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Polybrominated Diphenyl Ether (PBDE) Serum Concentrations in Children Ages 15 Months to 5 Years

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An abstract of
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Abstract

Polybrominated Diphenyl Ether (PBDE) Serum Concentrations in Children Ages 15 Months to 5 Years

By Emma Joanne Virginia

Polybrominated Diphenyl Ethers (PBDEs) are a class of brominated flame-retardants, and are ubiquitous in the environment and human serum globally. The United States population has especially high concentrations, approximately an order of magnitude above those seen in Europe. There is increasing evidence that PBDEs cause neurodevelopmental and behavioral toxicity and they are known endocrine disruptors. Studies have shown that young children may have serum concentrations up to five times those of the adult population, yet few studies have looked at individual child serum levels. The objective of this study was to measure individual serum PBDE concentrations in a diverse demographic of children from Atlanta, Georgia with ages of 15 months to five years. This study collected blood samples from 37 healthy children undergoing routine surgical procedures at Children's Hospital of Atlanta (CHOA) with a high participation rate of 93.7%. Serum samples were analyzed for six PBDE congeners (BDE-47, -85, -99, -100, -153, and -154) using a novel liquid/liquid extraction technique developed in house prior to GC/MS analysis. Summed PBDE concentrations ranged from 31.17 to 520.66 μmol/g lipid. BDE-47 was the dominant congener, followed by -99 and -100. Significant associations were found between age and BDE-154, race and BDE-100, and breastfeeding history and BDEs-47, -99, -100, and sumPBDEs. The mean BDE-47 concentration in our population was 38.73 ng/g lipid (mean used for study comparison; median 31.15 ng/g lipid), the second highest mean concentration recorded in children in this age range, only slightly below that measured in the CHAMACOS cohort in California in this population age group. Our study was the first to include a racially and social economically varied subject group in this age group in the United States. This study will be expanded in the future to include 81 subjects as well as corresponding house dust samples and child hand wipes.

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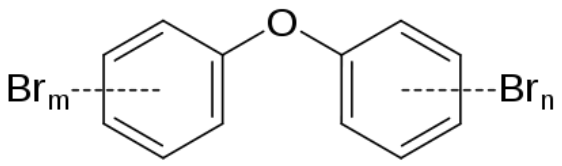
Table of Contents

1. Introduction & Background Significance

a. Background

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (Athanasiadou et al. 2008). Similar in structure and environmental disposition to polychlorinated biphenyls (PCBs), which were banned in the U.S. in 1978, PBDEs are persistent organic pollutants, which bioaccumulate and biomagnify up the food chain (Branchi et al. 2003; Chevrier et al. 2010). The general chemical formula of PBDEs is $C_{12}H_{(0-9)}BR_{(1-10)}O$, with 209 congeners possible; the general structure is illustrated in Figure 1 below (Branchi et al. 2003). PBDEs have low vapor pressures at room temperature, are highly lipophilic, have low water solubility, and are generally highly resistant to physical, chemical, or biological degradation (Branchi et al. 2003). The congeners are numbered using the IUPAC numbering system and are divided into 10 congener groups called homologs, mono- to deca-bromodiphenyl ethers (BDEs) (Branchi et al. 2003). Congeners with six or fewer bromines are the most biologically persistent, with half-lives in the human body ranging from 2-12 years; whereas congeners with 7-10 bromines have half-lives of 15-19 days in the human body (Castorina et al. 2011). Three major commercial mixtures of PBDEs have been used in the United States, decabromodiphenylether (Deca), octabromodiphenylether (Octa), and pentabromodiphenylether (Penta) composed of the following mixtures of PBDE congeners, respectively: 98% deca-BDEs and 2% nona-

Figure 1. General PBDE Physical Structure



BDEs; 10% hexa-BDEs, 40% hepta-BDEs, 30% octa-BDEs, and the remaining nona- and deca-BDEs; 40% tetra-BDEs, 45% penta-BDEs, and 6%hexa-BDEs (Branchi et al. 2003).

Because of their high performance efficiency and low cost, the use of PBDEs increased rapidly after coming onto the market in the 1970s (Athanasiadou et al. 2008; Gascon et al. 2011). PBDEs have been reportedly added to synthetic materials at approximately 5-30% by weight to retard flame ignition (Fischer et al. 2006). However, they are not chemically bound to the polymer matrices and can therefore easily leach out of products and enter the surrounding environment (Chen et al. 2011). The commercial Deca- mixture is most often found in polyurethane foam found in upholstery, carpet padding, etc., while Octa- and Penta- mixtures were primarily used in electronics, textiles, and plastics (Castorina et al. 2011). In response to increasing evidence of their ability to bioaccumulate and their increased toxicity relative to the Deca- mixture's congeners, the Octa- and Penta- mixtures were banned in 2004 from the European Union market as well as 10 U.S. states. Shortly after the ban, production companies voluntarily ceased production of the Octa- and Penta- mixtures that following year (Frederiksen et al. 2009; Lunder et al. 2010). The Deca- mixture is still produced and applied globally, with the exceptions of Sweden and Maine, who have banned its use completely and in residential upholstery, respectively (Frederiksen et al. 2009). The United States is tentatively planning on phasing-out all use of the Deca- mixture by 2013 (Lunder et al. 2010). However, because PBDEs are highly persistent in the environment, even after these mixtures are no longer being produced, there will still be large amounts of them in the environment from persistence and from existing products containing the compounds.

b. Toxicity & Health Effects

Research on the toxicology and health effects of exposure to PBDEs has grown rapidly over the last decade. While most of the research has been conducted using animal models, there are increasing numbers of epidemiologic studies investigating the potential health effects of PBDEs. While many of the toxicological mechanisms behind the health effects are still being investigated, PBDE exposure has been shown to interact with various hormones as an endocrine disruptor, alter thyroid homeostasis, cause low birth weight, cause reproductive problems in both males and females, cause liver toxicity, and can cause lasting neurodevelopmental effects such as hyperactivity, disruptions in learning and memory, as well as other cognitive and motor deficits in young children (Chen et al. 2011; Costa et al. 2008; Eskenazi et al. 2011; Harley et al. 2011; Harley et al. 2010). These health effects and their supporting studies will be discussed in detail below, as well as the general toxicology of PBDEs.

Absorption, metabolism, and excretion of PBDEs are congener-, species-, and gender-dependent (Costa et al. 2008). Fully brominated congeners (deca-BDEs) are poorly absorbed into the human body and are very rapidly eliminated or debrominate to other congeners, leading to very low bioaccumulation. Conversely, tetra- through hexa-BDEs are almost completely absorbed by the body and are eliminated very slowly from the body (Branchi et al. 2003). In rodent studies, male mice showed higher rates of urinary excretion compared to both female mice and all rats (Costa et al. 2008). The same studies show that young animals have a reduced ability to excrete PBDEs and that the compounds can cross the placenta and expose the fetus, making both pre- and postnatal exposure a particular concern for children (Chen et al. 2011; Costa et al. 2008). PBDEs have low acute toxicity with congener LD_{50s} under 5 g/kg and are not genotoxic

(Costa and Giordano 2007). Rodent studies indicate that during chronic exposures the liver, kidney, and thyroid gland are the main target organs in the body (Costa and Giordano 2007).

Endocrine Disruption & Reproductive Effects

PBDEs are endocrine disruptors making them particularly concerning for child and fetal exposure. They function as both agonists and antagonists when binding to estrogen and thyroid hormone receptors, and act as antagonists when binding with androgen and progesterone receptors (Chen et al. 2011). In experimental studies most congeners act as antiandrogens. Lower brominated congeners, up to hexa-BDEs, are estrogenic, while higher brominated congeners are antiestrogenic (Chen et al. 2011). Research shows that they inhibit estradiol sulfotransferase (E2SULT), increasing the bioavailability of endogenous estrogens (Chen et al. 2011).

When PBDEs enter the body they are often transformed into hydroxylated metabolites (OH-PBDEs) (Athanasiadou et al. 2008). OH-PBDEs inhibit aromatase activity that converts testosterone to estradiol (Chen et al. 2011). (Athanasiadou et al. 2008).

The majority of studies investigating the reproductive effects of PBDE exposure have utilized animal models. Rat and mice models have shown that both male and female reproductive systems are affected by prenatal exposures to multiple congeners. When rats were exposed prenatally to BDE-99 investigators found decreased sperm counts in adult males and alterations of females' ovarian cell ultrastructures (Kuriyama et al. 2005; Talsness et al. 2005).

Over the course of the past few years several epidemiologic studies have been conducted investigating reproductive effects in human populations. In 2010, Chao et al. investigated the effects of non-occupational PBDE exposure levels in women of childbearing age on menstrual cycles. The investigators found an association between higher levels of PBDEs and longer average menstrual cycles, as well as a delay in the age of onset of regular menstruation (Chao et al. 2010). However, Chen et al. found an association between higher PBDE levels and earlier age at menarche in a cohort of U.S. adolescent girls in 2011 (Chen et al. 2011). A study in 2010 found women who were more highly exposed to PBDEs to have increased time to pregnancy, compared to less exposed women (Harley et al. 2010). In 2011, the same research group reported a significant negative association between maternal serum PBDE levels during pregnancy and child birth weight, with a 10-fold increase in maternal PBDE concentrations associated with a 114-121 g decrease in child birth weight (Harley et al. 2011). In 2009, Meeker et al. found a relationship between PBDE concentrations and reduced testosterone levels in men (Meeker et al. 2009). The sample populations of these studies have generally been small and more studies are needed to confirm and further explore their findings.

Thyroid Effects

The effects of PBDEs and their endocrine disrupting effects on the thyroid have been investigated for the past decade in both animal models and human populations. The chemical structures of OH-PBDEs are very similar to those of thyroid hormones, especially thyroxin (T4) (Athanasiadou et al. 2008; Boas et al. 2006). This structural similarity could explain why most rodent studies have seen effects in both free and total

T4 as opposed to effects in thyroid-stimulating hormone (TSH) levels (Chevrier et al. 2010). The findings from both animal models and human studies are discussed in detail below.

Rodent studies have shown overall reductions in circulating levels of all thyroid hormones with exposures to PBDEs (Boas et al. 2006). OH-PBDEs have been shown to displace thyroid hormones from the thyroxin plasma transporter transthyretin (TTR) (Costa and Giordano 2007). Exposure to the commercial mixture DE-71 in rodents induced activity of hepatic enzymes as well as histopathologic changes such as increased follicular epithelial height and colloid depletion, indicative of a hypothyroid state (Boas et al. 2006). Similar exposures to the commercial mixtures Bromkal and DE-47 decreased both free and total T4 levels in rodents and induced microsomal enzyme activity (Boas et al. 2006). Studies of BDE-99 determined the congener was not as potent in reducing circulating thyroid hormone levels, illustrating that the effects on the thyroid system are congener dependent (Boas et al. 2006). Rodent studies suggest that the effects of PBDEs on thyroid hormone levels are due to the induction of thyroid hyperplasia and an alteration of thyroid hormone metabolism (Branchi et al. 2003). Further study illustrated that the lower brominated congeners were the most potent reducers of plasma T4 compared to higher brominated congeners (Boas et al. 2006). Similar hormone level effects have been found in both fish and avian models (Boas et al. 2006).

Proper thyroid hormone distribution is especially important during pregnancy for proper neurological development of the fetus. Even small changes in circulating maternal thyroid hormones can have lasting negative impacts on psychomotor skills and

the intelligence of the child and could explain some of the neurodevelopmental effects of PBDE exposure discussed in the next section (Branchi et al. 2003).

Early studies investigating the effects of PBDE exposure on thyroid hormones in humans were conducted in uniquely exposed populations. In 2001, Hagmar et al. measured thyroid hormone levels in 110 Baltic men exposed to organohalogens from consuming fish with elevated levels of the compounds. The study found a negative association between TSH and BDE-47 levels (Hagmar et al. 2001). In the early 2000s, an occupational cohort consisting of eleven workers in an electronic recycling facility, were followed for 1.5 years. Over the study period PBDE and thyroid hormone levels were measured routinely. The investigators noted that PBDE levels fluctuated over time, and although they saw a general trend towards increased T4 over time, the changes were small and statistically insignificant, likely due to the very small sample size (Julander et al. 2005).

Due to the importance of thyroid hormones during pregnancy and child development, many recent epidemiologic studies have focused on exposure to PBDEs during pregnancy and maternal and child thyroid hormone levels. One of the earliest of these studies, conducted by Mazdai et al., measured thyroid hormones and six BDE congeners in twelve pairs of maternal and cord blood samples. The study revealed no apparent correlation between serum PBDEs and thyroid hormone levels; however this was likely due to the small sample size. More recently similar studies with significantly larger sample sizes have found such associations. In 2010, a study involving a cohort of 270 primarily Latina women in California measured serum PBDE concentrations and serum thyroid hormone levels during the second trimester of pregnancy (Chevrier et al.

2010). The investigators found significant inverse associations between TSH and both individual and sum serum PBDE levels, primarily in the quartile of women with the highest PBDE concentrations (Chevrier et al. 2010). These results suggest a hyperthyroidic effect of PBDE exposure during pregnancy.

In 2011, the same researchers investigated the effects of maternal PBDE levels during pregnancy and neonatal TSH levels (Chevrier et al. 2011). Maternal serum samples were collected at the beginning of the third trimester and at delivery and were analyzed for PBDE and thyroid hormone levels. Cord blood samples were analyzed for neonatal TSH. Contradictory to rodent studies, there was no association between maternal serum PBDE concentrations and neonatal TSH levels. However, the study did not collect information on maternal iodine intake or OH-PBDE serum levels, possible covariates, and were unable to measure for free or total T4 in cord blood (Chevrier et al. 2011).

The first two studies measuring thyroid hormone levels and PBDE concentrations in young children were published in 2011. Gascon et al. followed the Menorca Birth Cohort (INMA Project) of 422 mother-child pairs in Spain. They measured PBDEs and thyroid hormones (TSH, total triiodothyronine [TT3], free T4) in 88 cord blood samples and 244 serum samples of children at age 4 (Gascon et al. 2011). TSH and free T4 levels were not associated with postnatal PBDE exposure, whereas TT3 was positively associated with postnatal BDE-47 exposure, however this was not statistically significant (Gascon et al. 2011). Another study published in 2011 was the first to show a significant association between PBDE levels and thyroid hormone levels in children. Han et al. measured serum PBDE and TSH levels in Chinese children, ages 6-8 (Han et al. 2011).

Children were enrolled from two different locations, representing a high exposure group from a computer E-waste recycling area, and control children from another district. The investigators found a significant positive correlation between PBDE levels and TSH levels in the control children, but not in the highly exposed children (Han et al. 2011).

Neurodevelopmental Effects

Neurodevelopmental effects of PBDEs are still not well understood by the scientific community, but are recognized important health endpoints of pre- and postnatal exposure to PBDEs. Both animal models and human epidemiologic studies have been conducted to investigate these effects and to try to understand the mechanisms. Neurochemical changes observed in rodents after PBDE exposure and altered thyroid homeostasis are possible mechanisms of PBDEs' developmental neurotoxicity, but there is not sufficient evidence or understanding of either (Costa and Giordano 2007).

In an early mouse study, investigators found that prenatal exposure to low doses of PBDEs during critical phases of brain development induced permanent changes in the resulting pups' adult brains (Branchi et al. 2003). Perinatal exposure to BDE-99 in mice produced significantly higher activity levels later in development, the same behavior has been observed in hypothyroid rats (Branchi et al. 2003). The same exposure produced motor developmental delay in mice, measured by a delay in screen climbing response in exposed animals (Branchi et al. 2003). Long lasting changes in spontaneous locomotor behavior was seen in mice and rats that had received oral doses of PBDEs as neonates (Costa and Giordano 2007). Alterations of learning and memory were observed in mice who were exposed as neonates to BDE-153, while those exposed to BDE-99 showed impaired hippocampal function measured using the Morris water maze test (Branchi et

al.). These rodent studies are older and more studies are needed to further investigate these observed effects as well as to investigate their mechanisms.

The first studies investigating neurodevelopmental effects of PBDEs in humans were published in the last three years. The first two studies were conducted in the Netherlands (Roze et al. 2009) and the United States (Herbstman et al. 2010). Both studies observed adverse neurodevelopmental effects in 4-6 year olds who had been prenatally exposed to PBDEs (Herbstman et al. 2010; Roze et al. 2009). In addition to investigating the association between prenatal PBDE exposure and thyroid hormone levels, Gascon et al. performed a neurodevelopmental assessment consisting of multiple measures of cognitive function, ADHD symptoms, and social competence on the 422 4 year olds in the Spanish Menorca Cohort (Gascon et al. 2011). Gascon et al. observed and association of higher risk of certain ADHD symptoms and social competence in children with postnatal exposure to BDE-47 (Gascon et al. 2011). Additionally, although it was not statistically significant, the observed decreasing cognitive and motor function scores with increasing pre- and postnatal exposures to BDE-47 (Gascon et al. 2011). While more studies are needed to validate and further investigate these findings, these initial studies illustrate the significance of minimizing and monitoring child exposures to PBDEs, discussed below.

c. Exposure Routes & Trends

Routes of Exposure

The main routes of PBDE exposure are ingestion and inhalation, although in recent studies dermal absorption has been demonstrated as well (Frederiksen et al. 2009; Eskenazi et al. 2011; Johnson-Restrepo and Kannan 2009). Ingestion of PBDEs can

occur from consumption of breast milk, household dust, and foodstuffs (most notably fish, meat, and dairy products) (Athanasiadou et al. 2008; Castorina et al. 2011; Costa et al. 2008; Eskenazi et al. 2011). Historically, breast milk and food stuffs were thought to be the most important route of exposure, however, in recent years studies have shown that household dust ingestion is a very significant route of exposure, and may explain the significant differences seen in serum concentrations between Europe and North America, as the concentrations in foodstuffs are relatively similar between continents (Castorina et al. 2011). Dust exposure is also of great significance in child PBDE exposures. The congener composition varies among breast milk, foodstuffs, dust, and air. The major congeners found in foodstuffs are BDE-47 and -99, those in indoor air are BDE-47, -99, and -209, the major congener in breast milk is BDE-47, while the major congener in household dust is BDE-209 (Frederiksen et al. 2009; Johnson-Restrepo and Kannan 2009).

BDE-209 comprises anywhere from thirty-two to ninety-seven percent of the total PBDE content of analyzed dust samples (Fischer et al. 2006; Frederiksen et al. 2009). BDE-209 has a half-life of approximately fifteen days in the body (Fischer et al. 2006). BDE-209 can debrominate to form BDE-153, potentially increasing levels of BDE-153 seen in the body (Fischer et al. 2006). Researchers are still uncertain how the PBDEs leach out of household products and into the dust. The main route of indoor dust exposure is ingestion; however inhalation of dust particles in indoor air also occurs. In addition, Webster et al. have shown that PBDEs can be directly absorbed through dermal contact with household dust (Webster et al. 2005). Their research estimated that dermal

exposure may be responsible for approximately ten percent of dust exposures in adults and up to thirty-five percent of dust exposures in children (Webster et al. 2005).

Exposure Studies & Trends

Global body burdens of PBDEs have been increasing at an exponential rate for the past thirty years (Sjodin et al. 2004; Thomsen et al. 2002). Early human PBDE exposure studies were focused on Europe and North America, however, more recently studies have been conducted in across Asia, Australia, New Zealand, and Russia (Athanasiadou et al. 2008; Eskenazi et al. 2011; Thomsen et al. 2002; Chen et al. 2010; Perez-Maldonado et al. 2009; Shen et al. 2010). The first large-scale monitoring of human serum PBDE concentrations in the United States began in 1973. At that time, serum PBDE levels were almost undetectable, contrastingly, in 2003-2004 the National Health and Nutrition Examination Survey found that the United States' mean total PBDE concentration in adult serum ranged from 40-60 ng/g lipid (Chen et al. 2011).

The majority of exposure monitoring and studies have been conducted in plasma and serum, with some in breast milk as well. These are all lipid-rich matrices and are therefore ideal for monitoring (Chen et al. 2010). In serum, the most commonly measured PBDE congeners are BDE-47, -99, -100, and -153, suggesting the majority of exposures are due to the penta-mixture of compounds (Castorina et al. 2011). Many studies report sumPBDE concentrations, however, these concentrations are not always good indicator for comparison between studies and populations as each study may measure a different array of individual congeners. However, global exposure studies have indicated that serum congener profiles vary by geographic region, and the most important congeners in one location may not be consistent with the most important

congeners in another location (Frederiksen et al. 2009; Chen et al. 2010). For this reason, we will use BDE-47 as a standard comparison measure between study populations. In most populations serum BDE-47 is consistently found at concentrations above the limit of detection (LOD), and is almost always included in exposure measurements, making it ideal for comparison (Frederiksen et al. 2009; Chen et al. 2010).

While measurable levels of PBDEs are detected in serum across the globe, the average concentrations vary greatly by region. Aside from special populations, such as those in E-waste recycling regions, the North Americans have the highest serum PBDE levels globally (Frederiksen et al. 2009; Costa et al. 2008). Average serum BDE-47 levels in North Americans are consistently measured at an order of magnitude higher than Europeans and most Asian populations (Frederiksen et al. 2009; Costa et al. 2008). Figure 2 illustrates the relative mean levels of serum BDE-47 across these populations in the early 2000s (Frederiksen et al. 2009). The United States has by far the highest average levels of serum BDE-47 across the globe. Potential explanations for this dramatic difference include more strict fire regulations, especially in California, demanding more widespread use of flame-retardants in products sold in the United States, as well as earlier banning in Europe (Frederiksen et al. 2009; Lunder et al. 2010). As previously stated, differences in food stuff concentrations are minimal between regions, and therefore cannot be the cause of these dramatic exposure differences (Castorina et al. 2011).

Within these populations there is significant variation between age groups, most notably between adults and young children (Athanasiadou et al. 2008; Lunder et al. 2010; Sjodin et al. 2004; Thomsen et al. 2002; Fangstrom et al. 2005; Lunder 2008; Toms et al.

2008; Toms et al. 2009). Children between the ages of one and five years have shown significantly higher serum PBDE concentrations than adults. Childhood exposures will be discussed in greater detail below.

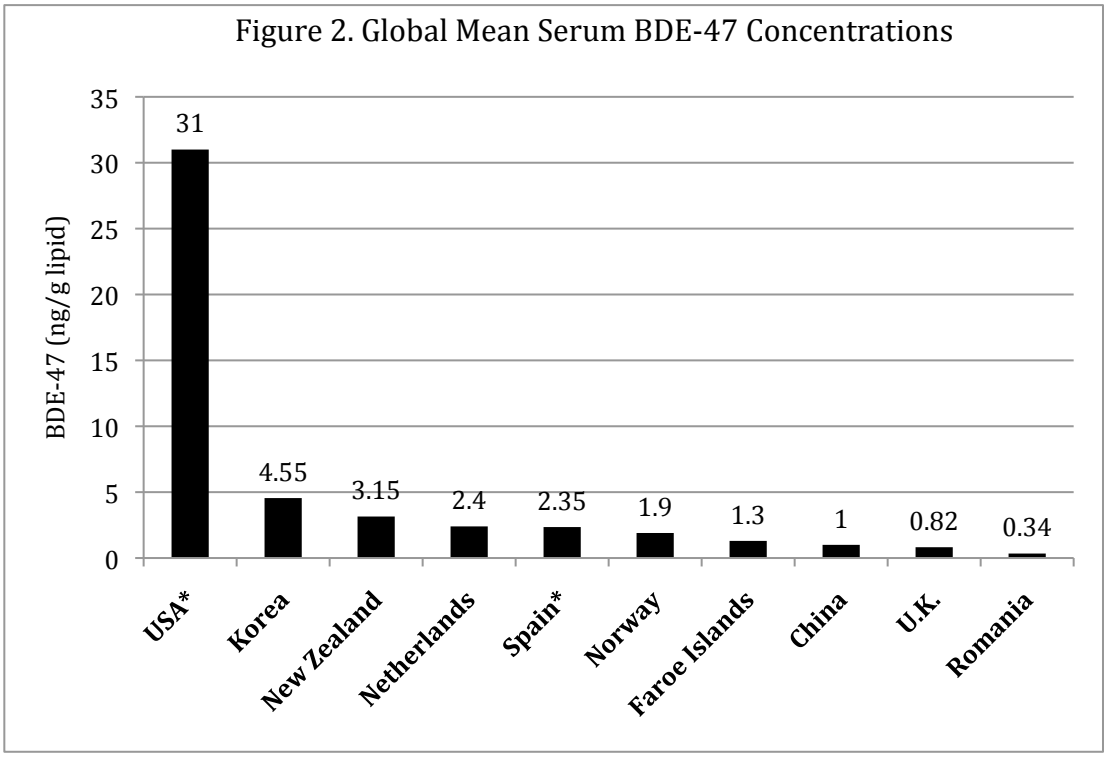


Figure 2. Illustrates mean serum BDE-47 concentrations (ng/g lipid) in adults from studies conducted in late-1990s through early 2000s.
*Indicates multiple study means averaged for ease of illustration and comparison.

Child Exposures

Globally, serum PBDE concentrations in children have been consistently up to two to three times higher than adult serum concentrations (Athanasiadou et al. 2008; Fischer et al. 2006; Lunder et al. 2010; Chen et al. 2010; Toms et al. 2008; Zuurbier et al. 2006). There are multiple potential explanations for the higher exposure levels in young children compared to adults. Children ingest significantly higher amounts of food based on body weight compared to adults, therefore their potential exposures from ingesting contaminated foodstuffs are relatively higher than adults per body weight (Chen et al.

2011). When children are breastfed they are exposed to the PBDEs secreted in their mothers breast milk, which can compose approximately ninety percent of a breastfed infant's PBDE body burden (Costa et al. 2008; Eskenazi et al. 2011). However, compared to food and breast milk exposures, recent studies suggest that indoor dust ingestion and inhalation is responsible close to eighty percent of toddlers' (1 to 5 years) exposure to PBDEs (Costa et al. 2008; Eskenazi et al. 2011). Children in this approximate one to five year age range exhibit the highest occurrence of hand-to-mouth behavior as they explore their surroundings and crawl close to the floor. During this hand to mouth behavior they ingest a significant amount of house dust containing high levels of PBDEs. In 2005, Jones-Otazo et al. estimated that due to their hand-to-mouth behavior, toddlers may be exposed to one hundred fold higher exposures to PBDEs from house dust than adults (Jones-Otazo et al. 2005).

In 2009, Johnson-Restrepo et al. estimated the average daily exposures to PBDEs of different age group based on various routes of exposure. Toddlers (ages one to five years) received an estimated average daily exposure of 0.29 ng/kg-bw (body weight) from indoor air inhalation, 9.5 ng/kg-bw from dust ingestion, 0.7 ng/kg-bw from dermal absorption, and 2.76 ng/kg-bw from food ingestion (Johnson-Restrepo and Kannan 2009). The increasing evidence of neurodevelopmental and behavioral toxicity of PBDEs, coupled with high body burdens of these compounds, make young children a particularly vulnerable and important population for study. While some studies assessing PBDE exposures in this population, many of them have severe limitations such as small sample sizes and pooled serum samples. This study aims to broaden the knowledge base of the exposures of young children by measuring individual body burdens in a larger population

than most previous studies. The current state of research on PBDE exposures in this age group can be seen in Table 1; each study will be discussed in detail below.

Table 1. PBDE Exposure Studies in Children

Study	Year	Location	Sample Size	Ages (years)	Sample Analysis
Thomsen et al.	2002	Norway	14	0-4	Pooled
Fisher et al.	2006	United States	1; 1	1.5; 5	Individual
Thomsen et al.	2007	Norway	29	0-4	Pooled
Toms et al.	2008	Australia	400	0-4	Pooled
Toms et al.	2009	Australia	NA	0-6	Pooled
Chen et al.	2010	China	29	0-11	Individual
Lunder et al.	2010	United States	20	1.5-4	Individual
Eskenazi et al.	2011	United States; Mexico	264; 283	7; 5	Individual
Gascon et al.	2011	Spain	244	4	Individual
Current Study	**2012**	**United States**	**37***	**1.25-5**	**Individual**

*The full study will include 81 individuals.

In 2002, Thomsen et al. conducted a wide scale PBDE exposure assessment in Norway comprised of banked serum samples of forty to fifty-year old men, as well as eight different age pools collected in 1998. The youngest age pool contained fourteen serum samples from children ages zero to four years. The serum BDE-47 concentration was 6.2 ng/g lipid, an average of 2.8 times the concentration of all other age groups. While the sample size was very small and the serum samples were pooled, this was the first study to include this age group in exposure analysis and provided a clear comparison with all other age groups up to fifty years old (Thomsen et al. 2002).

The first study displaying the high levels of PBDE exposure in young children in the United States was a case-study of a California family of four, conducted by Fisher et al. in 2006. Serum samples from all four family members, mother thirty-six years, father thirty-five years, daughter five years, and son eighteen-months, were collected twice, once in September and duplicate samples taken in December of the same year. At both time points BDE-47 levels in the five-year-old girl and the toddler were significantly higher than their parents. The five-year-old's levels were 137 ng/g lipid and 105;94 ng/g lipid (duplicate samples), the toddler's concentrations were even higher at 245 ng/g lipid and 209;186 ng/g lipid (duplicate samples), while the parents samples ranged from 29 to 62 ng/g lipid (Fischer et al. 2006). This is an extremely small sample from which to draw conclusions, but it illustrates the magnitude of difference between these age groups, and provided researchers with motivation to further explore the differences in exposures between these age groups.

In 2007, Thomsen et al. conducted an additional analysis of archived serum samples in Norway, and included a new 0-4 year old pool of twenty-nine individuals sampled in 2002. The concentration of BDE-47 in the 0-4 year old pool was 4.8 ng/g lipid, illustrating an increase in BDE-47 concentrations in this age group since 1998 (Thomsen et al. 2007). However, due to the small sample sizes of the pools and the fact that individual outliers may alter sample pool means, the increase may be due to the addition of new individuals with very high BDE-47 levels, and is not necessarily indicative of an increase in exposures.

The first large sample pooled serum study measuring PBDE exposures in this age range was conducted in 2008 in Australia by Toms et al. Like the Thomsen et al. studies,

multiple age pools, from 0-4 years through adulthood, were analyzed. There were four hundred serum samples total in the 0-4 year old age range, collected in 2004-2005. There were two hundred females and two hundred males, divided into two randomly selected pools per sex. The mean BDE-47 concentration across the 0-4 year pool was 24 ng/g lipid, higher than those seen in the Norway population, but dramatically lower than the California child cases. The sumPBDE of this age pool was 73 $\pm$ 7 ng/g lipid, four to five times the concentrations in adults over the age of sixteen (Toms et al. 2008).

In 2009, Toms et al. conducted a similar study reporting measured concentrations, breaking up the 0-4 year age range into three narrower age groups, newborns (using cord blood samples), 0-2 year olds, and 2-6 year olds, as well as pools through adulthood. The majority of the serum congener profile was made up of BDE-47 in all groups. Pool sample sizes and individual congener levels were unavailable at the time of this paper, however mean sumPBDE levels for the 0-2 year and 2-6 year groups were 31 ng/g lipid and 41 ng/g lipid, respectively (Toms et al. 2009).

The first study assessing exposures children in Asia was conducted in Dalian, China and published in 2010 by Chen et al. Twenty-nine children, ages 0-11 years, were randomly sampled. Plasma samples were analyzed individually, making this the first relatively broad exposure assessment measuring individual PBDE levels in children. Nineteen of the children fell in the 0-4 year range and ten in the 5-11 year range, however researchers did not see a significant different between the two age groups. This could be due to the small sample size between groups. Unlike studies in Europe, Australia, and the United States, the dominant congener in these samples was BDE-153, followed by -99 and -47, illustrating the geographic variability of congener profiles. The

concentrations of BDE-47 of all samples ranged from non-detectable (ND) to 19.44 ng/g lipid and the mean was 6.74 ng/g lipid, while the concentrations of BDE-153 ranged from ND-27.14 ng/g lipid with a mean of 9.21 ng/g lipid. While these concentrations are higher than those in Europe, they are still much lower than those seen in the United States and in Australia. These concentrations illustrate the wide range of concentrations in individuals, illustrating the importance of individually analyzed concentrations.

In 2010, the first study of PBDE concentrations focused on young children in the United States was published by Lunder et al., comparing twenty mothers with their firstborn children, ages 1.5-4 years old. The mother-child pairs were sampled from eleven U.S. states, were generally of higher socio-economic status, and were eighty percent Caucasian, and all were breastfed, most until they were at least one year old. Like Chen et al., the study measured individual serum PBDE levels, used to compare levels between mother and child. The mean serum BDE-47 concentration in children was 31 ng/g lipid, while the mean maternal serum BDE-47 concentration was 12 ng/g lipid (Lunder et al. 2010). The researchers tested for relationships between breastfeeding length, age, and PBDE levels, but did not find any significant associations. However, this could be due to the small sample size and the fact that all children were breastfed for at least some length of time.

Since the publication of Lunder et al. studies looking at PBDE exposures in young children have moved away from pooled sample analysis and have since used individual sample analysis in their research. Eskenazi et al. conducted the first large-scale PBDE exposure comparison study in young children, comparing 264 seven-year-old children from the CHAMACOS cohort in the United States to 283 five-year-old children with

similar demographics from the Mariposa cohort in Proyecto, Mexico. While the seven year olds are older than this study's target population and are well outside of the peak hand-to-mouth behavior age range, studies have shown that they have relatively similar levels to slightly younger children, in comparison with adults. The individual concentrations of serum BDE-47 in the CHAMACOS children ranged from 1.9-582.0 ng/g lipid with a geometric mean of 47.1 ng/g lipid, whereas the Mariposa serum BDE-47 concentrations ranged from 0.3-260.0 ng/g lipid with a geometric mean of 5.6 ng/g lipid. The levels in the CHAMACOS cohort were significantly elevated from those in the Mariposa cohort, despite the hypothesis that younger children would have higher levels. The Mariposa cohort's levels are more comparable to those of Europe and China. The extreme difference is likely at least partially attributable to the stringent flame retardant regulations in the state of California. To date the only population with PBDE body burdens greater than those seen in the CHAMACOS cohort children are those seen in children working at hazardous waste dumping sites in Nicaragua (Athanasiadou et al. 2008; Eskenazi et al. 2011).

The most recent study of exposures in young children assessed the body burden of PBDEs in four-year-olds and their mothers in the Menorca Birth Cohort (part of the INMA Project) in Spain. Cord blood samples were collected from 88 individuals at birth, followed by serum samples were collected from 244 individuals at age four. Serum samples were individually analyzed for PBDEs. The median serum BDE-47 level of the four-year-olds was 0.12 ng/g lipid, with a maximum concentration of 130.2 ng/g lipid (Gascon et al. 2011). The median concentration is consistent with a significantly lower level than those seen in the United States population.

The above studies illustrate the consistently elevated levels in young children compared to adult population levels. To date many of the studies are limited by small sample sizes, pooled analyses, and unrepresentative sample of the larger population. It is apparent that the levels measured in the United States to date are significantly higher than those of other regions. Within the United States studies have not been conducted whose results can be applied to the general population of young children. Despite the study's large sample size, the unique flame retardant regulations in California could potentially cause extreme exposures to California-native children, and thus the CHAMACOS cohort, compared to the rest of the country. The homogeneity in SES and race (White Caucasian in Lunder et al., Hispanic Caucasian in Eskenazi et al.) also make it difficult to generalize their results to the rest of the United States.

This study aims to improve upon the past PBDE exposure studies on young children by increasing sample size compared to many previous studies in the United States, analyze individual serum PBDE levels, and enroll a more demographically heterogeneous sample of children if possible.

2. Study Design & Methods

a. Study Population

Subjects were recruited from pediatric anesthesia patients, ages 15 months to 6 years old, at Children's Healthcare of Atlanta (CHOA) as part of the **B**rominated Flame Retardant **E**xposure **a**nd **T**hyroid Function (BEAT) study headed by Lyndsey Darrow, PhD (Dept of Epidemiology) and Dana Boyd Barr, PhD (Dept of Environmental Health) and supported by NIH grant ID 5R21ES019697-02. This age range was chosen based on

previous studies showing high serum levels of PBDEs in this age group (Athanasiadou et al. 2008; Gascon et al. 2011; Fischer et al. 2006; Lunder et al. 2010; Toms et al. 2009). The subjects required general anesthesia while undergoing one or more of the following routine procedures: myringotomy, adenoidectomy, or tonsillectomy. We targeted this population in order to more easily obtain blood samples from such a young cohort. The typical difficulty of blood draws in this age group due to the discomfort and anxiety for both children and parents were avoided as the children were under anesthesia. Children undergoing these three procedures are generally healthy without pre-existing or complicating conditions leading to these surgeries that could affect or be affected by blood PBDE levels. The study protocol was reviewed and approved by Emory's Institutional Review Board.

Children were excluded from the study if they were undergoing additional procedures not listed above, were outside of the target age range or had preexisting medical conditions that might affect PBDE or thyroid hormone levels. In addition, children who were initially enrolled in the study but could not provide the full 15 mL blood sample (e.g., because of dehydration) were excluded from further participation in the study. The total number of enrolled participants in the study was 80; 40 were included in this analysis.

Our research nurse (Christina Ryan, CHOA) gained parental informed consent and enrolled the children on the day of their surgery. The possible risks to the participants were clearly outlined and explained during the consent process. While risks were considered less than minimal, risks from the blood draw included infection and possible bruising at the blood draw site. At the time of enrollment additional

demographic and health information was gathered from the child's health records as well as a parental questionnaire. The questionnaire gathered important information about covariates such as age, race, ethnicity, breastfeeding history and duration, duration at current residence and address, time spent in the home each day, parental occupation, household smoking, insurance status, child medications, birth order, height and weight (BMI calculations), and family history of thyroid or other endocrine or auto-immune diagnoses.

b. Blood Samples

After the surgical procedures while the child was still under general anesthesia, the nurse performed the blood draw, collecting up to 15 mL of blood in two tiger-topped serum separator Vacutainer® tubes. The blood samples were transported to the Barr/Ryan laboratory at the Rollins School of Public Health, Emory University on the day of collection and were processed upon arrival. Samples were manually shaken and allowed to clot for 60 minutes, then centrifuged (IEC Medispin, Thermo Scientific®) for 30 minutes at 3000 rpm to separate the serum from the whole blood sample. The serum was then aliquoted into two freezer-safe storage vials of approximately equal volumes, one for PBDE analysis (see method below) and the other for future thyroid hormone analysis (method to be developed and performed in house). The serum samples were stored at -20°C prior to analysis.

c. Chemicals & Materials

The chemicals used during sample analysis are listed in Table 2. The Strata^{TM}-X 200 mg columns used in the extraction procedure were purchased from Phenomenex®. The ISOLUTE® brand bulk silica sorbent (United Kingdom) and ISOLUTE® 200 mg

Silica 100 mL XL columns (United Kingdom) used during the clean-up procedure were purchased from Biotage AB®.

d. Serum PBDE Extraction Method Development

The serum PBDE extraction method was developed based on three prominent methods reported in the literature, and was further refined to optimize extraction recovery and analytic precision (Hovander et al. 2000; Sandau et al. 2003; Zhang and Rhind 2011). Each of these methods utilized solid-phase extraction (SPE), however the reagents, columns, and methods differed. Hovander et al. utilized plasma in place of serum for PBDE extraction, while Sandau et al. and Zhang and Rhind utilized serum as their matrix. Hovander utilized 6M hydrochloric acid to denature samples, while Sandau and Zhang and Rhind denatured samples using a formic acid:water (v:v) solution and formic acid, respectively. We chose to utilize a 50% formic acid:water solution as this method performed as well as the methods using highly concentrated acid. All three methods utilized dichloromethane (DCM) as a key solvent. In their study in 2011, Zhang and Rhind tested various solvents, determining that DCM provided the best recovery. The same study tested popular commercially available SPE cartridges and determined that Strata^{TM}-X (200mg; 6mL) (Phenomenex®, Torrance, CA) provided the best recoveries (Zhang and Rhind 2011). We chose DCM as our main solvent and Strata^{TM}-X (200mg; 6mL) for those reasons. Zhang and Rhind also tested recoveries of extractions followed by various clean-up methods to remove coextracted chemicals that could potentially interfere with the analytical measurement such as lipids and other biogenic material and determined that a silica/acidic-silica clean-up method provided the best results. This

clean-up method was also used in both other methods (Hovander et al. 2000; Sandau et al. 2003; Zhang and Rhind 2011). Our initial hybrid extraction method is described below.

Initial Serum PBDE SPE Extraction Method

PBDEs were extracted from serum samples using SPE, followed by a clean-up method to remove lipids and other biogenic materials from the samples prior to analysis. Each set of samples analyzed was accompanied by a blank serum sample and low concentration and high concentration quality control serum sample, QCL and QCH respectively. The QCL standard concentration was 100 ng/mL of BDE-209 and 10 ng/mL of BDEs-47, -85, -99, -100, -153, and -154 in methanol. The QCH standard concentration was 250 ng/mL of BDE-209 and 100 ng/mL of BDEs-47, -85, -99, -100, -153, and -154 in methanol. A standard curve comprised of 8 concentrations (in ng/mL units; 0.2, 0.5 , 1 , 2.5 , 5 , 10 , 25 , 50) was included with all sets of samples in order to extrapolate PBDE concentrations during analysis and test for accuracy.

Each sample (1 ml of serum) was spiked with 100 µl ISTD (7.5 ng/mL in methanol) and vortex mixed to homogenize the mixture (Vortex-Genie 2, Scientific Industries, USA). 1 mL of 50% formic acid in water was added to each sample, which was then vortex mixed at 2000 rpm for 10 minutes followed by 10 minutes in the centrifuge at 3000 rpm for an additional 10 minutes. Samples were then loaded to Strata^{TM}-X (200 mg 6 ml) columns, preconditioned with 5 mL DCM and 5 mL 5% MeOH in 0.1M HCL in water. Columns were then dried using a vacuum, followed by elution with 15 mL DCM, which was collected in new 15 mL centrifuge tubes. The centrifuge tubes were brought to near dryness in the TurboVap® LV Evaporator

(Zymark®, USA) at 30°C at 15 psi. After evaporation the samples were reconstituted with 500 µL of hexane prior to the clean-up procedure.

Silica/Acidified-Silica Serum PBDE Clean-up

The serum clean-up procedure following PBDE extraction was performed using silica/acidified-silica columns prepared in house. Prepacked ISOLUTE® 200 mg silica columns were used as the basis for the prepared columns. 1.8 g of acidified silica, produced by combining 1 part 98% H_2SO_4 and 2 parts ISOLUTE® bulk silica, was added on top of the premade silica columns, followed by 0.5 g of anhydrous Na_2SO_4, preheated in an oven at 100°C overnight to ensure dryness. Columns were conditioned with 5 mL hexane, after which the reconstituted samples in hexane from the extraction procedure were loaded to the columns and eluted to waste. Columns were then eluted with 10 mL DCM and the elution was collected in clean 15 mL centrifuge tubes. Eluates were then brought to dryness in the TurboVap® at 30°C at 15 psi. Samples were reconstituted with 50 µL of toluene for analysis.

Extraction Method Recovery

The above methods were validated and altered based on multiple in-house recovery studies using pooled human serum from the Red Cross (Interstate Blood Bank, LLC; Memphis, TN). The recovery studies consisted of three groups of 10 samples of 1 mL of serum each, for a total of 30 samples and were extracted based on the above extraction and clean-up methods.

Group A consisted of 5 low concentration quality control serum samples (QCL) spiked with 100 µL 500 fg/µL native PBDE standard, an 5 high concentration quality

control serum samples (QCH) spiked with 100 µL 1 pg/µL native PBDE standard prior to the initial addition of 50% formic acid in water.

Group B consisted of 5 QCL serum samples spiked with 500 fg/µL native PBDE standard and 5 QCH serum samples spiked with 1 pg/µL native PBDE standard after the extraction and clean-up procedures after evaporation to dryness, and then brought to dryness again before reconstitution with toluene.

Group C consisted of 5 QCL and 5 QCH serum samples that were not spiked with native standard.

We saw poor recovery (20-60%) of PBDE congeners during our recovery study using our hybrid method. Therefore we conducted further experimental procedures to determine where the loss of compounds was occurring. We determined that the majority of the loss of compounds was occurring during the Strata™-X SPE procedure, and replaced this extraction step with a liquid/liquid extraction technique prior to the silica/acidified-silica procedure. The final extraction and amended clean-up procedures are listed below.

e. Final Liquid/Liquid Serum PBDE Extraction Method

As in our original method, each set of unknown samples was accompanied by one blank sample and four QC samples, two QCL and two QCH, using pooled human serum each. Each sample (1 mL serum) was pre-spiked with 100 µL ISTD. Additionally, each QC sample was spiked with 100 µL of their respective standard. Each sample was then spiked with 100 µL of straight formic acid and vortexed briefly. 5 mL of hexane was then added to each sample, which were then vortexed at 2000 rpm for 10 minutes. After

vortexing, an additional 5 mL of hexane was added to each sample, followed by an additional 5 minutes of vortexing.

f. Silica/Acidified-Silica Serum PBDE Clean-Up

The final serum clean-up procedure following PBDE extraction was performed using silica/acidified-silica columns prepared in house as described above. Columns were conditioned with 5 mL hexane. Samples were then loaded to the columns in two steps, briefly vortexing and manually shaking each sample immediately prior to loading it to the column. Sample tubes were then rinsed with 1 mL of hexane, briefly vortexed and shaken, and loaded to the columns. The sample breakthrough was collected in clean 15 mL glass centrifuge tubes. After samples finished loading, the 15 mL collection tubes were replaced by a new set of clean centrifuge tubes. The breakthrough collection tubes were inserted into the TurboVap® at 30°C at 15 psi to begin evaporation.

Columns were then eluted with 10 mL of 1:19 DCM:Hexane solution. The elutant was collected and combined with the sample breakthrough, which were then brought to total dryness in the TurboVap® at 30°C at 15 psi. Samples were reconstituted with 50 µL of toluene for analysis.

g. Method Validation

Abbreviated Extraction Recovery

Due to time constraints, an abbreviated extraction recovery experiment of the updated method was performed for the purposes of this paper. For the final study a full extraction procedure, as described above, will be performed and analyzed prior to publication. The abbreviated extraction procedure consisted of four quality control

samples and one blank sample. The quality control samples consisted of two QCH samples and two QCL samples. One sample of each concentration was spiked pre-extraction with 100 µL of its respective standard, while the other sample in each concentration was spiked after extraction with 100 µL of the same standard. All samples, QCs and the blank, were post-spiked with 100 µL of ISTD. Samples were extracted according to the final serum extraction method and clean-up method as described above. Concentrations between pre- and post-spiked samples were compared to determine relative extraction recovery at each concentration.

Quality Control Precision Validation

The precision of the extraction method was validated at both high and low quality control concentrations. The precision validation procedure was carried out as five separate runs with identical methods and samples. Each run was composed of three blank serum samples, three QCL samples, and three QCH samples. All samples were spiked pre-extraction with 100 µL of ISTD and QC samples were spiked with 100 µL of their respective native standards. All samples consisted of 1 mL of pooled blank serum. The nine samples were extracted according to the final extraction method, followed by the silica/acidified-silica clean-up method. Samples were then analyzed.

Method Accuracy Validation

Method accuracy was calculated based on concentrations within the same calibration curve analyzed with each sample run. The spiked concentration was compared to the quantified concentration and expressed as a percentage. Values ranging from 80-120% are considered acceptable for a quantitative method.

h. Serum PBDE Analysis

Prepared samples along with a calibration curve as described above, were analyzed for both total PBDEs and seven specific congeners (BDE-47, -85, -99, -100, -153, -154, -209) using gas chromatography-tandem mass spectrometry (GS-MS/MS) in house (Agilent Technologies; 7000 GC/MS Triple Quad). Specified GC-MS/MS procedures are shown in Appendix A. Congeners were identified chromatically and are illustrated in Figure 4. The limits of detection for all congeners using our method and instrumentation were determined prior to sample analysis and are listed in Table 3.

i. Lipid Determination

Due to time constraints the lipid content of the serum samples was not measured in the laboratory. For this study it was assumed that all samples had similar lipid content as all subject samples were taken under fasting conditions. In order to lipid adjust the serum BDE concentrations we assumed an average lipid content of 400 mg/dL based on average child serum lipid data (Tamimi et al. 2011). In the future, the total lipid content will be determined by measuring triglyceride and total cholesterol in each sample and calculating total serum lipid content using the short-form equation TL = (2.27*TC) + 62.3 mg/dL; where TC represents total cholesterol and TG represents total triglycerides (Bernert et al. 2007). Serum triglyceride content will be measured using the BioVision® Triglyceride Quantification Assay Kit (BioVision® Research Products; Mountain View, CA) and serum cholesterol content will be measured using the Cayman Cholesterol Assay Kit (Cayman Chemical Company; Ann Arbor, MI).

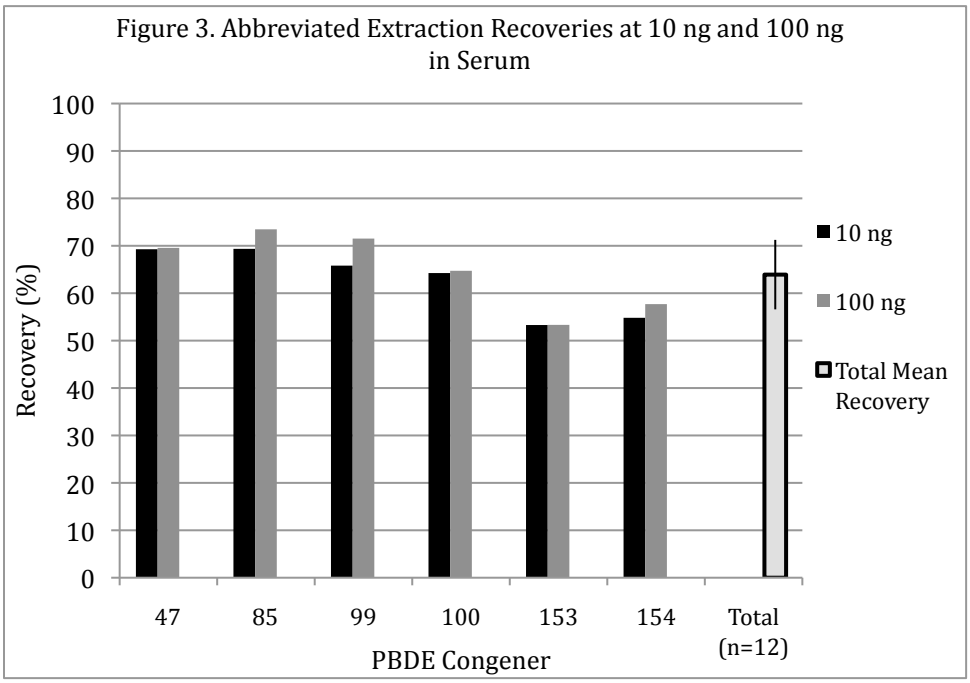
j. Data Analysis

All statistical analyses were performed using Microsoft® Excel 2008 Software (Version 12.3.2) with the Data Analysis add-on and SAS® Statistical Software (Version 9.3). One-way ANOVA tests, Student's T-tests, and simple linear regression analyses were used to investigate statistical associations between serum PBDE concentrations and covariates of interest.

3. Results

a. Method

The abbreviated extraction recovery shows consistent congener recoveries between 50-75% at both high and low concentrations, with decreasing percent recovery seen as bromination decreases, illustrated in Figure 3 below. While the percent recovery is not as high as was anticipated, the use of labeled internal standards in all of our samples, allows for 100% recovery correction after analysis.



Method accuracy was calculated based on a matrix calibration curve, using 1 ng and 25 ng levels, analyzed with both sets of unknown serum samples. Accuracy was calculated for all congeners at both quality control concentrations, as seen in Table 4. Except for BDE-100 at 1 ng, accuracies for all congeners at both concentrations were consistently with the FDA acceptable range of 100 ± 20% (FDA 2001). The accuracy of BDE-47 at 1 ng is high, at 119 ± 2.8.

Figure 5a. BDE-47 Curvlinear Calibration Curve

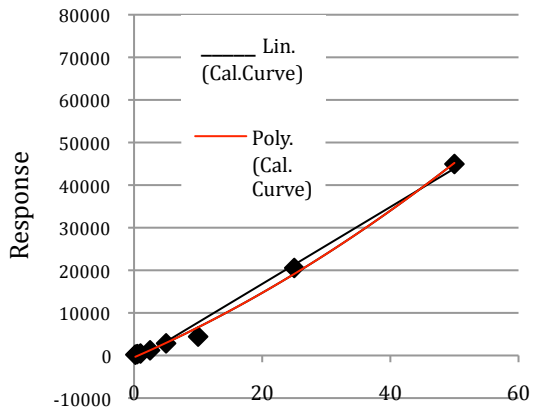


Figure 5b. BDE-47 Linear Calibration Curve

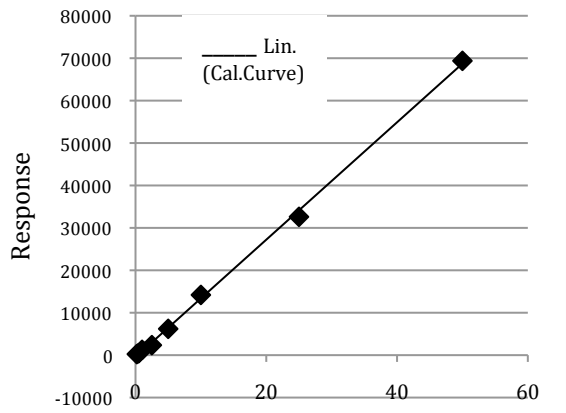


Figure 5a above illustrates the curvilinear calibration plot seen for BDE-47 with a polynomial fit, compared to the linear fit curves seen for all other congeners. Figure 5a was prepared using a more recent batch of Red Cross pooled human serum than Figure 5b. Figure 5a was utilized for the analyses in this study. Although it is unlikely that the curvilinear fit made a significant difference in concentration quantification, it is possible that there may be an upward bias of approximately thirty percent in the BDE-47 concentrations reported in this study. If this upward bias exists it brings the high accuracy of BDE-47 seen at 1 ng down to a more normal range. This phenomenon was only seen with BDE-47, all other congeners had linear curves with both serums. This illustrates the high sensitivity of BDE-47 to the serum matrix.

Method precision was calculated from the quality control validation experiments. The method precision was determined by congener within each of the five runs, among all the runs, and for all of the data points. The percent relative standard deviation were calculated for each category for both quality control concentrations, shown in Table 5 below. The results show that the method was very precise, with all percent relative standard deviations less than 8%.

Table 2. Quality Control Precision Data for 1 ng and 25 ng Concentrations in Serum

		BDE-	**47**	**100**	**99**	**85**	**154**	**153**
Within Runs	10 ng n=3/day	**% RSD**	1.85	1.99	5.32	5.11	6.26	6.47
	100 ng n=3/day	**% RSD**	1.85	2.76	4.24	4.55	4.66	2.90
Among Runs	10 ng n=5 days	**% RSD**	2.06	3.95	4.16	3.74	3.88	4.43
	100 ng n=5 days	**% RSD**	1.85	2.76	4.24	4.55	4.66	2.90
Total Points	10 ng n=15	**% RSD**	2.58	4.07	6.39	5.65	6.67	7.66
	100 ng n=15	**% RSD**	3.58	3.74	4.24	6.02	5.87	5.17

b. Subject Demographic Data

This study achieved extremely high enrollment with a refusal rate of only 6.3%. This is likely due to the fact that eligible participants' parents were approached by the research nurse regarding enrollment while the participants were already at the hospital for one or more routine surgical procedures. The blood sampling took place while the children were under general anesthesia, avoiding the general pain and discomfort experienced by children of this age group during blood sampling procedure while conscious. The full study recruited a total of 81 eligible participants, 37 of which were extracted for this analysis. The 37 samples were randomly selected from each age strata

to ensure a relatively even age distribution for analysis. A summary of subject demographic information can be found in Table 6 below.

Table 6. Subject Demographic Information

	n	Frequency (%) within n
Age (months)	n=37	n=37
15-23	6	16.22
24-35	10	27.03
36-47	6	16.22
48-59	8	21.62
60+	7	18.92
Sex	n=37	n=37
Male	23	62.16
Female	14	37.84
Race	n=37	n=37
African American	13	35.14
Caucasian	20	54.05
Mixed	4	10.81
# of Surgical Procedures	n=37	n=37
1	17	45.95
2	11	29.73
3	9	24.32
Household Smoking	n=32	n=32
Yes	6	18.75
No	25	78.13
Other (outside only)	1	3.13
Insurance Status	n=34	n=34
Medicaid	21	61.76
Private	13	38.24
Breastfeeding Status	n=37	n=37
Yes	24	64.86
No	13	35.14
BMI Age Related Percentile	n=31*	n=31
0>25	5	16.13
25>50	2	6.45
50>75	6	19.35
75>100	18	58.06

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

Subject BMI measurements were calculated using the "Children's BMI Group Calculator" Excel system developed by the Centers for Disease Control and Prevention (CDC). The calculator uses height, weight, sex, and age measurements to determine a child's BMI age-adjusted percentile that can be used to compare children of different

ages to one and other (CDC 2010). As children age, the amount of body fat changes, and differs for girls and boys, using age-adjusted percentiles allows for comparison between different age groups and sexes (CDC 2010). Child BMI's can be interpreted by their age-adjusted percentiles; children with BMI's less than the 5th percentile are classified as underweight, those with percentiles between the 5th and 85th percentile are classified as having healthy weights, those with BMI's between the 85th and 95th percentile are classified as overweight, and children with BMI's greater than the 95th percentile are classified as obese (CDC 2010). BMI cannot be accurately calculated for children less than two years of age and were therefore left out of BMI analyses.

As illustrated in Table 6, subjects were relatively evenly distributed across age strata with more two to three year olds than other age groups. There were more male subjects than female subjects in this sample. The majority of subjects were breastfed in the past and came from homes with no reported household smoking. This population included thirteen African American subjects, the largest number investigated for PBDE exposure in this age group to-date.

c. Serum PBDE Distributions

Individual serum congener data points measured at concentrations below the limit of detection were imputed by dividing the congener specific limit of detection by the square route of two. The congener specific frequencies of detection above the LOD can be seen in Table 8. SumPBDE concentrations were calculated by adjusting the individual congener values using their respective molecular weights, and summing the adjusted values together. Table 7 summarizes and characterizes the distribution of all six

congener concentrations and sumPBDE concentrations broken down by age, sex, insurance status, race, and breastfeeding history.

Table 8. Serum Congener Frequencies of Detection

BDE-	Frequency (%)
47	97.44
85	0
99	100
100	69.23
153	48.72
154	7.69

All congeners and sumPBDE distributions were skewed. After log-transformations, BDEs-47, -99, -100 and sumPBDE were normalized. BDE-153 and -154 distributions were less skewed as non log-transformed distributions.

As hypothesized, there was wide variation in serum BDE concentrations among the subjects, with one or two subjects with high concentrations often skewing the distribution. Serum BDE-47 concentrations were the highest of all congeners, as anticipated. Serum BDE-85 concentrations were all below the LOD.

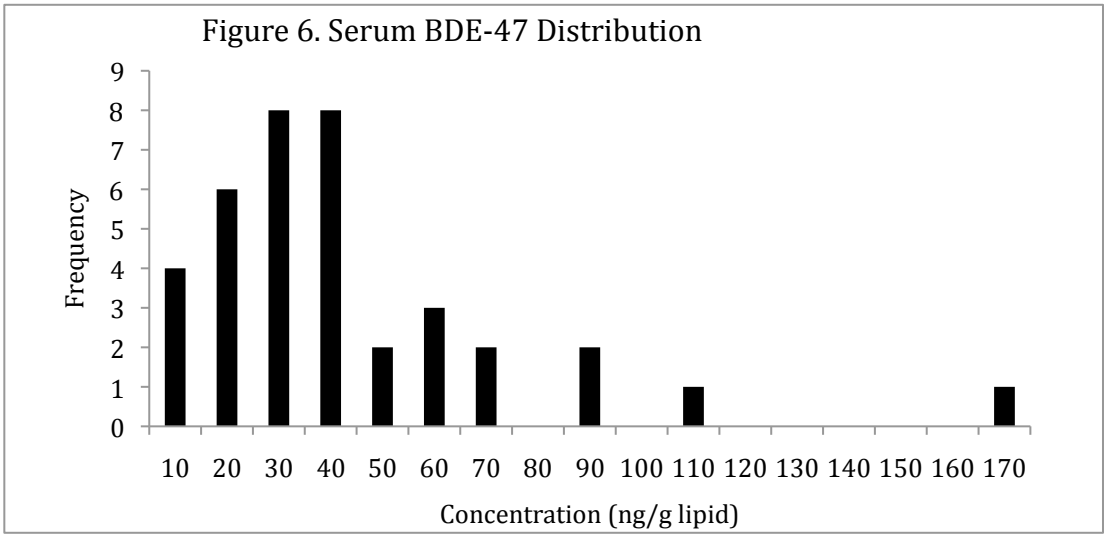


Figure 6 depicts the distribution of serum BDE-47 concentrations among the individuals. The vast majority of individuals had serum BDE-47 concentrations between 10 and 40 ng/g lipid, however a few individuals had very high levels over 100 ng/g lipid. The median concentration was 31.15 ng/g lipid, while the concentrations ranged from 8.04 ng/g lipid to 168.88 ng/g lipid. Figure 7 depicts the mean serum BDE-47 distribution by age group. The two-year-old age group has the highest mean concentration for all congeners except for BDEs-153 and -154.

Figure 7. Distribution of Serum BDE-Congeners by Age Group

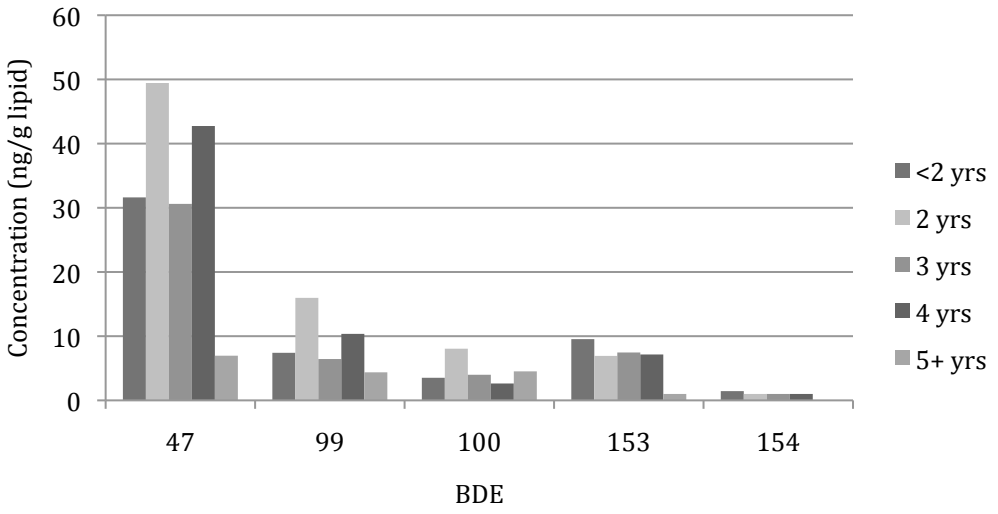
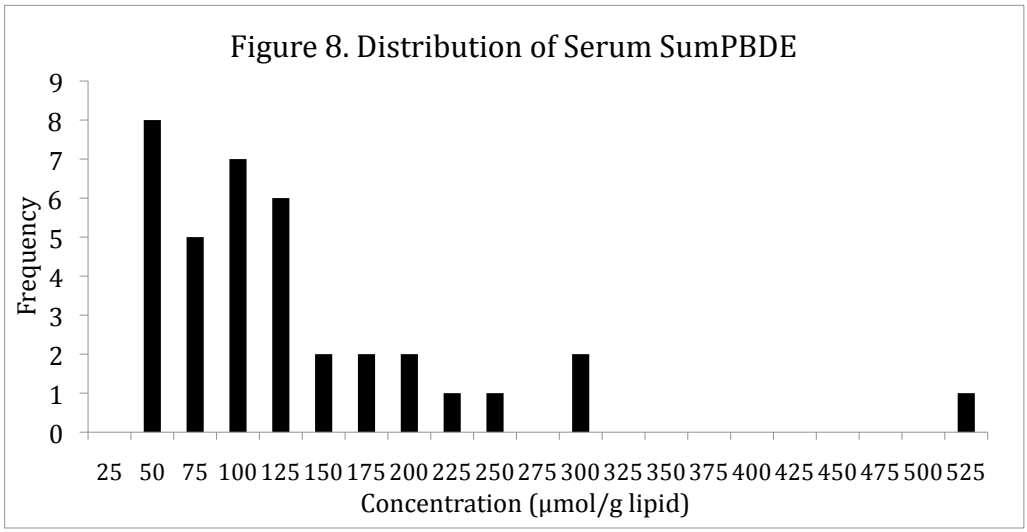


Figure 8 below, illustrates the sumPBDE serum concentration distribution. As seen in the serum BDE-47 distribution, a few individuals with very high concentrations are skewing the distribution of the subjects. The median sumPBDE concentration was 95.22 µmol/g lipid, concentrations ranged from 31.17 µmol.g lipid to 520.66 µmol.g lipid.



d. Statistical Analyses

Statistical analyses were performed on both log-transformed and non log-transformed congener and sum data. BMI age-adjusted percentile data was log-transformed and analyzed using both forms. Associations were tested for using both congener specific and sumPBDE concentrations. BDE-85 was not used in analyses, as all concentrations were the same, below the LOD. Analyses were performed using both continuous age and BMI percentile data and categorical age and BMI data. Age was data was categorized by year and BMI data was categorized into tertiles. Associations between congeners and sumPBDE concentrations and the following covariates were analyzed: age, BMI, sex, race, insurance status, and breastfeeding history.

Associations

Associations between serum congener concentrations and categorical variables with more than two categories, age group, BMI tertiles, and race, were tested using one-way ANOVA tests. Associations between serum concentrations and continuous age (months) and BMI percentile were tested using simple regression analyses. Two-sample

T-tests were used to test associations between serum concentrations and sex, breastfeeding history, and insurance status.

Age and Serum PBDE Concentrations

A significant association was found between age categories and BDE-154, when testing both the log-transformed and untransformed data. In the case of the more normal raw BDE-154 data, the F-statistic was 2.63, with a p-value of 0.026. Therefore, we reject the null hypothesis that all the mean BDE-154 concentrations in all five age groups are equal, and conclude that at least one of the groups' means is significantly different from the others at the α=0.05 level. The 15month–2 year group had a mean of 1.44 ng/g lipid, while all other age groups had means of 1.00 ng/g lipid.

Similarly, when investigated as a continuous variable, there was a significant negative association seen between an individual's age in months and their serum BDE-154 concentrations at the α=0.05 level. The R-squared for the association was 0.1157, with a p-value of 0.0394. For every month a child ages there is a 0.00649 ng/g lipid decrease in serum BDE-154 concentration.

BMI and Serum PBDE Concentrations

When tested as a continuous variable, there were no significant associations seen between BMI age-adjusted percentile and serum BDE concentrations. When categorized into tertiles, significant associations between BDE-47, -99 and sumPBDEs were observed. However, these distributions were skewed, and when the association was tested with their log-transformed normal distributions, no association was observed. It is likely that there is no association seen with serum concentrations and BMI due to the lipid adjustment of the serum PBDE concentrations themselves.

Race and PBDE Concentrations

Race was separated into four categories for statistical analyses, African American, Caucasian-White, Caucasian-Hispanic, and Mixed race. Significant associations between race and BDEs-47, -100, and sumPBDE concentrations were found at the α=0.05 level. However, the association only held for BDE-100 when the normal log-transformed distribution was analyzed. The ANOVA test determined that there was a significant difference between the mean logBDE-100 serum concentrations at the α=0.10 significance level, but not at the α=0.05 significance level. The F-statistic was 2.56 and the p-value was 0.0721. The non log-transformed BDE-100 means were 2.52 ng/g lipid for African Americans, 8.24 ng/g lipid for Caucasian-White, 4.15 ng/g lipid for Caucasian-Hispanic, and 1.96 ng/g lipid for Mixed race.

Breastfeeding History and PBDE Concentrations

Child history of breastfeeding was significantly associated with BDE-47, -99, -100, and sumPBDEs. All associations were seen with both the more normally distributed log-transformed data as well as the non log-transformed concentrations. All associations showed a positive association between history of breastfeeding and higher serum concentrations. The T-statistic for the logBDE-47 test was 2.12 and the p-value was 0.0414. Therefore, we conclude that there is a significant difference in mean logBDE-47 concentrations at the α=0.05 level. The group that was not breastfed had a higher mean concentration than that of the children who were breastfed; the non log-transformed means were 53.58 ng/g lipid and 30.69 ng/g lipid, respectively. The T-statistic for the logBDE-99 test was 2.89 and the p-value was 0.0066. There was a significant difference

in the mean logBDE-99 concentrations between those who were breastfed and those who were not at the α=0.05 significance level. The non-log transformed means of children who were not breastfed and those who were breastfed were 15.22 ng/g lipid and 7.35 ng/g lipid, respectively. The T-statistic for the logBDE-100 test was 1.86 and the p-value was 0.0713. There was a significance difference between the mean logBDE-100 concentrations of those who were breastfed and those who were not at the α=0.10 level, but not at the α=0.05 level. The non log-transformed means of the children who were not breastfed and those children who were breastfed were 8.29 ng/g lipid and 2.89 ng/g lipid, respectively. Finally, the T-statistic for the logsumPBDE test was 2.28 with a p-value of 0.0286. At the α=0.05 significance level, there was a significant difference between the mean of the children who were breastfed compared to the mean of the children who were not breastfed. The non log-transformed means concentration of those who were not breastfed and those who were breastfed were 165.5 µmol/g lipid and 94.96 µmol/g lipid, respectively.

Sex, Insurance Status, and Serum PBDE Concentrations

No significant associations between serum PBDE concentrations and sex or insurance status were found during analysis.

4. Discussion

a. Analysis Methods

We encountered many difficulties during extraction method development. In an attempt to improve upon past procedures used in the literature, we combined multiple extraction methods into one initial method. After multiple lab experiments and validation

trials, we discovered that we could not reproduce the recovery results previously reported using the Strata^{TM}-X SPE columns, even after switching to a wider pore size and switching extraction solvents from DCM to a 1:19 DCM:Hexane solution (Zhang and Rhind 2011). Therefore, we eliminated the Strata^{TM}-X SPE extraction from our method and replaced it with a liquid/liquid extraction using Hexane. The recovery was significantly higher using the new liquid/liquid method, however, only when using the fast speed (2000 rpm) single-vortexer, not the multi-shaker that was used for the full-recovery study. The low recovery using this method with the multi-shaker vortexer forced us to conduct the abbreviated recovery experiment discussed previously, due to time constraints. While even this technique did not provide the extraction percent recovery we hoped for, we corrected for this by utilizing labeled internal standards.

b. Data

This study is one of only three studies to date investigating the exposure of young children to PBDEs. The previous two studies have been in very homogenous groups, the first comprised of a high SES Caucasian population, and the other in a larger Mexican-American population in California, where strict fire codes/flame-retardant regulations are thought to increase exposures. This study is the first to include a more heterogeneous demographic population, with the first relatively large group of African American subjects in this age group (n=13). The subjects had mixed SES based on analysis of their insurance statuses. The results of this study, while still not necessarily applicable to the general population, can be more broadly applied to this age population than previous studies. The Lunder et al. study's population was composed exclusively of children who

were breastfed, while the population in the current study was mixed, allowing for analysis of concentrations by breastfeeding history (Lunder et al. 2010).

We measured all concentrations in ng/g lipid, except for the molecular weight adjusted sumPBDE measure, in order to correspond to previous and future studies on PBDE exposure levels to allow for comparison between studies. We utilized BDE-47 concentrations as our comparison, as this compound is almost always included in PBDE exposure studies, and is rarely measured at non-detectable levels in human serum. However, as discussed previously, BDE-47 appears to be more sensitive to the serum matrix than other congeners. This sensitivity, illustrated in Figures 4a and 4b, by both the curvilinear and linear curves found in two different batches of pooled human serum, suggests that this analysis, and potentially previous analyses in past studies where only neat standards were utilized, could be reporting upward biased serum BDE-47 concentrations. In this study it is estimated that there is a possible thirty percent upward bias in the BDE-47 concentrations, however, it is unclear if this was actually a factor in quantification. In the full study of this population, this phenomenon will be addressed through further laboratory and statistical analyses.

As anticipated, serum concentrations of BDE-47 were higher than all other congeners, followed by BDE-99. Individual subject concentrations varied greatly for all congeners, except for BDE-85, where all levels were below the limit of detection. This supports the hypothesis that using pooled data may over estimate PBDE exposure, as a few individuals with high concentrations pull up the mean. Individual sample analysis is necessary to determine accurate exposure levels using median exposures as mid-point

comparisons between studies instead of skewed means.

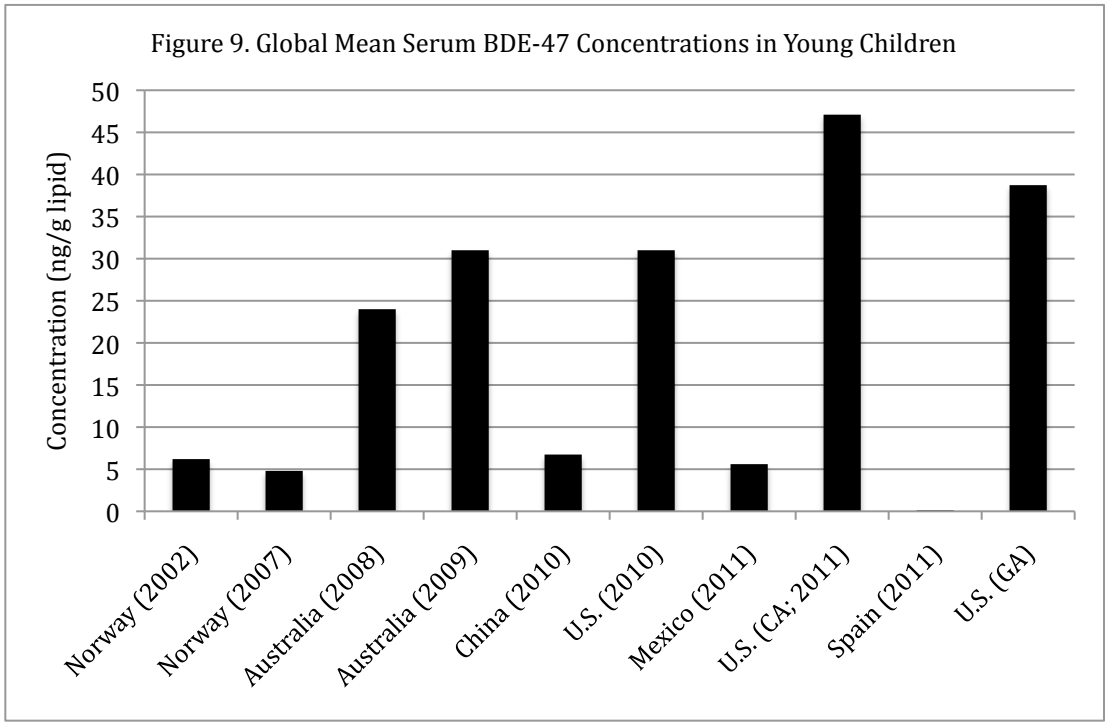


Figure 9 compares the mean serum BDE-47 levels in young children reported in global PBDE exposure studies listed in Table 1 (Gascon et al. 2011; Lunder et al. 2010; Eskenazi et al. 2011; Thomsen et al. 2002; Chen et al. 2010; Toms et al. 2008; Toms et al. 2009; Thomsen et al. 2007). The mean concentrations are used here instead of medians, despite the skewed distribution in this study, in order to facilitate comparison between individual sample analysis studies and pooled sample studies where only a mean concentration was reported. As illustrated in figure 9, the levels in this study's population are the second highest of all PBDE exposure studies in this age group, globally. Only the Mexican-American subjects in the CHAMACOS cohort have a higher mean serum BDE-47 concentration. As stated previously, the CHAMACOS cohort is located in California, which has very strict fire code and flame-retardant regulations in homes and products. It is likely that individuals in this state are exposed to much higher

levels of PBDEs than in other regions of the United States, and may explain the heightened mean concentration in this population compared to our study population.

Table 9. Significant Associations Found Between Serum PBDE Concentrations and Covariates of Interest

Covariate	**BDE-**	**Summary**
Age	154	Decrease with age
Race	100	Highest levels in Caucasian-White group
Breastfeeding History	47, 99, 100, sumPBDE	Higher levels in Breastfed group
*All associations described in detail in results section		

Table 9 summarizes the significant associations found in the study. As reported in previous studies, we found no association between subject sex and serum PBDE concentrations. This could be due to the lack of physical and behavioral differences between the sexes in this age range, compared to the adult population. However, the sample size is still small and simply may not be large enough to detect an association if it existed. The same could be true for the lack of association seen with insurance status, our proxy for SES.

The association seen with BDE-47, -99, -100 and the sumPBDE concentrations with subject history of breastfeeding is surprising. Across these congeners and the sumPBDEs we see a lower mean concentration in subjects who were not breastfed compared to those who were breastfed. However, in this analysis we were unable to control for possible confounders that could be causing the association. The uneven distribution of subjects who were breastfed versus those who were not breastfed, and the small sample size overall could also explain this association. Further analyses with large sample sizes are necessary to validate or refute the association. While we know that breastfeeding is a large source of exposure for infants, the exposures from breastfeeding,

especially in the older age categories, have likely been cleared from their bodies by this stage of their lives.

As mentioned previous, this cohort is especially interesting based on its racial distribution. Although it was still a small cohort, when stratified by race into four categories, African American, Caucasian-White, Caucasian-Hispanic, and Mixed race, we observed a significant difference in at least one of the categories mean serum BDE-100 concentration when analyzed using one-way ANOVA statistical test. We observed the highest serum BDE-47 mean concentration in the Caucasian-White race category (8.24 ng/g lipid), followed by the Caucasian-Hispanic group (4.15 ng/g lipid), the African American group (8.24 ng/g lipid), and finally the Mixed race group (1.96 ng/g lipid). We saw associations in the non-log transformed skewed serum BDE-47 and sumPBDE concentration means, however, when log-transformed, the associations did not hold. Race will be an especially interesting covariate to investigate with the larger full sample size in the future, as well as in future studies.

One of our hypotheses in this study was that the distribution of serum PBDE concentrations would vary by age group. After performing the statistical analysis between age and serum concentrations we only observed a significant association between age and serum BDE-154 concentrations, when analyzing age as a categorical variable and a continuous variable. There was a significant difference between one of the age groups mean serum BDE-154 concentrations compared to the others. The 15month >2 year age group was the only group with a mean above 1.00 ng/g lipid, of 1.44 ng/g lipid. However, this association is based on only two individuals in the 15month>2 year age group with concentrations above 1.00 ng/g lipid. When analyzed as a continuous

variable measured in months, age was negatively associated with BDE-154 concentrations. Again, however, this is due to two individuals less than two years of age with levels above 1.00 ng/g lipid.

We saw no significant associations with BMI when analyzed against the normal log-transformed congener concentrations. This is likely due to the lipid adjustment performed on the congener concentration data and is likely not an important variable of interest when looking at lipid-adjusted values.

All associations seen and unseen in this study are subject to scrutiny as they were performed on a small sample size. Running the same analyses on the full subjects group will give these analyses more power and may change the associations we see as significant in this study.

c. Strengths & Limitations

Although the sample size in this study is comparable to and greater than many of the previous PBDE exposure studies conducted on this age group, thirty-seven is still a relatively small sample size, especially when performing statistical analyses broken down by strata. However, this study has a much more demographically heterogeneous subject group than previous studies, especially in the United States, giving us the ability to test for associations on demographic covariates that previous studies have not been able to investigate, such as race, breastfeeding history, and SES. However, we were not able to have a fully even age-sex distribution, which may have influenced the results of our analyses.

The novel sampling and enrollment approach tested in this study allowed us to achieve a refusal rate of only 6.3%, which is extremely high, especially for a study requiring blood samples from young children.

Due to time constraints we were unable to measure individual lipid content of the serum samples and used an average lipid content for children in this age range. Despite the fact that all of the children were fasting for their surgical procedures, the use of an average lipid content for all individuals, could potentially alter the accuracy of our lipid-adjusted serum PBDE concentrations.

d. Conclusions & Future Directions

This study constituted only a portion of the larger BEAT study that is currently still underway, and will be used as guidance for the future analysis of the full 81 participants. This will increase the power of the study, and may provide the opportunity for more powerful statistical analysis and the possibility of more associations to found between the covariates and serum concentrations.

For the full study we will build on the abbreviated extraction recovery by performing a full recovery study and analysis in the laboratory. Further investigation of the matrix effect on BDE-47 will also be conducted to determine if we our current analysis is truly presenting an upward bias in concentrations, if so, we will correct for this in the final study report. Full lipid determination using enzymatic assays will also be conducted to more accurately lipid adjust the serum PBDE concentrations for the final analysis and report.

The full study is also analyzing serum thyroid hormone levels in each subject. Associations between hormone levels and serum PBDE concentrations will be analyzed,

and used to further investigate PBDEs and thyroid toxicity. In addition to serum samples, house dust and hand wipe samples are being collected from a portion of eligible participants to investigate the correlation between serum PBDE concentration and these other matrices to help us understand the routes of exposure of this population. The aim of all of these additional analyses will be to present our findings in the format of numerous peer-reviewed published papers in scientific journals, with the hopes of eventually continuing the research of this pilot study with a much larger full study in the future.

Beyond this study's future, it will be important for the field to begin monitoring exposures in this age group on a broader scale, as well as further investigating the health effects of these compounds in humans through further long-term epidemiologic study. It will be necessary to broaden the scope of analysis from PBDE exposures alone, to new and emerging flame-retardants such as Firemaster 550 and Chlorinated Tris, which have begun to replace PBDEs in the marketplace. Exposures and toxicity to these compounds are still unknown, but as their use begins to grow and the use of PBDEs begins to decline, they will become a major player in flame-retardant exposures.

In addition to the additional knowledge this study will add to the field of PBDE exposures in young children, the novel approach of blood sample collection used in this study, and our success in extremely high enrollment, has tremendous potential for use in future studies in all areas where blood samples are needed from this age group. The lack of pain and discomfort during blood sampling likely eases the minds of the parents, aiding with parental consent. Because the subjects underwent routine surgical procedures they presented a relatively healthy and demographically varied subset of the general

population, and did not have other underlying conditions or medical problems that would otherwise potentially confound the results of the exposure assessment.

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7. Tables & Figures

Table 2. Compounds Used in the Present Study: Sources and Grades

Compound	**Grade/Purity**	**Source**
Formic Acid	46.03%	Fisher Scientific™
Dichloromethane (DCM)	HPLC Grade/≥99.8%	Sigma Aldrich®
Methanol (Mesh)	HPLC Grade	Sigma Aldrich®
Hydrochloric Acid (HCL)	6N	Fisher Scientific™
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™

Table 3. GC-MS/MS PBDE Congener Analytical Limits of Detection

Congener	**LOD (pg/mol)**
47	0.499
85	3.399
99	1.497
100	1.603
153	15.806
154	5.531

Table 4. Quality Control Method Accuracy						
Concentration	**BDE-47**	**BDE-85**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**
1 ng/mL	119 ± 2.8	90 ± 9.0	80 ± 1.6	70 ± 3.5	87 ± 3.1	86 ± 5.0
25 ng/mL	99 ± 0.8	107 ± 10.0	102 ± 4.8	106 ± 0.2	106 ± 12.0	101 ± 2.7

Figure 4. GC/MS Congener Chromatogram Profile By Acquisition Time

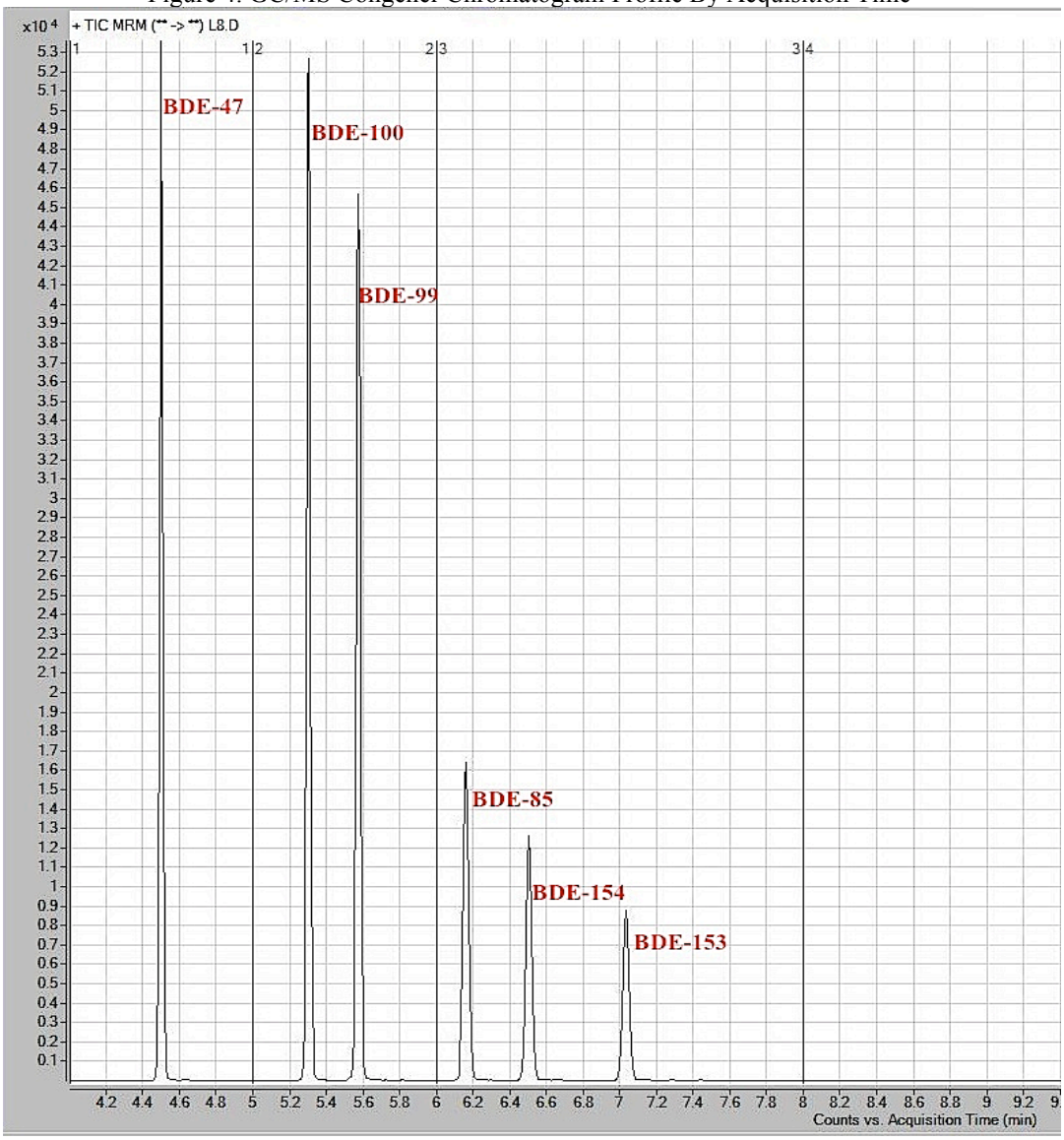

Table 7. Serum Congener (nag/g lipid) and Summed (µmol/g lipid) Concentrations by Strata

	BDE-	Mean	Std Dev	GM	SE	Median	Range		Percentile	
							Min	Max	5%	95%
Total	**47**	38.73	31.81	4.37	5.23	31.15	8.04	168.88	8.33	102.80
n=37	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	10.12	9.92	2.41	1.63	8.29	2.24	58.94	2.82	23.28
	100	4.79	6.11	1.36	1.00	3.04	0.29	26.61	0.29	25.15
	153	7.03	7.96	2.00	1.31	2.87	2.87	40.62	2.87	27.86
	154	1.07	0.33	1.02	0.05	1.00	1.00	2.86	1.00	1.75
	SUM	119.74	94.82	7.23	15.59	95.22	31.17	520.66	33.06	283.41
Age 15-2	**47**	31.62	15.45	4.20	6.31	34.70	8.04	52.60	8.04	52.60
n=6	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	7.41	3.58	2.27	1.46	7.67	3.12	11.32	3.12	11.32
	100	3.52	2.43	1.47	0.99	3.78	0.29	6.77	0.29	6.77
	153	9.54	15.25	2.00	6.23	2.87	2.87	40.62	2.87	40.62
	154	1.44	0.76	1.12	0.31	1.00	1.00	2.86	1.00	2.86
	SUM	102.55	46.77	7.09	19.09	110.10	33.06	150.95	33.06	150.95
Age 2-3	**47**	49.44	47.58	4.66	15.05	31.64	8.33	168.88	8.33	168.88
n=10	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	15.97	15.97	2.85	5.05	12.91	2.24	58.94	2.24	58.94
	100	8.05	9.94	1.56	3.14	4.92	0.29	26.61	0.29	26.61
	153	6.93	4.61	2.13	1.46	6.22	2.87	16.21	2.87	16.21
	154	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	157.62	145.77	7.83	46.10	107.37	31.17	520.66	21.17	520.66
Age 3-4	**47**	30.61	23.77	3.93	9.71	22.33	9.18	67.86	9.18	67.86
n=6	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	6.44	3.65	2.13	1.49	5.39	3.44	12.62	3.44	12.62
	100	3.99	4.33	1.30	1.77	2.22	0.29	9.64	0.29	9.64
	153	7.46	10.02	1.96	4.09	3.12	2.87	27.86	2.87	27.86
	154	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	95.69	70.92	6.56	28.95	63.22	37.26	189.37	37.26	189.37
Age 4-5	**47**	42.74	29.29	4.71	10.36	32.66	14.38	102.80	14.38	102.80
n=8	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	10.36	7.69	2.50	2.72	7.42	3.02	23.28	3.02	23.28
	100	2.63	4.13	0.94	1.46	0.29	0.29	12.03	0.29	12.03
	153	7.15	6.49	2.06	2.29	3.84	2.87	17.73	2.87	17.73
	154	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	124.69	80.26	7.58	28.38	104.73	50.43	283.41	50.43	283.41
Age 5+	**47**	31.90	24.92	4.15	9.42	23.77	14.78	86.27	14.78	86.27
n=7	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	6.96	5.19	2.15	1.96	4.92	2.82	18.07	2.82	18.07
	100	4.37	2.96	1.64	1.12	4.39	0.29	10.07	0.29	10.07
	153	4.52	2.86	1.81	1.08	2.87	2.87	9.48	2.87	9.48
	154	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	95.34	67.42	6.77	25.48	82.12	47.10	242.07	47.10	242.07
Male	**47**	38.48	36.08	4.29	7.52	31.34	8.04	168.88	8.33	102.80
n=23	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62

	99	10.53	12.00	2.38	2.50	5.80	2.24	58.94	2.84	23.28
	100	4.50	5.94	1.30	1.24	3.04	0.29	26.61	0.29	12.03
	153	5.63	4.60	1.92	0.96	2.87	2.87	17.73	2.87	17.73
	154	1.04	0.16	1.01	0.03	1.00	1.00	1.75	1.00	1.00
	SUM	117.22	106.95	7.07	22.30	94.94	31.17	520.66	33.06	283.41
Female	**47**	39.13	24.48	4.51	6.54	29.90	9.18	86.27	9.18	86.27
n=14	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	9.44	5.30	2.47	1.42	8.36	2.82	19.40	2.82	19.40
	100	5.26	6.58	1.45	1.76	3.57	0.29	25.15	0.29	25.15
	153	9.33	11.43	2.15	3.06	4.02	2.87	40.62	2.87	40.62
	154	1.14	0.50	1.03	0.13	1.00	1.00	2.86	1.00	2.86
	SUM	123.88	74.21	7.51	19.83	101.65	37.26	281.99	37.26	281.99
Medicaid	**47**	33.30	21.38	4.19	4.67	31.15	8.04	85.20	9.18	67.86
n=21	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	8.65	5.67	2.32	1.24	6.41	2.24	21.42	2.82	19.40
	100	4.19	5.82	1.25	1.27	2.63	0.29	25.15	0.29	12.03
	153	6.51	8.84	1.90	1.93	2.87	2.87	40.62	2.87	17.28
	154	1.13	0.43	1.04	0.09	1.00	1.00	2.86	1.00	1.75
	SUM	104.19	65.20	6.95	14.23	95.22	31.17	281.99	33.06	202.96
Private Insurance	**47**	47.97	45.50	4.64	12.62	29.77	8.33	168.88	8.33	168.88
n=13	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	12.81	15.06	2.56	4.18	8.44	2.84	58.94	2.84	58.94
	100	5.41	7.04	1.49	1.95	4.39	0.29	26.61	0.29	26.61
	153	6.59	4.67	2.07	1.29	5.48	2.87	17.73	2.87	17.73
	154	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	143.83	134.32	7.62	37.25	91.19	34.94	520.66	34.94	520.66
African American	**47**	24.70	12.48	3.82	3.46	21.26	9.18	52.60	9.18	52.60
n=13	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	6.50	3.41	2.13	0.95	4.92	2.82	12.35	2.82	12.35
	100	2.95	2.52	1.26	0.70	2.63	0.29	7.87	0.29	7.87
	153	6.63	10.40	1.87	2.88	2.87	2.87	40.62	2.87	40.62
	154	1.00	0.00	1.04	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	80.71	39.79	6.42	11.03	63.03	37.26	150.95	37.26	150.95
Caucasian White	**47**	54.72	42.13	5.03	10.88	44.24	8.33	168.88	8.33	168.88
n=15	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	14.59	13.96	2.77	3.60	10.46	2.24	58.94	2.24	58.94
	100	7.80	8.24	1.76	2.13	4.88	0.29	26.61	0.29	26.61
	153	8.47	7.29	2.22	1.88	5.93	2.87	27.86	2.87	27.86
	154	1.19	0.37	1.00	0.19	1.00	1.00	1.75	1.00	1.75
	SUM	168.03	126.39	8.29	32.63	119.34	31.17	520.66	31.17	520.66
Caucasian Hispanic	**47**	30.84	25.92	3.93	12.96	23.72	8.04	67.86	8.04	67.86
n=4	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	6.70	4.17	2.15	2.08	5.54	3.12	12.62	3.12	12.62
	100	3.98	4.15	1.38	2.08	3.00	0.29	9.64	0.29	9.64
	153	3.39	1.04	1.68	0.52	2.87	2.87	4.95	2.87	4.95
	154	1.19	0.37	1.06	0.19	1.00	1.00	1.75	1.00	1.75
	SUM	90.56	68.25	6.49	34.12	69.91	33.06	189.37	33.06	189.37

Mixed	**47**	33.54	15.14	4.43	6.77	28.64	16.02	56.78	16.02	56.78
n=5	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	8.82	4.89	2.43	2.19	7.03	3.44	15.01	3.44	15.01
	100	1.17	1.96	0.74	0.88	0.29	0.29	4.68	0.29	4.68
	153	6.67	6.17	2.02	2.76	3.37	2.87	7.28	2.87	17.28
	154	1.00	0.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00
	SUM	99.70	41.36	7.14	18.50	96.41	47.44	157.39	47.44	157.39
Breastfed	**47**	30.69	21.27	5.04	4.34	26.21	8.33	102.80	9.18	56.78
n=24	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	7.35	5.07	2.91	1.04	5.02	2.24	23.28	2.82	15.01
	100	2.89	2.81	1.76	0.57	2.62	0.29	9.29	0.29	9.20
	153	7.03	9.21	2.10	1.88	2.87	2.87	40.62	2.87	27.86
	154	1.08	0.38	1.02	0.08	1.00	1.00	2.86	1.00	1.00
	SUM	94.96	60.04	8.32	12.26	80.56	31.17	283.41	34.94	180.93
Not Breastfed	**47**	53.58	42.43	4.05	11.77	31.82	8.04	168.88	8.04	168.88
n=13	**85**	0.62	0.00	0.81	0.00	0.62	0.62	0.62	0.62	0.62
	99	15.22	14.22	2.18	3.94	12.35	3.12	58.94	3.12	58.94
	100	8.29	8.74	1.18	2.42	4.79	0.29	26.61	0.29	26.61
	153	7.03	5.28	1.95	1.46	4.95	2.87	17.28	2.87	17.28
	154	1.06	0.21	1.02	0.06	1.00	1.00	1.75	1.00	1.75
	SUM	165.51	128.70	6.70	35.69	110.50	33.06	520.66	33.06	520.66

8. Appendices

A. PBDE Instrumental Method Details GC-MS/MS

The method used for the analysis of PBDEs-47, 99, 100, 153, and was developed in-house in the Barr/Ryan Laboratory. The validation results indicate good precision, accuracy and limits of detection. Chromatographic separation was performed by Agilent 7890A Gas Chromatography (GC) with tandem mass spectrometry (Agilent Technologies, Waldbronn, Germany). The GC consisted of a temperature-stable column compartment. The GC and MS modules were programmed and controlled using Mass Hunter Software version B.03.01 (B2065) (Agilent Technologies, Waldbronn, Germany). PBDEs were analyzed by GC-QQQ-MS/MS (EI, -70eV). Analyses were carried out using a 7890A GC coupled to a 7000B MS (Agilent Technologies, Waldbronn, Germany). The system was fitted with a deactivated silica guard column (0.250 mm internal diameter (ID)) (Agilent Technologies, Santa Clara, CA USA) connected to a HP-5MS analytical column (15m x 0.250 ID x 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA USA). It was operated in pulsed splitless mode (24.656 PSI, 0.85 min) with an injector temperature of 250°C). The helium carrier gas flow rate was 1.8 mL/min and the oven temperature program was as follows: 100°C (0.1 min), 250°C (45°C/min), 275°C (5°C/min), and 315°C (45°C/min) held for 4 min. The interface, source, and quadropole temperatures were set to 315°C, 315°C, and 150°C, respectively. Multiple reactions monitoring (MRM) was used during the mass spectrometric analysis of the target compounds. The selected MRM transitions, including their associated parameters, are summarized in Table 8.

Table 8. Selected MRM Transitions and Associated Parameters

			Fragmentation from our Experiment (3 best transitions)								
PBDEs	**RT**	**MW**	**Quant**			**Qual_1**			**Qual_2**		
			Q1	**Q3**	**CE**	**Q1**	**Q3**	**CE**	**Q1**	**Q3**	**CE**
PBDE-47	4.56	207.3	485.6	326.0	25	325.7	217.0	25			
PBDE-100	5.37	284.8	563.6	404.0	20	565.5	406.0	25	403.6	137.0	55
PBDE-99	5.65	215.7	563.6	404.0	20	565.6	406.0	25	403.6	137.0	55
PBDE-85	6.25	209.2	565.6	406.0	20	563.6	404.0	40	405.6	137.0	25
PBDE-154	6.59	246.3	643.5	484.0	20	641.5	482.0	30	483.5	377.0	30
PBDE-153	7.12	223.2	643.5	484.0	20	483.5	324.0	40			