic of test chamber configuration for the abrasion induction experiments.

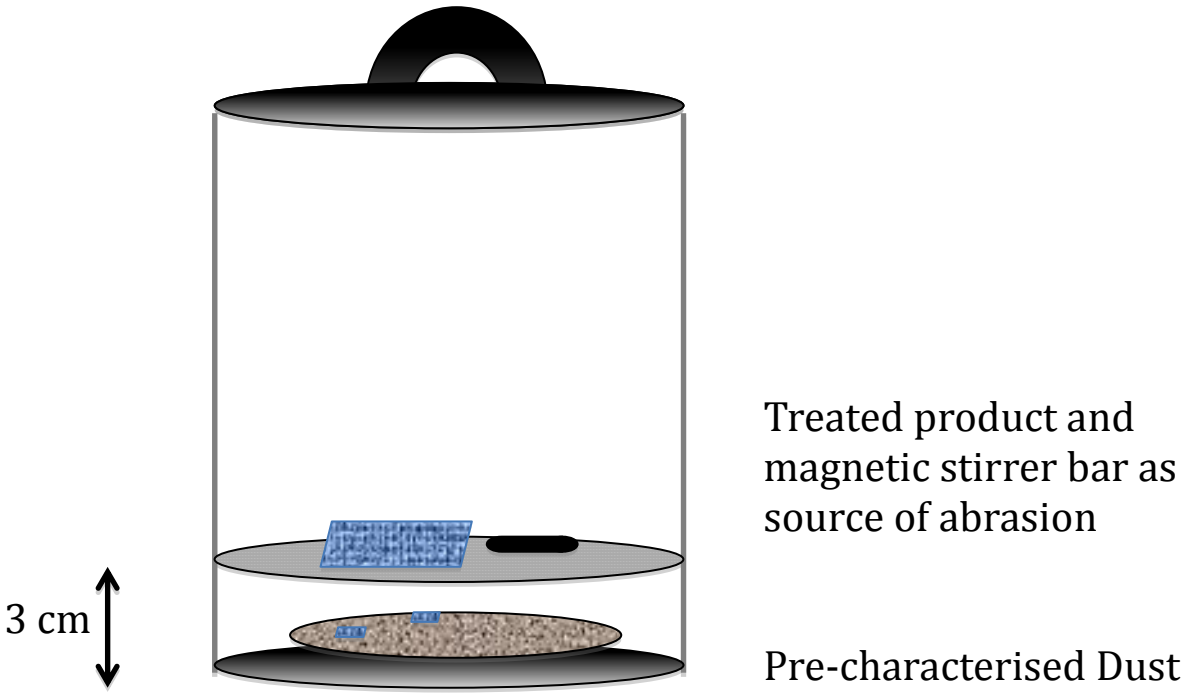


Figure 2: Concentration (ng g^{-1}) of HBCDs in dust both pre- and post-abrasion experiments (n=4)

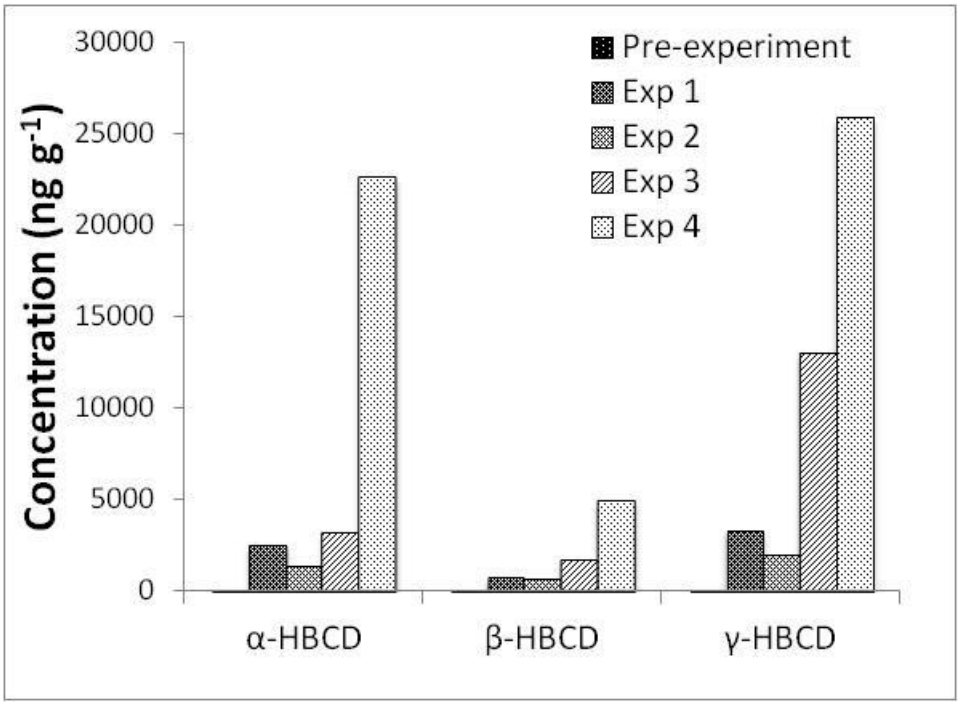
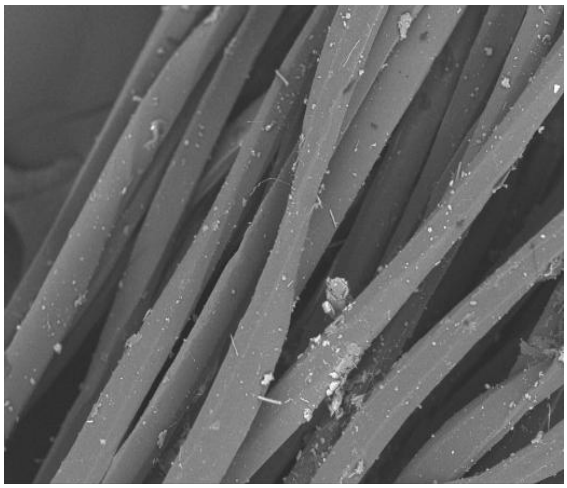


Figure 3: a) SEM backscattering image of fibres in post-abrasion induction experiment dust sample and b) EDS elemental profile in same fibres



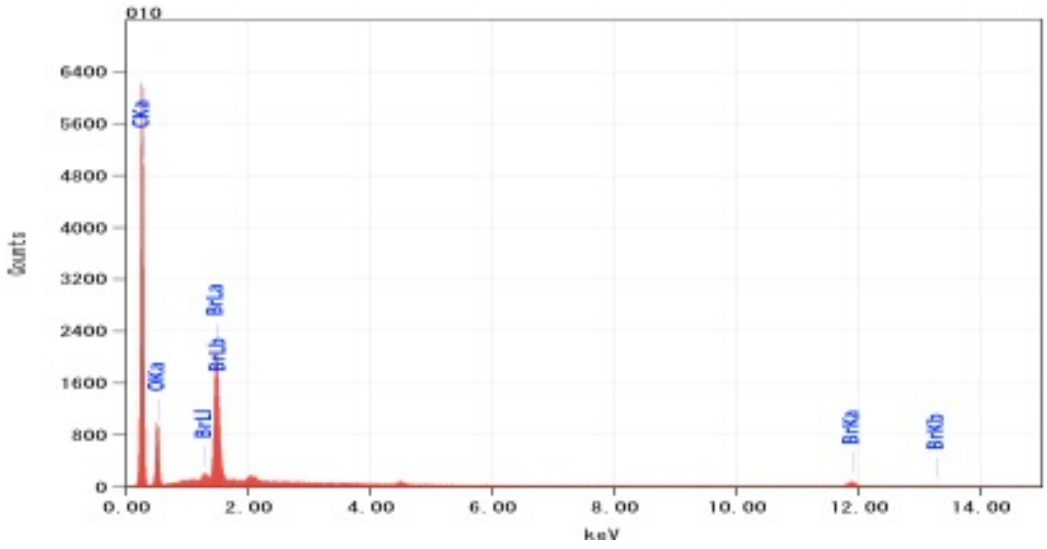


Figure 4: FTIR spectra of a fibre isolated from a post-abrasion induction experiment dust sample (top), with reference spectra for polyester (middle) and technical HBCD (bottom)

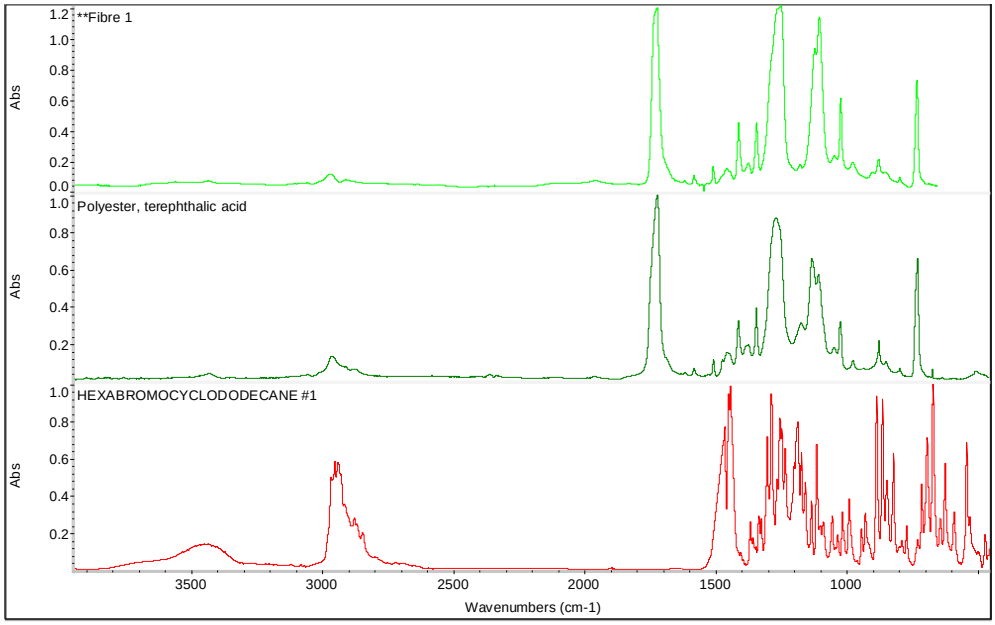


Figure 5: Micro XRFS images of areas/particles of high bromine content in dust samples containing elevated concentrations of BFRs:
Images of areas in dusts 1,2 and 3 from top to bottom respectively.
Left - Optical image of the mapped sample area and, Right - Bromine mapping image of the area, showing 50 μm square regions of high bromine in white/red/yellow

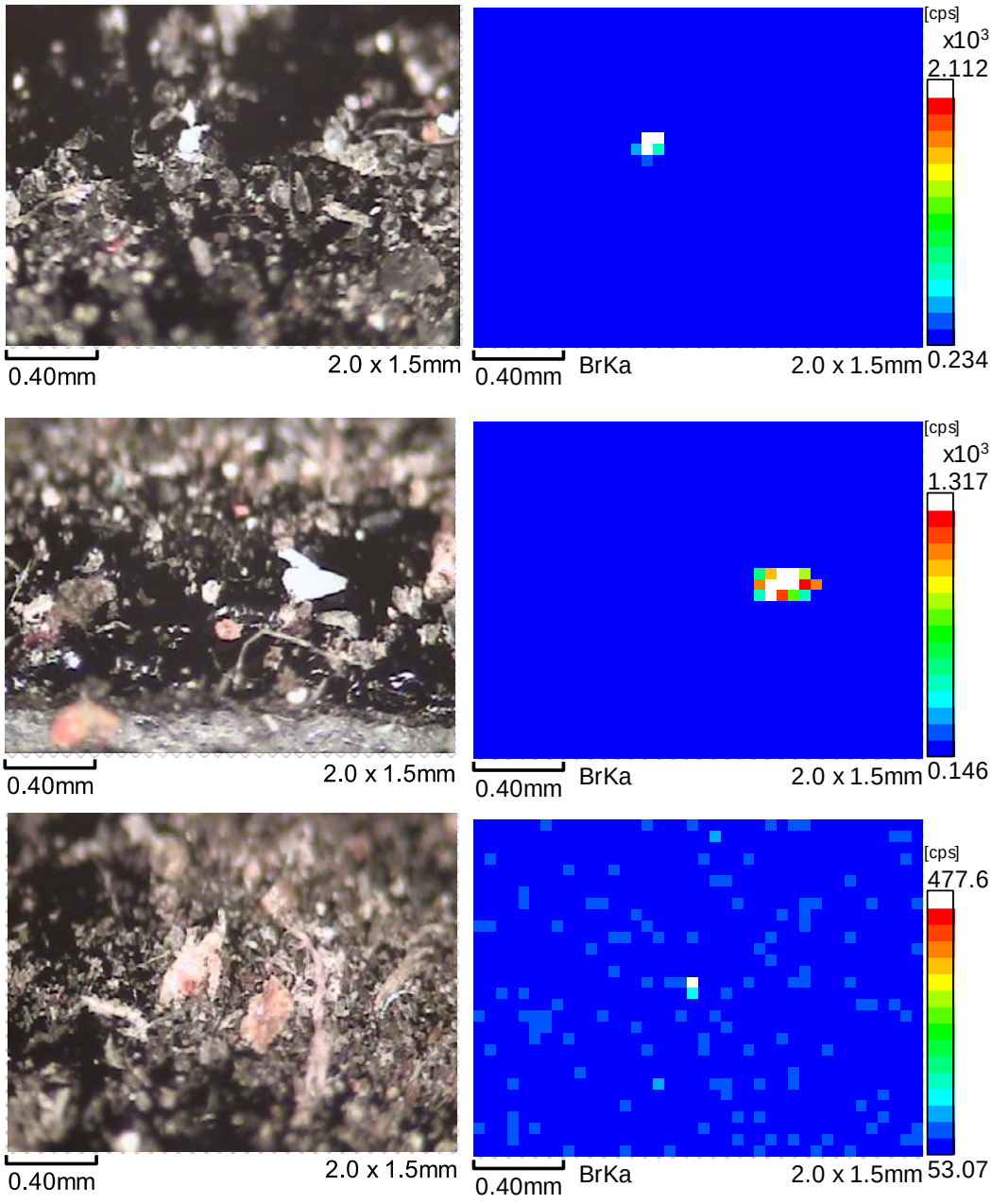
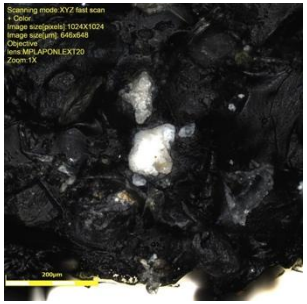


Figure 6: From top to bottom, bromine rich particles in dusts 1,2 and 3 respectively: Left – Laser microscopy optical images of particles with measured particle size. Right - SEM backscattering images of particles and related EDS elemental profiles.



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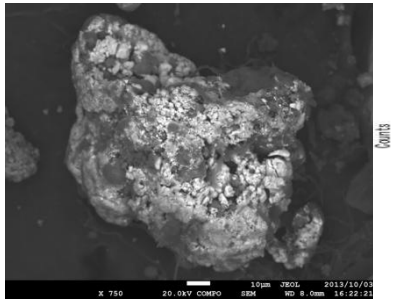
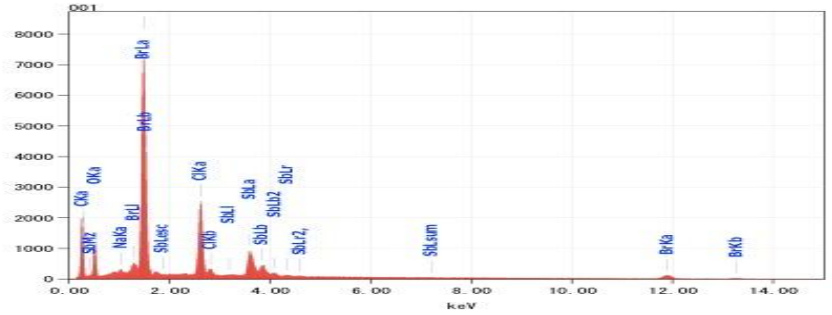
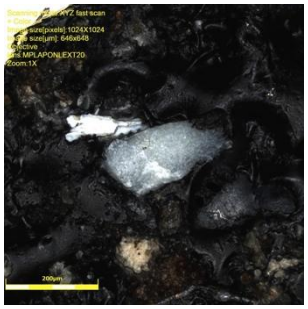
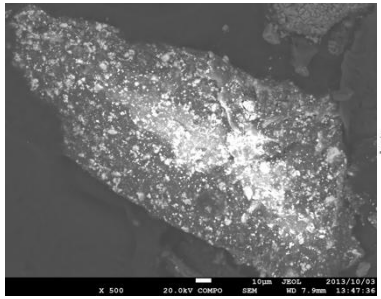
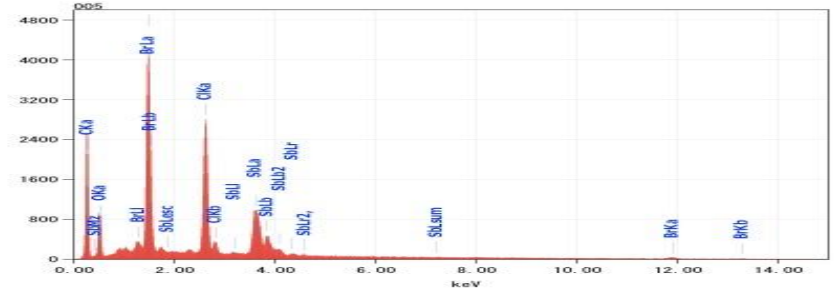


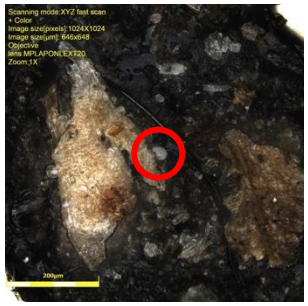




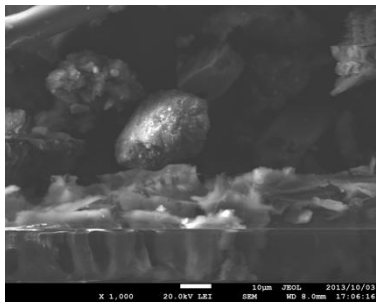
(260 µm)







(35 µm)



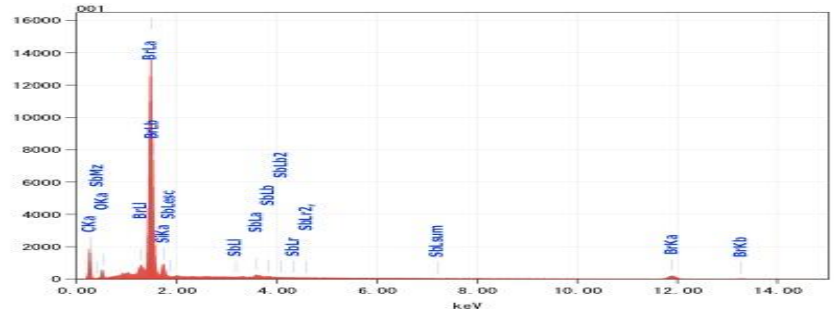
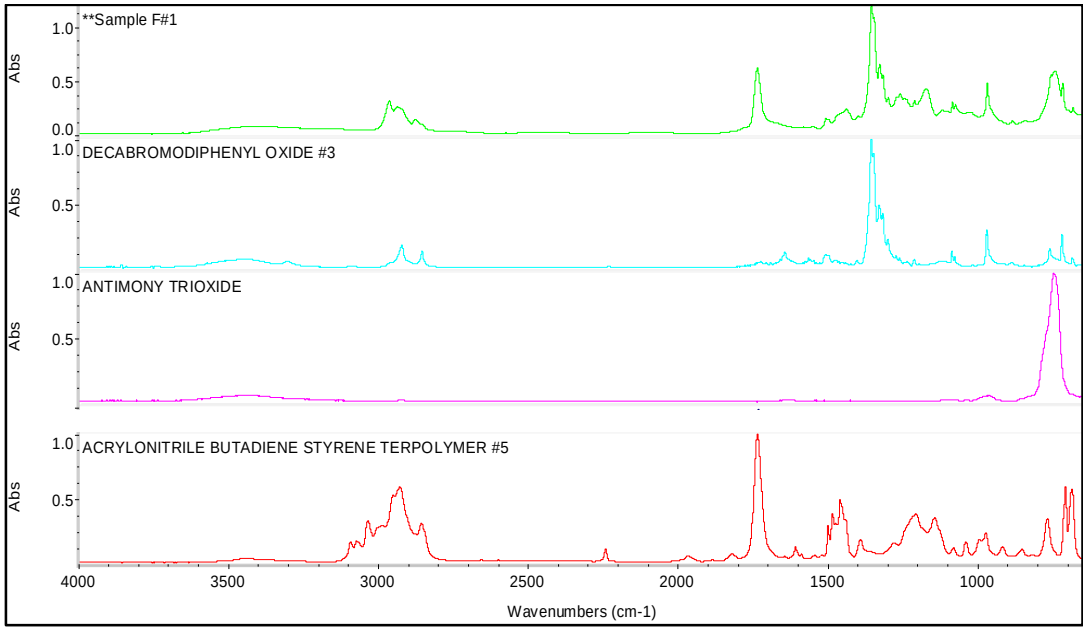


Figure 7: FTIR spectra of particles isolated from: Left - Dust 1 and Right - Dust 2, featuring from top to bottom: dust sample, and reference spectra for BDE-209, Antimony trioxide, ABS copolymer and (dust 2 only) calcium carbonate



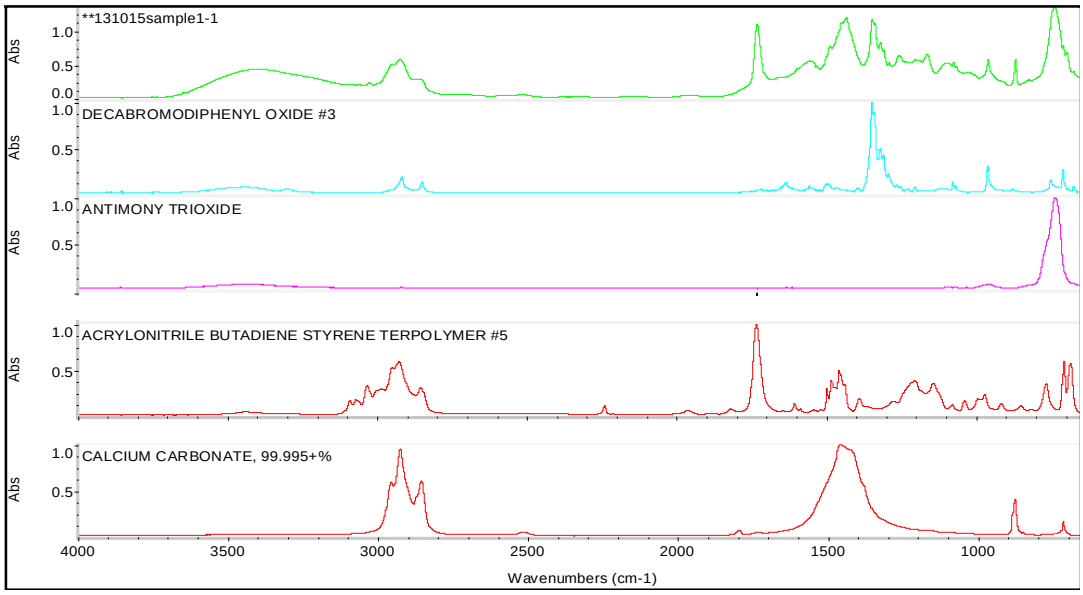


Table 1: Concentration (ng/g) of HBCDs and BDE-209 in archived dust samples (Harrad et al., 2010b; Harrad et al., 2008) and BDE-209 mass (ng) in combined isolated particles.

	Concentration in bulk dust (ng g^{-1})				**Mass in combined isolated particles (ng)**
	α-HBCD	**β-HBCD**	**γ-HBCD**	**BDE-209**	**BDE-209**
Dust #1	380	340	2 800	1 438 000	500
Dust #2	280	70	140	280 000	1 300
Dust #3	9 900	6 700	72 000	24 000	N/A

Supplementary Data

1.1 Sample preparation and extraction

Sample extraction and purification was performed using slight modifications of in-house published methods (Abdallah et al., 2009; Abdallah et al., 2008). Dust, PUFs and GFFs were extracted with pressurised liquid extraction (ASE-350, Dionex Europe, UK). PUFs and GFFs were packed into precleaned 66 mL cells using precleaned Hydromatrix (Varian Inc., UK) to fill the void. Dust samples were loaded into pre-cleaned 66 mL cells containing 1.5 g of pre-cleaned florisil and Hydromatrix. Each cell was spiked with 4 ng each of ^{13}C-labelled α-, β-, and γ-HBCD; 40 ng of ^{13}C-PBDE 47; 10 ng each of ^{13}C-labelled PBDE-99 and PBDE-153; and 20 ng of ^{13}C-PBDE 209 as internal (surrogate) standards prior to extraction with hexane:dichloromethane (1:1 v/v) at 90 ˚C and 1500 psi. The cell was heated for 5 min, held static for 4 min and purged for 90 s, with a flush volume of 50%, for 3 cycles.

1.2 Clean up

The ASE extracts and chamber inner surface solvent rinses were combined and concentrated to 0.5 mL using a Zymark Turbovap II (Hopkinton, MA, USA), then purified by loading onto SPE cartridges filled with 8 g of pre-cleaned acidified silica (44% concentrated sulfuric acid, w/w). The analytes were eluted with 30 mL of hexane:dichloromethane (1:1, v/v), with the eluate evaporated to dryness under a gentle stream of nitrogen. Samples were reconstituted to 100 μL with 2 ng of d_{18}-γ-HBCD and 20 ng of ^{13}C-PBDE 100 in HPLC grade methanol, used as recovery standards for internal standard recovery determination.

1.3 LC-MS/MS analysis

Target PBDEs and HBCDs were separated and analysed using modified, in-house published methods (Abdallah et al., 2009; Abdallah et al., 2008), using a dual pump Shimadzu LC-20AB Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a SIL-20A autosampler, and a DGU-20A3 vacuum degasser. Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an APPI (PBDEs) or ESI (HBCDs) ion source, operated in negative ion mode.

1.3.1 PBDE Analysis

A Varian Pursuit XRS3 (Varian, Inc., Palo Alto, CA) C18 reversed phase analytical column (250 mm x 4.6 mm i.d., 3 μm particle size) was used for separation of target PBDEs (47, 85, 99, 100, 153, 154, 183 and 209). A mobile phase programme based upon (mobile phase A) 1:1 methanol/water and (mobile phase B) 1:4 toluene/methanol at a flow rate of 0.4 mL min^{-1} was applied for elution of the target compounds; starting at 85% (mobile phase B), increased linearly to 100% (mobile phase B) over 20 min, and then held for 10 min. The column was equilibrated with 85% (mobile phase B) for 5 min between runs. MS/MS detection, operated in MRM mode, was used for quantitative determination of the PBDE congeners based on *m/z* 420.8→78.8, *m/z* 500.8→78.8, *m/z* 578.8→78.8, *m/z* 658.6→78.8, *m/z* 486.6→78.8. ^{13}C-labelled analogues were determined based on *m/z* 432.4→78.8, 512.4→78.8, 590.6→78.8, and *m/z* 494.7→78.8.

1.3.2 HBCD Analysis

A Varian Pursuit XRS3 C18 reversed phase analytical column (150 mm x 4.6 mm i.d., 3 μm particle size) was used for separation of target HBCDs (α-, β-, γ-). A mobile phase program based upon (mobile phase A) 1:1 methanol/water and (mobile phase B) methanol at a flow rate of 0.18 mL min^{-1} was applied for elution of the target compounds; starting at 50% (mobile phase B), then increased linearly to 100% (mobile phase B) over 4 min, held for 5 min before decreasing linearly to 88% (mobile phase B) over 1 min. The column was equilibrated with 50% (mobile phase B) for 4 min between runs. MS/MS detection, operated in MRM mode, was used for quantitative determination of the HBCD diastereomers, ^{13}C-, and d_{18}-labelled analogues based on *m/z* 640.4→79.0, *m/z* 652.4→79.0, and *m/z* 657.7→79 respectively.

1.4 Quality Assurance

Samples were analysed using established QA/QC procedures. Method blanks were run with each batch of samples. For ^{13}C-α-, β-, and γ-HBCDs, average recoveries ranged from 64 to 97% while for ^{13}C-PBDE 47, 99, 153, and 209, average recoveries ranged between 69 and 80%. Accuracy and precision of the analytical method was assessed *via* replicate analyses (n=7) of NIST SRM 2585 (organics in house dust). The results of these analyses compared with indicative and certified values as appropriate are supplied in Table SD-1.

Table SD-1: Average concentrations (ng g^{-1}) in 7 analyses of SRM 2585 and the reported certified PBDE (Stapleton et al., 2006) and indicative HBCD values (Keller et al., 2007)

	BDE-47	**BDE-85**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-209**	**α-HBCD**	**β-HBCD**	**γ-HBCD**
SRM Measured Value (n=7)	347 ± 39	35.1 ± 4.6	730 ± 93	133 ± 13	126 ± 13	78.6 ± 13	44.4 ± 5.0	2460 ± 400	19 ± 5.7	5.6 ± 2.2	98 ± 35
Certified/Indicative Values	*498 ± 46*	*43.8 ± 1.6*	*892 ± 53*	*145 ± 11*	*119 ± 11*	*83.5 ± 2.0*	*43.0 ± 3.5*	*2510 ± 190*	*19 ± 3.7*	*4.3 ± 1.1*	*120 ± 22*

Conflict of Interest Declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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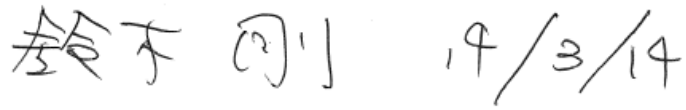
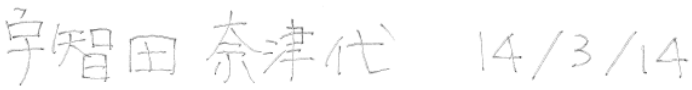


Hidetaka Takigami

Natsuyo Uchida



Kyouko Takata