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http://dx.doi.org/10.1289/ehp.1308028
Received: 18 December 2013
Accepted: 9 May 2014
Advance Publication: 13 May 2014

NIH National Institute of Environmental Health Sciences

Urinary Tetrabromobenzoic Acid (TBBA) as a Biomarker of Exposure to the Flame Retardant Mixture Firemaster® 550

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Running title: TBBA as a biomarker of Firemaster® 550 exposure

Acknowledgments: The authors would like to thank the study participants. This study was supported by a research grant to Dr. Heather Stapleton from the National Institute of Environmental Health Sciences (NIEHS), R01 ES016099 and by the intramural programs of NIEHS, the National Institutes of Health, and the National Cancer Institute. Dr. Hoffman was supported through a donation provided by Fred and Alice Stanback.

Competing financial interests: All authors declare no actual or potential competing financial interests.

Abstract

Background: Firemaster® 550 (FM550) is commonly added to residential furniture to reduce its flammability. Recent toxicological evidence suggests that FM550 may be endocrine disrupting and obesogenic.

Objectives: Our objectives were to develop methods to assess exposure to FM550 in human populations and to identify potential routes of exposure.

Methods: Using mass spectrometry methods, we developed a method to measure 2,3,4,5-tetrabromobenzoic acid (TBBA), a urinary metabolite of the major brominated FM550 component [2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB)]. The method was applied to a cohort of adult volunteers (n = 64). Participants completed questionnaires, provided urine and handwipe samples, and collected dust samples from their homes. We measured TBB and the other major brominated FM550 component, bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), in paired dust and handwipe samples.

Results: TBBA was detected in 72.4% of urine samples. Although TBBA is a rapidly formed metabolite, analyses indicated moderate temporal reliability (interclass correlation coefficient = 0.56; 95% confidence interval: 0.46, 0.66). TBB and TBPH were detected frequently in dust samples (geometric mean (GM) = 315.1 and 364.7 ng/g, respectively) and in handwipes (GM = 31.4 and 23.4 ng, respectively). Levels of TBB and TBPH in dust were positively correlated with levels in handwipes. Additionally, levels of TBB in handwipes were positively correlated with urinary TBBA. Results suggest frequent hand washing may reduce the mass of TBB on participants' hands and urinary TBBA levels.

Conclusions: Cumulatively, our data indicate that exposures to FM550 are widespread and the home environment may be an important source of exposure. Urinary TBBA provides a potentially useful biomarker of FM550 exposure for epidemiologic studies.

Introduction

Flame retardant (FR) chemicals are commonly applied to raw materials and consumer products to reduce their flammability and meet fire safety standards. Until recently, polybrominated diphenyl ethers (PBDEs) accounted for a large portion of FRs added to polymers and resins used in furniture, electronics, and building materials (de Wit 2002). However, concern over their persistence, bioaccumulation, and toxicity led to voluntary phase-outs and bans of several commercial PBDE mixtures from the marketplace in many countries, including the US (Costa et al. 2008; EPA 2009).

To maintain compliance with consumer product flammability standards, various PBDE replacements have been developed and introduced into commerce (Covaci et al. 2011; Stapleton et al. 2008a; van der Veen and de Boer 2012). In 2003, Chemtura (West Lafayette, IN, USA) introduced Firemaster® 550 (FM550) as a replacement for the commercial PentaBDE mixture used in polyurethane foam applications (Stapleton et al. 2008a). Stapleton et al. (2008a) identified four main components of FM550: (1) triphenyl phosphate (TPP); (2) a mixture of isopropylated triphenyl phosphate isomers (ITPs); (3) 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB); and (4) bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH). These components have been frequently detected in polyurethane foam samples from consumer goods, including furniture and baby products [e.g. nursing pillows and changing table pads (Stapleton et al. 2009; Stapleton et al. 2011)]. Based on these two studies, it appears that FM550 is the second most common FR mixture applied to foam containing products; however, with the expected phase-out of Tris (1,3-dichloroisopropyl) phosphate (TDCPP), use of FM550 may increase.

Like PBDEs, FM550 is an additive FR (i.e. not chemically bound to products) and its components leach into the environment over time, as evidenced by the prevalence of TBB and TBPH in indoor dust, outdoor air, and sewage (Ali et al. 2012; Covaci et al. 2011; Dodson et al. 2012; La Guardia et al. 2010; Ma et al. 2012; Stapleton et al. 2008a; Stapleton et al. 2009). These data suggest that the general population comes into contact with FM550 frequently and widespread exposure is likely; yet potential exposure sources and routes of human exposures have yet to be investigated. Based on similarities between FM550 and PBDEs (e.g. modes of application, presence of brominated aromatic backbones), similar exposure pathways could be expected. For PBDEs, indoor dust has been identified as an important contributor to overall body burden and data suggest that exposure via hands (e.g. incidental ingestion via hand-to-mouth contact) may in part explain how PBDEs get into the body (Johnson et al. 2010; Stapleton et al. 2012; Watkins et al. 2011).

Relatively little is known regarding the toxicokinetics of FM550 components. Recent *in vitro* evidence, however, suggests that TBB is metabolized to 2,3,4,5-tetrabromobenzoic acid (TBBA) in human hepatic tissues while TBPH appears to be recalcitrant to metabolism (Roberts et al. 2012). The toxicities of FM550 components, as well as those of their potential metabolites (e.g. TBBA) are largely unknown as few studies have investigated the impacts of exposure. However, Patisaul and colleagues recently reported that FM550 is an endocrine disrupter and an obesogen; pregnant rats exposed to FM550 across gestation and lactation had altered thyroid function and their female offspring entered puberty early (Patisaul et al. 2013). In addition, offspring were 30-60% heavier by weaning, an effect that persisted into adulthood.

To our knowledge there have been no assessments of FM550 exposure and associated health outcomes in human populations. Such studies rely on accurate means of assessing exposure;

however, there is no biomarker of exposure to FM550 and data on environmental contributions to the body burden remain sparse. Thus, our primary objective was to develop methods to assess exposure to FM550 in human populations. We focus on the brominated FM550 components, and TBB in particular, because its use is thought to be limited to FM550 and another flame retardant mixture, Firemaster® BZ-54, whereas TBPH and the organophosphates in FM550 (i.e. TPP and ITPs) are used in numerous other flame retardant and plasticizer applications (Covaci et al. 2011; van der Veen and de Boer 2012). To support this approach, we developed an assay to measure TBBA in urine samples. Furthermore, we exposed 6 adult rats to a one time dose of FM550 and measured TBBA in their urine over a 24 hour period. Using the newly developed method, we measured TBBA in urine samples from 64 healthy, North Carolina adults. Additionally, we assessed temporal variability in TBBA measures to inform exposure assessments in future epidemiologic studies. Finally, building on our previous work investigating environmental pathways of exposure to FRs, we assessed relationships between TBB and TBPH in household dust and handwipe samples and evaluated associations of environmental TBB and urinary TBBA as first steps in identifying possible sources and potential routes of human exposure to FM550.

Methods

Study design

Two groups of healthy adult volunteers were recruited to the National Institute of Environmental Health Sciences (NIEHS) Clinical Research Unit (CRU) from central North Carolina. Eligible participants were at least 18 years of age and had never been diagnosed with a kidney problem (not including kidney stones). One group of participants (n=53) completed demographic and behavioral questionnaires, provided handwipe and urine samples, and collected dust samples in their homes (from February 2012 to March 2012). All samples from each participant were

collected within approximately one week of each other. Another group of adults was recruited to provide repeated urine samples on 5 consecutive days (11 participants with 53 total urine samples, all collected in August of 2011). All study protocols were approved by the NIEHS Institutional Review Board and participants provided informed consent.

Questionnaires

We designed a questionnaire to collect information on personal characteristics, including age; sex; race and ethnicity; height and weight; as well as information on personal habits such as average number of hours spent active in the home and average number of times participants washed their hands per day. Information on hand washing was collected as never, 1-2 times/day, 3-5 times/day, 6-8 times/day, and >8 times/day. For analyses we collapsed hand washing into two categories: <8 times/day (low) and ≥8 times/day (frequent), with the categorization determined based on the distribution of responses. Participants also provided information about whether they used hand sanitizing gels (categorized as yes or no). Response categories for average time spent active in the home and average time spent driving each day were also dichotomized to ≤8 hours/day and >8 hours/day and ≤1 hour/day and >1 hour/day, respectively).

Dust collection

Each participant was provided with a kit and instructions for the collection of house dust samples (See Supplemental Material, Figure S3 for a description of the instructions). Participants inserted a dust collection unit, which contains a nylon thimble, into the hose attachment of their home vacuum cleaner and followed instructions to vacuum the floor in the main living area of their home for two minutes. The dust collection unit, containing the thimble, was them removed from the vacuum, sealed in a plastic bag, and mailed back to the CRU. The nylon thimbles were never

in contact with the plastic bag. Upon receipt in the lab, the thimbles were removed, the dust sieved to <500 microns, and then stored in amber glass vials at room temperature until analysis.

Handwipe collection

Handwipe samples were collected at the CRU using previously described methods (Stapleton et al. 2008b). Briefly, sterile gauze wipes were soaked in 3.0 mL isopropyl alcohol and the entire surface of each participant's hand was wiped two times from the fingers to the wrist, including the space between fingers and both sides of the participants' hands, by clinical staff. Wipes were sealed in individual plastic bags and stored at -20ºC until analysis.

Dust and handwipe analyses

Handwipe and dust samples were extracted in the laboratory and analyzed for TBB and TBPH using previously published methods with minor modifications (Stapleton et al. in press; Van den Eede et al. 2012). Detailed information on the methods and standards used can be found in Supplemental Material (see "Dust and handwipe analyses" in Supplemental Material). Handwipes and dust samples (~100 mg) were spiked with internal standards and extracted with 50:50 dichloromethane:hexane using either Soxhlets or sonication, respectively. Extracts were concentrated to 1.0 mL and further cleaned using a solid-phase extraction cartridge (Supelclean ENVI-Florisil, 6 mL, 500 mg bed weight, Supelco). TBB and TBPH were eluted with hexane, reduced in concentration to 1.0 mL, spiked with recovery standards and analyzed using gas chromatography mass spectrometry operate in electron capture negative ionization mode (GC/ECNI-MS). Analysis of laboratory blanks (n=5) and an indoor dust Standard Reference Materials (SRM 2585, NIST, Gaithersburg, MD) were employed for quality assurance and quality control.

Urinary elimination in rats

To further investigate metabolism of TBB *in vivo*, we conducted an assessment of TBB metabolism in rats (mean weight=281 g, range 257-336 g; mean age=4.3 months, range 4.0-4.4 months). An ethanol solution of FM550 was prepared from a commercial stock in the same manner as described previously to obtain a final concentration of 0.05 mg/µL (Patisaul et al. 2013). Adult female Wistar rats (n=6/treatment), obtained from the same in house colony as for our prior study, were used for these experiments to ensure consistency with that previous work (Patisaul et al. 2013). As shown previously (Patisaul et al. 2013), the animals used for the present study can develop an obese phenotype. The sample size (n) is consistent with, or higher than, what is typically used to examine the pharmacokinetics of endocrine disrupting chemicals, such as BPA, in rodents (Churchwell et al. in press; Taylor et al. 2011). Animals were maintained on a soy-free diet (Teklad 2020, Harlan) in an environment that minimizes exogenous EDC exposure (including use of glass water bottles, wood chip bedding, polysulfone caging, and filtered water) at the North Carolina State University (NCSU) Biological Resources Facility (Patisaul et al. 2013; Patisaul et al. 2012). Study animals (pair housed until day of exposure and testing) were kept in a climate controlled room at 25°C and 45-60% average relative humidity and a 12-h light cycle (lights on from 12:00 to 24:00). Rats (n=6) were exposed at 10:00h to FM550 by dispensing 20 µL of the FM 550 solution (1 mg) onto ¼ of a soy-free food treat pellet (apple or chocolate flavored AIN-76A Rodent Diet Test Tabs, Test Diet, Richmond, IN). Control rats (n=6) were exposed to the treat pellet spiked with 20 µL of ethanol alone. Over the 24 hours following exposure, urine was collected from all 12 rats (at 0, 1 3, 6 and 24h). For each collection, rats were stimulated to urinate by moving them from their home cage to an unfamiliar clean cage with no bedding. All urinated within 3 minutes of being moved and the urine was

collected via pipette and stored at -20ºC until analysis. Animal care and maintenance were conducted in accordance with the applicable portions of the Animal Welfare Act and the U.S. Department of Health and Human Services ''Guide for the Care and use of Laboratory Animals'' (available at http://www.nap.edu/catalog/12910.html) and were approved by the NCSU Institutional Animal Care and Use Committee (IACUC). All procedures were approved and monitored by the supervising veterinarian for the duration of the project to ensure all animals were treated humanely and with regard for the alleviation of suffering.

Human urine collection and analysis

Study participants provided spot urine samples during visits to the CRU (all samples were collected between 0830 and 1630). Samples were collected in standard polypropylene specimen containers and were stored at -20ºC until analysis. 2,3,5 triiodo-benzoic acid (TIBA; Sigma Aldrich, St. Louis, MI) was used as the internal standard for quantification. The extraction and cleanup of TBBA in rat urine (<1 mL for most samples) was based on our previous study (Roberts et al. 2012) in which 6 mL Agilent SampliQ OPT SPE columns were used to concentrate and clean the urine samples. However, a matrix effect, (i.e., ion suppression of TBBA and TIBA) in human urine was observed, possibly due to the large volume of urine (~10 mL) extracted. Therefore, method modifications to reduce matrix effects were developed, and are described in Supplemental Material (see "Method development of TBBA analysis in human urine" in Supplemental Material and Supplemental Material, Figure S1), along with more details on the quantification method. Briefly, urine was thawed at room temperature and 10 mL was transferred to a pre-cleaned 50 mL glass centrifuge tube and 5 ng TIBA was spiked in as an internal standard. The urine was further diluted with 10 mL phosphate buffer (pH: 7.4, 0.1 M) and 1 mL concentrated sulfuric acid (95–98%, J.T. Baker) was added. After reaching room

temperature in the hood, 10 mL hexane was added to extract TBBA from urine. The mixture was vortexed for 1 minute and centrifuged at 3000 revolutions per minute (rpm) for 5 minutes. The supernant hexane layer was transferred into another empty 50 mL glass centrifuge tube and the extraction step was repeated twice and all hexane was combined. To completely remove sulfuric acid residue and further clean the matrix, the extract was then washed twice by 10 mL deionized water that was acidified with 1 M acetic acid to pH 2~3. The final extract in hexane was blown down to ~1 mL using an automated nitrogen evaporation system. Extracts were then dried and reconstituted with 0.5 mL methanol/H_2O (1/1, v/v) in LC vials and analyzed using liquid chromatography tandem mass spectrometry (LC/MS-MS) (Agilent 6410 Triple Quad LCMS) based on our previous study (Roberts et al. 2012). In brief, TBBA and TIBA were analyzed in negative ESI (electrospray ionization) mode using multiple reaction monitoring (MRM). Ion transition m/z 436.6 to 392.6 was used for quantification of TBBA and m/z 436.6 to 79 was used for the qualifier ions. Ion transition m/z 498.7 to 127 was used for quantification of TIBA and m/z 498.7 to 454.8 was used for the qualifier ions. 40 μL of the extract was injected and separated by a Synergi Polar-RP column (50×2.0 mm, 2.5 μm; Phenomenex) with water and acetonitrile mobile phases with 5 mM acetic acid. In a matrix spike recovery evaluation, the average recovery of TBBA was 79±9 % in 10 urine samples. Due to the lack of a proper recovery standard, no additional chemical was used to calculate the recovery of TBBA in each urine sample. As part of our quality control, the inter-day and intra-day variability of one TBBA detected urine sample were also tested and was less than 10%.

Sample specific gravity (SG) was also measured in each urine sample using a digital handheld refractometer (Atago, Bellevue, WA, USA). SG measurements were used to produce TBBA concentrations corrected for urine dilution (Boeniger et al. 1993). Analyses were then conducted

using both specific-gravity corrected and raw measurements. As the results were very similar with both methods, we present only the SG-corrected analyses. Creatinine, an alternative means of adjusting for dilution, was not measured in samples, as it is known to vary considerable by age and gender (James et al. 1988).

Data analyses

Quality control

Handwipe and house dust measurements were blank subtracted using the average field (handwipe) or laboratory (dust) blank measurement. The method detection limits (MDL) were calculated using three times the standard deviation of the appropriate blanks. MDLs for TBB and TBPH measured in handwipes were 3.8 and 1.1 ng, respectively. MDLs for TBB and TBPH in house dust were 8.9 and 33.5 ng/g, respectively. Recovery of the internal standard, F-BDE-69, averaged 91±18% in the handwipe samples, and 88±6% in the house dust samples. Measurements of TBB and TBPH in SRM 2585 averaged 35.2±4.0 and 545±74.5 ng/g, respectively, which are very similar to measurements made by Ali et al. (2011).

Statistical analyses

Concentrations below the MDL were substituted with the MDL/√2. Preliminary analyses indicated that concentrations of TBB and TBPH in handwipes and dust, and concentrations of TBBA in urine were log-normally distributed. We calculated Pearson correlation coefficients using $\log_{10}$-transformed concentrations assess associations between continuous ($\log_{10}$-transformed) measures of TBB and TBPH in handwipes and dust, and TBBA in urine. We also calculated Spearman correlation coefficients using non-transformed values.

We used linear regression models to assess bivariate associations between demographic, behavioral, and environmental variables and TBB and TBPH in handwipes and TBBA in urine samples (outcomes were $\log_{10}$-transformed). To aid in the interpretation of results, we exponentiated beta coefficients (10^{β}), producing the multiplicative change in outcome. As predictors of TBB and TBPH in handwipes, dust concentrations were categorized (dichotomized at the median and categorized into tertiles), and as predictors of urinary TBBA, both dust and handwipe concentrations were categorized to minimize the effect of skewed data and outliers in regression analyses.

We assessed temporal variability in TBBA concentrations by calculating interclass correlation coefficients (ICCs)(Shrout and Fleiss 1979). ICCs, which are calculated by taking the ratio of the between-subject variability to the sum of the between- and within-subject variability, provide a measure of the reliability of repeated measures over time (Rosner 2000). ICC values range from 0, indicating no reproducibility, to 1, indicating perfect reproducibility. All statistical analyses were performed in SAS (version 9.2; SAS Institute Inc, Cary, NC), with statistical significance defined as $\alpha \leq 0.05$ for main effects and $\alpha \leq 0.20$ for interactions.

Results

TBB metabolism

TBBA was detected in urine from FM550 exposed rats and significantly increased during the first 2-3 hours following exposure, reaching a maximum concentration of 1105 ng/mL. This was followed by a rapid decrease in concentration which then appeared to level off around 6-8 hours post exposure (Figure 1; see also Supplemental Material, Figure S2). These data indicate that urinary TBBA is rapidly formed from metabolism of FM550.

FM 550 in environmental samples

Study participants average 44 years of age (range 19-67). Approximately half were male (49.1%) and the majority reported white race (75.5%) and non-Hispanic ethnicity (94.3%). Table 1 presents summary statistics for TBB and TBPH concentrations in house dust and handwipes. Both compounds were detected in nearly all house dust samples (n=93.9%). Geometric mean concentrations were 315.1 and 364.7 ng/g for TBB and TBPH, respectively (Table 1), with considerable variability among samples. TBB and TBPH levels in dust were highly correlated (r_p=0.81, p<0.001; r_s=0.78, p<0.001; Figure 2A; analysis robust to the exclusion of extreme observations). TBB and TBPH were also detected with high frequency in handwipe samples (92.5% and 98.1% of all samples), with a geometric means of 31.4 and 23.4 ng, respectively. As with dust, TBB and TBPH levels in handwipes were correlated, although the magnitude of the correlation was lower (r_p=0.73, p<0.001; r_s=0.56, p<0.001; Figure 2B).

Dust and handwipe associations

In correlation analyses, continuous measures of TBB in handwipes and dust were positively correlated (r_p=0.48; p<0.001; r_s=0.34; p=0.01) as were levels of TBPH (r_p=0.38; p<0.001; r_s=0.35; p=0.01). We used linear regression models to further explore bivariate associations between dust and handwipe concentrations. Table 2 presents exponentiated β-coefficients for these models, representing the multiplicative change in handwipe concentrations. For example, those with the highest dust levels (3rd tertile) in their homes averaged 3.64 times (95% confidence interval (CI): 1.27, 10.39) the levels of TBB in handwipe samples compared to those with the lowest TBB dust level (Table 2). Similarly, higher levels of TBPH in dust also contributed to higher mass of TBPH on participants hands (10^{β}=2.81 for the 3rd tertile compared to the 1st; 95% CI: 1.09, 7.22). For both TBB and TBPH, results were similar when we

considered dichotomized versions of the dust (results not shown). Participant reporting frequent hand washing also tended to have decreased TBB mass on their hands (Table 2). Conversely, using hand sanitizing gel had little effect on TBB levels in handwipes.

Handwipe and urine associations

TBBA was also detected frequently (76.9% detect) in urine samples from participants with paired house dust and handwipe samples, with a geometric mean of 5.6 pg/ml (specific-gravity corrected; Table 1). We observed a positive correlation between TBB levels in handwipes and TBBA in urine (r_p=0.38; p<0.001; r_s=0.31; p=0.02; Figure 3). Using linear regression models we investigated bivariate associations between categorical handwipe TBB levels and TBBA (Table 3). Participants with the highest TBB handwipe levels had 1.99 times the urinary TBBA concentrations of participants with low handwipe levels, although associations were imprecisely estimated (comparing the 3rd tertile to the 1st; 95% CI: 0.73, 5.41). In bivariate models, TBBA concentrations also tended to decrease with age (10^{β}=0.97; 95% CI: 0.94, 1.00) and were lower for participants that spent >1 hour/per day in their cars (10^{β}=0.54; 95% CI: 0.24, 1.21), although associations were imprecisely estimated.

Dust and urine associations

The correlation between continuous measures of TBB in dust and TBBA in urine was weakly positive, but not statistically significant (r_p=0.24; p=0.10; r_s=0.20; p=0.16). In bivariate regression models, participants with the highest levels of TBB in dust samples had urinary TBBA levels 1.55 times those of participants with lower dust levels, although results were not statistically significant (comparing the 3rd tertile to the 1st; 95% CI: 0.55, 4.22; Table 3). Results were similar when dichotomized dust levels were included in analyses (results not shown). We

hypothesized that the relationship between dust TBB and urinary TBBA concentrations may be modified by hand washing frequency which bivariate results suggest may be inversely associated with TBBA. We conducted analyses with an interaction term consisting of hand washing and dichotomized house dust. Although the interaction term was not statistically significant (p=0.18), our result indicated that hand washing may modify the association between dust and internal exposure (Figure 4).

Temporal variation in urinary TBBA

TBBA levels in repeated urine samples were similar to those from the larger cohort (GM=5.1 pg/mL; 66.0% detect). Examining temporal variability in specific-gravity-corrected TBBA levels using ICCs, we observed moderate reliability over the course of five consecutive days (ICC=0.56; 95% CI: 0.46, 0.66)(Rosner 2000).

Discussion

Cumulatively, our results indicate that exposures to FM550 are widespread and the home environment may be an important source of exposure. These data, coupled with recent studies in animals linking exposure to adverse health outcomes (e.g. McGee et al. 2013; Patisaul et al. 2013), prompt the need for additional epidemiologic studies to identify potential impacts of FM550 exposure. Such studies rely on accurate and reliable methods of assessing exposure. Based on toxicokinetic investigations using human and animal tissues, we hypothesized that TBBA may be a novel biomarker of FM550 exposure and we developed a method for measuring urinary TBBA using liquid chromatography tandem mass spectrometry.

We investigated the validity of using TBBA as an indicator of exposure to TBB. In rats, TBBA in urine peaked between exposure and 3 hours after a single administered dose of FM550,

corroborating *in vivo* metabolism studies reporting the rapid metabolism of TBB to TBBA in human and rat tissues (Bearr et al. 2012; Roberts et al. 2012). Rapid formation of TBBA indicates a reduced bioaccumulative potential for TBB and suggests that TBBA may be a useful biomarker of recent FM550 exposure in future studies.

TBBA was frequently detected in urine samples from North Carolina adults (overall detection 72.4%). Levels of TBBA were highly variable between study participants; one sample had TBBA levels that were 60 times the geometric mean concentration, indicating potential differences in exposure patterns or in TBB metabolism between subjects. Because we hypothesize TBB is rapidly metabolized to TBBA, it is likely that the spot urine samples we assessed are reflective of recent exposure (<1 day previous). Nonetheless, although there was day-to-day variability of TBBA, repeated urine samples collected from the same participants on five consecutive days indicate moderate temporal reliability (ICC = 0.56; 95% CI: 0.46, 0.66). While higher than what might be expected for a rapidly formed metabolite, the estimated ICC for TBBA is similar to what has been reported previously for phthalates (Fromme et al. 2007; Hauser et al. 2004). These data suggest that the exposure sources (e.g. dust), or behaviors related to TBB exposure, such as the average hand washing frequency, may be stable over time.

Sources and pathways of FM550 exposure

Collecting paired house dust, handwipe and urine samples from study participant allowed us to examine associations between sample types and to explore potential pathways of exposure. Our results suggest that higher levels of TBB in house dust may be associated with higher levels of TBBA in urine, indicating that the home environment contributes to the overall body burden of TBB. Although associations were generally imprecisely estimated, several lines of evidence

point to exposures via the hands as an important pathway of exposure: 1) higher TBB in dust was associated with a greater mass on participants' hands, a measure of personal exposure (Table 2); 2) higher mass of TBB on participants' hands was also associated with higher levels of TBBA in urine (Table 3); and 3) frequent hand washing may mitigate the effects of TBB in dust on overall body burden as participants that reported frequent hand washing had a lower mass of TBB on their hands and had lower levels of TBBA in urine samples (Figure 1). Although we did not measure internal exposure to TBPH, our results suggest that higher levels of TBPH in household dust may also lead to increased personal exposure. We did not find associations between average hand washing frequency and the levels TBB or TBPH on participant's hands; however handwipe levels may be more closely related to the time since a participant last washed their hands, rather than their average behavior.

Although our results suggest TBB on hands may capture individual exposure information and may be an important exposure pathway, we are unable to determine the mechanism by which TBB gets into the human body in the current study. Incidental ingestion via hand-to-mouth contact and dermal absorption are both consistent with our findings, and may be similar to an exposure pathway for PBDEs (Johnson et al. 2010; Stapleton et al. 2012; Watkins et al. 2011). However, data also suggest that diet can also be a route of exposure for PBDEs (Schecter et al. 2006), which was not investigated in this study. To our knowledge, there are no published assessments of FM 550 in food.

Comparison with previous studies

We measured detectable levels of TBB and TBPH in the vast majority of household dust samples, suggesting widespread use of FM550 and probable human exposure. Dust TBB and

TBPH levels were highly variable, ranging over several orders of magnitude, and were slightly higher than those reported in samples collected in 2006 and 2011 (Dodson et al. 2012; Stapleton et al. 2008a). Levels of TBB and TBPH were approximately one order of magnitude lower than the sum of PentaBDE congeners found in household dust samples (congeners included in the PentaBDE sum varied across previous studies; (Dodson et al. 2012; Stapleton et al. 2012; Watkins et al. 2011)); however levels in house dust may be expected to increase as older household products containing PBDEs are replaced with newer versions containing FM550, particularly as products age and degrade.

TBB and TBPH were also detected on the hands of nearly all study participants. On average, the levels of TBB on handwipes were slightly lower than those reported in our previous work investigating the levels of TBB on toddler's hands (GM=31.4 vs 4.1 ng; Stapleton et al. in press); difference in the behavior or in hand surface area between the two studies may explain differences in the levels of TBB observed. Additionally, PBDEs have been measured in handwipes in several studies. In contrast to dust, the mass of TBB and TBPH on handwipes in our analyses was similar to previous reports of the mass the sum of PentaBDE congeners (Stapleton et al. 2012; Watkins et al. 2011).

Although both are components of FM550, it is possible that the TBB and TBPH that we measured are from an alternate source. For example, both compounds are also used in BZ-54, a flame retardant mixture that is also manufactured by Chemtura. However, our previous research, which consistently found TPP in foam samples containing TBB and TBPH, suggests that FM550 is used more frequently in household foam applications than BZ-54 (which does not contain TPP)(Stapleton et al. 2009; Stapleton et al. 2011). TBPH is also used in polyvinyl chloride, neoprene, wire insulation, carpet backing, coated fabrics, wall coverings and another flame

retardant mixture, DP-45 (Covaci et al. 2011). However, the strong correlations between TBB and TBPH in environmental samples suggest a common source. Further data are needed to identify FR mixtures and products containing TBB and THBP.

Our results should be interpreted within the context of several limitations. Our small sample size limited the number of predictive variables that we could include in multivariate regression analyses at the same time and may have limited our power to detect meaningful associations. Additionally, paired dust, handwipe, and urine samples were each collected only once. Multiple samples taken over time and in different micro environments (e.g. workplaces and car) may provide additional insights as to important routes of TBB and TBPH exposure (Watkins et al. 2012). Similarly, household dust samples were collected by participants. Detailed instructions for dust collection were provided, however, variability in the areas sampled, the types of flooring in those areas, and the types of vacuums used may have introduced measurement error into our analyses. Finally, although participants were recruited from the North Carolina general population, the cohort was comprised of a relatively homogeneous group; participants were primarily white and there was little variability in behavioral characteristics. Although this may limit our ability to generalize results to the broader US population, it does not impact the internal validity of our study.

Conclusions

Cumulatively, our results indicate that the general population is exposed to FM550 in the home environment. These results are of concern given recent toxicological data suggesting that FM550 exposure may have the potential to adversely impact health. Our results indicate that urinary TBBA provides a useful biomarker of FM550 exposure for future epidemiologic studies. In

addition, handwipe levels of TBB were significantly associated with both dust levels and urinary TBBA levels, suggesting that collection and analysis of handwipes may also provide a good measure of exposure.

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Table 1. Geometric mean and selected percentiles in household dust, handwipes, and urine samples from North Carolina adults contributing paired samples (n=53).

Matrix and compound	N[a]	% Detect	Geometric mean	25th percentile	50th percentile	75th percentile	Maximum
Dust							
TBB (ng/g)	49	93.9	315.1	159.1	275.5	487.0	18148.7
TBPH (ng/g)	49	93.9	364.7	165.7	473.8	860.6	4814.2
Handwipes							
TBB (ng)	53	92.5	31.4	14.8	26.9	55.5	2633.1
TBPH (ng)	53	98.1	23.4	9.9	23.0	40.8	655.1
SG[b]-corrected urine							
TBBA (pg/mL)	52	76.9	5.6	2.3	5.3	10.8	340.6

[a]Insufficient sample volume for 4 dust and 1 urine sample. [b]SG: specific gravity.

Table 2. Regression analyses for predictors of handwipe TBB and TBPH.

Predictor	TBB handwipes coefficient[a] (95% CI)	p-Value	TBPH handwipes coefficient[a] (95% CI)	p-Value
Sex				
Male	Reference		Reference	
Female	1.75 (0.75, 4.06)	0.19	1.62 (0.76, 3.55)	0.19
Age (years)	0.98 (0.94, 1.01)	0.18	0.99 (0.96, 1.02)	0.62
Average times hands washed per day				
≤8 times/day	Reference		Reference	
≥8 times/day	0.46 (0.18, 1.12)	0.08	0.81 (0.37, 1.78)	0.59
Handgel use				
No	Reference		Reference	
Yes	1.45 (0.58, 3.62)	0.41	1.62 (0.76, 3.55)	0.21
Average time active in the home/day				
≤8 hours/day	Reference		Reference	
>8 hours/day	0.91 (0.34, 2.47)	0.85	1.51 (0.65, 3.55)	0.32
Average time driving in car/day				
≤1 hour/day	Reference		Reference	
>1 hour/day	0.79 (0.34, 1.85)	0.58	0.95 (0.46, 1.95)	0.88
Dust TBB or TBPH				
Low – tertile 1	Reference		Reference	
Mid – tertile 2	1.09 (0.39, 3.06)	0.87	1.61 (0.64, 4.09)	0.31
High – tertile 3	3.64 (1.27, 10.39)	0.02	2.81 (1.09, 7.22)	0.03

[a]Exponentiated beta-coefficients are presented and represent the multiplicative change in handwipe levels relative to the reference group for categorical variables, or the per unit change for continuous variables (age).

Table 3. Regression analyses for predictors of urinary TBBA.

Predictor	SG-corrected TBBA coefficient[a] (95% CI)	p-Value
Sex		
Male	Reference	
Female	0.99 (0.44, 2.24)	0.98
Age (years)	0.97 (0.94, 1.00)	0.09
Average times hands washed/ day		
≤8 times/day	Reference	
≥8 times/day	0.61 (0.25, 1.47)	0.27
Handgel use		
No	Reference	
Yes	0.88 (0.36, 2.14)	0.78
Average time active in the home/day		
≤8 hours/day	Reference	
>8 hours/day	1.40 (0.55, 3.59)	0.23
Average time driving in car/day		
≤1 hour/day	Reference	
>1 hour/day	0.54 (0.24, 1.21)	0.13
Handwipe TBB		
Low – tertile 1	Reference	
Mid – tertile 2	1.16 (0.42, 3.16)	0.77
High – tertile 3	1.99 (0.73, 5.41)	0.17
Dust TBB		
Low – tertile 1	Reference	
Mid – tertile 2	1.19 (0.43, 3.35)	0.73
High – tertile 3	1.55 (0.55, 4.22)	0.40

[a]Exponentiated beta-coefficients are presented and represent the multiplicative change in urine concentrations relative to the reference group for categorical variables, or the per unit change for continuous variables (age).

Figure legends

Figure 1. Mean (with standard error) TBBA in urine samples collected from 6 treated and 6 control adult rats. Exposed animals were administered a one-time dose of 1 mg Firemaster® 550 (via treat pellet) at time zero. TBBA concentrations were measure at 1, 3, 6, and 24 hours in urine sample from exposed animals and controls.

Figure 2. Spearman correlation coefficients (r_s) between TBB and TBPH in 49 dust samples (A) and 53 handwipe samples (B).

Figure 3. Spearman correlation coefficients (r_s) between TBB in handwipes and TBBA in urine samples.

Figure 4. GM and 95% CI of TBBA measurements in urine by household dust and hand-washing frequency. * Indicates significant difference between low-dust/high-wash and high-dust/low-wash groups ($p < 0.05$) in regression analyses including an interaction term.

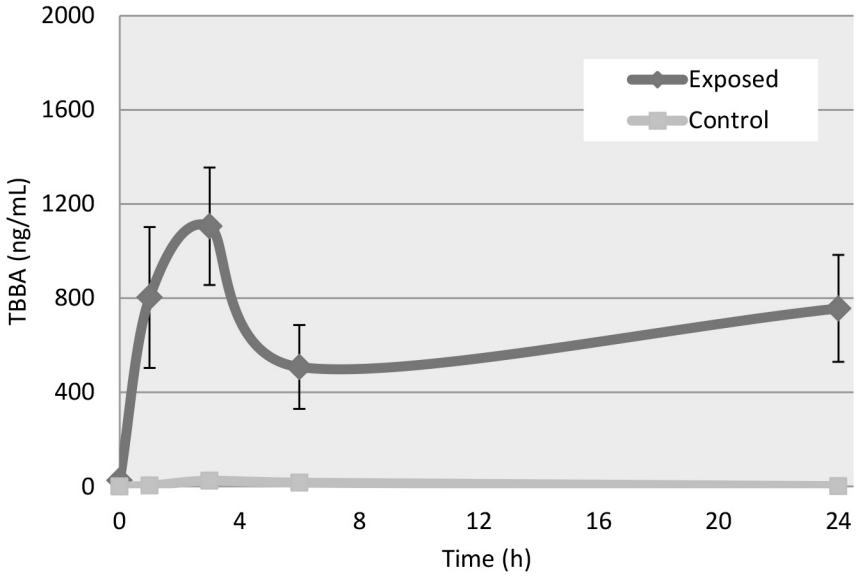
2000
1600
1200
800
400
0
TBBA (ng/mL)
0
4
8
12
16
20
24
Time (h)
Exposed
Control

Figure 1

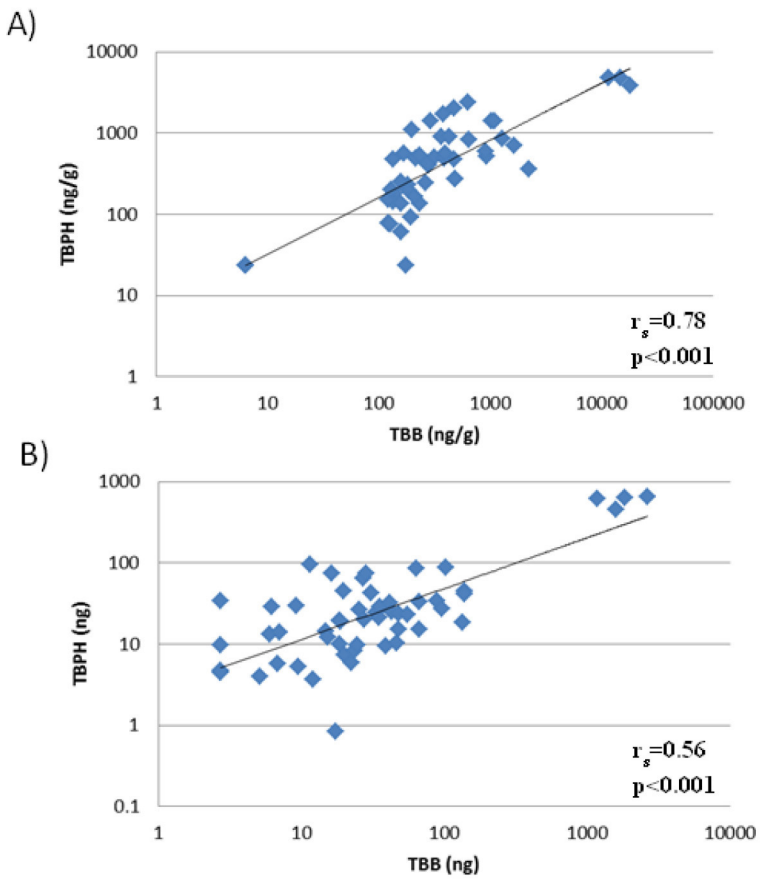
A)
10000
1000
100
10
1
TBPH (ng/g)
1
10
100
1000
10000
100000
TBB (ng/g)
r_s=0.78
p<0.001
B)
1000
100
10
1
0.1
TBPH (ng)
1
10
100
1000
10000
TBB (ng)
r_s=0.56
p<0.001

Figure 2

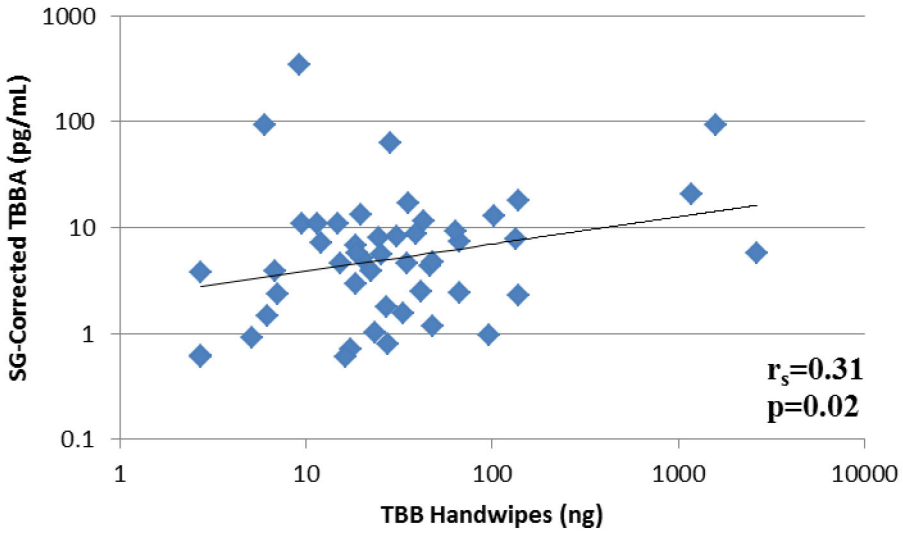
1000
100
10
1
0.1
SG-Corrected TBBA (pg/mL)
1
10
100
1000
10000
TBB Handwipes (ng)
r_s=0.31
p=0.02

Figure 3

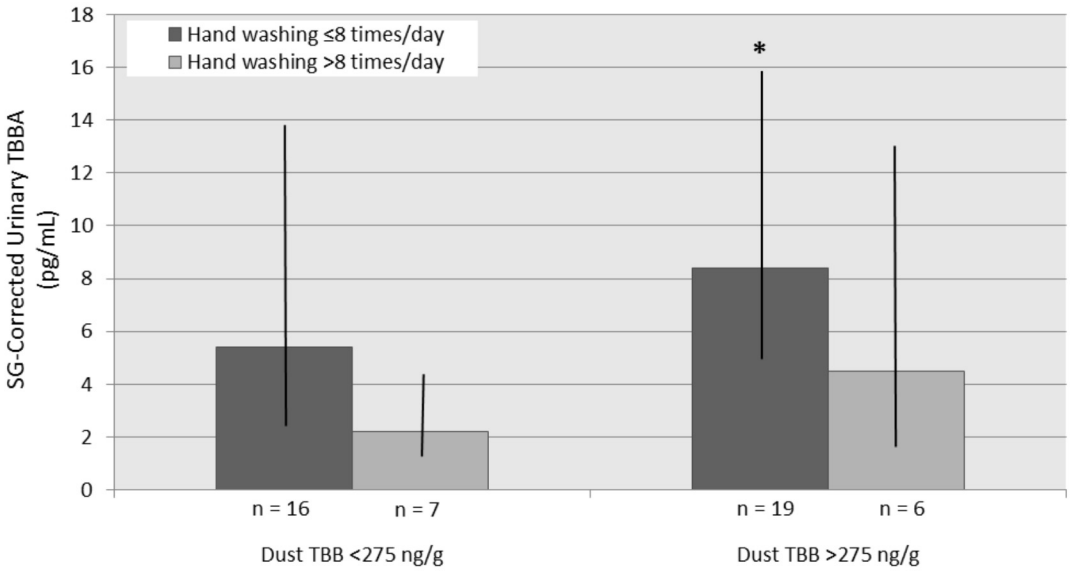
Hand washing ≤8 times/day
Hand washing >8 times/day
SG-Corrected Urinary TBBA (pg/mL)
18
16
14
12
10
8
6
4
2
0
*
n = 16
n = 7
n = 19
n = 6
Dust TBB <275 ng/g
Dust TBB >275 ng/g

Figure 4