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Predicting Body Burden of Polybrominated Diphenyl Ethers in Children Aged 15 Months to 5 Years Using Household Dust and Handwipe Levels and Selected Demographic Variables

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B.A.
Kenyon College
2009

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An abstract of
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Abstract

Predicting Body Burden of Polybrominated Diphenyl Ethers in Children Aged 15 Months to 5 Years Using Household Dust and Handwipe Levels and Selected Demographic Variables

By Grace Evelyn Lee

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that are ubiquitous in the environment and in human biological samples. Despite bans on two of the three major commercial mixtures of PBDEs, the United States has the highest serum concentrations in the world due to historically high use. PBDEs are known endocrine modulators and have been linked to numerous neurodevelopmental and behavioral disabilities. Studies have suggested that children may be particularly sensitive to PBDE exposures. Simultaneously, they may have serum concentrations up to 10-fold higher than adults. Few studies have looked at individual child serum levels or attempted to correlate them with environmental matrices.

The aim of this study was to determine child serum, handwipe and household dust levels of PBDEs for a group of demographically diverse children. Blood samples were obtained from 81 children enrolled at the Children's Hospital of Atlanta (CHOA) while undergoing a routine surgery. At a later date, a subset of participants had household dust (n=15) and handwipes (n=13) collected.

Serum, dust, and handwipes were extracted and the concentrations of six congeners (BDE-47, -85, -99, -100, -153, -154) and ΣPBDE determined in-house by GC/MSD analysis. BDE-47 and -99 were dominant congeners in all three matrices. In serum, significant associations were found between sex and BDE-47 and ΣPBDE, race and BDE-100, breastfeeding history and BDE-47, and BMI and BDE-153. Handwipe and serum were correlated for BDE-47 and ΣPBDE, suggesting that handwipes may be a good personal exposure measure for the Penta DBE mixture.

To date, only one other study has investigated child serum, child handwipes, and household dust in the same individuals for PBDEs. Additionally, this is one of the largest and most diverse groups studied in this age demographic for PBDE exposures.

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1. INTRODUCTION

A. Background

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame

retardants frequently used in consumer products such as electronics and textiles. PBDEs

have a chemical structure similar to polychlorinated biphenyls (PCBs), which were

banned in the United States in 1978. Like PCBs, PBDEs are persistent organic pollutants

that are environmentally persistent, bioaccumulate, and biomagnify (Birnbaum and Hubal

2006). 209 PBDE congeners exist, differing in structure by the number and location of

bromines around two aromatic rings and are numbered according to the IUPAC system

(Birnbaum and Hubal 2006). The congeners are divided into 10 homologous groups

dependent on the total number of bromines that they contain (mono- to

decabromodiphenyl ethers). The general structure is shown in Figure 1.
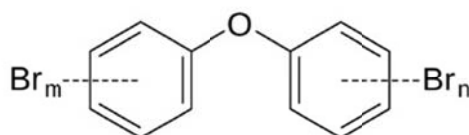
Figure 1: PBDE General Structure

Three major commercial mixtures of PBDEs have been used in the United States

and internationally: pentabromodiphenylether (Penta), octabromodiphenylether (Octa),

and decabromodiphenylether (Deca), so named for the average number of bromines

present (Birnbaum and Hubal 2006; Krol et al. 2012). Penta-BDE consists primarily of

BDE-47 (tetrabrominated) and BDE-99 (pentabrominated) and is applied mainly to

polyurethane foam in furniture. Octa-BDE is composed mainly of BDE-153

(hexabrominated) and BDE-183 (octabrominated) and is used in office equipment and plastic housing. Deca-BDE consists mainly of BDE-209 (decabrominated) and is used primarily in electronics (Birnbaum and Hubal 2006; Frederiksen et al. 2009).

PBDEs are additives mixed into polymers and are not chemically linked to the polymer; thus they can leach out and enter the environment (Talsness 2008). Because of rising concerns about their health effects, Octa- and Penta- mixtures were phased out by production companies in 2005 in the United States. The Deca- mixture is still currently in use in all states except Maine, with a nationwide voluntary phase out by 2013 (BSEF 2012). However, even if PBDEs are no longer being produced, there is the potential for exposure because of PBDEs' previous widespread use, persistence in the environment, and continued use of pre-ban products.

B. Health Effects

The health effects of PBDEs in humans are not well defined as most of the research has been limited to animal models. While the toxicological mechanisms are not well understood, PBDE exposure has been linked to numerous health outcomes including liver toxicity, endocrine modulation, altered thyroid homeostasis, reproductive problems in women and men, and behavioral and neurodevelopmental alterations such as hyperactivity (Birnbaum and Hubal 2006; Chen et al. 2011; Branchi et al. 2003; Boas et al. 2009).

The health effects of PBDEs may be congener-dependent. These differences in health effects are likely due to changes in biochemical properties associated with additional bromines. Lower molecular weight congeners (six bromines or fewer) are

almost completely absorbed, highly bio-accumulate, and have half-lives on the order of 2 to 12 years (Branchi et al. 2003; Trudel et al. 2011). Fully brominated deca-BDE congeners are poorly absorbed and are rapidly eliminated (Branchi, Capone et al. 2003).

Endocrine Modulation

PBDEs are endocrine modulators that cause disruption of thyroid hormone homeostasis (Talsness 2008; Boas et al. 2009). Proper development and regulation of body systems is dependent upon appropriate levels of thyroid hormones. Perturbations of thyroid hormone levels may be particularly harmful during critical development phases, such a neurodevelopment (Birnbaum 2004). Developing fetuses and infants are especially responsive to small changes in thyroid hormone and represent an extremely vulnerable subpopulation (Branchi et al. 2003).

In rat studies, perinatal maternal and postnatal PBDE exposure reduced circulating thyroid hormones concentrations in fetuses. Similarly, studies in other species have consistently shown thyroxine (T_4) and triiodothyronine (T_3) reductions after exposure at dosage levels comparable to environmental human exposure (Boas et al. 2009). Potential mechanisms of PBDE-induced thyroid hormone dysregulation are increased metabolism of T_4 by induction of hepatic enzymes, downregulation of thyroid hormone transport proteins, or competition for transthyretin (Birnbaum and Hubal 2006; Talsness 2008; Boas et al. 2009).

Few human studies exist regarding PBDE and thyroid function. In a study of 308 adult male fishers, PBDE concentration was positively associated with T_4 and negatively associated with T_3 and thyroid-stimulating hormone (TSH). Hormone levels varied by

congener, suggesting that there are congener-specific mechanisms (Turyk et al. 2008).

Similarly, other epidemiological studies found that BDE-47 was associated with

decreased TSH (Hagmar et al. 2001) and occupationally exposed workers had slight T_4

elevation over time (Julander et al. 2005). Studies of maternal PBDE levels found

negative associations with TSH and individual and sum PBDE levels, but no association

with neonatal TSH (Chevrier et al. 2011; Chevrier et al. 2010). The general trend

revealed by these studies is not consistent with laboratory animal data that consistently

saw T_4 depression and no effect on TSH. It remains unclear why these differences exist.

The current understanding of the effects of PBDEs on human thyroid hormone levels is

very limited, with information on children sorely lacking despite their vulnerability.

Neurotoxic Effects

PBDE is a known developmental neurotoxicant in mice. Gestational and neonatal

low doses of PBDE caused irreversible changes in adult mouse brain function; mice

displayed hyperactivity as adults that worsened with age (Talsness 2008). BDE-99 in

particular altered the cholinergic system and caused deficits in learning and memory in

mice.

Human studies investigating neurodevelopmental effects of PBDEs have observed

adverse neurodevelopmental effects in 4-6 year olds prenatally exposed (Roze et al.

2009; Herbstman et al. 2010). Postnatal BDE-47 exposure was associated with higher

risk of ADHD symptoms and decreased mental development (Gascon et al. 2012).

Mechanisms underlying this neurotoxicity have not been fully elucidated, but

there is evidence of thyroid hormone-mediated effect. Thyroid hormone is an integral

component of brain development, and alterations in normal hormone levels can cause distinct changes in neuro-behavioral development. Relatively small changes in maternal T_4 negatively impact intelligence and psychomotor skills in children (Branchi et al. 2003). As discussed above, thyroid hormone regulation is a likely target of PBDE toxicity.

There is a critical window of sensitivity to PBDE in mice that corresponds to the period of rapid brain growth (Talsness 2008). Fetal exposure and infant exposures via breastfeeding would occur during this developmental stage. Therefore protection of pregnant women and young children is a priority when dealing with PBDE, and there is a clear importance of minimizing childhood exposures.

C. Human Exposure to PBDE

Global Exposure Trends

The total world-wide production of PBDEs in 2001 was approximately 67,400 metric tons/year, with the Americas consuming just over half of it (Birnbaum 2004). PBDE concentrations in humans have increased by approximately 100 times during the last 30 years, with North Americans having approximately 20 times more PBDE in serum compared to Europeans (Hites 2004). Differences in PBDE utilization and exposure levels are likely due to stringent flammability standards in the United States, and the European Union's ban of Penta and Octa in 2003 (Whitehead et al. 2013). Recent studies in China have suggested that Chinese exposure levels may be elevated due to the import and recycling of other nations' electronic waste (Frederiksen et al. 2009).

Within the U.S., there are wide variations in environmental and biological measures of PBDEs. In California, PBDE dust concentrations were 4-10 higher and serum 2-fold higher than other regions in the United States (Zota et al. 2008). These differences were likely attributable to California's strict furniture flammability standards that resulted in widespread use of Deca and Penta (Whitehead et al. 2013; Zota et al. 2008).

Child Serum PBDE

Exposure studies have primarily used plasma, serum, or breast milk to measure PBDE body burden and have focused on adults (Frederiksen et al. 2009). Studies of PBDE levels in children have been extremely limited due to difficulties associated with collecting blood samples. However, the toxicological data suggests that children may be more sensitive to PBDE exposures. Simultaneously, children aged one to five years appear to be more 2- to 10-fold more exposed to PBDEs than adults (Lunder et al. 2010; Toms et al. 2009; Rose et al. 2010; Eskenazi et al. 2011).

Peak concentrations of PBDEs in children occur around 2 to 3 years of age as a result of increased hand-to mouth behavior and dust exposure, discussed more below (Lunder et al. 2010; Toms et al. 2009). Infants (<1 years old) also have high PBDE exposure, but their exposure comes almost exclusively from breastmilk (Johnson-Restrepo and Kannan 2009).

The results produced by child serum PBDE studies in the U.S. have been severely limited by sample size, pooled analyses, congener choice, and unrepresentative study populations. Only recently have studies moved away from using pooled serum samples

and started using individual samples. This is problematic because it has been well documented that serum PBDE concentrations may vary greatly, with 5% of the population having concentrations of PBDE at least 10 times the mean, and 1% as much as 50 times the mean (Birnbaum and Hubal 2006). Pooled serum samples therefore may not be a good representation of average exposures and cannot identify individuals with high body burdens. There were also problems with inconsistent congener choice; there is no consensus among researchers what congeners are important to report. The most commonly reported congeners were BDE-47, -99, -100, -153, and -154 (Birnbaum and Hubal 2006; Hites 2004). BDE-209 was often not measured due to difficulties in measurement methodology and lack of comparative data (Lorber 2008).

The study populations looked at in child serum studies were often not generalizable to the American public. Lunder *et al.* (2010) investigated a primarily higher socioeconomic status (SES), white Caucasian population. Eskenazi *et al*. (2011) used the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS), a primarily low-income Hispanic population. Both of these studies were extremely homogenous in terms of socioeconomic status and ethnicity. Rose *et al.* (2009) studied children born in California, a state known to have high mean concentrations of PBDE compared to the rest of the United States. The limitations of these studies make it difficult to generalize their findings to the larger population of American children.

Exposure Routes and Potential Measurement Methods

In nations with low to moderate body burdens of PBDE, the dominant exposure is through foodstuffs, particularly dairy and meat products (Lorber 2008). In highly exposed

nations, dust is the primary pathway of exposure and can account for about 82% total PBDE intake (Trudel et al. 2011; Lorber 2008). In the United States, household dust levels have been found to be good estimators of exposure (Johnson et al. 2010; Wu et al. 2007; Stapleton et al. 2012; Watkins et al. 2011a, b). Dust sample collection is less invasive than biological sampling, making it an appealing alternative matrix.

PBDEs volatilize from the product and adsorb onto dust. Dust can then enter the body via inhalation, inadvertent ingestion from hand-to-mouth contact, or direct absorption through the skin (Whitehead et al. 2013). Very little data currently exists about the relative importance of each of these routes to total exposure levels, but it is estimated that ingestion is responsible for ~80% and dermal 10-30% of total PBDE exposure (Frederiksen et al. 2009).

For young children (approximately one to five years old), dust exposure is increased by hand-to-mouth behaviors and proximity to dust covered surfaces. Daily intakes of dust for small children (1-4 years) have been estimated to be 100-200 mg/d while adult intake is estimated around 50 mg/d (U.S. EPA 2011). Therefore, PBDE dust measurements may be of particular use in determining child exposure levels. Currently, only one study has attempted to link child serum PBDE levels and household dust levels. Stapleton *et al.* (2012) found that serum ΣPenta levels were associated with house dust ΣPenta levels in a study of 83 children 12 to 36 months of age.

Several problems have been identified with dust sampling. Dust PBDE concentrations have been found to vary depending on the room selected for sampling, probably due to differences in the furniture and electronics present (Krol et al. 2012; Allen et al. 2008; Muenhor and Harrad 2012). Body levels of PBDE may be dependent

on the room the majority of time is spent in, and for adults this is complicated by the fact that many work non-residentially (Watkins et al. 2011a, b).

Handwipes may be a more direct measure of personal exposure than dust measurements, particularly among children. To date, four studies have measured PBDEs in handwipes and have found good agreement with dust concentrations (Stapleton et al. 2012; Watkins et al. 2011a, b; Stapleton et al. 2008). Handwipe concentrations were found to be better predictors of serum Penta-BDE levels than dust (Stapleton et al. 2012; Watkins et al. 2011a, b).

D. Aims and Hypotheses

Aim 1: Provide needed reference values for total and congener-specific blood PBDE levels in children, particularly the range and variability of exposure levels. Define the levels of dust and handwipe PBDEs in a subset of the participants.

Aim 2: Examine potential predictors of PBDE exposure levels including age, sex, race, SES assessed by insurance status (Medicaid vs. private), breastfeeding status, and BMI.

Aim 3: Determine if dust or handwipe PBDE concentrations are good predictors of serum PBDEs levels in children.

I hypothesize that lower SES, breastfeeding, and higher BMI will be associated with higher levels of serum PBDE.

I also predict that handwipe PBDE levels will be more strongly correlated with serum levels of PBDE than household dust levels.

2. METHODS AND MATERIALS

A. Study Population and Enrollment

Subjects between the ages of 15 months and 6 years were recruited to be in the **B**rominated Flame Retardant **E**xposure **a**nd **T**hyroid Function (BEAT) study. The study primary investigators were Lyndsey Darrow, PhD (Dept of Epidemiology) and Dana Boyd Barr, PhD (Dept of Environmental Health), and it was funded by NIH grant ID 5R21ES019697-02. The study age range was chosen because previous studies have shown high serum levels of PBDEs in this age group, which may represent a vulnerable subpopulation (Lunder et al. 2010; Toms et al. 2009)

Eighty-seven children were recruited from Children's Healthcare of Atlanta (CHOA) when they were admitted for a routine myringotomy, adenoidectomy, or tonsillectomy surgery. This population was chosen for ease of blood draw. Anxiety and discomfort associated with blood draws in this age group were avoided because of general anesthesia administered for the surgery. The study protocol was reviewed and approved by Emory's Institutional Review Board.

Criteria for exclusion from the study included undergoing any additional procedures not listed above, being outside the target age range, or having any preexisting condition that may affect PBDE or thyroid hormone levels. Enrolled children were also excluded from further participation if they were unable to provide a full 15 mL of blood during the blood draw. The total number of participants in the study was 81.

The BEAT study nurse, Christina Ryan, R.N. (CHOA), obtained parental consent and enrolled the children on the day of their surgery. Demographic and health information was gathered through a parental questionnaire and from the child's health records while the child was undergoing surgery. The questionnaire included questions about child date of birth, sex, race, ethnicity, breastfeeding history, duration at current residence, parental occupation, household smoking, insurance status, child medications, height and weight, and family medical history of endocrine or autoimmune diagnosis. Approximately one-fourth of parents indicated that they were interested in being involved in home dust and child handwipe sampling at a later date. Families who resided within a 25-mile radius of Atlanta were considered for future sampling. A total of 15 households were sampled for dust, representing 17 child dust exposure measurements as some participants lived in the same household. In addition, 13 handwipes were collected at the time of dust sampling from the participating children.

B. Chemicals and Materials

Chemicals used for sample extraction and analysis are listed in Table 1. For serum extractions, internal standard (ISTD) solution was 10 ng/mL of $^{13}C_{12}$ isotopically labeled BDE-47, -99, -100, -153, and -154 in methanol. For dust and handwipe extractions, ISTD solution was 20 ng/mL of $^{13}C_{12}$ isotopically labeled BDE-47, -99, -100, -153, and -154 in hexane. Low (QCL) and high (QCH) concentration quality control solutions were 100 ng/mL and 300 ng/mL, respectively, of BDE-47, -85, -99, -100, -153, and -154 in hexane. ISOLUTE brand bulk silica sorbent (United Kingdom) and ISOLUTE 200 mg Silica 10 mL XL columns (United Kingdom) used during sample clean-up were purchased from

Biotage AB. Supelclean ENVI-Florisil 500mg/3mL SPE cartridges for handwipe

extraction were purchased from Supelco (Bellefonte, PA). Ottawa sand, 20-30 mesh, for

accelerated solvent extraction was purchased from Fisher Scientific (Fair Lawn, NJ).

C. Blood Samples

Blood Sample Collection

While the child was still under general anesthesia after surgery, the nurse

collected 15 mL of blood in two tiger-topped serum separator Vacutainer tubes. Blood

samples were transported to the Barr/Ryan laboratory at the Rollins School of Public

Health, Emory University on the day of collection and immediately processed. As

described by Virginia (2012), serum was separated, aliquoted into freezer-safe storage

vials, and stored at -20°C until analysis.

Serum PBDE Extraction and Quantification

PBDEs were extracted from serum using solid phase extraction (SPE) and a

clean-up method to remove biological contaminants before analysis. 1 mL of each serum

sample was spiked with 100 µL of ISTD (10 ng/mL) and 100 µL of formic acid and

vortex mixed briefly. 5 mL of hexane was added to each sample and then vortex mixed at

2000 rpm for 10 minutes. An additional 5 mL of hexane was added, and samples were

vortex mixed for an additional 5 minutes.

Serum clean-up used acidified-silica columns prepared in-house. Pre-packed

ISOLUTE 200 mg silica columns were used as the base. Acidified silica was produced by

combining 98% H_2SO_4 and ISOLUTE bulk silica (1:2). 1.8 g of acidified silica was layered on top of the premade silica columns, followed by 0.5 g of anhydrous Na_2SO_4. The columns were dried overnight at 100°C. Columns were conditioned with 5 mL of hexane before addition of samples. Samples were briefly vortex mixed and manually shaken prior to loading in the column. Samples tubes were rinsed with 1 mL of hexane, vortex mixed and shaken, and loaded into the column. The sample breakthrough was collected in clean 15 mL tubes. The eluates were evaporated at 30°C at 15 psi. The 15 mL collection tubes were replaced by clean tubes, and the columns eluted again with 10 mL of 1:19 dichloromethane (DCM)/hexane solution. The eluate was collected and combined with the sample breakthrough in the TurboVap, and the samples brought to total dryness at 30°C at 15 psi. Samples were reconstituted with 50 μL of toluene for analysis. GC-MS/MS instrument parameters for serum PBDEs were developed by Virginia (2012).

As PBDEs are lipophilic, serum PBDE levels were lipid adjusted. Total lipid (TL) content of each sample was calculated using the formula TL= (2.27*TC) + TG + 62.3 mg/dL, where TC is total cholesterol and TG is total triglyceride (Bernert et al. 2007). Serum triglyceride content was measured using BioVision Triglyceride Quantification Assay Kit (BioVision Research Products; Mountain View, CA), and serum cholesterol content was measured using Cayman Cholesterol Assay Kit (Cayman Chemical Company; Ann Arbor, MI) according to manufacturer instructions. Serum PBDE levels are reported in ng of PBDE per gram of lipid (ng/g).

D. Household Dust Samples

Dust Sample Collection

15 households agreed to allow a field technician enter their homes and vacuum to collect a dust sample. When arranging a sampling time, participants were asked to refrain from vacuuming so there would be enough dust volume to collect. Parents identified the room that the participant spent the most time in and this room was selected for sampling. Using a solvent-cleaned high volume small surface sampler (HVS3) vacuum (CS3 Inc.; Bend, OR) and solvent-cleaned Teflon® catch bottle, a 1 m^3 square of floor was sampled according to a standard operating procedure (Appendix A). If available, dust from the household vacuum was also collected for future comparison. Samples were transported to the Barr/Ryan laboratory the same day they were collected and were processed within 7 days of sampling. Dust samples were sieved twice to a particle size of less than 250 μm and stored at room temperature in a solvent-cleaned amber jar until analysis.

Dust PBDE Extraction

PBDEs were extracted from dust using accelerated solvent extraction (ASE) and a clean-up method to remove contaminants before analysis. Dust was extracted in duplicate using the ASE 350 (Dionex; Sunnyvale, CA) system. The ASE was operated using the following parameters: 100°C, two 5 min static cycles, 5 min heat time, 60% rinse volume, 60 sec purge time, at approximately 1500 psi, using an extraction solution of 10% (v/v) DCM/*n*-hexane. Cells were packed with 50 mg of dust and 20-30 mesh Ottawa Sand (Fisher Scientific; Fair Lawn, NJ). All runs included a blank sample (sand only) and duplicate QCHs (100 μL of QCH spiked into a blank cell). The extract was blown to

dryness at 40°C using the TurboVap and reconstituted with 5 mL of 10% DCM/hexane solution. Acidified silica clean-up columns, prepared as described in serum extraction section, were preconditioned with 5 mL of hexane. The sample extracts were loaded into the columns and the breakthrough collected. The columns were eluted with 10 mL of hexane, and the eluate added to the breakthrough. Gentle negative pressure was applied to the columns to aid elution. 100 μL of ISTD was added and the samples were dried in the TurboVap. Samples were reconstituted in 50 μL of toluene.

E. Child Handwipe Samples

Handwipe Collection

In the same visit as the dust collection, handwipe samples were collected if the child was present. A total of 13 samples were collected. The child's hands were wiped with a sterile gauze pad that had been soaked in isopropyl alcohol, one pad per hand. The child's hands were carefully wiped up to the wrist and the two gauze wipes combined and stored in a solvent-cleaned brown amber jar. The samples were transferred to the Barr/Ryan laboratory the same day and stored at room temperature until analysis.

Handwipe Extraction

Handwipes were extracted following a modified protocol from Stapleton *et al.* (Stapleton et al. 2008). QCL and QCH were prepared by spiking 100 μL of QCL or QCH standard solutions onto two pieces of gauze soaked with isopropanol. Sample and QC gauze pads were spiked with 100 μL of ISTD immediately prior to extraction. The

combined gauze (right and left hand) were submerged in 30 mL of DCM and sonicated for 20 minutes. This extraction was repeated two times, for a total extraction volume of 90 mL. The extract was dried at 40°C in the TurboVap. 500 mg/3 mL SupelClean ENVI-Florisil SPE cartridges were used to purify the handwipe extracts. The cartridges were preconditioned with 2 mL of hexane. The samples were reconstituted with 2 mL hexane and loaded onto conditioned cartridges. The breakthrough was caught and the cartridge was eluted with 6 mL hexane. The extract was dried in the TurboVap and reconstituted in 50 μL of toluene.

F. Dust and Handwipe PBDE Quantification

A calibration curve (0.1, 0.25, 0.5, 1.25, 2.5, 12.5, 35, and 37.5 ng) of BDE-47, -85, -99, -100, -153, and -154 mixture was prepared. The calibration curve and samples were analyzed for total PBDE (ΣPBDE) and six congeners (BDE-47, -85, -99, -100, -153, and -154) using gas chromatography/mass selective detection (GC/MSD) in house (5975 GC/MSD System; Agilent Technologies). GC parameters are show in Appendix B. The congeners were identified by mass and chromatographic retention time, as shown in Figure 2. The limits of detection, derived from the standard curve, were 0.10 ng for BDE-47, -85, -99, and -100 and 0.25 ng for BDE-153 and -154. Dust PBDE concentrations were blank subtracted and duplicates were averaged for the final concentration.

G. Data Analysis

BMI measurements were calculated using "Children's BMI Group Calculator" Excel system developed by the Centers for Disease Control and Prevention (CDC). The

calculator produces a BMI percentile that is adjusted by height, weight, sex, and age measurements (CDC 2012). Body fat composition varies by gender and age in children. The "age-adjusted BMI percentile" allows for comparison of children regardless of age and gender. BMI cannot be accurately calculated for children less than two years old, so those individuals were removed for BMI analysis.

Statistical analyses were performed using Microsoft Excel 2011 Software (Version 14.3.2) and SAS Statistical Software (Version 9.3). Serum and handwipe measurements below the limit of detection (LOD) were computed by dividing the congener-specific LOD by the square root of two. All data were $\log_{10}$-transformed to correct for skewedness. Log-transformation produced more normal distributions for all congeners and ΣPBDE; therefore, all statistical tests were performed using the log-transformed serum data. One-way ANOVA tests, Student's T-tests, and simple linear regression analyses were used to investigate associations between serum PBDE concentrations and covariates of interest. Correlation analyses were performed to determine associations between serum/dust, serum/handwipe, and dust/handwipe congener and total PBDE levels.

3. RESULTS

A. Demographic Data

The BEAT study had high enrollment with a refusal rate of only 6.3% for participation in the blood draw. The study recruited a total of 81 eligible participants that were all included in analysis. Subject demographic information is presented in Table 2.

Subject age was relatively evenly distributed with two to three year olds representing the largest age group (Table 2). There were slightly more male participants than females. There were a total of 33 African American participants, representing one of the largest numbers in a PBDE exposure study. The majority of participants was breastfed as infants and came from smoke-free homes. Almost half of the participants were in the highest quartile (75>100) for age-adjusted BMI.

B. Serum PBDE Distributions

Lipid-adjusted serum congener levels are presented in Table 3. Frequency of detection above LOD for the various congeners is reported in Table 4. Congener and ΣPBDE concentrations statistics are presented stratified by age, sex, race, insurance status, breastfeeding history, and age-adjusted BMI in Table 5.

Table 3: Serum PBDE Congener and Summed Concentrations (ng/g lipid), n=81					
BDE-	**Mean**	**Median**	**Std Dev**	**Min**	**Max**
47	54.01	36.33	76.38	0.07	642.13
85	1.09	0.64	2.03	0.31	16.41
99	15.03	9.84	22.90	0.31	193.57
100	9.43	5.99	15.00	0.24	125.79
153	10.40	5.73	12.69	1.65	78.81
154	1.65	1.06	2.66	0.51	22.89
SUM	91.61	62.86	125.32	5.74	1079.61

There was wide variation in serum BDE concentrations between subjects. All congener and ΣPBDE distributions were right-skewed, caused by a few subjects with high concentrations. BDE-47 concentrations were the highest of all congeners (Figure 3), while BDE-85 and -154 both had low frequency of detection in serum samples (Table 4).
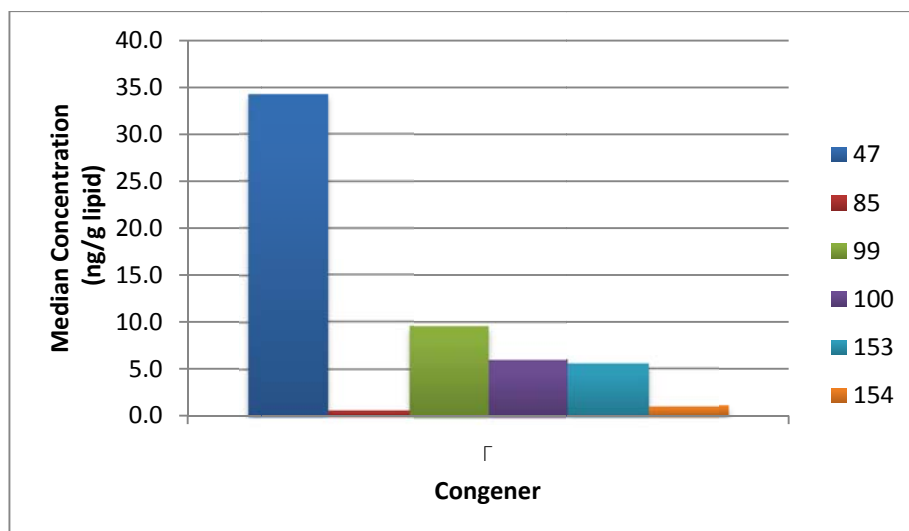
Figure 3: Median Concentration of Serum Congeners

Due to its high frequency of detection and its establishment in the literature as a sentinel BDE, BDE-47 was chosen for further examination. Its distribution was right-skewed with one individual having an extreme value of 642.13 ng/g lipid (Figure 4). 90% of the values were between 6.07 and 141.61 ng/g lipid and the median value was 36.33 ng/g lipid. Participant who were five years old or under two years old had the highest median BDE-47 serum concentrations (Figure 5). Those under two years of age also has the highest median concentrations of BDE-99, -100, and -153.
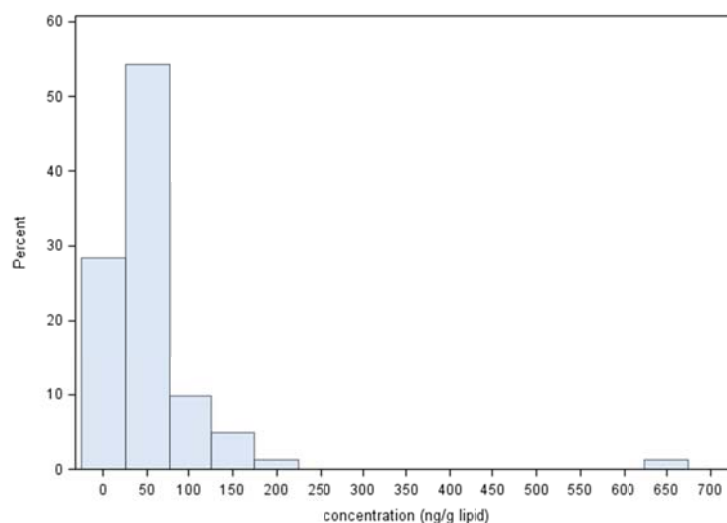
Figure 4: Serum BDE-47 Distribution of Concentrations
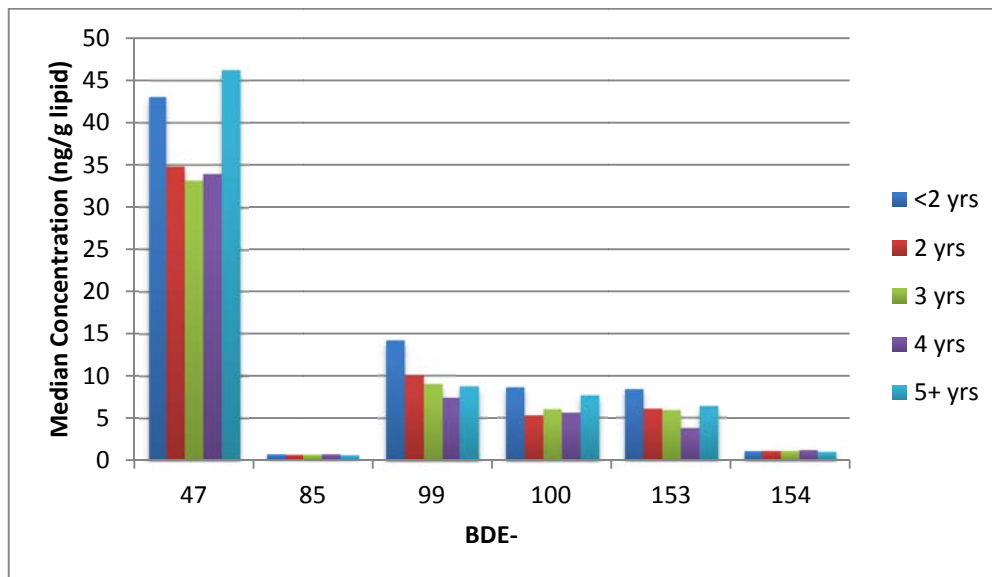
Figure 5: Distribution of Serum Congeners by Age Group

ΣPBDE serum concentrations followed a pattern similar to BDE-47 as the summed value was driven mostly by BDE-47. The data were right-skewed with one extreme high value at 1079.61 ng/g lipid (Figure 6). The median ΣPBDE concentration was 62.86 ng/g lipid, with 90% of values falling between 15.44 and 236.91 ng/g lipid.
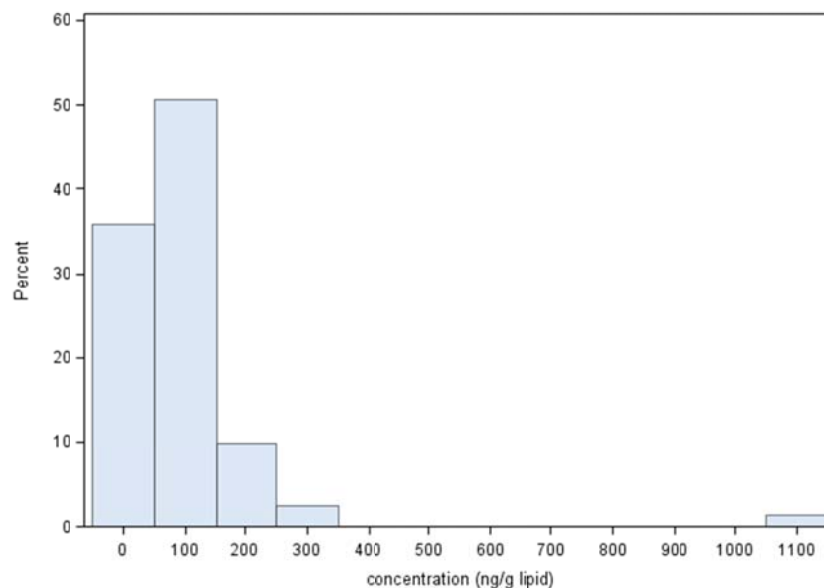
Figure 6: Serum ΣPBDE Distribution of Concentrations

C. Serum PBDE Associations

Statistical Analysis

Congener specific and ΣPBDE associations with age, sex, race, insurance status, breastfeeding status, and age-adjusted BMI were tested. Age and BMI were tested both as continuous and categorical variables. Age was categorized by years of age, and BMI data were divided into quartiles to produce categorical variables. Continuous variables, age and BMI, were tested for associations with serum PBDE concentrations using simple linear regression. One-way ANOVA tests were performed for categorical variables with more than two groups (age, BMI, and race). Student's T-tests were used to test for associations between serum concentrations and sex, breastfeeding history, and insurance status.

Age and Serum PBDE Concentrations

Age (in months) was relatively normally distributed. Simple linear regression found that there was no association between age in months and individual congeners or ΣPBDE concentrations. Categorical age was also not associated with specific congener or ΣPBDE concentrations.

Sex and Serum PBDE Concentrations

Being a female was associated with higher levels of BDE-47 (t=-2.77, p=0.007) and ΣPBDE (t=-2.61, p=0.0109). Females had a mean BDE-47 concentration of 47.79 ng/g lipid compared to 23.16 ng/g lipid for males. Mean ΣPBDE concentrations were

82.30 ng/g lipid for females and 51.44 ng/g lipid for males. While not significant at the $\alpha=0.05$ level, BDE-99 and -100 approached significance, with females having higher mean concentrations.

Race and Serum PBDE Concentrations

Race was divided into four categories for statistical analysis: African American, Caucasian-White, Caucasian-Hispanic, and other. BDE-100 serum concentrations differed between the racial groups, with a F-value of 6.16 and p=0.0008. Mean BDE-100 values were 8.51, 3.16, 1.86, and 1.41 ng/g lipid for African American, Caucasian-White, Caucasian-Hispanic, and other, respectively.

Insurance Status and Serum PBDE Concentrations

Insurance status was not found to be associated with serum PBDE concentrations.

Breastfeeding and Serum PBDE Concentrations

Child history of breastfeeding was significantly associated with BDE-47 concentration. Non-breastfed children had higher mean BDE-47 concentrations than breastfed children (t=2.02, p=0.0465). Mean BDE-47 was 43.66 ng/g lipid for non-breastfed children and 25.39 ng/g lipid for breastfed children.

BMI and Serum PBDE Concentrations

Age-adjusted BMI percentile was normally distributed and did not need to be log adjusted. When tested as a continuous variable, there was a significant negative association between BDE-153 and BMI percentile at the α=0.05 level. The R^2 for the association was 0.0911, with a p-value of 0.0111. For every increase in BMI percentile, there is a 1.007 ng/g lipid decrease in BDE-153. A similar trend was observed when BMI was tested as a categorical variable. BDE-153 concentrations differed between BMI quartiles, with a F-value of 3.87 and a p-value of 0.0130. BDE-153 mean concentrations were 8.51, 11.75, 6.03, and 5.13 ng/g lipid for the 0>25, 25>50, 50>75, and 75>100 BMI groups, respectively.

D. Dust PBDE Distributions

A convenience sample of eligible children was selected for household dust and handwipe sampling. The desired sample size was 20, but there was difficulty in obtaining permission for home visits. To boost participation, all participants' families within a 25 mile radius of Emory were contacted about home sampling. The final sample size for household dust was 15. Refusal for participation in the dust sampling portion of the study was extremely high, likely from difficulties in scheduling and the invasiveness of a home visit.

All congeners were detectable in household dust (Table 4). All congeners and ΣPBDE were right-skewed, with a few extreme high values causing the tailing. There was wide variation in congener and total PBDE concentrations between samples (Figure 7). Table 6 presents the descriptive statistics for the concentration of individual congeners

and ΣPBDE in dust. BDE-99 and -47 were the most prevalent congeners in dust,

accounting for approximately 49% and 28% of total measured PBDE, respectively. BDE-

99 had a median concentration of 579.20 ng/g of dust, and BDE-47 was 405.26 ng/g.
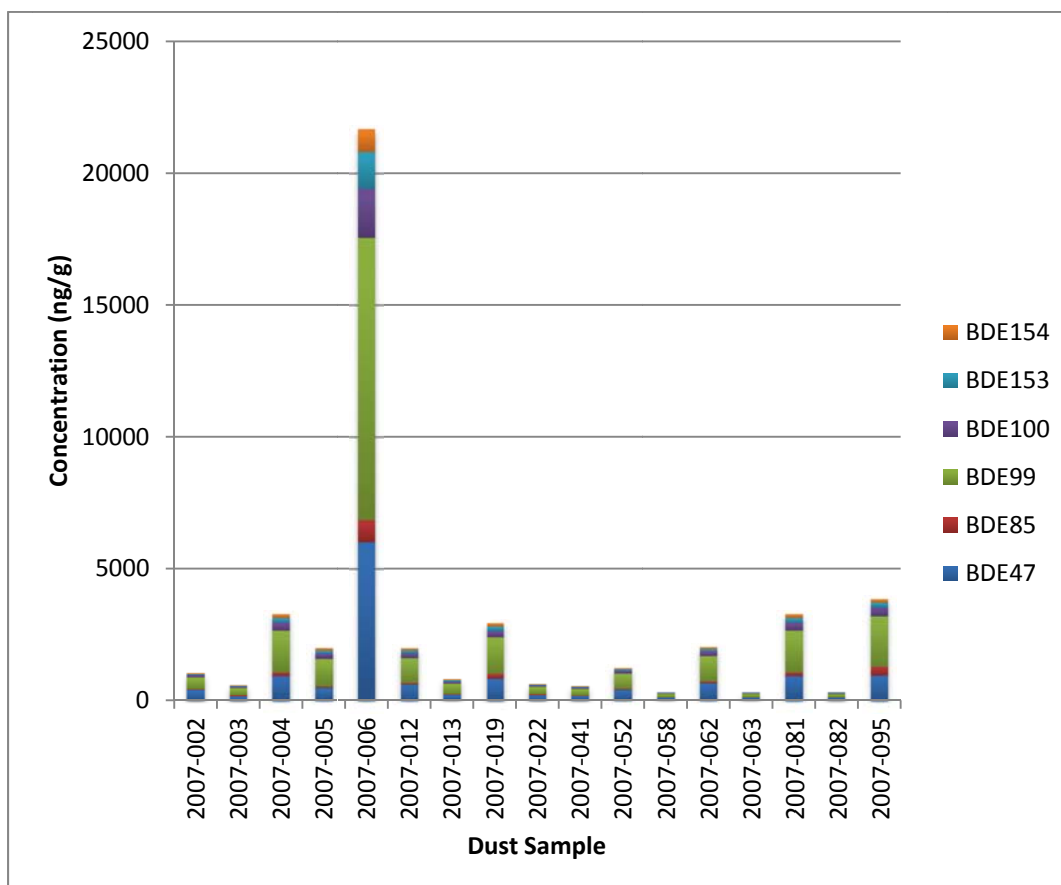
Figure 7: Congener and ΣPBDE Concentrations in Dust by Sample

Table 6: Dust PBDE Congener and Summed Concentrations (ng/g), n=15					
BDE-	**Mean**	**Median**	**Std Dev**	**Minimum**	**Maximum**
47	813.91	405.26	1469.70	95.02	6012.43
85	123.44	39.48	217.25	7.62	840.98
99	1397.19	579.20	2631.46	127.25	10701.50
100	248.55	101.35	457.35	23.92	1862.65
153	170.91	60.77	343.56	13.41	1393.50
154	115.16	40.93	213.40	10.25	868.65
Sum	2869.16	1221.80	5323.64	293.00	21679.71

E. Handwipe PBDE Distributions

Handwipes were collected at the same time as household dust sampling so handwipe levels could be correlated with dust levels. Only thirteen handwipe samples were collected, because either the protocol had not yet been implemented or some children were not present at the house at the time of the visit.

Handwipe congener detection was good, with 100% detection rate for BDE-47, -99, and -100 and over 60% for all other congeners (Table 4). Handwipe PBDE concentrations were right-skewed and varied widely by sample (Figure 8). Descriptive statistics for the congeners and ΣPBDE are presented in Table 7. BDE-99 and -47 were the most prevalent congeners in the handwipes, accounting for approximately 43% and 39% of total measured PBDE (Table 7).
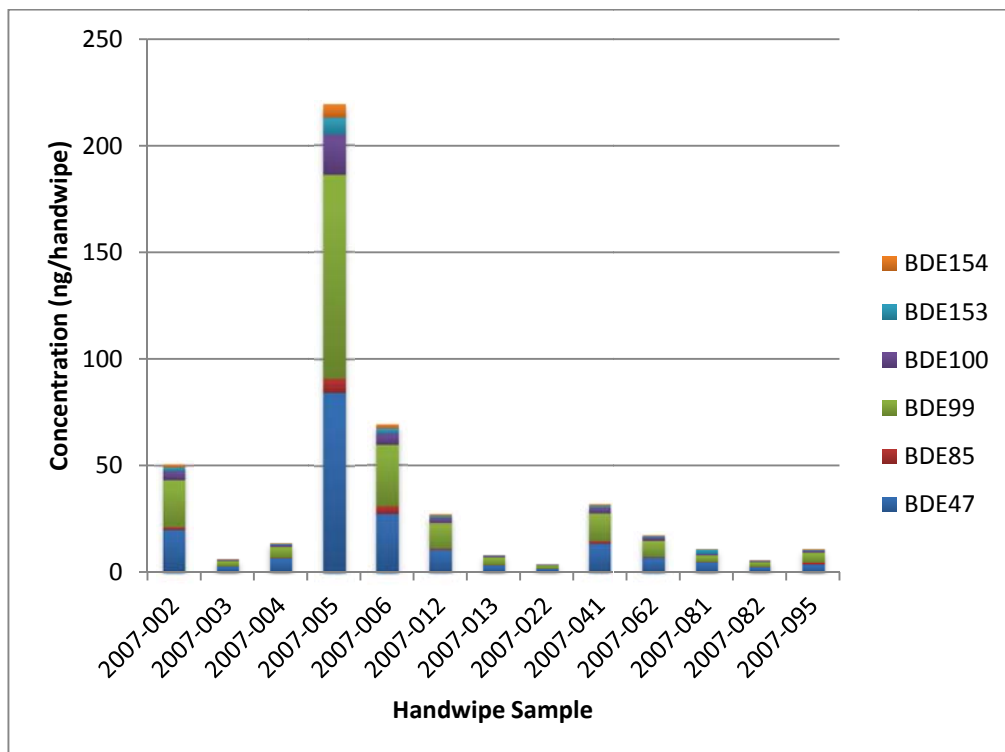
Figure 8: Congener and ΣPBDE Concentrations in Handwipes by Sample

Table 7: Handwipe PBDE Congener and Summed Concentrations (ng/handwipe), n=13					
BDE-	**Mean**	**Median**	**Std Dev**	**Minimum**	**Maximum**
47	14.31	6.46	22.40	1.44	84.27
85	1.17	0.42	1.89	0.07	6.67
99	15.44	5.01	25.34	1.48	95.20
100	2.97	0.97	4.98	0.23	18.73
153	1.43	0.63	2.11	0.18	7.95
154	1.04	0.35	1.70	0.18	6.39
Sum	36.27	13.46	58.35	3.14	219.22

F. Serum, Dust, Handwipe Correlations

All serum, dust, and handwipe PBDE data were non-normal. Therefore $\log_{10}$ transformed data were used for all correlation tests. While there were 15 dust samples, there were 17 children with dust PBDE data available for comparison because some children lived in same household.

Serum and dust individual congeners and ΣPBDE were not correlated. Dust and handwipes PBDE levels were also not correlated at the α=0.05 level. However, BDE-153 approaches significance (r=0.50, p=0.0818).

Serum and handwipes were correlated for BDE-47 and ΣPBDE. For BDE-47, the Pearson's correlation coefficient, r, is equal to 0.652 and p is 0.0158. For ΣPBDE, r=0.607 and p=0.0278. The correlation between handwipe and serum BDE-99 concentrations was significant at the α=0.10 level (r=0.518, p=0.0640).

4. DISCUSSION

A. Serum PBDE Study Design

Only four studies investigating serum concentrations of PBDEs in young American children currently exist (Lunder et al. 2010; Rose et al. 2010; Eskenazi et al. 2011; Stapleton et al. 2012). Three of the studies were undertaken in study populations that are not representative of the general American public. Lunder *et al.* (2010) studied 20 mother-child pairs, the majority of whom were high SES, white Caucasians. Eskenazi *et al.* (2011) evaluated PBDEs in 7 year olds from a low-income, Mexican-American agricultural group as a part of the Center for Health Assessment in Mothers and Children of Salinas (CHAMACOS) study. Rose *et al.*'s (2009) study population was selected from the Childhood Autism Risks from Genetics and the Environment (CHARGE) study and included autism and developmental delay cases and matched controls from California.

This study adds to the literature by providing a more diverse study population with a large sample size to test for potential predictors of exposure. 33 African American children were enrolled, representing the largest number to date. SES, as determined by insurance status, was mixed for this population with approximately 63% on Medicaid. In Lunder *et al.* (2010), all children were breastfed; in this current study 60% of the children were breastfed, allowing for analysis of PBDE concentrations by breastfeeding history. The results of this study can be more widely applied to the general population of young children than previous studies.

Additionally, the current study utilized a novel blood draw technique that was well accepted by parents. By drawing blood while the child was under general anesthesia, complications associated with blood draws in young children, such as discomfort, were

avoided. There was no additional time burden on parents as children were already at the hospital, and participants were enrolled and sampled the same day. The subjects were undergoing routine surgeries unrelated to the health outcome of interest, and therefore represented a relatively health and demographically diverse subset of the general population. This enrollment and sampling technique has tremendous potential for use in future studies requiring blood samples from similar age groups.

B. Serum PBDE Distribution and Associations

Serum congener and ΣPBDE levels were highly varied between individuals (Table 3). BDE-47 ranged from 0.07 to 642.13 ng/g lipid, and ΣPBDE from 5.74 to 1079.61 ng/g lipid. Studies in adults have found there is approximately a 100-fold range in variability, and children have been noted to have greater variability in PBDE serum concentrations than adults (Birnbaum and Hubal 2006; Lunder et al. 2010). BDE-47 and -99 had the highest median concentrations and were the most frequently detected congeners, which is consistent with the literature (Rose et al. 2010; Stapleton et al. 2012). When compared to values found in the previous four studies, BDE-47 median concentration fell in the middle (Figure 9). Both the CHARGE and CHAMACOS groups had higher median concentrations of serum BDE-47 (Rose et al. 2010; Eskenazi et al. 2011). This is likely due to both study populations being located in California, where exposure tends to be higher because of strict fire regulations resulting in the high use of flame retardants (Zota et al. 2008).
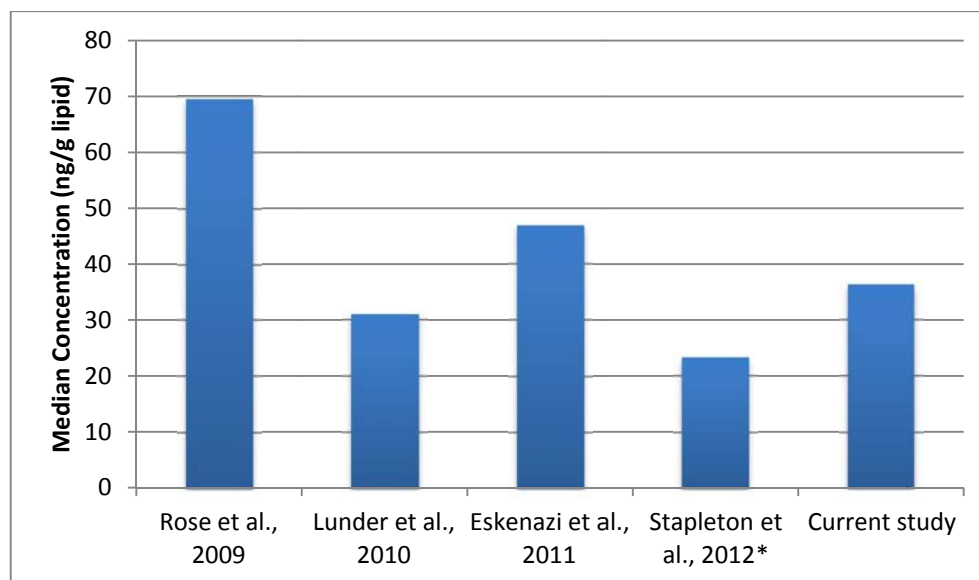
Figure 9: Median serum BDE-47 concentrations from literature.
*Stapleton *et al.* (2012) was reported as geometric mean.

Table 8: Significant Associations between Serum PBDE Concentrations and Covariates of Interest		
Significant Covariates	**Congener**	**Association**
Sex	-47, ΣPBDE	Higher levels in females
Race	-100	Highest levels in African Americans
Breastfeeding History	-47	Higher levels in non-breastfed
BMI	-153	Concentration decreases with BMI; Highest levels in BMI 25>50

A list of covariates that were significantly associated with serum PBDE levels are listed in Table 8. Age was not a significant predictor of exposure. Previous studies have reported mixed results with regard to age. Toms *et al.* (2009) reported peak concentrations at 2.6 to 3 years of age corresponding to increased toddling behaviors. In Stapleton *et al.* (2012), ΣBDE-47, -99, -100 (ΣBDE_3) and BDE-153 burdens increased with age, perhaps because of accumulation over time of increased hand-to-mouth activity, but Rose *et al.* (2009) found no association with age.

In the current study, being a female was associated with higher BDE-47 and ΣPBDE concentrations. Gender was not identified as a potential predictor of PBDE exposure in any of the other studies, although small sample size in the Lunder *et al.* (2010) and CHARGE studies may have limited the statistical power to detect such an association. It is unknown why female children would have higher body burdens of BDE-47 and ΣPBDE, but could be attributable to specific gender activities.

This is one of the first studies including a substantial African American subset, so no good comparison group exists. BDE-100 differed between racial groups, with African American children having the highest concentrations. Similarly, non-white children had higher ΣBDE_3 compared to white children (Stapleton et al. 2012). Interestingly, Rose *et al.* (2009) found that Hispanic children had higher levels of lower brominated congeners but low levels of higher brominated congeners compared to non-Hispanic children. In our study, Hispanic children had the lowest concentrations for BDE-47, -99, -100, -153, and ΣPBDE of any racial group. Clearly, racial trends are not well defined and will need to be further elucidated by future studies.

It was hypothesized that being breastfed would result in a child having higher PBDE body burden as a result of maternal transfer of PBDEs via breast milk. However, BDE-47 was negatively associated with breastfeeding. Breastfeeding status associations vary greatly in the literature. For CHARGE, BDE-47 and the sum of lower brominated congeners (ΣBDE-28-153) were significantly lower in breastfed children younger than 3.7 years of age, but significantly higher in children older than 3.7 years of age (Rose et al. 2010). Lunder *et al.* (2010) found that age of weaning and duration of exclusive breastfeeding were not associated with child serum levels.

Review of the literature suggests that child BDE-153 exposures differ from the rest of the Penta congeners (BDE-47, -99, -100) by pathway. Duration of breast-feeding was a strong predictor of BDE-153 but not sum BDE-47, -99, and -100 (Stapleton et al. 2012). In breastfed children, BDE-153 was positively associated with age; conversely, in non-breastfed children, BDE-153 was negatively associated with age (Rose et al. 2010). Also BDE-153 comprised a larger portion of ΣBDE-28-153 in breastfed children than non-breastfed children (Rose et al. 2010). BDE-153 is enriched in maternal adipose tissue compared to other Penta congeners, and is found in greater levels in breast milk despite only being 5-8% of the commercial mixture (La Guardia et al. 2006). Taken together, the data suggest that the primary exposure to BDE-153 in children is via breast milk and to BDE-47,-99, and -100 via some other pathway, likely dust. However, we did not observe any association between serum BDE-153 and breastfeeding history.

It has been posited that low-income individuals may have greater PBDE exposure because they are more likely to have older furniture that was treated with Penta and Octa before the ban (Zota et al. 2008; Quiros-Alcala et al. 2011). Therefore we hypothesized that lower SES would be associated with higher PBDE body burdens. In our study, we used insurance status (private vs. Medicaid) as a proxy for SES and found no associations. However, higher paternal education level was strongly and inversely related to ΣBDE$_3$ (Stapleton et al. 2012), while maternal education level is negatively associated with BDE-47, -66, -85, -99, 100, -153, -197, and -209 levels (Rose et al. 2010). If SES is truly associated with PBDE serum levels, insurance status may not be the appropriate proxy measurement.

PBDEs are lipophilic chemicals, and therefore we expected them to be elevated in higher BMI groups. The inverse was found with BDE-153, with concentration decreasing with increasing age-adjusted BMI. When tested as a categorical variable, the highest BDE-153 level was found in the 25>50 percentile group. PBDE concentrations were lipid-adjusted, likely obscuring the relationship between BMI and PBDE levels. In Rose *et al.* (2009), neither BMI nor weight-for-age were found to be important predictors. BMI is likely not an important covariate for predicting child serum PBDE levels.

C. Dust and Handwipe PBDE Study

Participation in the household dust sampling was very low, and we were unable to reach the desired subset sample size of 20. This was likely due to difficulties scheduling home visits, and the inherent invasiveness of having a stranger in your home. Anecdotally, participants were more willing to provide a used vacuum bag than having the technician vacuum. Participants were asked not to vacuum three days prior to the house visit, but not all complied, raising problems with standardization.

Congener and total BDE levels in dust were extremely variable for different households. The cause of such wide variability is not know currently, although there are several studies attempting to correlate the number of PBDE-treated items with dust PBDE levels in homes. In household dust, BDE-47 and -99 had the highest concentrations of all congeners and accounted for approximately 77% of ΣPBDE. BDE-47 and -99 have been routinely found to be major constituents of household dust. However, when measured, BDE-209 was also a major component of dust (Frederiksen et al. 2009; Allen et al. 2008). In the United States, reported median dust concentrations for

BDE-209 are at least 1 mg/g and 0.1 mg/g for BDE-47 and -99 (Whitehead et al. 2013). Therefore our dust measurements may greatly underestimate ΣPBDE without inclusion of BDE-209.

During dust sample quantification, significant differences between duplicates were noted for two samples (2007-005 and 2007-006). Samples were reextracted, and a value between the two original values found, although closer to the higher value. These samples had large total sample volumes, and we suspect that the dust may not have been completely homogenous. The 50 mg of dust sampled was such a small proportion of the total volume that it may have resulted in a non-representative sample. Future study protocol should take this potential into account and ensure that samples are well mixed prior to extraction.

In our study, dust was poorly correlated with serum levels. The majority of literature has found good correlations for residential dust and serum levels (Zota et al. 2008; Johnson et al. 2010; Stapleton et al. 2012). Problems may be from non-standardized sampling; not all families complied with the non-vacuum request. Also it is well established that PBDE concentrations can vary by room, so we may not have sampled the room that accounts for the majority of child exposure (Watkins et al. 2011a, b; Allen et al. 2008; Muenhor and Harrad 2012).

Handwipe collection was relatively easy and quick in comparison to dust collection. Overall, handwipes had good detection of congeners. BDE-99 and -47 were the congeners most frequently detected and had the highest median concentrations, in agreement with current literature (Stapleton et al. 2012; Watkins et al. 2011a, b; Stapleton et al. 2008). Again BDE-209 was not measured but it appears to be an important

contributor to handwipe PBDE levels (Stapleton et al. 2012; Watkins et al. 2011b; Stapleton et al. 2008).

Handwipes were correlated with serum for BDE-47 and ΣPBDE and approached significance BDE-99. Likewise, the four existing handwipe studies found that handwipes were better at predicting serum levels than dust. Therefore it appears that handwipes may be a good surrogate measure for Penta exposure in children. Dust and handwipe levels were not correlated, which was not unexpected as the literature has shown only weak correlations. Handwipes are a more integrative measure of exposure as it represents all the locations a child has recently been, whereas dust only provides information about one particular microenvironment. However, a potential issue with handwipes is that handwashing immediately prior to sampling may reduce handwipe levels so they are no longer representative of body burden (Watkins et al. 2011b). It was noted that one child had washed his hands before sampling, and we are unsure what effect this had on his handwipe levels. Despite this problem, handwipe sampling has the advantage of being faster, easier, and easier to standardize than dust collection.

D. Limitations

While sample size for our study was much larger than those previously studied, power was still limited when the group was broken down into strata. Likewise, the sample size for the dust and handwipes was very small. We did not have appropriate statistical power, and we may have missed some correlations. Dust sample size was intended to be larger, but there was great difficulty gaining subject participation. It is unclear what can be done to boost participation in dust sampling.

Another major limitation of this study was that BDE-209 was not measured, despite evidence that it is an important contributor to ΣPBDE. As BDE-209 is the principle component of the Deca commercial mixture, we have no data on Deca exposure. Knowing Deca exposure is important because it is only PBDE still currently produced in the United States and there is increasing evidence that it may be more biologically relevant than first thought (Frederiksen et al. 2009).

E. Future Studies

Another branch of the BEAT study will be the investigation of serum thyroid hormone levels in the study population. We want to determine if there are any associations between serum PBDE and thyroid hormone levels, as that has been an experimentally determined health outcome for PBDE exposure.

Due to time constraints, several proposed components of the current study were not completed. In the full study, dust and handwipe samples will be reanalyzed for BDE-209. Also, technician collected dust samples will be compared to home vacuum dust samples to see if they are well correlated. If so, home vacuum bags could be an easy way to boost participation in the study.

A potential future study is determination of the relative contributions direct absorption (dermal), inhalation, and ingestion to dust exposures, and if there are differences in congener composition between these three exposure scenarios. In the future, we may try to sieve particles into fractions that would represent inhalation, ingestion, and dermal exposures.

Beyond the scope of this study, little is known about the exposure potential of the flame retardants replacing Penta and Octa. Studies on exposure and toxicity to new flame retardants like Firemaster 550 and Chlorinated Tris must be undertaken as PBDE exposures begin to decline.

F. Conclusions

The data generated by BEAT project is intended for publication in peer-reviewed scientific journals. For young child serum PBDE levels, our study represents one of the largest and most diverse groups studies to date. Additionally, only one other study has investigated child serum, child handwipes, and household dust in the same individuals for PBDEs. This study represents a much need addition to the current scientific literature.

The future aim of BEAT is to scale-up this pilot study in order to better identify trends and associations between serum, dust, handwipes, and thyroid hormone levels. In general, more studies are needed to test predictors of exposure as the current understanding is very limited. Likewise, handwipes appear to have potential as a new matrix, but more studies and validation are needed to fully determine the variability and factors that may affect results.

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7. TABLES & FIGURES

Table 1. Study Compounds, Sources and Grades		
Compound	**Grade/Purity**	**Source**
Dichloromethane (DCM)	HPLC Grade/≥99.8%	Sigma Aldrich®
Hexane (Hex)	98.5%	Sigma Aldrich®
Sulfuric Acid	98.08%	Macron Fine Chemicals™
Anhydrous Sodium Sulfate	>99.0%	Sigma Aldrich®
Toluene	92.14%	Macron Fine Chemicals™
Isopropyl Alcohol	99.5%	EMD Chemicals Inc.

Figure 2: GC/MSD PBDE Congener Chromatogam Profile by Acquisition Time
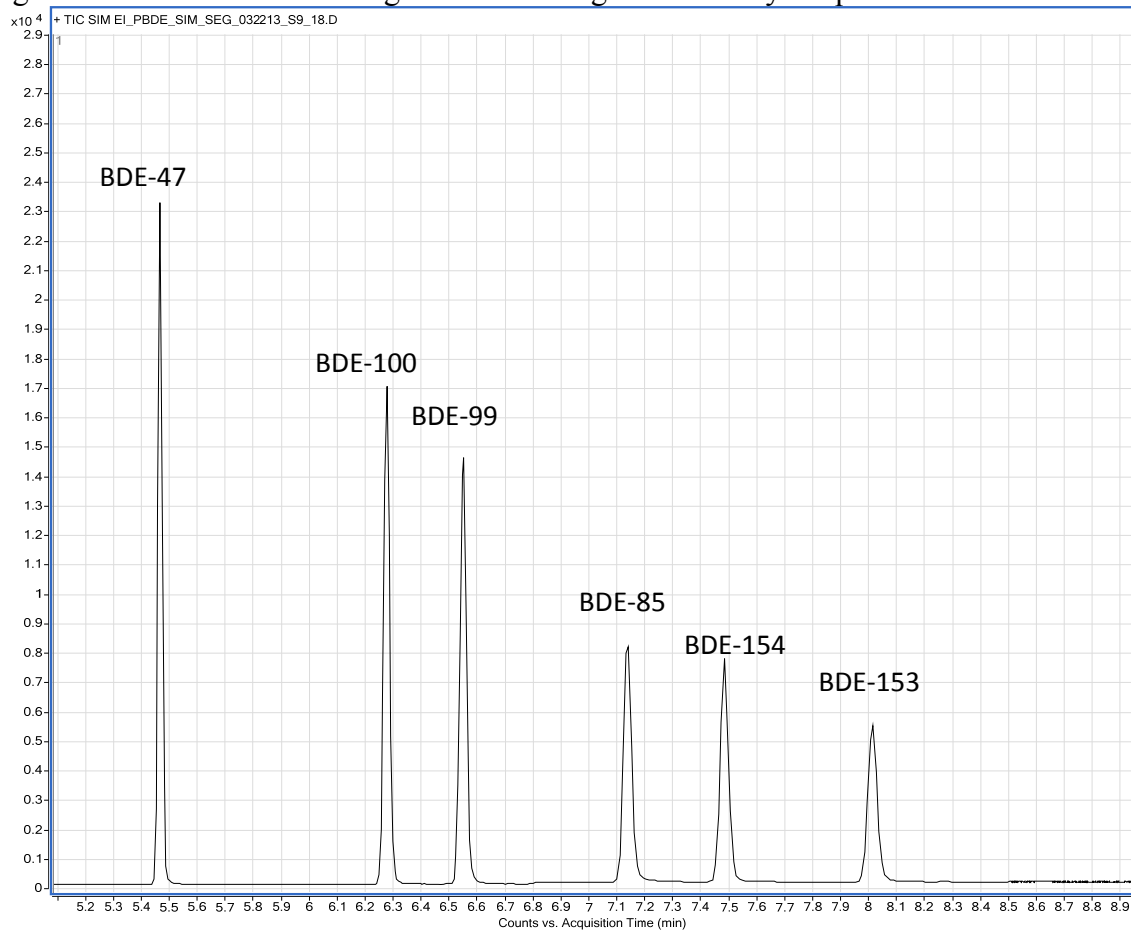
Table 2: Subject Demographic Information		
	n	**Percent**
Age (years)	81	
>2	10	12.35
2-3	26	32.1
3-4	18	22.22
4-5	13	16.05
5+	14	17.28
Sex	81	
Male	46	56.79
Female	35	43.21
Race	81	
African American	33	40.74
Caucasian	39	48.15
Other	9	11.11
# of Surgical Procedures	81	
1	36	44.44
2	30	37.04
3	15	18.52
Household Smoking	70	
No	56	80
Yes	14	20
Insurance Status	81	
Medicaid	51	62.96
Private	30	37.04
Breastfeeding Status	81	
No	33	40.74
Yes	48	59.26
BMI Age-Related Percentile	70*	
0>25	16	22.86
25>50	9	12.86
50>75	13	18.57
75>100	32	45.71

* BMI measurements for children under 2 years of age cannot be reliably compared. Subjects with incomplete height and/or weight data or under 2 years were not included in BMI analyses.

Table 4: Congener Frequency of Detection in Different Matrices		
Sample Type	**BDE-**	**Frequency (%)**
Serum (n=81)	47	89.77
	85	11.11
	99	98.77
	100	82.72
	153	66.67
	154	14.81
Dust (n=15)	47	100
	85	100
	99	100
	100	100
	153	100
	154	100
Handwipes (n=13)	47	100
	85	86.67
	99	100
	100	100
	153	69.23
	154	61.54

Table 5: Stratified Serum PBDE Congener and Summed Concentrations (ng/g lipid)							
	N	BDE-	Mean	Median	Std Dev	Min	Max
Age (years)							
<2	10	47	119.49	43.05	190.02	7.45	642.13
		85	2.21	0.66	4.99	0.41	16.41
		99	33.18	14.17	57.64	3.04	193.57
		100	21.45	8.61	37.62	0.29	125.79
		153	14.61	8.35	22.98	2.33	78.81
		154	3.21	1.07	6.92	0.66	22.89
		SUM	194.15	72.34	319.00	16.28	1079.61
2-3	26	47	41.01	34.82	30.41	0.10	141.61
		85	0.90	0.61	0.93	0.38	4.16
		99	13.16	10.07	11.87	0.31	55.37
		100	6.06	5.29	5.44	0.25	24.82
		153	7.67	6.06	4.72	2.46	18.28
		154	1.66	1.06	1.62	0.62	8.16
		SUM	70.47	58.13	49.83	5.74	251.99
3-4	18	47	51.47	33.24	50.13	0.07	193.85
		85	1.23	0.65	1.82	0.37	8.06
		99	12.82	9.03	10.51	3.55	36.05
		100	9.99	6.00	8.62	0.30	29.21
		153	9.60	5.88	8.42	1.65	28.15
		154	1.45	1.06	1.32	0.68	6.22
		SUM	86.55	62.92	69.04	24.60	275.40
4-5	13	47	37.25	34.01	23.64	7.68	75.76
		85	0.95	0.67	0.92	0.39	3.95
		99	10.22	7.41	7.75	2.22	24.43
		100	6.43	5.60	6.39	0.28	17.88
		153	9.20	3.79	10.14	2.78	36.54
		154	1.40	1.15	0.74	0.63	3.55
		SUM	65.47	59.44	41.33	15.44	126.03
5+	14	47	50.20	46.24	37.18	4.54	149.36
		85	0.61	0.58	0.18	0.31	1.01
		99	12.85	8.78	12.65	1.46	52.13
		100	9.19	7.66	8.38	0.24	26.27
		153	14.58	6.38	18.58	2.53	69.37
		154	0.99	0.95	0.30	0.51	1.64
		SUM	88.41	72.09	62.43	11.19	237.33
Sex							
Male	46	47	38.36	29.11	32.61	0.07	149.36
		85	0.80	0.63	0.70	0.38	3.95
		99	12.03	8.00	11.94	0.31	55.37
		100	6.71	5.01	6.69	0.24	24.82
		153	9.36	4.89	11.56	1.65	69.37
		154	1.40	1.06	1.20	0.63	8.16
		SUM	68.67	57.58	54.22	5.74	251.99

Female	35	**47**	74.57	47.65	107.49	4.54	642.13
		85	1.48	0.65	2.97	0.31	16.41
		99	18.97	12.55	31.89	1.46	193.57
		100	13.01	7.68	21.14	0.25	125.79
		153	11.76	8.06	14.10	2.49	78.81
		154	1.97	1.06	3.81	0.51	22.89
		SUM	121.77	79.74	177.24	11.19	1079.61
Race							
African American	33	**47**	52.96	41.54	41.61	0.07	193.85
		85	1.18	0.65	1.54	0.38	8.06
		99	15.46	12.44	11.55	3.60	55.37
		100	11.16	9.25	7.49	0.29	29.21
		153	10.90	9.69	8.46	2.49	37.47
		154	1.75	1.15	1.67	0.62	8.16
		SUM	93.41	79.74	61.20	26.68	275.40
White Caucasian	30	**47**	61.74	40.07	115.00	0.10	642.13
		85	10.01	4.97	22.76	0.24	125.79
		99	16.68	7.92	34.78	0.31	193.57
		100	1.28	0.66	2.93	0.31	16.41
		153	1.87	1.10	4.00	0.51	22.89
		154	11.84	4.82	18.24	2.33	78.81
		SUM	103.42	61.15	191.79	5.74	1079.61
Hispanic Caucasian	7	**47**	34.83	26.33	35.39	4.54	110.78
		85	5.04	2.17	7.12	0.28	20.18
		99	8.76	6.20	7.20	1.46	23.18
		100	0.57	0.60	0.18	0.37	0.87
		153	1.34	1.05	0.79	0.63	2.49
		154	4.78	3.34	2.69	2.78	9.52
		SUM	55.33	37.16	52.01	11.19	166.52
Other	9	**47**	39.49	32.56	22.77	15.56	86.40
		85	0.67	0.61	0.29	0.42	1.42
		99	9.50	8.78	5.24	3.55	19.57
		100	3.31	2.75	3.48	0.25	9.88
		153	8.90	6.67	8.16	1.65	26.12
		154	0.93	0.95	0.13	0.68	1.06
		SUM	62.81	61.51	33.82	24.60	122.48
Insurance							
Medicaid	51	**47**	56.09	33.92	89.63	0.07	642.13
		85	1.21	0.64	2.34	0.37	16.41
		99	16.20	9.91	27.23	1.46	193.57
		100	10.57	6.82	17.95	0.25	125.79
		153	12.31	7.68	15.07	1.65	78.81
		154	1.97	1.06	3.31	0.63	22.89
		SUM	98.35	63.39	149.31	11.19	1079.61
Private	30	**47**	50.47	40.33	47.13	0.10	193.85
		85	0.89	0.65	1.37	0.31	8.06
		99	13.04	8.31	12.71	0.31	52.13
		100	7.51	5.37	7.71	0.24	29.21

		153	7.14	4.82	5.95	2.33	26.12
		154	1.10	1.08	0.35	0.51	1.97
		SUM	80.15	62.18	68.33	5.74	275.40
Breastfeeding Status							
No	33	47	74.28	47.55	111.75	7.68	642.13
		85	1.43	0.64	3.02	0.31	16.41
		99	19.18	11.18	33.06	2.22	193.57
		100	12.18	6.81	21.97	0.25	125.79
		153	9.60	5.73	13.28	2.49	78.81
		154	1.95	1.07	3.89	0.51	22.89
		SUM	118.63	69.95	184.79	15.44	1079.61
Yes	48	47	40.07	32.32	30.67	0.07	149.36
		85	0.86	0.64	0.83	0.38	4.16
		99	12.18	8.58	11.31	0.31	55.37
		100	7.55	5.98	6.74	0.24	26.27
		153	10.94	6.18	12.38	1.65	69.37
		154	1.44	1.06	1.27	0.63	8.16
		SUM	73.04	60.18	51.14	5.74	251.99
BMI (Age Adjusted Percentile)							
0>25	16	47	42.28	27.07	38.38	0.07	129.09
		85	0.78	0.65	0.61	0.39	3.00
		99	11.23	8.58	10.10	0.31	32.85
		100	9.78	7.62	8.89	0.31	26.27
		153	16.02	10.26	17.89	2.49	69.37
		154	1.48	1.06	1.36	0.63	6.22
		SUM	81.57	68.57	60.84	5.74	211.61
25>50	9	47	71.04	61.60	54.66	8.53	193.85
		85	0.94	0.57	1.13	0.37	3.95
		99	17.79	18.06	11.09	2.98	36.05
		100	12.70	11.72	9.06	0.26	29.21
		153	14.85	12.49	10.27	2.53	36.54
		154	1.25	1.07	0.60	0.72	2.49
		SUM	118.58	119.33	73.86	24.20	275.40
50>75	13	47	43.26	28.64	39.03	5.47	141.61
		85	1.18	0.64	1.26	0.41	4.16
		99	13.74	6.89	15.06	1.87	55.37
		100	5.51	3.80	6.93	0.25	24.82
		153	7.45	5.69	5.12	1.65	18.28
		154	1.92	1.07	2.19	0.66	8.16
		SUM	73.06	46.20	65.10	12.60	251.99
75>100	32	47	39.75	38.19	26.15	4.54	149.36
		85	0.92	0.65	1.34	0.31	8.06
		99	11.17	8.60	9.32	1.46	52.13
		100	6.23	5.85	5.01	0.24	23.54
		153	6.24	4.09	4.54	2.46	22.19
		154	1.26	1.06	0.68	0.51	3.55
		SUM	65.57	62.01	40.14	11.19	237.33

8. APPENDICES

A. Standard Operating Procedure for BEAT Dust Collection

1. Title of Standard Operating Procedure

   Emory University/RSPH Standard Operating Procedures:
   F01   BEAT STUDY Collection of House Dust Samples for Pesticide Analysis, Rev. 1.0.

2. Overview and Purpose

   This standard operating procedure reviews the procedures for house dust field collection for the BEAT (Body Burden) Study. The collection method uses a high volume small surface sampler (HVS3) for dust collection from bare and carpeted floors.

3. Discussion

    The HVS3 built by CS3 of Bend, Oregon, has a sampling train made from aluminum.   For sampling pesticides, Teflon gaskets and tube connections are used. The HVS3 may be connected to normal household outlets. House dust more than 5 μm in diameter will be collected. Particles larger than 150 μm in diameter will be sieved out in the laboratory before analysis, as larger particles are less likely to adhere to skin or to be ingested inadvertently by the age group studied. Pesticides will be measured in units of ng/g. In addition, the area sampled will be recorded, so loadings can also be expressed as mass per square meter.

4. Personnel Responsibilities

Sampler Preparation and Maintenance

   Teflon catch bottles and all metal parts of the HVS3 will be cleaned at RSPH according to SOP "L01 Trace cleaning Vacuum and collection bottle rev. 01."

   Sampler preparation is the responsibility of the field technicians. The field technicians are also responsible for preparing Field Packets and equipment box.

Sample Collection

   The field technicians are responsible for all aspects of sample collection. Also responsible for contacting and setting appointments.

Storage

   Immediate storage of the sample upon return to RSPH is the responsibility of the field technicians. Custody will be turned over to the lab technician(s) for extraction and/or long-term storage.

Sieving and Division

   The field technicians are responsible for sieving and division of samples prior to extraction (see SOP L02 Beat Sieving and Division of Dust Samples rev. 1).

5. Required Equipment and Reagents

RSPH Laboratory (before field sampling)

> 2 catch bottles (1 for samples + 1 extra): trace-cleaned Teflon FEP wide mouth bottles, 8 oz./250 mL capacity, pre-weighed, with weight recorded on House Dust Sampling – Field Log (attached)
> lab tape
> plastic bags, sealable, 1-quart (about 6" x 10", to hold catch bottle)

Field Sampling

> tape measure, approximately 25 feet long (8 m), Craftsman 1" wide recommended
> easy peel-off laboratory tape (lab tape)
> 1 high volume small surface samplers (HVS3) (metal and Teflon parts of sampling train trace cleaned; nozzle covered in acetonitrile-rinsed aluminum foil; 1 trace-cleaned HVS3's will be used at each house)
> HVS3 maintenance record (kept with sampler)
> heavy duty extension cord, at least 25 feet long
> 3-prong outlet adaptor
> tools: screwdriver, wrench
> wipes, Kimwipe or equivalent
> plastic bottle of ethanol (for wiping HVS3 wheels prior to sampling)
> spare filter bag for HVS3
> Teflon catch bottles with caps, trace-cleaned, in Ziploc bags
> disposable powder-free gloves, in Ziploc bags
> two sheets of aluminum foil (for covering Teflon catch bottles to keep out light after sampling)
> plastic box for toting supplies

RSPH Laboratory (after field sampling)

> freezer
> materials and supplies for trace-cleaning metal and Teflon HVS3 parts

Sample Tracking and Paperwork

> Field Packet for household: field log/floor plan, chain-of-custody form
> Field Manual (SOPs)
> clipboard
> pens, ballpoint, 2 black or blue for field log/floor plan

6. Procedure

   6.1 Preparation for Collection

       6.1.1 Assembly, Calibration, and Maintenance of HVS3

           The field technicians are responsible for assembly, calibration, and maintenance of the HVS3. They will record assembly, calibration, and filter bag changes on the maintenance record sheet that is kept for each HVS3.

Assembly: The HVS3 will be assembled and tested no more than 2 weeks prior to the beginning of study sampling. For assembly instructions, see section 3.4 of the HVS3 Manual.

Calibration: A full flow calibration will be conducted no more than two weeks prior to the beginning of study sampling. For directions, see the HVS3 Manual, section 3.3. Zero the gauge in the position in which it will be used.

Filter bag: Bags will be changed immediately prior to beginning of study sampling and the changed logged in the maintenance records (attached). Later in the study, if the flow rate cannot be adjusted for sampling, change the bag and record the change on the maintenance record.

6.1.2   Reminder to Respondent

The field technicians will contact the subject and remind her/him about the sampling appointment at least 3 days ahead of the appointment. Include instructions that respondent refrain from vacuuming for at least three days prior to sampling.

6.1.3   Identification Labels

The field technicians will:

- Hand write ID labels for each sample to be collected from a particular house that day.

- For each sample to be collected, record ID on the floor plan sheet.

- Assemble the Field Packet for the household. The Field Packet should include one floor plan sheet for each sample to be collected and this SOP.

- Weigh the trace-cleaned, empty Teflon catch bottle with the lid screwed on to the nearest 0.1 gram. Record the weight in the box marked, "Before sampling (empty)" on the House Dust Sampling – Field Log.

- If the weights of the 3 empty Teflon bottles going to the same house differ by more than 1 gram, then put a piece of lab tap marking each bottle 1, 2 or 3 and record the bottle number on the field log.

6.1.4   Daily Maintenance of HVS3 – for each vacuum used:

At RSPH, after sampling, the field technicians will:

- Place any unused catch bottles in a designated storage area to be relabeled.

- Feel the filter bag. If it feels almost full, change it and record the change on the maintenance record.

- Decontaminate nozzles and tubing, following SOP L02: Trace Cleaning of

vaccum and parts.

6.1.5   Teflon Catch Bottle – for each sample collected:

- Weigh the dust-filled Teflon catch bottle with its lid on to the nearest 0.1 gram. Record the weight in the box marked, "After sampling (full)" on the House Dust Sampling – Field Log.

- Enter the full bottle weight again on the "House Dust and Soil – Sieving Log" and complete sieving tasks.

- Decontaminate catch bottle following SOP L02: Trace Cleaning of vaccum and parts.

- If there are clean bottles that were taken to the field but not used, remove the labels and treat them like new clean bottles.

6.2   Selection of Sampling Locations

One floor dust sample will be collected from the room the child spends most of their time in.

6.2.1   Dust sampling

The field technician will ask the caregiver which room the child spends the most time in.

For each room sampled, the technician will:

- Remove her shoes prior to entering the room to be sampled; store shoes outside the room.

- Choose a sampling area in the room that:
    – is in the main footpath or play area
    – is accessible by the HVS3 apparatus
    – is at least 1 meter from an outside door
    – does not require moving furniture

   Areas with carpet or rug are preferred.

- On the floor plan form for that room, sketch a floor plan with measurements. (It does not have to be exactly to scale; see example.) Note all significant features, but especially the location of windows, doors, and the area sampled. The location of the sampling area should be measured with the meter tape and documented on the floor plan form.

- Choose the area to be sampled and measure the distances from two walls. On the floor plan, sketch the location of the area and record the sampling location and type(s) of floor covering.

6.3   Sample Collection Procedure

    6.3.1   General – for each room to be sampled:

- Enter the HVS3 number, your initials and date onto the House Dust Sampling – Field Log

- Place the template on the area to be sampled and secure it with tape.

- Note the type of surface and/or type of rug (if relevant) on the floor plan.

- Ask the caregiver what date that area was last vacuumed or swept and enter the information on the floor plan.

- Put on a clean pair of gloves.

    6.3.2   Pre-Sampling Checks

    6.3.2a Leak Check (section 4.2 of HVS3 Manual)

- Seal the inlet tubing by placing the back of the clipboard across the end. Turn on the HVS3 with the switch at the top of the handle. The flow Magnehelic gauge should read between 0-0.02 inches of water.

- If the gauge reads more than 0.02 inches of water, check that all connections of gauge tubing are correct and sealed tightly.

- If all tubing is connected properly and the flow through the system still exceeds 0.02 inches of water, check all gaskets and tightness of clamps, catch bottle, and the plug sealing the inlet tubing.

- If the flow gauge continues to read more than 0.02 inches of water, note the gauge reading in the "Technician comments" section of the floor plan.

    6.3.2b   Setting Nozzle Pressure Drop [section 5.2 of HVS3 manual]

- Clean the plastic wheels with a Kimwipe and alcohol. Make sure the wheels are dry before beginning sampling.

- Remove the foil from the nozzle and place the HVS3 in the lower left corner of the sampling area. Adjust the flow rate and pressure drop according to the surface (wood or carpet). The two factors that affect the efficiency of a sampling system are the flow rate and the pressure drop at the nozzle. The pressure drop at the nozzle is a function of the flow rate and the distance between the surface and the nozzle flange.

- Pressure drops and flows will change as you vacuum. Try to keep the average values at least equal to those in Table 1. Slightly higher is better than slightly lower.

Table 1 -- Target HVS3 Flow Rate and Nozzle Pressure Drop During Sampling (cfm = cubic feet per minute)

Surface	Flow Rate		Nozzle Pressure Drop (inches of water)
	cfm	inches of water	
Carpet, level loop	16	5	10
Carpet, other (plush, multilevel, shag)	20	8	9
Hard (wood, tile, etc.)	20	8	9

- To regulate the nozzle position, use the height control knob on the back of the HVS3 and the nozzle level adjustment knob on the front of the nozzle. (See Figure 5-3 on page 15 of the HVS3 manual.)

- To regulate the flow rate, use the butterfly valve on the control tube on the downstream side of the cyclone. The flow is measured by the pressure drop across the cyclone. The higher the flow, the higher the pressure drop.

- On level loop carpet:

  - Adjust the height of the nozzle until the bubble level is centered. If the HVS3 is close to the position required but the nozzle bubble is not centered, use the leveling knob on the nozzle.

  - Once it is leveled, set the flow rate with the butterfly valve. Check the flow rate on the flow gauge (marked   Q).

  - Tip the HVS3 forward for a few seconds so that the carpet will seal to the nozzle. Set the flow so that the flow gauge reads 5".

  - Read the pressure drop across the nozzle. It should be approximately 10". If it is not, recheck the flow and/or check that the nozzle is still level.

  - If the pressure drop still does not read 10", note the gauge reading in the "Technician comments" section of the floor plan.

- On other carpet (plush, multilevel, shag):

  - Read the pressure drop across the nozzle. Set the pressure drop at approximately 9" on the nozzle gauge by using the height adjustment knob and the level knob to keep the nozzle level.

  - Check the flow rate. Use the butterfly valve to set it at about 8".

  - Check the pressure drop across the nozzle again. It will have changed from 9" because of the increased flow rate, which increased the pressure

drop across the nozzle and vice versa.

- Set the nozzle pressure drop to 9" again, using the height adjustment.

- You will probably need to make a few small adjustments three or four times until it is set right. It need not be exact. The flow rate should be between 8" and 9", and the nozzle pressure drop should be between 9" and 9.5".

- If the pressure drop still does not read 9-9.5", note the gauge reading in the "Technician comments" section of the floor plan.

- If the carpet is thin and the vacuum sucks it up, open the butterfly valve and readjust.

- If the flow rate cannot be adjusted for sampling, change the filter bag. Record the change on the maintenance record sheet.

- Check that the lever at the bottom is in the notch next to the lowest, so that the handle is at a 45º angle with the floor. With the handle at this angle and a firm downward pressure, the HVS3 is much less likely to nosedive.

6.3.3 Sample Collection

- Begin sampling by moving the nozzle forward and back along the left edge of the area marked by the template, covering an area 7.5 cm (3") wide and 1 m long. Vacuum this area 8 times (4 back-and-forth passes). Move the sampler at about 0.5 m per second, so it should take 2 seconds to travel 1 m. Move in a straight line between the numbers on the tape.

- After 4 back-and-forth passes on the first strip, gradually angle over to the next 7.5 cm wide strip and repeat 4 back-and-forth passes. Repeat until you have sampled about half the area of the template.

- Take care not to step inside the template on areas that you have not yet sampled; it is OK to step on places you have already vacuumed.

- When you have sampled 1 square meter, turn the vacuum off and check the amount of collected material in the catch bottle. (Don't count hair and fluff.)

- If it appears that at least 6 mm (1/4 inch) of material has been obtained, stop vacuuming. Calculate the area sampled by noting the width of the cleaned area on the measuring tapes. An area 1 m by 1 m is 1 m^2. Record the area on the logsheet.

- After vacuuming 1 m^2, if less than 6 mm has been obtained, mark off another 1-m^2 area next to the first and vacuum it. When enough material is collected, record the area on the logsheet.

- If the bottle is more than 25% full, or if material in the bottle swirls near the top of the bottle when the vacuum is on, take the bottle off and cap it with its own

cap from the plastic storage bag. Secure the bottle cap with lab tape. Place the bottle in its plastic storage bag and place it in the cooler with cold packs. Record the number of bottles used on the logsheet. Install a new bottle and continue sampling.

- When you have enough dust, turn off the HVS3. Tap on the U-tube and cyclone vigorously to dislodge any particles trapped in the sampling train.

- Unscrew the catch bottle. Holding the catch bottle under the cyclone, gently tap the threads on the cyclone to be sure any particles trapped in the threads are dislodged into the catch bottle.

- Gently tap the catch bottle a few times to allow particles to settle and cap it with its own cap from the plastic storage bag. Screw the cap on tightly and secure it with lab tape. Wrap the bottle tightly with foil to protect it from light. Place the bottle in its plastic Ziploc and place it in the cooler with cold packs.

- Remove the gloves and discard them in the trash bag.

- Finish the floor plan sketch if necessary and fill in any missing information on the floor plan form.

- Tell the caregiver you are finished sampling that room and thank her/him for her/his help.

6.4   Decontamination and Reassembly – at RSPH

- Unfasten the clamps and disassemble each HVS3 from the nozzle to the control valve tube (see Figure 3-1 of HVS3 manual). You do not need to clean the control valve tube or anything between it and the vacuum bag.

- You do not need to trace clean the metal clamps.

- Wipe each wheel with a Kimwipe to remove any dirt or dust.

- Separate the Teflon joints from the metal parts and the gaskets from their corresponding parts.

- Rinse all the loose parts under the faucet to remove the dirt.

- Scrub each part (including the flexible joints and gaskets) with a plastic test tube brush in a warm water-Liquinox solution. Clean each part separately, rotating it so that each internal surface is washed and brushed.

- Rinse off the Liquinox then rinse each part 3 times with tap water, 3 times with DI water and then one final time with MilliQ water.

- Inspect each part to be sure that there is no visible trace of dust. If any dust is visible, wash the part again.

- Let the parts dry in the fume hood on acetonitrile-rinsed aluminum foil.

- When parts are dry, rinse all sampling surfaces with acetonitrile using a Teflon squirt bottle. Be sure that all surfaces that will come into contact with the sample are rinsed with acetonitrile.

- Let the acetonitrile evaporate under the fume hood; when parts are dry, the vacuums can be reassembled.

6.5   Reassembly – at RSPH

- Put on a clean pair of nitrile gloves.

- Secure a piece of trace clean aluminum foil over the mouth of the cyclone where the catch bottle attaches. Use a rubber band or lab tape to be sure the foil stays on tight even when the HVS3 is being loaded/unloaded into the car.

- Secure a piece of trace clean aluminum foil over the nozzle. Use a rubber band or lab tape to be sure the foil stays on tight even when the HVS3 is being loaded/unloaded into the car.

- Wrap the other trace-clean parts in acetonitrile-rinsed foil (OK to use the foil from the floor of the fume hood as long as it is try), and bring them to the HVS3 assembly area.

- Put the HVS3 back together using Figure 3-1 of the HVS3 user manual as a guide.

- Be sure the trace-clean parts do not touch the floor or any other contaminated surface while you are reassembling each HVS3.

- Assemble only one HVS3 at a time, taking care to keep the parts trace clean while not using them.

6.6   Storage Prior to Sieving

Dust samples should be sieved in the laboratory within a week of collecting. They can be stored, tightly sealed, in a draw before processing. If a dust sample cannot be sieved on the same day it is collected, the field technician may transfer the sample to a trace-clean amber glass jar for temporary storage:

- Wipe the balance area clean with a paper towel, and water if necessary. Use the camel's hair brush to clean the balance pan and inside the balance.

- Bring the filled Teflon catch bottle, a trace-clean 120 mL amber glass jar, a piece of acetonitritile-rinsed foil, a pen, and the House Dust and Soil – Sieving Log over to the balance area.

- Enter the sample ID, UID, cycle, and sampling day on the Sieving Log.

- Weigh the sample-filled catch bottle with lid to the nearest 0.1 g and record the weight on the sieving log in the row labeled, "Mass of full Teflon bottle w/lid

(g)."

- Put on a clean pair of nitrile gloves.

- Place a sample ID label on the amber glass jar, and an identical label on the lid (a handwritten label on lab tape is OK, just be sure the ID number is correct).

- Zero the balance. Weigh the empty amber glass jar with its lid on to the nearest 0.1 g and record the weight on the sieving log in the row labeled, "Mass of empty amber glass jar w/lid (g)."

- Remove the lid from the amber glass jar and set it aside on the foil, taking care to not get any lab dust or dirt on it. Gently tap the dust from the catch bottle into the amber glass jar making sure you get as much sample as possible into the jar. Some residue will remain on the walls of the catch bottle. That is OK.

- Screw the lid tightly back on the amber glass jar, weigh it to the nearest 0.1 g, and record the weight on the sieving log in the row labeled, "Mass of full amber glass jar w/lid (g)."

- Write is the Sample was stored before sieving.

- Trace clean the Teflon catch bottle and lid following the procedures outlined in Section 6.4.

- Put the Sieving Log back into the binder labeled, "BEAT STUDY"

6.7   Sample Tracking

The ID number will allow tracking of each sample. A data base management system will ensure knowledge of the status and location of any sample at any time including retrospectively.

The chain-of-custody form will accompany samples through all transactions. Anyone who receives, transfers, or ships samples will sign and date the form, and keep a photocopy. It must clearly contain all necessary information so that the custody of the sample can be determined at any time.

7.   Quality Assurance Procedures

7.1   Field and Laboratory Blanks

Field blanks will be prepared by using 10ml of Hexane in the Dust collection bottle. The dust residue left in the bottle will be removed with the hexane and stored in a amber jar with the regular dust samples. Solvent blanks will also be analyzed with every analytical batch.

7.2   Tolerance Limits, Detection Limits, and Sensitivity Limits

The tolerance limits, detection limits, and sensitivity limits for the method will be determined through analysis of catch bottle rinse blank, solvent blanks and internal standards of analyte.

8. References

CS3, Inc. High Volume Small Surface Sampler (HVS3) Operation Manual. CS3, Inc: Bend, OR. 2001-2004 [Available:www.cs-3.com/HVS3.htm, accessed 20 April 2008].

Harvard University/Emory University/Johns Hopkins University Standard Operating Procedures for the National Human Exposure Assessment Study – Maryland (NHEXAS-MD), F04 Dust Sampling, September 1995.

Emory University/RSPH Standard Operating Procedures:
F03   Collection of House Dust Samples for Pesticide Analysis, CEEP Study, Rev. 1.0.

B. PBDE GC/MSD Instrument Method Details

The method used for analysis of BDE-47, -85, -99, -100, -153, and -154 was developed in-house in the Barr/Ryan laboratory. Chromatographic separation was performed by Agilent 5975 Series Gas Chromatography (GC)/Mass Selective Detector (MSD) system (Agilent Technologies; Waldbronn, Germany). The GC consisted of a temperature-stable column compartment, The GC/MSD was programmed and controlled using MSD ChemStation Software version E.02.02.1431 (Agilent Technologies; Waldbronn, Germany). PBDEs were analyzed by GC/MSD (EI, -10eV). Analyses were carried out using a 7890A GC coupled to a 5975C MSD (Agilent Technologies; Waldbronn, Germany). The system was fitted with a deactivated silca guard column (0.250 mm internal diameter (ID)) (Agilent Technologies; Waldbronn, Germany)) connected to a HP-5MS analytical column (15 m x 0.250 ID x 0.25 μm film thickness, Agilent Technologies; Santa Clara, CA). It was operated in splitless mode (7.1442 psi, 0.38703 min) with an injector temperature of 250°C. The helium carrier gas flow rate was 1.5 mL/min and the oven temperature program was as follows: 100°C (1 min), 250°C (45°C/min), 275°C (5°C/min), and 315°C (45°C/min) held for 8 min. The interface, source, and quadropole temperatures were set to 100°C 230°C, and 150°C, respectively. Selective ion monitoring (SIM) was used during the mass spectrometric analysis of the target compounds. The selected SIM are summarized in Table 9.

Table 9: Congener-specific GC/MSD parameters						
			Selective Ion Monitoring m/z			
Congener	RT	MW				
BDE-47	5.47	485.8	497.7	483.7	485.7	487.7
BDE-85	7.14	564.69	577.7	563.6	565.6	
BDE-99	6.55	564.7	577.7	563.6	565.6	
BDE-100	6.28	564.69	577.7	563.6	565.6	
BDE-153	8.02	643.62	655.5	643.6	645.5	
BDE-154	7.45	643.59	655.5	643.6	645.5	