Human Exposure to Brominated Flame Retardants and Reproductive Health

by

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"On you will go though the weather be foul.
On you will go though the Hakken-Kraks howl.
Oh, the places you'll go!"
- Dr. Seuss

This dissertation is dedicated to all the other "first generation" college graduates considering a PhD. Know that you can do it, even if no one ever told you that you could.

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Table of Contents

Dedication ii
Acknowledgements iii
List of Figures v
List of Tables vi
Abstract vii
Chapter I: Introduction 1
Chapter II: Relationships between Polybrominated Diphenyl Ether Concentrations in House Dust and Serum 12
Chapter III: Brominated Flame Retardants in House Dust are Related to Hormone Levels in Men 36
Chapter IV: Serum and Follicular Fluid Concentrations of Polybrominated Diphenyl Ethers and *In vitro* Fertilization Outcome 77
Chapter V: Conclusion 101

List of Figures

Figure 2.1. PBDE Congener by proportion in dust and serum……...30
Figure 2.2. Within-couple serum PBDE correlation (sum of BDE 47, 99, 100)…32
Figure 3.1. Scatterplot of HBCD in house dust and ln-transformed free androgen index……68
Figure 3.2. Scatterplot of BTBPE in house dust and serum total T3……..69
Figure 3.3. Scatterplot of TBPH in house dust and serum total T3……70
Figure A-1. Example histogram of dependent variable……...73
Figure A-2. Example histograms of transformed dependent variable…….73
Figure A-3. Example histograms of independent variable……..74
Figure A-4. Bivariate relationships……..74
Figure A-5. Residual plot for model 4: FreeT4 = ln(sum of pentaBDEs) + age + bmi……..75
Figure A-6. Residual plot for model 1: ln(FSH) = ln(sum of pentaBDEs) + age + bmi…….75
Figure A-7. Smoothing plot for FreeT4 = s(sum of pentaBDEs) + age + bmi…...76
Figure A-8. Smoothing plot for ln(FSH) = s(sum of pentaBDEs) + age + bmi…..76

List of Tables

Table 2.1. Distribution of PBDE Congeners in House Dust.....28
Table 2.2. Distribution of PBDE Congeners in Serum……29
Table 2.3. Spearman correlation coefficients for selected PBDE concentrations in dust and serum…….....31
Table S1. Spearman correlation coefficients for PBDE concentrations in dust……..33
Table S2. Spearman correlation coefficients for PBDE concentrations in serum……...34
Table S3. Spearman correlation coefficients for PBDE concentrations in lipid adjusted serum of males and females…….35
Table 3.1. Distribution of brominated flame retardants in house dust……...65
Table 3.2. Spearman correlation coefficients among select BFRs in house dust……...66
Table 3.3. Percent change in hormone level (95% confidence intervals), relative to population median, associated with an interquartile range (IQR) increase in house dust BFR concentration……67
Table 3.4. Factor pattern for 8 independent variables, representing weightings of each congener, for all PBDE congeners detected in house dust……..71
Table 4.1. Characteristics of study population……96
Table 4.2. Distribution of PBDEs (ng/g wet weight) in serum (n=63) and follicular fluid (n=64)…….….97
Table 4.3. Kendall's Tau-beta correlation coefficients for PBDE concentrations in serum and follicular fluid……...98
Table 4.4. Distribution of follicular fluid:serum ratios……..99
Table 4.5. Odds ratios for implantation failure associated with elevated (above median, or, if <50% detected, detectable) levels in serum or follicular fluid……...100

ABSTRACT

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Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and other compounds are used in the manufacture of a variety of materials and consumer products to meet fire safety standards. BFRs persist in the environment and have been detected in wildlife, humans and indoor dust and air. Some BFRs have demonstrated adverse endocrine and reproductive effects, but human studies are limited. We investigated markers of exposure to BFRs using serum, ovarian follicular fluid and house dust collected from men and women attending infertility clinics. House dust concentrations of the major pentaBDE commercial formulation congeners (BDE 47, 99 and 100) were highly correlated (r=0.65-0.89) to serum concentrations of the same congeners, suggesting that dust is a major exposure source of these PBDEs. Serum concentrations of these congeners were also strongly correlated (r=0.85) between males and females, indicating that adults living in the same household have similar exposures. PBDE congeners in dust were grouped into penta-, octa- and deca-BDEs, resembling commercial mixtures, and alterations in hormone levels in men were modeled in relation

to PBDE exposure. Significant positive associations ($p<0.05$) were found between dust concentrations of pentaBDEs and serum levels of thyroid hormones T4 and T3, estradiol, sex hormone binding globulin (SHBG) and prolactin, along with an inverse association with follicle stimulating hormone. Positive associations between octaBDE concentrations and serum T4, thyroid stimulating hormone, luteinizing hormone and testosterone, and an inverse association between decaBDE concentrations and testosterone, were also found. Relationships between alternate BFRs and hormone levels were examined. Hexabromocyclododecane was associated with decreased SHBG and increased free androgen index. The association between serum and follicular fluid concentrations of PBDEs and failed embryo implantation was investigated. Women with detectable levels of BDE 153 in follicular fluid had elevated odds (adjusted OR=10.0, 95%CI: 1.9-52) of failed embryo implantation following *in vitro* fertilization (IVF), compared with women who had non-detectable concentrations. There was only a moderate correlation ($T_\beta<0.4$) between serum and follicular fluid concentrations of PBDEs; therefore follicular fluid PBDEs, which may be a more biologically relevant measure of exposure when studying IVF endpoints, may not be well-estimated by serum concentrations of PBDEs.

CHAPTER I

Introduction

Endocrine disrupting chemicals, substances that impede normal hormone synthesis, metabolism or action, have been implicated by a growing amount of animal and human evidence as a significant public health concern. Temporal and geographical disease trends point to environmental factors associated with endocrine disruption. For instance, male testosterone levels and semen quality have declined over the past several decades without a known cause (Swan et al. 2000; Travison et al. 2007), and rates of hypospadias and crytorchidism (abnormal reproductive tract development) (Paulozzi 1999) and testicular cancer (Huyghe et al. 2003) have increased, particularly in industrialized nations. Rates of congenital hypothyroidism and thyroid cancers in the US have been rising and cannot be explained simply by increased medical diagnoses (Enewold et al. 2009). Additionally, rates of impaired fecundity are on the rise according to US national statistics and reduced fertility remains a challenge worldwide (Chandra et al. 2005). Brominated flame retardants (BFRs) have been implicated as endocrine disruptors in a limited but growing body of scientific studies.

BFRs are a group of chemicals added to many types of consumer products (e.g. furniture, carpet padding, draperies, and electronics) and other applications (e.g. automobile seats and aircraft) to increase their fire resistance. BFRs release bromine atoms at high temperatures, capturing free radicals and halting the chemical reactions of

fires. In response to increasing fire safety regulations, the global use of BFRs has increased exponentially since the 1970s, to about 410,000 metric tons annually (Shaw et al. 2010). There are numerous types of flame retardants, and BFRs are one subset, with several different types comprising this subset. Most BFRs are additive, or mixed into, rather than chemically bound to the polymer material of the end product. BFRs commonly comprise up to 5% of the weight of the polyurethane foam in furniture or padded baby products, and 20% or more of the weight of plastics in electronic devices (Allen et al. 2008). BFRs can leach into the environment from the products or the material containing the BFR may physically degrade into dust particles.

BFRs are persistent bioaccumulative compounds that have become ubiquitous in the environment, in wildlife and in people, particularly in North America where their use is greatest and fire safety standards are strict. Some BFRs, such as certain polybrominated biphenyl ethers (PBDEs) have been banned or voluntarily phased out by manufacturers because of their toxicity and environmental persistence. Despite restrictions, older products, both still in use and in waste or recycling streams, continue to be a source of exposure. Several decades of high-volume use has resulted in pound levels of PBDEs in indoor and transportation environments (Shaw et al. 2010). This level of indoor contamination makes PBDEs unique among toxic chemicals. Additionally, as PBDEs are phasing out, they are being replaced by alternative chemicals with similar structure and little or no information on health or environmental impacts. Moreover, the public and scientific communities have begun to question the use of flame retardants, with respect to their benefits versus risks (DiGangi et al. 2010).

PBDEs share a similar structure to polychlorinated biphenyls (PCBs) which were banned from production in the US in 1979. Epidemiological studies provided evidence that PCBs impair thyroid homeostasis, cognitive function and sexually dimorphic behavior (as reviewed by Talsness 2008). Most of the available BFR monitoring and toxicity data is for PBDEs. PBDEs have demonstrated neurotoxic and adverse endocrine and reproductive effects in animal studies, but human health studies are limited despite evidence of exposure through dietary intake and contact with or inhalation of indoor dust (as reviewed by Costa and Giordano 2007; Darnerud 2008; Talsness 2008).

Because PBDEs have been measured in various foods, intake of contaminated food had been assumed to be the primary source of human exposure (Bocio et al. 2003; Ohta et al. 2002; Schecter et al. 2008), similar to the exposure scenario for PCBs. However, additional studies acknowledged that because US body burdens of PBDEs were so high compared to other countries with a similar degree of food contamination, food could not be the main source, and the indoor environment must play a major role in the exposure of Americans to PBDEs (Johnson-Restrepo and Kannan 2009; Lorber 2008; Schecter et al. 2006). It was estimated that 91% of a breast-fed infant's body burden is acquired via breast milk, but by the age of 1 to 5 years, 77% of the body burden is a result of increased hand-to-mouth contact with indoor dust (Johnson-Restrepo and Kannan 2009). Exposure to PBDEs in house dust was estimated, using pharmacokinetic modeling, to account for 82% of the body burden of American adults (Lorber 2008). Body burdens of young children are typically higher than those of adults in the same household (Lunder et al. 2010). Concentrations of PBDEs measured in people in the US and Canada are generally an order of magnitude higher than those from Europe or Asia,

which follows commercial use patterns for those countries (Hites 2004; Schecter et al. 2003). Occupational exposures are also of concern, particularly in electronic dismantling environments where workers have elevated levels of specific PBDE congeners associated with the types of materials in those environments (Qu et al. 2007; Sjödin et al. 1999).

The majority of the toxicological studies on BFRs have focused on effects on the thyroid system. In particular, several rodent studies demonstrated lower levels of the thyroid hormone thyroxine (T4), some with a measurable corresponding increase in thyroid stimulating hormone (TSH), in response to administration of PBDEs (Ellis-Hutchings 2006; Fowles 1994; Hallgren 2001; Kuriyama 2007; Skarman et al. 2005; Stoker et al. 2005; Zhou 2001, 2002). PBDEs are thought to disrupt thyroid homeostasis mainly by competing with T4 for binding to transthyretin (TTR) (Meerts et al. 2000), or possibly by binding directly to thyroid hormone receptors (Marsh et al. 1998). Human epidemiological studies do not seem to be consistent with animal models, as most studies found positive associations between PBDE exposure and T4 levels (Bloom et al. 2008; Dallaire et al. 2009; Gascon et al. 2011; Meeker et al. 2009a; Turyk et al. 2008; Wang et al. 2010). However, the mechanism of thyroid hormone disruption in rodents may differ from humans due to differences in thyroid hormone transport proteins. In humans, thyroxine-binding globulin (TBG) is the major protein that binds circulating T4 (Klaassen, ed. 2001). TBG has approximately a thousand times the binding affinity for T4 than does TTR. While TTR is also present in humans at a lesser percent, it is less important to the transport of T4 as compared to rodents which do not have TBG. This difference may be one reason why rodents may be more sensitive to thyroid perturbations by the stimulation of TSH. Nevertheless, alterations in thyroid hormone levels in humans

may be an important indicator of potential disease, and rodent studies may still be informative, particularly if performed using exposure concentrations relevant to humans. Thyroid hormone homeostasis is very important to physiological processes such as normal brain development, metabolism and reproductive function, and the alteration of this homeostasis is one possible mechanism by which PBDEs may affect the health of humans and other animals.

Animal studies have shown that BFRs can also alter reproductive hormone homeostasis. Specifically, several studies demonstrate anti-androgenic activity of BFRs both *in vitro* and *in-vivo*, including alterations of hormone levels, delayed puberty, and decreased growth of androgen-dependent tissues (Hamers et al. 2006; Kuriyama 2005; Stoker et al. 2005; Stoker 2004; Ven et al. 2008). The endocrine disrupting properties may be dependent on the specific structure of the compound or its metabolites. For instance, depending on the degree of bromination, PBDEs may have either estrogenic or antiestrogenic effects (Hamers et al. 2006; Meerts et al. 2001). Because different PBDE congeners may have different effects, studying PBDE exposure becomes complicated by the numerous congeners that are measurable in environmental and biological samples. Comparing between studies is difficult, especially when experimental studies expose animals to a congener mixture or when epidemiological studies only report analyses using summed PBDE congeners.

The present research utilizes data and environmental and biological samples from two large ongoing studies of environmental exposures among couples undergoing assisted reproductive technologies. Serum, follicular fluid and house dust are examined as markers of exposure to BFRs for environmental epidemiology studies. Alterations in

serum thyroid and reproductive hormones in men and early pregnancy loss (failed implantation) in women undergoing *in vitro* fertilization (IVF) are the health outcomes investigated in relation to BFR exposure. The validity of using house dust as an exposure marker is examined in Chapter 2, where house dust concentrations of PBDEs are compared to serum concentrations in male-female couples residing in the homes. Correlations are examined in detail on a congener by congener basis, as well as correlations within couples. The relative importance of house dust to body burdens of PBDEs and different dust collection methods are discussed. The findings on dust and serum correlations in Chapter 2 are used to inform the methods of data analysis in Chapter 3, in terms of synthesizing PBDE congener groupings for the statistical modeling of the relationships between PBDE exposure and hormone levels in men. Several methods were employed to analyze these relationships, including a factor analysis of all congeners detected in dust. Additionally, concentrations of alternate BFRs that are replacing PBDEs and their relationships to hormone levels are investigated, which has not previously been studied. Chapter 4 explores the relationship between serum and ovarian follicular fluid concentrations of PBDEs, which is a novel measurement in humans. Finally, the association between PBDE concentrations in both matrices and failed embryo implantation is investigated.

This research is important to improve our understanding of the exposure routes and health risks associated with BFRs and to provide guidance on the use of environmental and biological markers of exposure in environmental epidemiology studies. The use of BFRs is a current topic widely under debate, and this research adds to the growing evidence that BFR exposure may have adverse implications for humans. The

use of infertility clinic cohorts provides a unique perspective from which to study human exposure to environmental contaminants relevant to reproductive function.

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CHAPTER II
Relationships between Polybrominated Diphenyl Ether Concentrations in House Dust and Serum

Abstract

Polybrominated diphenyl ethers (PBDEs) have been measured in the home environment and in humans, but studies linking environmental levels to body burdens are limited. This study examines the relationship between PBDE concentrations in house dust and serum from adults residing in these homes. We measured PBDE concentrations in serum and in house dust from 12 male-female couples enrolled in an ongoing study of male reproductive health. Detection rates, dust-serum and within-matrix correlations varied by PBDE congener. There was a strong correlation ($r = 0.65\text{-}0.89$, $p < 0.05$) between dust and serum concentrations of several predominant PBDE congeners (BDE 47, 99 and 100). Dust and serum levels of BDE 153 were not correlated ($r < 0.01$). Serum concentrations of the sum of BDE 47, 99, and 100 were also strongly correlated within couples ($r = 0.85$, $p = 0.0005$). This study provides evidence that house dust is a primary exposure pathway of PBDEs and validates the use of dust PBDE concentrations as a marker for exposure to PBDEs.

1. Introduction

Polybrominated biphenyl ethers (PBDEs) are a group of flame retardant chemicals that have been added to numerous consumer products, such as home electronics (e.g. televisions, computers), textiles (e.g. carpeting, drapery) and items containing polyurethane foam (e.g. mattresses, upholstered furniture) to meet fire safety standards and to slow burning in case of fire. There are 10 different homologue groups of PBDEs (mono- to deca-), that consist of 209 possible congeners, or combinations of the number and position of bromine atoms on the diphenyl ether backbone. These different compounds have different chemical, exposure and toxicological properties (1). Commercial formulations of PBDEs consist of a mixture of congeners and are mainly described as Penta-, Octa- and Deca- BDE.

Due to concerns over the persistence of PBDEs in the environment and bioaccumulation in wildlife and particularly in human milk, the European Union banned the use of Penta- and Octa-BDEs in 2004 (2). The sole U.S. manufacturer phased out production of Penta- and Octa-BDEs in 2004. There is currently no U.S. federal regulation on the use of PBDEs, but several states have issued their own restrictions (3). DecaBDE has been the least studied formulation, in terms of exposure and health effects in humans, and it is still widely in use. BDE 209, which is the major component of the Deca commercial mixture, has the shortest half-life in the body (approximately 2 weeks), and it is more readily transformed or eliminated than the lower-brominated congeners (4). However, BDE 209 is found in the environment and can break down into the lower-brominated congeners that are more bioaccumulative (5, 6). Although Penta- and Octa-BDEs once in mass production are now banned in Europe and discontinued in the United

States, the general population continues to be exposed to these compounds due to their persistence and continued release into home environments from older products. Importation of products from countries that continue to use certain PBDEs is another potential exposure source (7).

PBDEs are additive, or not chemically bound, and can leach out or physically degrade into particles and thus may end up in indoor air and house dust. Potential routes of exposure to these compounds include ingestion, inhalation and dermal uptake. Ingestion may include dietary exposure, particularly meats and dairy products that have accumulated PBDEs, but PBDE exposure has been estimated to be primarily from inhaling and ingesting dust (8, 9, 10, 11). In support of these assessments, a recent U.S. study found a stronger association of PBDE concentrations in house dust with PBDE levels in human breast milk than with reported consumption of meat or dairy products (12). However, that study's analysis did not include BDE 209 due to low detection rates in milk and dust.

PBDEs have been shown to disrupt endocrine functions (13, 14), but human studies are limited. The few human studies that have been conducted to date have reported associations between PBDE exposure and altered hormone levels (15, 16, 17, 18). Specifically, we recently reported that PBDE concentrations in dust from participant-collected vacuum bags were associated with altered serum hormone levels in 24 men (18). In the present study, we measured PBDE concentrations in serum and in participant vacuum bag dust from couples (male and female partners living in same household) participating in the same ongoing study. The objective was to examine the relationship between dust and serum PBDE concentrations, as well as explore whether serum PBDE

concentrations were strongly correlated within-couple. Strong relationships between dust and serum PBDE concentrations may help identify dust as a primary exposure pathway and provide validation for the use of dust PBDE concentrations as estimates of exposure in epidemiological studies.

2. Methods

Couples living in the same household who were seeking fertility treatment were recruited from the Massachusetts General Hospital Fertility Center as part of an ongoing study of environmental exposures and reproductive health (18). Participants included men and women from infertile couples due to a male factor, a female factor, or a combination of both male and female factors. The study protocols were approved by the committees on research ethics at all participating institutions, and all participants signed an informed consent.

2.1 House dust PBDEs

Participants donated the current-use vacuum bags from their home between 2002 and 2008. Participants wrapped the vacuum bags in aluminum foil and then sealed them in labeled plastic bags. In the one case where a bagless vacuum was used, the participant emptied vacuum dust directly into the plastic bag. Dust samples were stored at -20C until analysis. Dust was sieved using a 150 mesh sieve to obtain the fine fraction and analyzed for PBDEs using the method by Stapleton et al. (19, 20). Samples were spiked with internal and recovery standards, and four laboratory blanks were also spiked and analyzed alongside samples. PBDEs were quantified using an Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometer (Agilent Technologies,

Santa Clara, CA, USA) operated in negative chemical ionization mode (GC/ECNI-MS). Laboratory blanks were low enough (<1%) that blank correction was not needed. Surrogate recoveries averaged 135%.

2.2 Serum PBDEs

Serum samples (5 mL) collected from 12 male/female partners recruited into the ongoing study in 2007-2008 were sent on dry ice to the CDC Combustion Products and Persistent Pollutants Biomonitoring Laboratory in Atlanta, GA. The methodology for the analysis of PBDEs in serum has been published by Sjodin et al. (21). Briefly, samples were denatured with formic acid, diluted with water and fortified with internal standards prior to solid phase extraction (SPE) using a Rapid Trace modular SPE system (Caliper Life Sciences; Hopkinton, MA, USA). Determination of the target analytes was performed by gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS) employing a MAT95XP instrument (ThermoFinnigan MAT; Bremen, Germany). The serum lipid concentrations were determined using test kits from Roche Diagnostics (Indianapolis, IN) for total triglycerides and total cholesterol. Final determinations were made on a Hitachi 912 Chemistry Analyzer (Hitachi; Tokyo, Japan). All concentrations of PBDEs are reported as background subtracted.

2.3 Data analysis

Descriptive statistics were calculated for all PBDE congeners with at least 50% detection rates to examine the distributions by congener in house dust and serum. One half the detection limit was assigned to non-detect levels for the calculation of geometric means. Spearman's correlation coefficients were calculated to assess bivariate relationships between PBDE concentrations in house dust and serum, and between

different PBDE congeners within the same matrix. Spearman's correlation coefficients were also calculated to assess within-couple (female-male) correlations of serum PBDE concentrations.

3. Results

Table 2.1 presents the distribution and detection rates of PBDE congeners measured in house dust. Results are presented for 50 dust samples, although only the 12 most recently collected dust samples were from couples who also provided serum samples for PBDE analysis. The distribution of PBDE concentrations of the 12 matched dust samples is similar to the distribution of the entire 50 samples analyzed. Concentrations of PBDEs (sum of all congeners) ranged from 980 to 44,546 ng/g dust. The geometric mean of summed PBDEs was 4,742 ng/g. All of the PBDE congeners detected were log-normally distributed. BDE 209, the major congener in DecaBDE commercial mixtures, was the dominant PBDE congener, accounting for 43% on average of total PBDEs by weight in the dust samples. BDE 47 and 99, the two major constituents of the PentaBDE commercial mixture, made up 16% and 22% respectively, of total PBDEs on average. There were strong correlations (Spearman $r \geq 0.80$, $p < 0.05$) among dust concentrations of PBDE congener groups with the same or close degree of bromination. A complete table of correlation coefficients for all detectable congeners in dust can be found in the Supporting Information (Table S1).

Table 2.2 presents the distribution and detection rates of PBDEs measured in serum, shown as both total serum and lipid-adjusted values. BDE 47 was found at the highest median concentration in serum, followed by BDE 153 at the next to highest

median concentration. Several congeners, including BDE 209, had low detection rates (less than 30%). BDE 209 had the highest detection limit and was only detected in 2 of 24 serum samples. The congeners with high detection rates in serum (BDE 28, 47, 99, 100 and 153), components of the PentaBDE commercial mixture, were correlated with one another ($r = 0.70\text{-}0.96$, $p < 0.05$), with the exception of BDE 153, which is also a component of the OctaBDE mixture. A complete table of correlation coefficients for all detectable congeners in serum can be found in the Supporting Information (Table S2).

Figure 2.1 compares the overall PBDE congener composition profiles of dust and serum samples. The compositions are represented as percent, by mass, of combined dust and serum samples. The dust and serum samples followed the same pattern with some exceptions. BDE 209 was the dominant congener in the majority of dust samples, although 11 of 50 samples had levels of BDE 99 higher than the other congeners, and 7 of these samples also had levels of other congeners higher than BDE 209. In serum, BDE 47 was found at the highest concentration in 17 out of 24 samples, while in 4 females and 3 males, BDE-153 was found at a higher concentration than other congeners.

Table 2.3 shows Spearman's correlations between dust and serum concentrations of PBDEs. Several congeners (BDE 17, 66, 85, 154, 183 and 209) had low detection rates (see Table 2.2) and were therefore not included in correlation matrices with dust concentrations. There was a strong correlation ($r = 0.65\text{-}0.89$, $p < 0.05$) between dust and serum concentrations for most BDE congeners with high detection rates (BDE 47, 99 and 100). However, dust and serum levels of BDE 153 were not correlated ($r < 0.01$). There were strong correlations between several BDE congeners measured in dust and lower-

brominated congeners measured in serum. For example, BDE 100 in dust was correlated with BDE 28 in serum ($r = 0.79$, $p = 0.002$).

There was a strong correlation ($r = 0.85$, $p = 0.0005$) for serum concentrations of the major Penta formulation BDEs (sum of BDE 47, 99 and 100) between males and females of couples residing in the same household (Figure 2.2). However, serum levels of BDE 153 were not significantly correlated within couples ($r = 0.40$, $p = 0.19$). A complete table of correlations between PBDE concentrations in male and female serum which includes all detectable congeners (BDE 28, 47, 99, 100, and 153) can be found in the Supporting Information (Figure S3).

4. Discussion

PBDE concentration ranges in dust and serum in the present study were similar to concentrations found in other studies conducted among North Americans (22, 23, 19, 36), and therefore the PBDE exposures among individuals in this study are likely representative of exposures of the general US population. There were strong correlations between dust concentrations of PBDE congener groups with the same or close degree of bromination. These relationships resemble the congener mixtures (PentaBDE, OctaBDE, or DecaBDE) in commercial products and suggest that the congeners originated from the same sources within the home. Because BDE 202 is not present at detectable levels in commercial formulations, its detection in dust samples may be indicative of environmental debromination of BDE 209 (24). BDE 209 was measured at the highest concentrations in dust, followed by BDE 99 and BDE 47. In contrast, BDE 47 and BDE 153 were measured at the highest concentrations in serum. BDE 47 may dominate serum

samples due to dietary intake, as it was often found as the dominant congener in food items analyzed as part of a market basket survey (25). The predominance of BDE 47 in serum samples may also be due in part to gaseous inhalation, as BDE 47 has been found to be a dominant congener in indoor air (11, 26). However, it is not possible to evaluate possible contributions to exposure from diet or inhalation of volatile gases in the present study.

Several congeners, including BDE 209, had low detection rates in serum and therefore conclusions regarding dust-serum or within-couple relationships for these congeners were not possible. Several congeners (BDE 28, 47, 99, 100, 153) had high detection rates, and therefore associations with PBDE concentrations in dust could be evaluated. There were strong correlations between dust and serum concentrations of the major Penta formulation BDE congeners 47, 99 and 100, which suggests that dust is a good measure of exposure to these congeners. Serum concentrations of these congeners were also strongly correlated between males and females of couples (Figure 2.2), which supports the use of a serum or dust measurement from one member of a couple living together to represent their partner's exposure to these congeners. This estimate of exposure may not apply to children living in the same household, as a child's exposure to dust is expected to be greater. Dust and serum levels of BDE 153 were not correlated, which may be due to differences in exposure sources (e.g. diet or exposures outside the home), transformation, distribution or metabolism of this congener. BDE 153 has a long half-life (approximately 2 years) as compared to the other congeners (27). Serum concentrations of BDE 153 were also not correlated between males and females within couples. Conversely, BDE 153 in dust was correlated to lower brominated congeners in

serum, which may support the argument that transformation is occurring. As observed by Qiu, et al. (28), different PBDE congeners may have different rates of hydroxylation, and this may be an explanation why human serum may exhibit different congener profiles than dust or commercial product mixtures. Huwe et al. (29) demonstrated that PBDE congeners have different degrees of bioconcentration in rats, possibly due to metabolism differences between congeners. The lack of BDE 209 measured in serum may be due to higher detection limits and/or to its short biological half-life. Stapleton et al. (5) demonstrated that when carp were fed BDE 209, only lower-brominated congeners, and not BDE 209, bioaccumulated in the fish. Further research into the transformation processes of individual PBDE congeners is needed to understand patterns in the biomarker profiles of these compounds.

A recent study utilizing NHANES dietary questionnaire responses and serum PBDE data concluded that intake of poultry and red meat is a source of PBDE exposure in the US population (30). In particular, BDE 153 was the only congener associated with total fat intake, and although vegetarians had lower total PBDE serum levels, they did not have significantly reduced levels of BDE 153. Higher levels of dietary exposure to BDE 153 may explain higher BDE 153 levels in serum in the present study population. However, US market basket surveys (25, 31) do not indicate that certain foods are higher in BDE 153. Fraser et al. (30) also found similar results to the present study in terms of serum congener correlations, with the Penta formulation congeners (BDE 28, 47, 99, 100 and 153) being strongly correlated with one another. However, the authors reported that BDE 153 had a weaker association than the other congeners. In the present study, the

Penta formulation congeners were also strongly correlated, but BDE 153 was not associated with the other congeners in serum.

Limited studies have assessed relationships between dust and serum concentrations of PBDEs. A study of only five Swedish homes reported a correlation between researcher-collected house dust and plasma levels of PBDEs, although the association was dependent on one of the five households (32). A recent study conducted in Germany found no significant correlation between dust and serum concentrations of PBDEs, and the authors concluded that diet is the main exposure pathway (33). However, although European food samples were reported to have the same level of PBDE contamination as US samples, (8, 25), dust levels in European countries are orders of magnitude lower than US levels (22), and therefore these data may not be comparable to the data in the present study. Based on pharmacokinetic modeling, Lorber (8) concluded that dietary and inhalation exposures could not explain US body burdens of PBDEs, and that exposure to indoor dust is the primary pathway.

Various studies on indoor environmental contaminants employ different methods in the collection of house dust as a measure of exposure. Specifically, researcher-collected dust has been compared to vacuum bag dust (34, 35, 36). The studies by Colt et al. concluded that there was a high level of agreement between researcher-collected dust (high-volume surface sampler, HVS3) and vacuum bag dust for pesticides and other organic contaminants, including polychlorinated biphenyls (PCBs). The study by Allen et al. found that researcher-collected dust had varying degrees of correlation with vacuum bag dust concentrations of PBDEs ($r = 0.39$-0.77), depending on the room in the home and the sampling round. Furthermore, PBDE concentrations in researcher-collected dust

were significantly different between rooms of the same home. Future studies on dust collection methods should focus on the validation of these methods and include biomarkers as evidence of biological relevance. It is possible that the use of vacuum bag dust in exposure assessment may be a superior method of dust collection, provided that the dust collected is a measure of longer term integrative exposure representative of the total home environment, and thus total exposure, and not limited to a specific area or time. The use of vacuum bags may also provide a much more time- and cost-efficient method for measuring dust contamination in large-scale epidemiological studies.

This study is the first to provide empirical evidence of the association between house dust and serum concentrations of PBDEs in the US. For PBDE congeners that do not show strong correlation between dust and serum, such as BDE 153, dust may not be a good indicator of body burden. However, for other PBDE congeners such as the major Penta formulation BDEs, which were strongly correlated between dust and serum concentrations, house dust may be a good measure of exposure. This observation serves to further validate our recent finding of significant relationships between dust concentrations of PentaBDEs and circulating hormone levels in men (18). Furthermore, house dust may provide a satisfactory estimate of human exposure to BDE 209 due to its high concentrations in dust and current limitations of measuring BDE 209 in serum. The relatively short biological half-life of BDE 209 may prevent reliable measurement in serum, but because BDE 209 concentrations in dust are high, people are likely continuously exposed. Thus, dust concentrations may currently be the best marker of exposure to BDE 209 in the absence of other biomarkers.

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Table 2.1. Distribution of PBDE Congeners in House Dust
ng/g (n=50)

Congener	Geometric Mean	Selected Percentiles				Maximum	DL	Detection rate
		25th	50th	75th	90th			
30	NC	<DL	<DL	<DL	<DL	5.20	0.06	4%
17	2.62	1.57	2.67	5.49	8.86	486	0.10	94%
25	NC	<DL	<DL	<DL	<DL	3.95	0.20	24%
28/33[a]	13.0	6.8	11.7	21.1	35.2	84.0	0.10	100%
75	7.5	5.9	14.6	24.6	51.3	100	0.70	76%
49	21.4	12.8	19.6	41.5	74.7	195	0.80	98%
71	NC	<DL	<DL	<DL	<DL	<DL	0.30	0%
47	543	288	390	1122	2299	8627	4.17	100%
66	9.76	5.79	7.94	20.6	35.4	134	0.05	98%
100	135	63.3	99.9	228	608	2164	0.19	100%
119	NC	<DL	<DL	<DL	<DL	77.3	0.05	2%
99	643	260	427	1140	3241	12967	0.47	100%
116	NC	<DL	<DL	<DL	<DL	<DL	0.10	0%
85/155[a]	33.6	14.4	24.6	54.2	169	544	0.10	100%
154	63.2	25.4	51.3	131	359	1093	0.10	100%
153	78.6	31.2	55.9	188	380	1352	0.10	100%
138	4.28	3.10	5.45	13.0	32.7	81.5	0.10	86%
156	NC	<DL	<DL	<DL	<DL	<DL	0.10	0%
183	20.0	11.3	17.4	33.9	61.8	688	0.10	100%
190	NC	<DL	<DL	<DL	<DL	<DL	0.10	0%
202	0.86	0.05	3.40	6.29	10.3	33.6	0.10	58%
201	12.6	7.37	12.6	22.8	39.6	494	0.10	98%
197	NC	<DL	<DL	<DL	<DL	<DL	0.20	0%
203/200[a]	12.1	5.90	11.2	27.5	36.4	243	0.25	100%
196	NC	<DL	<DL	<DL	<DL	<DL	0.20	0%
205	NC	<DL	<DL	<DL	<DL	64.1	0.20	10%
208	35.3	18.5	30.1	49.9	93.0	853	0.89	100%
207	63.1	31.9	54.9	97.3	242	1492	1.06	100%
206	163	85.5	156	250	505	3772	0.50	100%
209	1906	1146	1482	2840	5816	32366	14.1	100%
Total PBDEs	4742	2651	4458	7879	15360	44546		

[a] Congeners are listed together due to coelution in GC/MS analysis.

DL = Detection limit. One-half the DL was used for measurements below the DL.

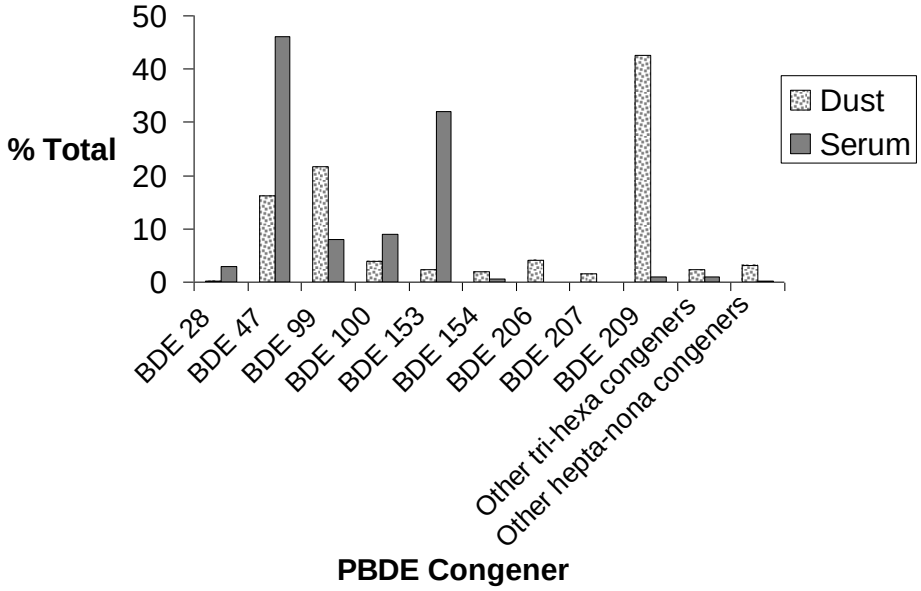
NC = Not calculated. Geometric means and percentiles were not calculated for congeners with detection rates below 50%.

Table 2.2. Distribution of PBDE Congeners in Serum
(n=24)

			Selected Percentiles						
	Congener	Geometric Mean	25th	50th	75th	90th	Max	DL	Detection rate
Serum (pg/g)	17	NC	<DL	<DL	<DL	<DL	4.1	2.5	8%
	28	6.7	4.1	7.6	11	17	39	2.5	88%
	47	95	50	96	187	245	511	10.9	100%
	66	NC	<DL	<DL	<DL	<DL	5.8	2.5	17%
	100	18	11	19	22	49	176	2.5	100%
	99	16	8.9	15	34	41	72	9.8	75%
	85	NC	<DL	<DL	<DL	<DL	6.1	2.5	25%
	154	NC	<DL	<DL	<DL	<DL	8.6	2.5	29%
	153	43	24	36	74	137	1151	2.5	100%
	183	NC	<DL	<DL	<DL	<DL	4.8	2.5	13%
	209	NC	<DL	<DL	<DL	<DL	25.1	25.0	8%
	Total PBDEs	255	157	219	341	523	1691		
Serum, Lipid-adjusted (ng/g)	17	NC	<DL	<DL	<DL	<DL	0.7	0.3-0.6	
	28	1.1	0.7	1.3	1.8	2.8	6.4	0.3-0.6	
	47	16	8.7	17	34	41	83	1.4-2.5	
	66	NC	<DL	<DL	<DL	<DL	0.9	0.3-0.6	
	100	3.0	2.1	3.0	4.3	8.1	24	0.3-0.6	
	99	2.6	1.4	2.4	5.0	6.9	12	1.3-2.3	
	85	NC	<DL	<DL	<DL	<DL	0.9	0.3-0.6	
	154	NC	<DL	<DL	<DL	<DL	1.2	0.3-0.6	
	153	7.1	4.3	7.0	11	20	154	0.3-0.6	
	183	NC	<DL	<DL	<DL	<DL	0.6	0.3-0.6	
	209	NC	<DL	<DL	<DL	<DL	6.0	3.3-5.8	
	Total PBDEs	40	28	39	61	76	225		

DL = Detection limit. One-half the DL was used for measurements below the DL.
NC = Not calculated. Geometric means and percentiles were not calculated for congeners with detection rates below 50%.
Lipid-adjusted serum detection limits are ranges due to varying sample volumes.

Figure 2.1. PBDE Congener by proportion in dust and serum



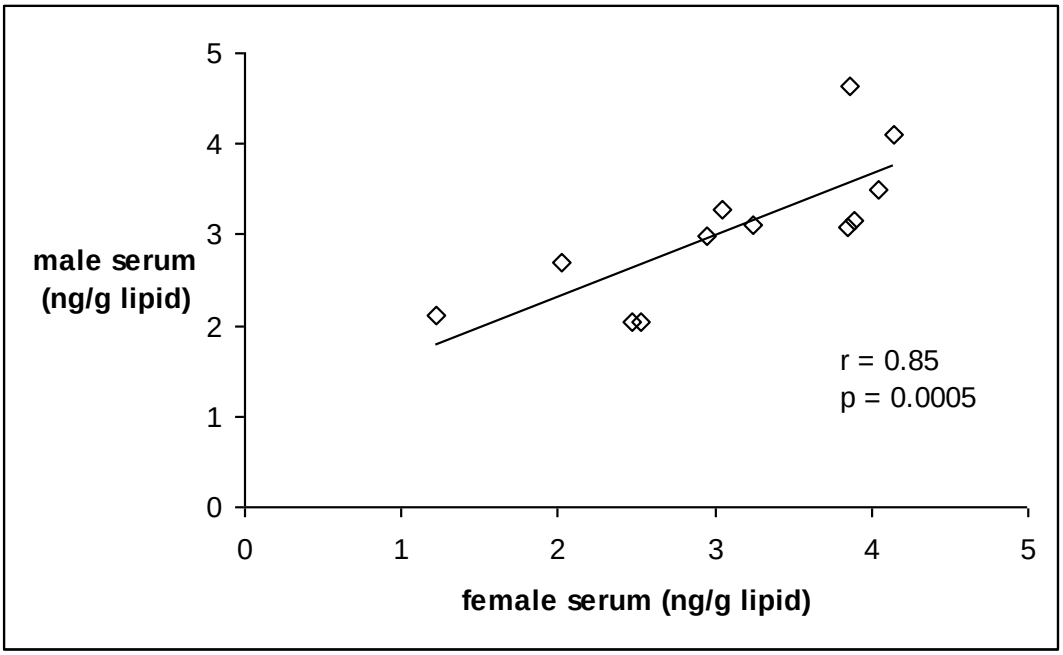
Dust: n = 50, except those congeners with <100% detection (some of "other tri-nona congeners"). See Table 2.1 for detection rates.
Serum: n = 24, except those congeners with <100% detection (BDE 28, 99, 154, 209 and "other tri-nona congeners"). See Table 2.2 for detection rates.
Other tri-hexa and hepta-nona congeners are those listed in Tables 2.1 and 2.2.
BDE 206 and 207 were not measured in serum.

Table 2.3. Spearman correlation coefficients for selected PBDE concentrations in dust and serum (n=12)

			Dust PBDE Congener												
			BDE 17	BDE 28	BDE 75	BDE 49	BDE 47	BDE 66	BDE 99	BDE 100	BDE 154	BDE 153	BDE 138	BDE 183	BDE 209
Male Serum (lipid-adjusted) PBDE Congener	BDE 28	r	0.81	0.49	0.83	0.77	0.82	0.75	0.89	0.79	0.87	0.87	0.79	0.55	0.04
		p-value	0.001	0.10	0.0008	0.004	0.001	0.005	0.0001	0.002	0.0002	0.0002	0.002	0.06	0.91
	BDE 47	r	0.84	0.53	0.79	0.71	0.81	0.70	0.85	0.69	0.81	0.82	0.69	0.54	-0.004
		p-value	0.0006	0.08	0.002	0.009	0.002	0.01	0.0004	0.01	0.001	0.001	0.01	0.07	0.99
	BDE 99	r	0.87	0.60	0.76	0.75	0.84	0.73	0.89	0.72	0.85	0.83	0.73	0.36	0.07
		p-value	0.0003	0.04	0.004	0.005	0.0007	0.007	0.0001	0.008	0.0004	0.0008	0.008	0.25	0.84
	BDE 100	r	0.78	0.64	0.88	0.82	0.72	0.70	0.81	0.65	0.71	0.72	0.58	0.55	0.16
		p-value	0.003	0.02	0.0002	0.001	0.008	0.01	0.001	0.02	0.01	0.008	0.05	0.07	0.61
	BDE 153	r	0.02	0.05	0.25	-0.01	0.02	-0.14	-0.04	-0.24	-0.05	0.00	-0.15	0.01	0.33
		p-value	0.96	0.87	0.44	0.97	0.97	0.66	0.91	0.46	0.87	1.00	0.65	0.97	0.29

			BDE 17	BDE 28	BDE 75	BDE 49	BDE 47	BDE 66	BDE 99	BDE 100	BDE 154	BDE 153	BDE 138	BDE 183	BDE 209
Female Serum (lipid-adjusted) PBDE Congener	BDE 28	r	0.82	0.51	0.70	0.73	0.85	0.82	0.90	0.84	0.94	0.84	0.85	0.40	0.01
		p-value	0.001	0.09	0.01	0.007	0.0004	0.001	0.0001	0.0007	0.0001	0.0007	0.0005	0.20	0.97
	BDE 47	r	0.78	0.45	0.62	0.64	0.80	0.76	0.87	0.80	0.90	0.80	0.85	0.34	0.24
		p-value	0.003	0.14	0.03	0.03	0.002	0.005	0.0002	0.002	0.0001	0.002	0.0004	0.30	0.46
	BDE 99	r	0.56	0.32	0.34	0.31	0.64	0.50	0.69	0.55	0.67	0.62	0.71	0.13	0.45
		p-value	0.06	0.32	0.28	0.33	0.03	0.10	0.01	0.06	0.02	0.03	0.01	0.69	0.14
	BDE 100	r	0.57	0.31	0.51	0.47	0.70	0.66	0.78	0.71	0.80	0.71	0.78	0.29	0.16
		p-value	0.05	0.33	0.09	0.12	0.01	0.02	0.003	0.01	0.002	0.01	0.003	0.35	0.62
	BDE 153	r	0.05	0.22	0.10	0.07	0.13	0.03	0.08	0.03	-0.11	0.02	0.06	0.03	0.16
		p-value	0.88	0.48	0.76	0.83	0.68	0.91	0.80	0.91	0.73	0.95	0.86	0.91	0.62

Figure 2.2. Within-couple serum PBDE correlation (sum of BDE 47, 99, 100)



Values in graph are transformed to the natural log. Spearman correlation coefficient, n = 12 couples.

Table S1. Spearman correlation coefficients for PBDE concentrations in dust (n = 50)

		BDE 17	BDE 28/33	BDE 75	BDE 49	BDE47	BDE66	BDE99	BDE100	BDE85/155	BDE154	BDE153	BDE138	BDE183	BDE202	BDE201	BDE200	BDE208	BDE207	BDE206	BDE209
BDE 17	r	1.00	0.85	0.38	0.76	0.85	0.79	0.76	0.75	0.70	0.63	0.65	0.51	0.28	0.06	0.14	-0.04	-0.05	-0.05	-0.07	-0.08
	p-value		<0.0001	0.007	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.05	0.70	0.35	0.80	0.74	0.72	0.65	0.60
BDE 28/33	r	0.85	1.00	0.40	0.73	0.86	0.81	0.73	0.76	0.68	0.65	0.64	0.50	0.32	0.00	0.16	-0.16	-0.09	-0.09	-0.07	-0.08
	p-value	<0.0001		0.004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.02	1.00	0.27	0.28	0.51	0.56	0.61	0.57
BDE 75	r	0.38	0.40	1.00	0.29	0.38	0.36	0.27	0.24	0.26	0.21	0.27	0.29	0.25	0.03	0.22	0.07	-0.14	-0.13	-0.18	-0.11
	p-value	0.007	0.004		0.04	0.006	0.01	0.06	0.09	0.07	0.15	0.06	0.04	0.09	0.86	0.13	0.61	0.33	0.39	0.20	0.47
BDE 49	r	0.76	0.73	0.29	1.00	0.76	0.77	0.70	0.70	0.68	0.61	0.65	0.50	0.36	0.09	0.24	0.13	0.16	0.14	0.13	0.16
	p-value	<0.0001	<0.0001	0.04		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.009	0.52	0.10	0.38	0.27	0.33	0.38	0.27
BDE 47	r	0.85	0.86	0.38	0.76	1.00	0.91	0.93	0.93	0.89	0.80	0.87	0.63	0.46	0.17	0.29	0.00	0.06	0.09	0.04	0.01
	p-value	<0.0001	<0.0001	0.006	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	0.24	0.04	1.00	0.67	0.52	0.76	0.93
BDE 66	r	0.79	0.81	0.36	0.77	0.91	1.00	0.87	0.87	0.84	0.74	0.80	0.57	0.46	0.18	0.33	0.01	0.14	0.17	0.11	0.09
	p-value	<0.0001	<0.0001	0.01	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0007	0.22	0.02	0.96	0.35	0.24	0.45	0.55
BDE 99	r	0.76	0.73	0.27	0.70	0.93	0.87	1.00	0.96	0.96	0.86	0.95	0.72	0.43	0.23	0.26	0.01	0.17	0.17	0.10	0.08
	p-value	<0.0001	<0.0001	0.06	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.002	0.11	0.06	0.95	0.25	0.23	0.49	0.56
BDE 100	r	0.75	0.76	0.24	0.70	0.93	0.87	0.96	1.00	0.96	0.93	0.94	0.73	0.48	0.28	0.30	0.02	0.18	0.20	0.12	0.11
	p-value	<0.0001	<0.0001	0.09	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	0.0005	0.05	0.04	0.90	0.20	0.16	0.40	0.46
BDE 85/155	r	0.70	0.68	0.26	0.68	0.89	0.84	0.96	0.96	1.00	0.92	0.98	0.75	0.50	0.26	0.32	0.00	0.22	0.23	0.14	0.11
	p-value	<0.0001	<0.0001	0.07	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	0.0002	0.07	0.02	0.98	0.13	0.11	0.34	0.43
BDE 154	r	0.63	0.65	0.21	0.61	0.80	0.74	0.86	0.93	0.92	1.00	0.92	0.70	0.54	0.36	0.38	0.04	0.29	0.30	0.23	0.22
	p-value	<0.0001	<0.0001	0.15	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	0.01	0.006	0.77	0.04	0.03	0.12	0.12
BDE 153	r	0.65	0.64	0.27	0.65	0.87	0.80	0.95	0.94	0.98	0.92	1.00	0.72	0.54	0.30	0.38	0.01	0.26	0.27	0.18	0.15
	p-value	<0.0001	<0.0001	0.06	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	0.03	0.006	0.95	0.07	0.05	0.20	0.29
BDE 138	r	0.51	0.50	0.29	0.50	0.63	0.57	0.72	0.73	0.75	0.70	0.72	1.00	0.16	0.09	0.00	-0.10	0.12	0.07	0.05	0.19
	p-value	0.0002	0.0002	0.04	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.27	0.51	0.99	0.47	0.40	0.61	0.73	0.19
BDE 183	r	0.28	0.32	0.25	0.36	0.46	0.46	0.43	0.48	0.50	0.54	0.54	0.16	1.00	0.32	0.88	0.28	0.23	0.36	0.24	0.13
	p-value	0.05	0.02	0.09	0.009	0.0009	0.0007	0.002	0.0005	0.0002	<0.0001	<0.0001	0.27		0.02	<0.0001	0.05	0.11	0.01	0.09	0.36
BDE 202	r	0.06	0.00	0.03	0.09	0.17	0.18	0.23	0.28	0.26	0.36	0.30	0.09	0.32	1.00	0.48	0.37	0.77	0.80	0.70	0.61
	p-value	0.70	1.00	0.86	0.52	0.24	0.22	0.11	0.05	0.07	0.01	0.03	0.51	0.02		0.0004	0.008	<0.0001	<0.0001	<0.0001	<0.0001
BDE 201	r	0.14	0.16	0.22	0.24	0.29	0.33	0.26	0.30	0.32	0.38	0.38	0.00	0.88	0.48	1.00	0.38	0.41	0.55	0.44	0.30
	p-value	0.35	0.27	0.13	0.10	0.04	0.02	0.06	0.04	0.02	0.01	0.01	0.99	<0.0001	0.0004		0.007	0.003	<0.0001	0.002	0.03
BDE 200/203	r	-0.04	-0.16	0.07	0.13	0.00	0.01	0.01	0.02	0.00	0.04	0.01	-0.10	0.28	0.37	0.38	1.00	0.45	0.49	0.40	0.43
	p-value	0.80	0.28	0.61	0.38	1.00	0.96	0.95	0.90	0.98	0.77	0.95	0.47	0.05	0.008	0.007		0.001	0.0003	0.004	0.002
BDE 208	r	-0.05	-0.09	-0.14	0.16	0.06	0.14	0.17	0.18	0.22	0.29	0.26	0.12	0.23	0.77	0.41	0.45	1.00	0.97	0.93	0.85
	p-value	0.74	0.51	0.33	0.27	0.67	0.35	0.25	0.20	0.13	0.04	0.07	0.40	0.11	<0.0001	0.003	0.001		<0.0001	<0.0001	<0.0001
BDE 207	r	-0.05	-0.09	-0.13	0.14	0.09	0.17	0.17	0.20	0.23	0.30	0.27	0.07	0.36	0.80	0.55	0.49	0.97	1.00	0.93	0.83
	p-value	0.72	0.56	0.39	0.33	0.52	0.24	0.23	0.16	0.11	0.03	0.05	0.61	0.01	<0.0001	<0.0001	0.0003	<0.0001		<0.0001	<0.0001
BDE 206	r	-0.07	-0.07	-0.18	0.13	0.04	0.11	0.10	0.12	0.14	0.23	0.18	0.05	0.24	0.70	0.44	0.40	0.93	0.93	1.00	0.88
	p-value	0.65	0.61	0.20	0.38	0.76	0.45	0.49	0.40	0.34	0.12	0.20	0.73	0.09	<0.0001	0.002	0.004	<0.0001	<0.0001		<0.0001
BDE 209	r	-0.08	-0.08	-0.11	0.16	0.01	0.09	0.08	0.11	0.11	0.22	0.15	0.19	0.13	0.61	0.30	0.43	0.85	0.83	0.88	1.00
	p-value	0.60	0.57	0.47	0.27	0.93	0.55	0.56	0.46	0.43	0.12	0.29	0.19	0.36	<0.0001	0.03	0.002	<0.0001	<0.0001	<0.0001	

Table S2. Spearman correlation coefficients for PBDE concentrations in serum (n = 24)

			Serum PBDE congener					Lipid adjusted serum PBDE congener				
			BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153
Serum PBDE congener	BDE 28	r	1	0.96	0.83	0.88	0.05	0.96	0.93	0.80	0.86	0.06
		p-value		<0.0001	<0.0001	<0.0001	0.83	<0.0001	<0.0001	<0.0001	<0.0001	0.79
	BDE 47	r	0.96	1	0.92	0.92	0.15	0.95	0.97	0.90	0.90	0.16
		p-value	<0.0001		<0.0001	<0.0001	0.49	<0.0001	<0.0001	<0.0001	<0.0001	0.46
	BDE 99	r	0.83	0.92	1	0.85	0.18	0.80	0.88	0.97	0.81	0.19
		p-value	<0.0001	<0.0001		<0.0001	0.40	<0.0001	<0.0001	<0.0001	<0.0001	0.37
	BDE 100	r	0.88	0.92	0.85	1	0.29	0.88	0.89	0.83	0.96	0.32
		p-value	<0.0001	<0.0001	<0.0001		0.18	<0.0001	<0.0001	<0.0001	<0.0001	0.13
	BDE 153	r	0.05	0.15	0.18	0.29	1	0.11	0.07	0.13	0.25	0.98
		p-value	0.83	0.49	0.40	0.18		0.62	0.74	0.55	0.24	<0.0001
Lipid adjusted serum PBDE congener	BDE 28	r	0.96	0.95	0.80	0.88	0.11	1	0.96	0.81	0.91	0.14
		p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.62		<0.0001	<0.0001	<0.0001	0.50
	BDE 47	r	0.93	0.97	0.88	0.89	0.07	0.96	1	0.90	0.91	0.11
		p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.74	<0.0001		<0.0001	<0.0001	0.62
	BDE 99	r	0.80	0.90	0.97	0.83	0.13	0.81	0.90	1	0.83	0.16
		p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.55	<0.0001	<0.0001		<0.0001	0.45
	BDE 100	r	0.86	0.90	0.81	0.96	0.25	0.91	0.91	0.83	1	0.30
		p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.24	<0.0001	<0.0001	<0.0001		0.16
	BDE 153	r	0.06	0.16	0.19	0.32	0.98	0.14	0.11	0.16	0.30	1
		p-value	0.79	0.46	0.37	0.13	<0.0001	0.50	0.62	0.45	0.16	

Table S3. Spearman correlation coefficients for PBDE concentrations in lipid adjusted serum of males and females
(n = 12 couples)

			Males				
			BDE 28	BDE 47	BDE 99	BDE 100	BDE 153
Females	BDE 28	r	0.93	0.90	0.92	0.76	0.07
		p-value	<0.0001	<0.0001	<0.0001	0.004	0.83
	BDE 47	r	0.81	0.81	0.83	0.64	-0.06
		p-value	0.001	0.001	0.0008	0.02	0.86
	BDE 99	r	0.54	0.65	0.70	0.44	-0.08
		p-value	0.07	0.02	0.01	0.15	0.81
	BDE 100	r	0.76	0.75	0.76	0.63	0.08
		p-value	0.004	0.005	0.004	0.03	0.80
	BDE 153	r	0.10	0.14	0.09	0.33	0.40
		p-value	0.76	0.66	0.78	0.29	0.19

CHAPTER III

Brominated Flame Retardants in House Dust are Related to Hormone Levels in Men

Abstract

Brominated flame retardants (BFRs) are used in the manufacture of a variety of materials and consumer products in order to meet fire safety standards. BFRs may persistent in the environment and have been detected in wildlife, humans and indoor dust and air. Some BFRs have demonstrated endocrine and reproductive effects in animals, but human studies are limited. We measured serum hormone levels and flame retardant concentrations [31 polybrominated diphenyl ether (PBDE) congeners and 6 other flame retardants that are replacing PBDEs in some applications] in house dust from 38 men recruited through a US infertility clinic. PBDE congeners in dust were modeled as 1) individual congeners, 2) grouped into penta-, octa- and deca-BDEs, and 3) independent variables generated by a factor analysis representing 90 percent of the variability of all congeners detected in dust. In multivariable linear regression models adjusted by age and body mass index (BMI), significant positive associations were found between house dust concentrations of pentaBDEs and serum levels of free T4, total T3, estradiol, sex hormone binding globulin (SHBG) and prolactin, along with an inverse association with follicle stimulating hormone (FSH). Positive associations between octaBDE concentrations and serum free T4, thyroid stimulating hormone (TSH), luteinizing

hormone (LH) and testosterone and an inverse association between decaBDE concentrations and testosterone were also found. Hexabromocyclododecane (HBCD) was associated with decreased SHBG and increased free androgen index. Dust concentrations of bis-tribromophenoxyethane (BTBPE) and tetrabromo-diethylhexylphthalate (TBPH) were positively associated with total T3. These findings are consistent with our previous report of associations between PBDEs (BDE 47, 99 and 100) in house dust and hormone levels in men, and further support the consensus that indoor dust is an important source of exposure to BFRs.

1. Introduction

1.1 Background

Brominated flame retardants (BFRs) are a group of chemicals that are used in the production of consumer goods, such as home electronics and numerous items containing polyurethane foam padding and other thermoplastics, in order to meet fire safety standards. Polybrominated diphenyl ethers (PBDEs) have been one of the most widely used groups of BFRs. Commercial formulations of PBDEs consist of a mixture of congeners and are described as penta-, octa- and deca- brominated diphenyl ethers (BDEs). The European Union banned the use of penta- and octa-BDEs in 2004 due to their persistence and bioaccumulation. These formulations were voluntarily phased out of production in the United States in 2004. The U.S. has no federal regulation on the use of PBDEs, but several states have issued their own restrictions (BSEF.com, accessed Nov.3, 2011). DecaBDE is currently still in use, although there are plans to begin phasing out in December, 2012 (EPA 2009). BDE 209, the major component of the decaBDE

formulation, has the shortest half-life (approximately 15 days, as estimated by Thuresson et al. 2006) in the body, but it is widely found in the environment and can break down into the more bioaccumulative lower-brominated congeners commonly found in wildlife and humans (Noyes et al. 2011; Söderstrom et al. 2004; Stapleton et al. 2004).

Despite use restrictions, the general population continues to be exposed to PBDEs due to their persistence and through the continued use of existing products containing PBDEs. Few studies on the human health effects of PBDEs exist, despite evidence of widespread exposure through contact or inhalation of house dust or from dietary sources. Additionally, there are alternate flame retardants entering the marketplace or increasing in use as others are phased out. As alternative flame retardants replace discontinued compounds and production volumes increase, concern over potential exposure to these alternates and possible health effects is rising (DiGangi et al. 2010; Shaw et al. 2010).

Hexabromocyclododecane (HBCD) is another large volume BFR used primarily in polystyrene insulation foam, but also in certain textiles and electronics (EPA 2008). HBCD is also an alternative to decaBDE in some plastics applications (BSEF 2009). Other alternatives such as 1,2- bis (2,4,6-tribromophenoxy)ethane (BTBPE) is in the formulation that replaced Octa-BDE, while 2,3,4,5-ethylhexyltetrabromobenzoate (TBB) and 2,3,4,5-tetrabromo di (2-ethylhexyl) phthalate (TBPH) are in the replacement formulation for pentaBDE (manufacturer announcement, available online at http://www.pu2pu.com/htdocs/customers/greatlakes/Firemaster.htm – accessed July, 2009). These BFRs can leach into the environment in the same manner as PBDEs and have also been measured in house dust (Stapleton et al. 2008; Zhu et al. 2007). However,

few or no studies exist on the toxicity and potential human health effects of these replacement compounds. The use of TBPH is a concern because it is a brominated analogue of di(ethylhexyl)phthalate (DEHP), which is listed under California's Proposition 65 as a chemical known to cause cancer and reproductive and developmental toxicity (OEHHA 2008). Dechlorane Plus (DP) (bis(hexachlorocyclopentadieno)cyclooctane) is a large volume highly chlorinated flame retardant recently identified in lake sediments (Hoh et al. 2006), house dust (Zhu et al. 2007), and humans (Ren et al. 2009; Siddique et al. 2012) with growing concern for human exposure.

1.2 Evidence for endocrine disruption

Animal studies have established that PBDEs are endocrine disruptors, altering reproductive and thyroid hormone homeostasis. A number of animal studies report reduced thyroxine (T4) levels after administration of pentaBDE mixtures (Ellis-Hutchings et al. 2006; Fernie et al. 2005; Fowles et al. 1994; Hallgren et al. 2001; Skarman et al. 2005; Stoker et al. 2004; Zhou et al. 2001, 2002). Some of these studies also found decreases in triiodothyronine (T3) and increases in thyroid stimulating hormone (TSH), which is consistent with the biological feedback relationship of these hormones, but others found no effects on these hormones. Displacement of thyroid hormones from the hormone receptor or the transport protein, which was shown in vitro (Meerts et al. 2000), has been postulated as a possible mechanism by which thyroid hormone homeostasis is disrupted (reviewed by (Darnerud 2008).

There may be a number of different mechanisms involved in the endocrine disrupting activity of PBDEs. Experimental studies have demonstrated that the endocrine disrupting

properties of PBDEs are congener dependent. For example, several lesser brominated congeners, such as the tetra- and penta-brominated compounds, acted as estrogen receptor agonists, while some hexa- and hepta-brominated congeners had antiestrogenic effects *in vitro* (Meerts et al. 2001). Tetra-, penta- and hexa-brominated congeners had estrogenic activity, while hepta-brominated congeners and a metabolite of BDE 47 had anti-estrogenic activity in another *in vitro* study by (Hamers et al. 2006). Most of the congeners tested in that study displayed anti-androgenic activity. Gregoraszczuk et al. (2008) demonstrated that steroid hormone secretion was either induced or suppressed depending on whether an individual congener or mixture of congeners was tested *in vitro*. There is also evidence that PBDE metabolites cause induction of steroidogenic gene expression (Song et al. 2008).

The commercial pentaBDE mixture and individual congener constituents BDE 47 and 100 inhibited binding of androgen receptors *in vitro* (Stoker et al. 2005). This same study found a significant increase in luteinizing hormone (LH) and a non-significant increase in testosterone after exposing adult male rats to the same congeners. Exposure of male rats to a pentaBDE mixture also resulted in delayed puberty, decreased growth of androgen-dependent tissues such as the prostate (Stoker et al. 2004), and a dose-dependent increase in organ weights and sperm head deformities (Van der Ven et al. 2008). Exposure to BDE 99 specifically resulted in other reproductive effects such as reduced sperm counts (Kuriyama et al. 2005) and a decrease in sexual behavior (Lichtensteiger et al. 2004). Exposure to BDE 209 decreased epididymal sperm functions but sperm count and other parameters were not affected (Tseng et al. 2006). Finally, several avian studies have reported reduced reproductive success after exposure to PBDEs, which may be a

consequence of endocrine disruption (Fernie et al. 2009; Henny et al. 2009; Johansson et al. 2009; Van den Steen et al. 2009).

HBCD has been much less studied in relation to altered endocrine function. HBCD has displayed estrogenic (Dorosh et al. 2011) as well as anti-estrogenic and anti-androgenic (Hamers et al. 2006) activity in different *in vitro* experiments. Similar to PBDEs, exposure of rats to HBCD resulted in decreased T4 and increased TSH levels in serum (Ema et al. 2008). To our knowledge, there are no studies to date on endocrine function in relation to exposure to other flame retardants measured in the present study.

1.3 Human exposure

Studies of potential human health effects of BFRs are limited and focus primarily on thyroid hormone disruption in relation to PBDE exposure. Contrary to most of the animal experiments, several human epidemiological studies reported increases in T4 and T3 levels associated with exposure to PBDEs (Bloom et al. 2008; Dallaire et al. 2009; Gascon et al. 2011; Meeker et al. 2009a; Turyk et al. 2008; Wang et al. 2010). Others have reported both increases and declines in TSH levels in relation to PBDE exposure, which may be dependent on exposure level, population, or specific congener measured (Chevrier et al. 2010; Hagmar et al. 2001; Yuan et al. 2008; Zota et al. 2011).

Although consumption of contaminated foods is an important exposure route for PBDEs (Fraser et al. 2009), PBDE exposure in North America is estimated to be mainly from the inhalation and ingestion of indoor dust (Johnson-Restrepo and Kannan 2009; Jones-Otazo et al. 2005; Lorber 2008; Webster et al. 2005). These estimates are supported by studies that link body burdens to indoor dust concentrations. Wu et al. (2007) found that indoor dust PBDE concentrations were more strongly correlated with

breastmilk PBDE concentrations than with estimated dietary intake of PBDEs. Similarly, Roosens et al. (2009) found that serum concentrations of HBCD were correlated with indoor dust concentrations but not dietary HBCD. Additionally, concentrations of DP in dust and hair have been strongly correlated (Zheng et al. 2010). We recently reported a strong correlation between several BDE congeners in serum and vacuum bag dust (Johnson et al. 2010), supporting the use of dust as a marker of exposure. We found that the major Penta formulation BDE congeners (BDE 47, 99, and 100) were strongly correlated between dust and serum, but that low detection rates of other congeners in serum (such as BDE 209) prevented reliance on serum measurements as a marker of exposure. The objective of the present study was to explore concentrations of a variety of BFRs in house dust and whether BFR exposure is associated with serum hormone levels in men.

We recently reported that dust concentrations of BDE 47, 99, and 100 were positively associated with serum levels of free T4 in 24 men. These congeners were also associated with alterations in levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), Inhibin B, sex hormone binding globulin (SHBG) and free androgen index (FAI) (Meeker et al. 2009a). The present study aims to expand our previous work to additional samples and analytes, including 31 BDE congeners and 6 alternate flame retardants.

2. Methods

2.1 Subject Recruitment

The present study utilizes serum hormone data collected from participants in a study on environmental exposures and male reproductive health. Men between 18 and 54

years of age were recruited from the Vincent Memorial Andrology lab at Massachusetts General Hospital. Male participants were from couples seeking infertility treatment due to a male factor, a female factor, or a combination of both male and female factors. Exclusionary criteria included prior vasectomy or current use of exogenous hormones. Research ethics committees at participating institutions approved the study protocols, and all participants signed an informed consent.

2.2 Dust sample collection and analysis

Participants donated existing vacuum bags in the home between years 2002 and 2003. Participants wrapped the used vacuum bag in aluminum foil and sealed it in a labeled plastic bag. Dust samples were stored at -20C until analysis. Dust was sieved using a 150 μm mesh sieve to obtain the fine fraction. Determination of the target analytes was performed by a gas chromatograph (Agilent 6890) coupled to an Agilent 5975 mass spectrometer (Agilent Technologies, Santa Clara, CA) operated in negative chemical ionization mode (GC/ECNI-MS) using the method by Stapleton et al. (2005, 2006). Laboratory blanks were low enough (<1%) for most analytes that blank correction was not needed except as follows. The average concentration of four blanks was subtracted from each sample for TBB and TBPH. The separate stereoisomers of HBCD were not distinguished. The two stereoisomers of Dechlorane Plus, syn-DP (sDP) and anti-DP (aDP) were quantified.

2.3 Serum hormones

One non-fasting blood sample was drawn and centrifuged, and the serum was stored at -80C until analysis. The hormone analytical methods were described previously (Meeker et al. 2008). The methods employed were as follows: Follicle stimulating

hormone (FSH), serum luteinizing hormone (LH), estradiol, prolactin, free T4, total T3, and thyrotropin (TSH) concentrations were determined by microparticle enzyme immunoassay using an automated Abbott AxSYM system (Abbott Laboratories, Chicago, IL,USA); inhibin B was measured using a double-antibody, enzyme-linked immunosorbent assay (Oxford Bioinnovation, Oxford, UK); a Coat-A-Count RIA kit (Diagnostics Products, Los Angeles, CA, USA) was used to measure testosterone; sex hormone binding globulin (SHBG) was measured using an Immulite fully automated chemiluminescent immunometric assay (DPC, Inc., Los Angeles, CA, USA). The free androgen index (FAI) was calculated as the ratio of testosterone to SHBG. Additionally, free unbound testosterone was estimated using the equation by Vermeulen et al. (1999).

2.4 Data analysis

Descriptive statistics were calculated for dust concentrations of BFRs. One half the limit of detection (LOD) was assigned to non-detect levels. Further statistical analysis was conducted for the alternate flame retardants and for PBDE congeners that were detected in over 85% of the dust samples. Spearman's correlation coefficients were calculated, using SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA), to assess bivariate relationships between different BFRs and between BFR concentrations in house dust and serum hormone levels. These relationships were assessed using multivariable linear regression to control for potential confounding variables in R version 2.8.1 (R Foundation for Statistical Computing, Vienna, Austria). A description of how the data was examined prior to arriving upon the final models is provided in the Appendix following this chapter. Regression models for PBDEs were run several ways: 1) using individual PBDE congener concentrations, 2) using summed concentrations of

PentaBDEs (BDE 47, 99, 100), OctaBDEs (BDE 183 and 201), and DecaBDEs (BDE 206, 207, 208 and 209), and 3) using independent factor variables generated by a factor analysis of all detectable congeners performed using SAS. The factor analysis utilized data on all congeners detected in dust and eliminated the potential for collinearity arising from any correlation between variables in the same model. The congener grouping in method 2) was based on three factors: the congener prevalence in commercial mixtures (ATSDR 2004; La Guardia et al. 2006), the Spearman's correlation coefficients between congeners within group in dust ($r > 0.80$, $p < 0.05$) and the Spearmans' correlation coefficients between congeners we detected in serum and matched dust samples ($r > 0.60$, $p < 0.05$; Johnson et al. 2010). Note that because fewer congeners were detected in serum, these data could only inform the pentaBDE congener grouping. All variables were analyzed as continuous variables. The distributions of several hormone levels (estradiol, testosterone, inhibin B, free T4 and total T3) approximated normality and were not transformed in statistical models. Several other hormones (prolactin, FSH, LH, SHBG, FAI, and TSH) were skewed right and were transformed to the natural log (ln) for statistical analyses. BFR concentrations in house dust were also transformed to the natural log. All multivariable models were adjusted for age and body mass index (BMI) because these parameters are known to be associated with changes in hormone levels. Age and BMI may also be associated with differences in BFR levels, although the ranges of these parameters were not large in this cohort of men.Dust concentrations of BDE 47, 99 and 100 were combined with concentrations from a previous analysis of 24 men from the same study cohort (Meeker et al. 2009a) and models were additionally computed for

the pooled total of 62 men. The previous study measured dust concentrations of only these three congeners.

3. Results

3.1 Distributions and bivariate relationships

Table 3.1 presents the distribution of selected PBDE groupings and alternate BFRs measured in house dust. The distribution and detection limits of all individual PBDE congeners measured were previously reported (Johnson et al. 2010). There were a total of 18 PBDE congeners that were detected in over 85% of the dust samples and thus were included in further analyses. BTBPE was detected in 100% of the dust samples, while HBCD, TBPH and TBB were detected in 92, 64 and 48% of samples, respectively. All samples contained aDP and 96% contained sDP, although concentrations of these chlorinated flame retardants were relatively lower than the alternate BFRs. All analyte concentrations were highly skewed (log-normal distribution).

Concentrations of PBDE congeners with similar degrees of bromination were strongly correlated (Spearman $r \geq 0.80$, $p<0.05$) (Johnson et al. 2010). The correlation coefficients for the PBDE congeners included in the congener groupings for data analysis are shown in Table 3.2, along with coefficients for the alternate BFRs. Concentrations of tetrabromobenzoate (TBB) and tetrabromo phthalate (TBPH), which are both found in the same commercial products that replaced pentaBDE, were also strongly correlated with one another (Spearman $r = 0.79$, $p<0.0001$). As expected, there were also strong correlations between concentrations of the two stereoisomers of Dechlorane Plus, sDP and aDP (Spearman $r = 0.83$, $p<0.0001$). There was also some degree of correlation

among alternate BFRs (r = 0.32-0.47) and between alternate BFRs and lower-brominated PBDEs (r = 0.31–0.49), octaBDE formulation congeners (r = 0.34–0.60), and decaBDE formulation congeners (r = 0.31-0.43).

In preliminary analyses, positive correlations were found (p-values < 0.05) between concentrations of pentaBDE congeners and serum levels of prolactin and total T3. OctaBDE congeners were positively correlated with free T4 (r = 0.36, p = 0.03), luteinizing hormone (r = 0.36, p = 0.03) and prolactin (p = 0.01). Several bivariate relationships between alternate BFRs and hormone levels are presented in scatterplots (Figures 3.1 through 3.3). There was a positive correlation between BTBPE and total T3 (r = 0.33, p = 0.04). TBPH, which was only detected in 64% percent of the samples, was also positively associated with total T3 (r = 0.30, p = 0.07). HBCD was positively correlated with free androgen index (FAI) (r = 0.46, p = 0.004) and inversely correlated with sex hormone binding globulin (SHBG) (r = -0.35, p = 0.03).

3.2 Multivariable linear regression

Table 3.3 presents results from multivariable linear regression models, as percent change in hormone level associated with an interquartile range (IQR) increase in BFR dust concentration adjusted for age and BMI. The age and BMI adjusted results were similar to the bivariate relationships between single BFR compounds and serum hormone levels. An IQR increase in pentaBDEs (n=38) was associated with (p<0.05) a 22% increase in prolactin and a 7% increase in total T3. Additonally, there were statistically significant positive associations with T3, T4, estradiol and SHBG, and a suggestive (p=0.13) relationship with inhibin B in the pooled total pentaBDE samples (n=62). Inverse associations between the pentaBDE congeners and FSH (p < 0.05) and FAI (p =

0.13) were also found. The association with prolactin was weakened in the pooled analysis. These relationships were consistent with our earlier report among the original 24 samples (Meeker et al. 2009a).

There were significant positive associations between dust concentrations of octaBDEs and serum T4, TSH, LH and testosterone and a significant inverse association between dust concentrations of decaBDEs and testosterone (Table 3.3). There were also suggestive positive relationships between OctaBDEs and estradiol, prolactin, and total T3.

Associations between dust concentrations of several alternative BFRs (BTBPE, TBPH and HBCD) and hormone levels were observed. An IQR increase in BTBPE was associated with a statistically significant 6% increase in total T3. After adjusting for age and BMI, the positive relationship between BTBPE and prolactin became statistically suggestive ($p=0.09$). An IQR increase in TBPH was associated with a statistically significant 9% increase in total T3. An IQR increase in HBCD was associated ($p<0.05$) with decreased SHBG (15%) and increased FAI (19%).

When analyzed as individual congeners (data not shown) in multivariate linear regression models adjusted for age and BMI, IQR increases in BDE 153 and 154 were associated with statistically significant 24% and 22% increases, respectively in SHBG, and with 31% and 38% increases in prolactin. There were also several other PBDE congeners that were positively associated, when modeled individually, with total T3: BDE 28/33, 47, 49, 66, 85/155, 99, 100 and 153. The sum of all congeners was also associated with a 6% increase in total T3.

Table 3.4 describes the factor pattern of 8 independent variables generated by the factor analysis. The cumulative proportions of the variability explained by each factor, as determined by eigenvalues of the correlation matrix from the factor analysis, were used in our decision to choose 8 factors to use in the regression models. These eight factors account for 90 percent of the variability of all the detectable congeners. Table 3.4 indicates that most of the variability of the congeners is accounted for in the first 2 factors (high number of weightings >0.75 under Factor 1 and 2 in the first two columns), and factor 1 is most heavily weighted by pentaBDE congeners and factor 2 is heavily weighted by decaBDE congeners. The congeners that we grouped into our octaBDE group (BDE 183 and BDE 201) were not as clearly delineated by the factor pattern. Linear regression models were run for each hormone outcome with all 8 factors in the models. Results from these models, which are not easily interpreted and are thus not shown, were compared to the results of the other two methods of analysis (individual congeners and congener groupings). The regression model results using the 8 factors corroborate the findings from models using our congener groupings. For example, factors 2 and 3, which are mainly weighted by decaBDE congeners, were inversely and significantly associated with testosterone levels. Factor 1, which is heavily weighted by pentaBDE congeners, had a significant positive association with prolactin and total T3. These results are consistent with the associations shown for the congener groupings presented in Table 3.3.

4. Discussion

4.1 Concentrations of BFRs in dust

The BFR concentrations in house dust in the present study were similar to those found in other studies in the United States (Allen et al. 2008; Sjödin et al. 2008; Stapleton et al. 2005, 2008). Similar to other studies, DecaBDE was the dominant mixture found in house dust, followed by pentaBDEs. Due to the decline in use of penta- and octaBDE mixtures since 2002-2003 when the dust samples were collected, it is reasonable to expect that indoor levels of these congeners may have declined. However, because of continued use of older products, it is uncertain. It is also possible that concentrations of alternate BFRs may be increasing due to the increased use of alternate BFRs as substitutes for PBDEs. As expected, there were strong correlations found among BFRs that comprise the same commercial formulations. There was also some degree of correlation between different formulations, suggesting that these BFRs may have originated from the same sources within the home.

4.3 Thyroid hormone effects

Although several animal studies show decreased T3 and T4 levels following dosing with PBDEs, our findings of increased T4 levels associated with PBDE exposure are consistent with most other human epidemiological studies (Bloom et al. 2008; Turyk et al. 2008; Wang et al. 2010). A more recent study, however, found that lower levels of a pentaBDE mixture increased T4 levels in perinatally exposed rats (Blake et al. 2011), which is consistent with the human studies to date. The authors note that the relatively higher doses of PBDEs that decrease T4 levels in animal studies are also associated with increased liver weights (Stoker et al. 2004; Zhou et al. 2001, 2002) and may be the result of a different mechanism of action. However, Kuriyama et al. (2007) reported decreased T4 in rats exposed to BDE 99 at levels similar to those used by Blake et al. (2011). It is

difficult to draw conclusions about potential animal and human differences in thyroid hormone effects based on a few studies, particularly due to differences in study design such as specific congener or congener mixture tested, time of dosing, and timing of effects measurement.

The positive association between many of the penta formulation PBDEs and T3 levels we found is consistent with a study by Dallaire et al. (2009) which found higher T3 levels associated with serum BDE 47 among a population of 623 Inuit adults. However, another large study of 308 men found lower T3 and TSH levels associated with serum PBDEs (sum of BDE 47, 99, 100 and 153) (Turyk et al. 2008). The associations between PBDE exposure and thyroid hormones in humans are not consistent across studies, which may be due to inherent differences in population, exposure levels or other study characteristics. While Chevrier et al. (2010) found lower TSH levels associated with serum concentrations of pentaBDE formulation congeners, Zota et al. (2011) found that the same congeners in a different population were associated with higher TSH levels. Zota et al. (2011) also reported lower TSH levels associated with BDE 207, a congener in the decaBDE mixture. These two studies were of populations of pregnant women, and as Zota et al. (2011) point out, thyroid hormones fluctuate in women over the course of pregnancy, and therefore the two studies may not be comparable. Additionally, because our study population was male, it may not be meaningful to compare our results to those of studies of pregnant women. We observed higher TSH levels with increased exposure to PBDEs, and this association was statistically significant for the octaBDE group. Because TSH regulates the production of T3 and T4 through negative feedback, an inverse relationship between TSH and both T3 and T4 is expected. However, we did not

observe this inverse relationship, and the present study is not the first to observe unexpected thyroid hormone level alterations in relation to PBDE exposure (Gascon et al. 2011; Chevier et al. 2010; Zota et al. 2011). This could mean that thyroid hormones are being affected at the hypothalamus or pituitary gland, rather than responding to fluctuations in T4. Thyroid hormone homeostasis is critical to numerous physiologic processes including metabolism, neurodevelopment, cardiovascular health and reproduction. Further study of the implications of subclinical alterations in thyroid hormone levels is needed to better understand the effects of BFRs on human health.

A Norwegian study did not find an association between PBDE or HBCD concentrations in human breast milk and serum TSH levels in newborns (Eggesbø et al. 2011). However, as the authors point out, most European environmental concentrations of these compounds are orders of magnitude lower than levels in the United States. We also did not find a significant association between HBCD exposure and thyroid hormone levels in the present study.

4.4 Reproductive hormone effects

There are very few human studies of BFR exposure and reproductive hormones, and only one other study among adult men. Meijer et al. (2008) reported an inverse association between prenatal BDE 99 exposure and testosterone and SHBG measured in male infants at 3 months of age and positive associations between BDE 154 and testosterone, SHBG, inhibin B and estradiol. The authors also reported anti-androgenic or androgenic effects which were congener-dependent. BDE 47 was associated with lower testis volume and penile length, while BDE 154 was associated with higher volume and length. We also found positive associations between the pentaBDEs and inhibin B,

SHBG and estradiol, with statistical significance for SHBG and estradiol when pooling all 62 samples. We also observed a significant positive association between the individual congener BDE154 and SHBG. Main et al. (2007) reported a positive association between the sum of 14 PBDE congeners in breastmilk and serum LH among newborn males, along with an association with congenital cryptorchidism. We also observed a significant positive association between octaBDE exposure and LH. However, our findings do not appear consistent for pentaBDEs. We previously found a negative association in our preliminary analysis of only 24 men (Meeker et al. 2009a). In the present study, there was an inverse relationship when all 62 samples were pooled, although this association was not statistically significant. The findings of Turyk et al. (2008) included a positive association between BDE 47 and testosterone in men. We also found a positive relationship between the pentaBDEs and testosterone, but the association was statistically significant for octaBDEs only. There was a negative association between decaBDEs and testosterone in the present study. Testosterone is important to several physiologic functions in adult men, including sex drive and spermatogenesis. A reduction in LH is expected in relation to increases in testosterone. However, we observed changes in the same direction for LH and testosterone.

The pentaBDE group and several individual congeners, including BDE 153 and BDE 154, were positively associated with prolactin. Prolactin is involved in reproductive, metabolic and other functions, and may be used as a measure of neuroendocrine/ dopaminergic function (Meeker et al. 2009b).

Animal studies on BFRs that measured reproductive hormone responses are limited. Lilienthal et al. (2006) reported reduced levels of estradiol and testosterone in

male rats in response to in utero BDE 99 exposure. Gestational exposure to BDE 47 resulted in reduced estradiol in females (Talsness et al. 2006, 2008) and reduced FSH in males (Andrade et al. 2004). Our findings are consistent with the Andrade et al. findings of reduced FSH related to pentaBDE exposure. As FSH has a negative feedback relationship with inhibin B, our observation of increased inhibin B in relation to pentaBDE exposure, although not statistically significant ($p=0.13$), is expected. Our findings do not appear to be consistent with the directions of alterations in estradiol and testosterone levels in these animal studies, as we did not find reductions in testosterone levels and found significantly increased estradiol levels associated with pentaBDE exposure in men. Unlike our finding of lower testosterone levels associated with exposure to BDE 209, Kim et al. (2009) did not observe any effect on testosterone levels of male rats after gestational exposure to BDE 209. It is again difficult to compare results between studies, however. For example, many animal studies measured effects of gestational exposure while the present study estimates current exposure to adult men. Our findings appear consistent with the direction of testosterone level alterations found by Stoker et al. (2005) in adult male rats, although both studies found non-significant increases in testosterone levels in relation to pentaBDE exposure.

Marteinson et al. (2011) found higher testosterone levels associated with HBCD exposure in American kestrels. We did not find significantly higher testosterone levels in relation to HBCD exposure; rather, we found higher free androgen index (FAI), an estimation of free testosterone, which is influenced by levels of SHBG. We observed reduced SHBG levels in relation to HBCD exposure.

Because the endocrine system is complex, and there are relatively few studies examining the endocrine effects of BFRs, it is difficult to speculate on the mechanisms or clinical significance of the hormone alterations in the present study. Further research on these topics is needed, particularly if subclinical hormone alterations may have an impact on health at the population level.

4.5 Limitations and considerations

There may be certain limitations when comparing our findings to other studies using biomarkers of BFR exposure, considering we estimated exposure to BFRs by measuring concentrations in dust. However, we expect good agreement between serum and dust concentrations for at least the pentaBDE congener grouping, as we previously demonstrated (Johnson et al. 2010). Additionally, our previous report also showed that dust concentrations of higher-brominated congeners tended to be correlated to serum concentrations of lower-brominated congeners, which may have implications for exposure assessment in terms of debromination and congener-specific body burdens. Because PBDEs can debrominate within an organism (Noyes et al. 2011; Stapleton et al. 2004), it is reasonable to predict that exposure to higher-brominated congeners in dust may result in body burdens of lower-brominated congeners. Because there was some degree of correlation between some PBDEs and BTBPE and TBPH, and these BFRs were associated with the same hormone effects, further study is needed to confirm the associations involving these two alternate BFRs.

The present study is the first to explore human exposure and associated effects of some of these compounds. It is also the first study to relate dust concentrations of decaBDEs and other alternate BFRs to hormone levels. This work expands upon our

previous study where we found hormone level alterations associated with exposure to BDE 47, 99 and 100 as measured in house dust (Meeker et al. 2009a). The use of vacuum bag dust as a marker of exposure had the advantages of low cost and efficiency. Furthermore, as compared to spot sampling, vacuum bag dust may be a measure of longer term integrative exposure representing the total home environment.

Future study should address the validation of house dust as a marker of exposure for flame retardants other than PBDEs. Although we previously found a strong correlation between concentrations of the major penta formulation congeners in dust and serum (Johnson et al. 2010), BDE 153 was not correlated. Other flame retardants may have characteristics similar to BDE 153, such as longer biological half-lives, which could affect the relationship between body burdens and environmental measures such as dust. BTBPE has a relatively higher vapor pressure than the other compounds measured, and therefore inhalation may also be an important exposure route. However, in the absence of biomarkers of exposure for these compounds, dust may be an adequate surrogate estimate of exposure that likely underestimates body burden to some degree.

This study had a relatively small sample size, and a large number of relationships were investigated due to its exploratory nature. Further studies of these exposures and outcomes should be larger to increase confidence and reduce the possibility that some of the findings are due to chance. We used a factor analysis to generate independent exposure variables and eliminate the potential problem of collinearity when including multiple variables in the same regression model. These results supported those of our *a-priori* congener groupings, suggesting that it was appropriate to group the congeners using our criterion for at least the pentaBDEs and decaBDEs. Additionally, because

house dust may contain a variety of chemical compounds to which people are potentially exposed, we cannot rule out the possibility that our reported findings may be due to unmeasured coexposures or confounders. Furthermore, the present study has a cross-sectional perspective in terms of environmental exposure and hormone levels. However, because exposure to these compounds is expected to be relatively constant if they originate from consumer products present in the home, our exposure estimates are likely representative of longer term exposure.

5. Conclusion

In conclusion, the present study provides further evidence of altered hormone levels in relation to BFR exposure, and that house dust is an important source of human BFR exposure. Further research is needed to confirm these findings and to determine sources of human exposure. More reliable biomarkers are needed for some BFRs. Research is also needed to determine the public health implications of alterations in hormone levels by environmental exposures.

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Table 3.1. Distribution of brominated flame retardants in house dust
ng/g (n = 50)

		Percentiles				
	Mean[a]	25th	50th	75th	90th	Maximum
PentaBDEs	1337	667	862	2,385	5,431	22,300
OctaBDEs	34.1	21.2	28.8	60.4	103	1,181
DecaBDEs	2,192	1,352	1,681	3,228	6,669	38,483
TBB	464	68.4	68.4	2,714	23,934	72,460
HBCD	136	96.9	242	396	1,045	2,213
BTBPE	21.2	9.05	19.9	41.4	110	953
TBPH	426	47	416	1,532	10,944	47,110
sDP	2.82	2.47	4.26	8.22	14.2	43.1
aDP	9.29	5.64	8.85	15.8	29.2	68.4

[a]Geometric Mean

Table 3.2. Spearman correlation coefficients among select BFRs in house dust (n=38)

	BDE	47	99	100	183	201	206	207	208	209	TBB	TBPH	BTBPE	HBCD	aDP	sDP
BDE 47	r	1.00	0.93	0.94	0.52	0.40	0.17	0.22	0.14	0.13	0.31	0.38	0.37	0.27	0.11	0.18
	p-value		<0.0001	<0.0001	0.0009	0.01	0.30	0.19	0.40	0.44	0.06	0.02	0.02	0.11	0.5	0.28
BDE 99	r		1.00	0.97	0.49	0.36	0.19	0.26	0.21	0.17	0.26	0.31	0.35	0.19	0.10	0.12
	p-value			<0.0001	0.002	0.03	0.27	0.11	0.21	0.29	0.12	0.06	0.03	0.26	0.57	0.47
BDE 100	r			1.00	0.54	0.41	0.22	0.29	0.23	0.22	0.31	0.37	0.39	0.17	0.13	0.15
	p-value				0.0004	0.01	0.19	0.08	0.16	0.19	0.05	0.02	0.01	0.3	0.45	0.36
BDE 183	r				1.00	0.87	0.21	0.36	0.18	0.16	0.50	0.44	0.47	0.05	0.29	0.34
	p-value					<0.0001	0.20	0.02	0.28	0.33	0.002	0.006	0.003	0.75	0.08	0.03
BDE 201	r					1.00	0.39	0.53	0.37	0.30	0.60	0.55	0.43	0.12	0.40	0.52
	p-value						0.02	0.0005	0.020	0.07	<0.0001	0.0003	0.007	0.49	0.01	0.0008
BDE 206	r						1.00	0.93	0.94	0.84	0.28	0.28	0.28	-0.24	0.22	0.37
	p-value							<0.0001	<0.0001	<0.0001	0.09	0.09	0.09	0.14	0.19	0.02
BDE 207	r							1.00	0.96	0.83	0.40	0.35	0.32	-0.16	0.24	0.43
	p-value								<0.0001	<0.0001	0.01	0.03	0.05	0.33	0.15	0.008
BDE 208	r								1.00	0.85	0.31	0.30	0.28	-0.20	0.23	0.36
	p-value									<0.0001	0.05	0.07	0.09	0.23	0.16	0.02
BDE 209	r									1.00	0.29	0.19	0.27	-0.16	0.22	0.36
	p-value										0.08	0.26	0.11	0.33	0.19	0.03
TBB	r										1.00	0.79	0.31	0.04	0.30	0.39
	p-value											<0.0001	0.06	0.80	0.07	0.01
TBPH	r											1.00	0.43	0.16	0.32	0.46
	p-value												0.01	0.35	0.05	0.004
BTBPE	r												1.00	-0.09	0.45	0.47
	p-value													0.59	0.004	0.003
HBCD	r													1.00	0.20	0.15
	p-value														0.23	0.36
aDP	r														1.00	0.83
	p-value															<0.0001
sDP	r															1.00
	p-value															

Table 3.3. Percent change[a] in hormone level (95% confidence intervals), relative to population median, associated with an interquartile range (IQR) increase in house dust BFR concentration

	PentaBDE[c] (n=38)			OctaBDE[d] (n=38)			DecaBDE[e] (n=38)			PentaBDE[c] (n=62)[f]		
			p-value			p-value			p-value			p-value
FSH	-10.4	(-3.6, 10.7)	0.31	4.4	(-13.2, 25.6)	0.65	1.8	(-15.5, 22.7)	0.85	-20.2	(-34.7, -2.5)	0.03
LH	7.4	(-9.2, 26.9)	0.41	15.5	(0.6, 32.6)	0.05	-6.6	(-19.2, 8.1)	0.37	-9.4	(-24.1, 8.2)	0.28
Inhibin B	6.8	(-20.4, 33.9)	0.63	-5.8	(-29.4, 17.7)	0.63	-6.2	(-29.9, 17.5)	0.61	18.2	(-4.8, 41.3)	0.13
Testosterone[b]	5.2	(-5.2, 15.6)	0.33	9.0	(0.8, 17.2)	0.03	-9.4	(-17.6, -1.2)	0.02	3.6	(-7.4, 14.7)	0.52
SHBG	14.3	(-3.8, 35.8)	0.13	9.2	(-6.1, 27.0)	0.26	-10.2	(-22.8, 4.4)	0.17	16.8	(0.7, 35.4)	0.05
FAI	-7.3	(-21.2, 9.1)	0.36	-0.7	(-13.9, 14.5)	0.92	0.4	(-13.0, 16.0)	0.95	-10.6	(-22.5, 3.2)	0.13
Estradiol	16.1	(-3.9, 36.2)	0.12	14.4	(-2.8, 31.7)	0.11	-8.5	(-26.3, 9.3)	0.36	17.1	(0.0, 34.2)	0.05
Prolactin	21.6	(1.6, 45.5)	0.03	13.8	(-2.9, 33.4)	0.12	4.5	(-11.4, 23.3)	0.60	10.8	(-6.0, 30.7)	0.23
Free T4	1.4	(-1.5, 4.3)	0.36	3.3	(1.0, 5.6)	0.01	-1.7	(-4.2, 0.9)	0.20	3.6	(0.6, 6.5)	0.02
Total T3	6.9	(1.9, 12.0)	0.01	4.3	(-0.2, 8.8)	0.07	1.7	(-3.1, 6.4)	0.50	5.4	(0.0, 10.7)	0.05
TSH	16.3	(-6.7, 45.0)	0.18	21.2	(0.8, 45.8)	0.05	11.1	(-8.5, 34.9)	0.29	14.1	(-4.7, 36.7)	0.16

[a] Adjusted for age and BMI. [b] Free testosterone estimated using equation by Vermeulen et al. 1999.

[c] PentaBDE is sum of BDE 47, 99 and 100. IQR = 2268 ng/g for n=38 and 2985 ng/g for n=60.

[d] OctaBDE is sum of BDE 183 and 201. IQR = 39 ng/g.

[e] DecaBDE is sum of BDE 206, 207, 208 and 209. IQR = 1876 ng/g.

[f] Includes additional samples from prior preliminary analysis, and models are also adjusted for difference in dust analytical method.

Figure 3.1. Scatterplot of HBCD in house dust and ln-transformed free androgen index (FAI)
(n = 38, r = 0.46, p = 0.004)
One outlier with a concentration of HBCD less than the detection limit was removed and did not affect the positive association.

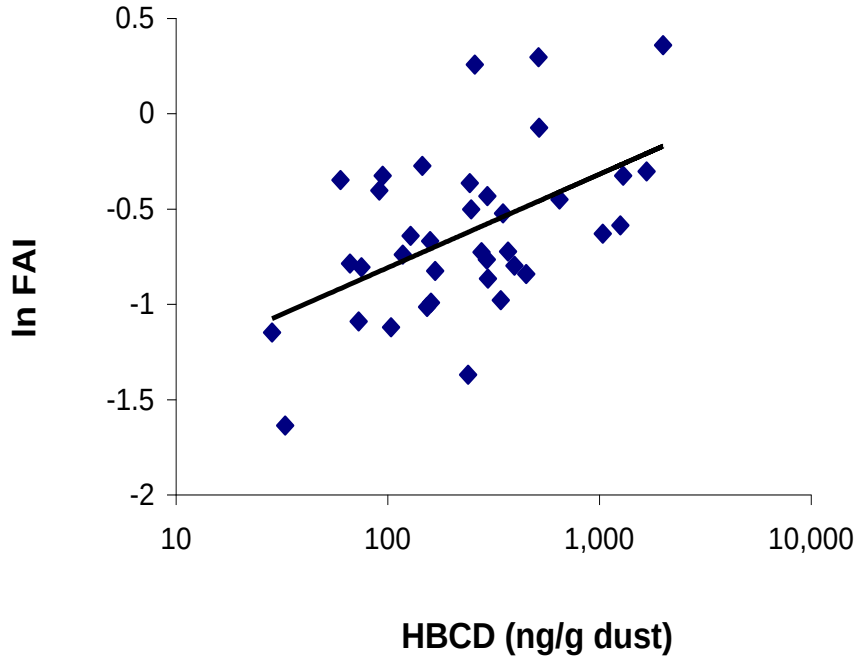


Figure 3.2. Scatterplot of BTBPE in house dust and serum total T3
(n = 38, r = 0.33, p = 0.04)

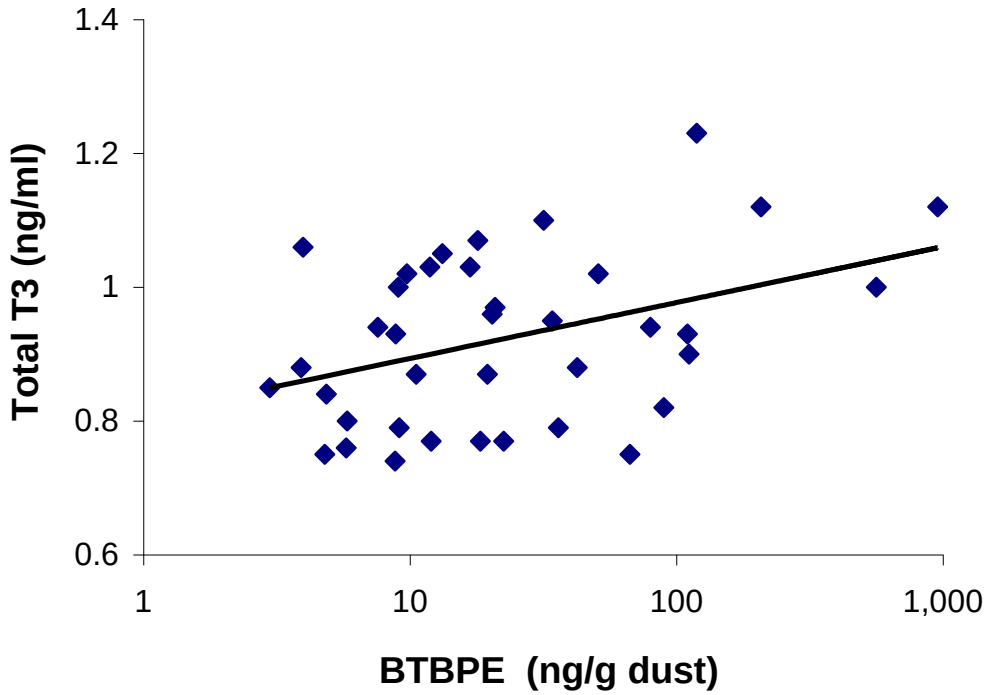


Figure 3.3. Scatterplot of TBPH in house dust and serum total T3
(n = 38, r = 0.30, p = 0.07)

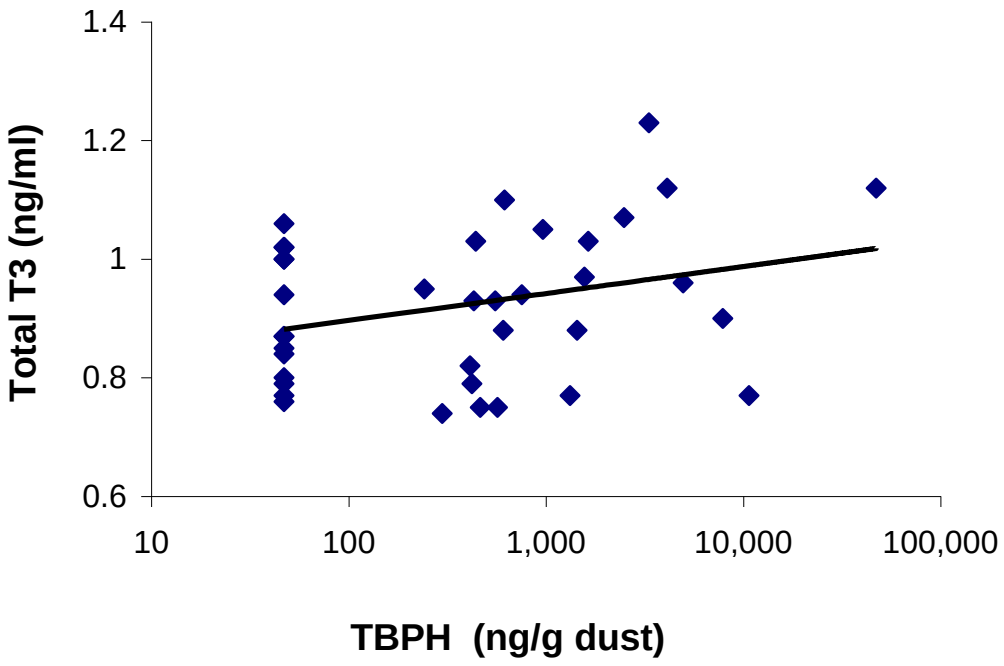


Table 3.4. Factor pattern for 8 independent variables, representing weightings of each congener, for all PBDE congeners detected in house dust.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
BDE 17	0.61921	-0.27052	0.19168	0.17357	-0.41984	-0.04351	0.33098	-0.07932
BDE 25	0.19201	-0.36883	-0.12747	0.39082	-0.30498	-0.46457	-0.01396	0.49010
BDE 28/33	0.81550	-0.38798	0.06208	0.07015	-0.03569	0.07351	-0.06067	-0.07033
BDE 30	-0.13225	-0.02905	0.20550	-0.53665	0.12428	-0.28102	0.68385	0.01557
BDE 47	0.95770	-0.21810	0.02513	0.01519	-0.00523	0.04297	-0.00737	-0.08296
BDE 49	0.42602	-0.00713	0.15241	-0.25128	-0.50311	0.52132	0.07574	0.15202
BDE 66	0.73432	-0.32019	0.08162	0.18837	-0.29711	0.08202	0.20863	-0.16743
BDE 75	0.23279	-0.28282	0.03633	0.49519	0.36456	0.55011	0.15913	-0.17713
BDE 85/155	0.96164	-0.17075	0.02102	-0.06266	0.03537	-0.10549	-0.07274	-0.01817
BDE 99	0.96213	-0.21373	0.04277	-0.03854	-0.00481	-0.08907	-0.02396	-0.03014
BDE 100	0.97057	-0.17617	0.00468	-0.06570	0.04365	-0.07927	-0.07759	-0.02223
BDE 138	0.38529	-0.17239	0.54641	-0.17250	0.36090	0.23085	-0.26340	0.15432
BDE 153	0.95584	-0.14743	-0.06018	-0.08995	0.03895	-0.11845	-0.05213	0.02262
BDE 154	0.84431	-0.01778	-0.07175	-0.22286	0.24656	-0.19706	-0.22573	0.13240
BDE 183	0.53488	0.19800	-0.77153	-0.11729	0.11291	0.00159	-0.03000	-0.03953
BDE 201	0.43644	0.42526	-0.73047	-0.00085	0.09663	-0.00121	-0.00375	-0.10207
BDE 202	0.40385	0.63789	-0.02366	-0.06827	0.06280	-0.17517	0.17920	-0.29929
BDE 203/200	0.06711	0.61525	-0.46298	0.28807	-0.22872	0.26425	0.08367	0.23107
BDE 205	0.13333	-0.01979	0.18782	0.66479	0.46476	-0.23387	0.27388	0.09357
BDE 206	0.30397	0.85720	0.31233	0.08345	-0.05574	-0.05673	-0.03512	-0.00946
BDE 207	0.40369	0.87831	0.12270	0.10306	-0.05670	-0.06045	0.02305	-0.06370
BDE 208	0.35133	0.84867	0.31784	0.10512	-0.07158	-0.04356	0.02223	-0.04902
BDE 209	0.31736	0.76528	0.42895	0.02329	-0.00433	0.02991	-0.17121	0.20390
Variability[a]	0.3631	0.5547	0.6483	0.7123	0.7695	0.8234	0.8691	0.9025
Color coding:	> 0.75	0.50-0.74	0.25-0.49					

[a] Cumulative proportions of the variability explained by each factor, as determined by eigenvalues of the correlation matrix from the factor analysis. Eight factors account for 90 percent of the variability of all congeners.

Appendix

The following information is to show how data was examined and transformed prior to analyses. Several examples are provided, but not all data is shown.

Hormones with distributions that approximated normality were left untransformed, as in the case of free T4 (Figure A-1). Several hormones had skewed distributions and were transformed to the natural log (ln) to satisfy the normality assumptions of the regression models, as in the case of FSH (Figure A-2). All BFR distributions were skewed and were also ln-transformed (Figure A-3).

Bivariate relationships were examined prior to running models with additional variables (Figure A-4). Plots of residuals were examined to ensure that the points were randomly dispersed and therefore linear regression models were appropriate for the data. Figure A-5 is the residual plot for model 4: FreeT4 = ln(sum of pentaBDEs) + age + bmi. Figure A-6 is the residual plot for model 1: ln(FSH) = ln(sum of pentaBDEs) + age + bmi. Because the data appear randomly dispersed about the horizontal axis, these plots indicate that linear models are appropriate.

The use of splines in the regression models was investigated as a means to improve model fit by smoothing the exposure variable. Models were built using GAM in R with penalized splines. Smoothing of the exposure variable was examined, allowing mgcv to choose the number of knots. Figure A-7 is the plot of the smoothing of the variable (sum of pentaBDEs) for the model: FreeT4 = (sum of pentaBDEs) + age + bmi. Because the relationship between (sum of pentaBDEs) and free T4 was linear (estimated degrees of freedom = 1), smoothing was not used in the final model. Likewise, the

relationship between (sum of pentaBDEs) and FSH was linear (estimated degrees of freedom = 1.28), and smoothing was not necessary in the final model (Figure A-8).

Figure A-1. Example histogram of dependent variable

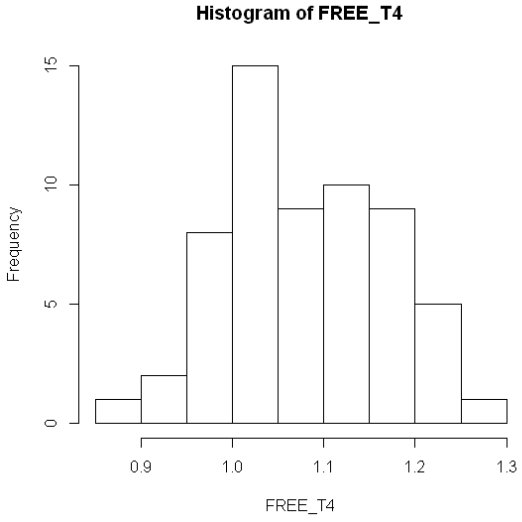


Figure A-2. Example histograms of transformed dependent variable

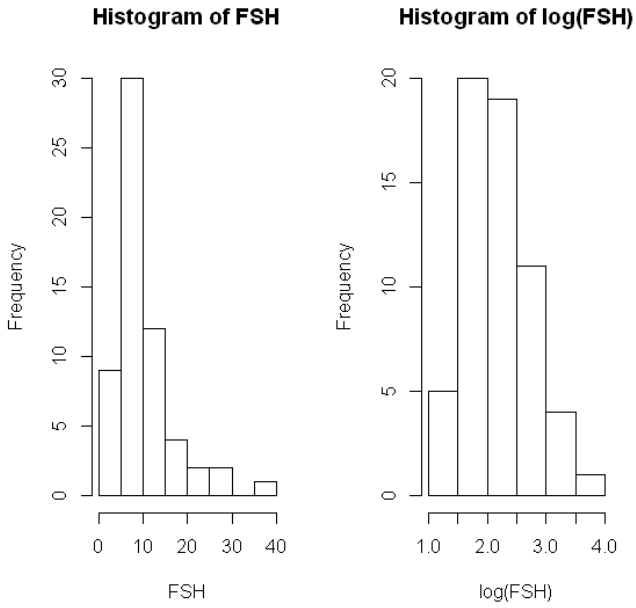


Figure A-3. Example histograms of independent variable

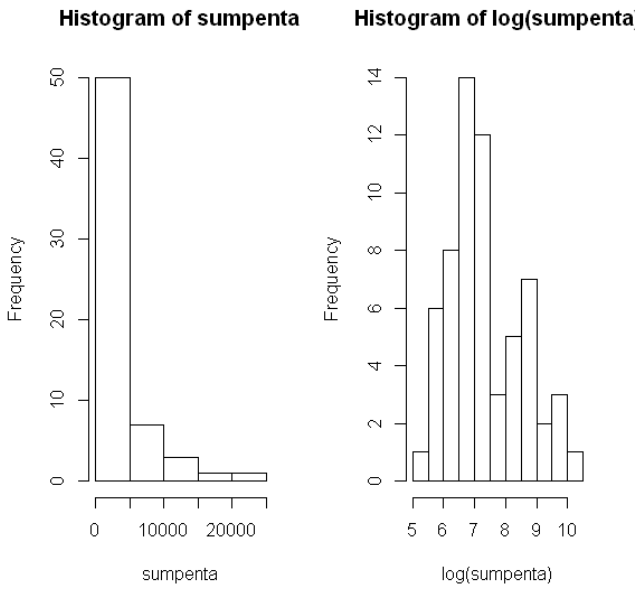


Figure A-4. Bivariate relationships.

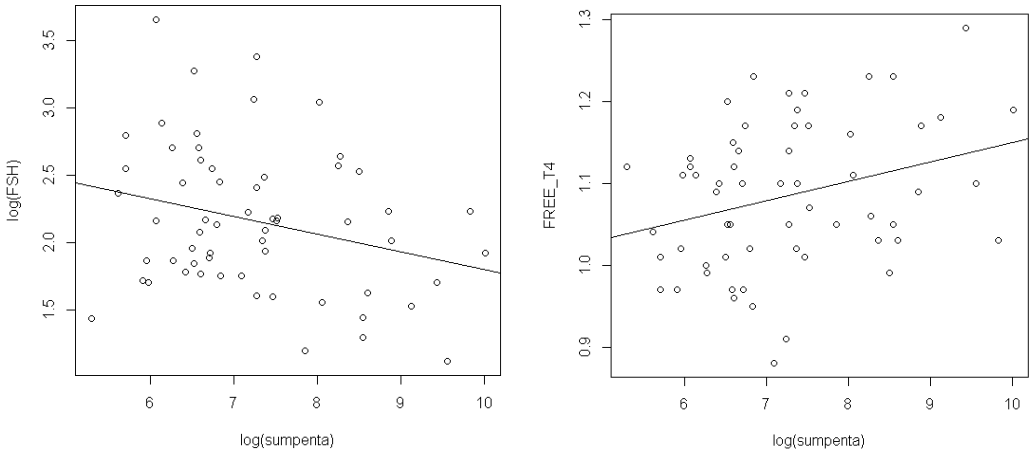


Figure A-5. Residual plot for model 4: FreeT4 = ln(sum of pentaBDEs) + age + bmi.

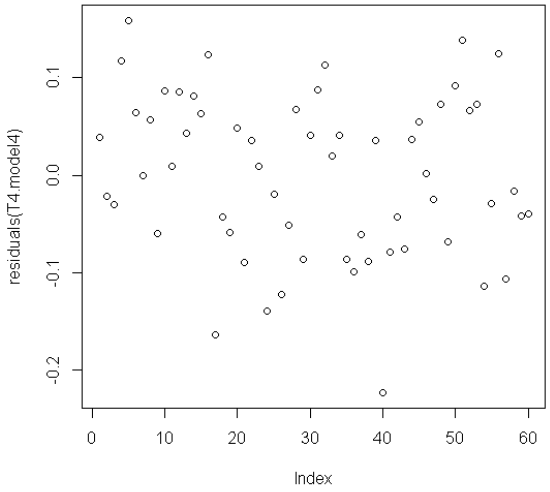


Figure A-6. Residual plot for model 1: ln(FSH) = ln(sum of pentaBDEs) + age + bmi.

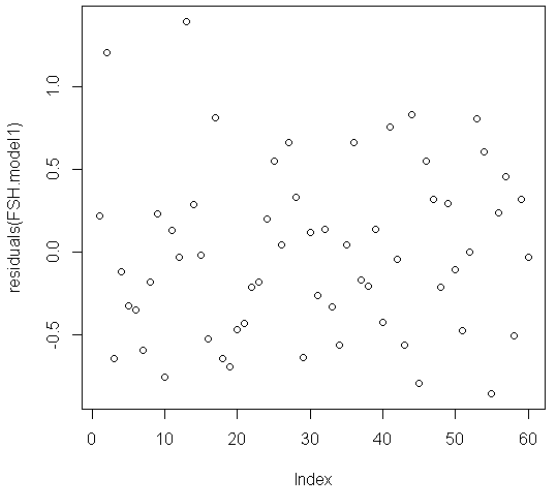


Figure A-7. Smoothing plot for FreeT4 = s(sum of pentaBDEs) + age + bmi.

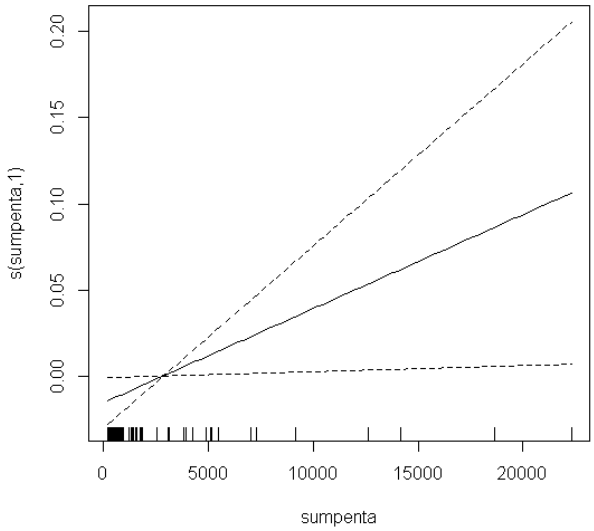
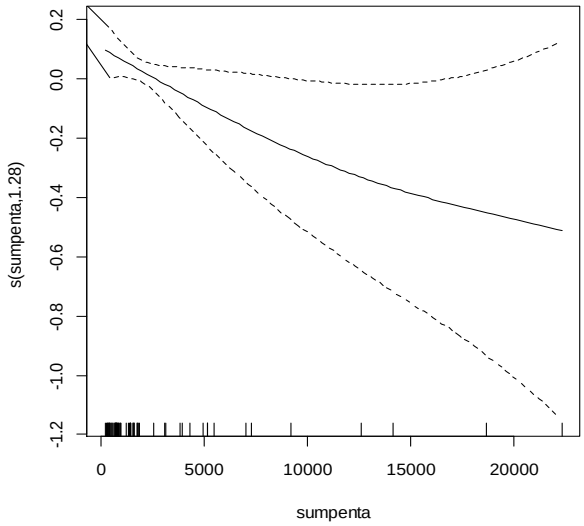


Figure A-8. Smoothing plot for ln(FSH) = s(sum of pentaBDEs) + age + bmi.



CHAPTER IV

Serum and Follicular Fluid Concentrations of Polybrominated Diphenyl Ethers and *In vitro* Fertilization Outcome

Abstract

There is evidence of endocrine disruption and reproductive effects in animals following exposure to certain PBDEs, but human studies are limited. The goal of this study was to investigate the use of serum and follicular fluid as biomarkers of exposure to PBDEs and to explore whether a relationship between PBDE exposure and early pregnancy loss exists. We measured 8 PBDE congeners in archived serum and ovarian follicular fluid samples from 65 women undergoing *in vitro* fertilization (IVF). Logistic regression models were used to predict the odds of failed embryo implantation associated with higher levels of PBDEs among the women in the study. There were moderate Kendall's Tau-beta correlations between serum and follicular fluid concentrations of BDE 28, 47, 100 and 154 (T_β=0.29-0.38, all p-values<0.005), but BDE 99 and 153 were not correlated between the two matrices (T_β<0.2, p-values>0.05). Women with detectable concentrations of BDE 153 (39% had detectable levels) in follicular fluid had elevated odds of failed implantation compared with women who had non-detectable concentrations (adjusted OR=10.0; 95%CI: 1.9 to 52; p=0.006; adjusted by age and body mass index). These findings suggest that exposure to BDE 153 may be associated with failed embryo implantation. Due to our observation of only moderate correlations between matrices, serum PBDE

concentrations may not be a good indicator of follicular fluid concentrations when studying early pregnancy endpoints in women undergoing IVF.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of flame retardants used in the manufacture of a variety of consumer products, including home electronics, upholstered furniture, carpeting, and other items containing polyurethane foam or plastics. Flame retardants are added to these products with the intention to slow the rate of burning in case of fire and meet fire safety standards such as Technical Bulletin 117 which requires that certain articles do not ignite when exposed to 12 seconds of open flame (CA Dept. of Consumer Affairs, 2000). PBDEs are not chemically bound, and thus may leach out or physically degrade and end up in indoor air and house dust. PBDEs have been measured in the indoor environment (Sjodin et al., 2008a; Stapleton et al., 2005), and house dust is expected to be a primary exposure pathway (Johnson et al., 2010; Johnson-Restrepo and Kannan, 2009; Lorber, 2008; Wu et al., 2007). Human exposure to PBDEs is widespread and body burdens in North Americans are orders of magnitude higher (around 35 ng/g lipid) than those in European countries where most PBDEs have been banned (Hites, 2004; Sjodin et al., 2008b).

Three commercial formulations of PBDEs have been produced, designated as penta-, octa-, and deca- BDE. These formulations consist of mixtures of specific PBDE congeners, and are named according to their degree of bromination. Penta- and octa-BDEs have been banned in Europe and phased out of production in the United States, and deca-BDE will begin phasing out in 2012 (EPA, 2009). However, the general population continues to be exposed to all of these

compounds due to their persistence in the environment and continued release from older products.

PBDEs are established endocrine disruptors. PBDEs have been shown to alter reproductive and thyroid hormone homeostasis in animal studies, even at environmentally relevant levels to which humans may be exposed. Fernie et al. (2005) found inverse associations between exposure to BDE 47, 99 and 100, but not 153, and plasma thyroxine (T4) in captive American kestrels. Exposure to pentaBDE commercial mixtures decreased T4 levels in rodent studies, possibly by inducing hepatic enzymes that increase thyroid hormone clearance (Ellis-Hutchings et al., 2006; Fowles et al., 1994; Hallgren et al., 2001; Skarman et al., 2005; Stoker et al., 2004; Zhou et al., 2001, 2002). Zhou et al. (2001) also tested a decaBDE mixture and did not find the same effects on the measured outcome parameters. However, thyroid and liver tumors have resulted from chronic exposure to decaBDE (NTP, 1986). Although Zhou et al. (2002) found decreased T4 levels and increased liver weights and hepatic enzyme activity in dams and fetuses in response to gestational pentaBDE exposure, maternal weight gain, litter size and sex ratio, and offspring viability or growth were not significantly affected. Studies of the reproductive system have revealed adverse effects in both male and female rats after exposure to PBDEs. For instance, Talsness et al. (2006, 2008) found that gestational exposure to BDE 47 decreased circulating levels of estradiol and the number of ovarian follicles in offspring. Lilienthal et al. (2006) reported effects on the number of ovarian follicles resulting from exposure to BDE 99. A recent *in vitro* study suggested that PBDEs may disrupt ovulation by stimulating progesterone secretion by ovarian follicles (Karpeta et al., 2010). Talsness et al. (2005) found that a single dose gestational exposure to BDE 99 resulted in structural abnormalities of ovaries and greater fetal resorption rates among female offspring. Their results

were suggestive of a dose-dependent effect on resorption, but the differences in resorption rates between exposure groups were not statistically significant. On the other hand, Hardy et al. (2002) did not find differences in rates of pregnancy or uterine implantation in rats after 20 days of gestational exposure to a decaBDE mixture.

Studies of potential human health effects of PBDEs are limited. A few human studies have reported associations between PBDE exposure and altered levels of the thyroid hormones thyroxine (T4), triiodothyronine (T3) or thyroid stimulating hormone (TSH) (Bloom et al., 2008; Chevrier et al., 2010; Dallaire et al., 2009; Hagmar et al., 2001; Turyk et al., 2008; Wang et al., 2010; Yuan et al., 2008). Thyroid hormone homeostasis is important for regular ovulation, fertilization and maintaining pregnancy (Cramer et al., 2003; Krassas et al., 2010; Zoeller and Meeker, 2010). We recently reported that concentrations of BDE 47, 99 and 100 in house dust were associated with increased levels of serum T4 in 24 men (Meeker et al., 2009a). PBDEs in house dust were also inversely associated with luteinizing hormone and follicle stimulating hormone in these men. Human studies involving PBDE exposure and female reproductive function are even more limited, but two studies suggest PBDEs may adversely impact fertility. Chao et al. (2007) found evidence of shorter menstrual cycle length in relation to elevated levels of PBDEs in breast milk, although this association was not statistically significant in their small sample size. Harley et al. (2010) recently reported significantly reduced fecundability, in terms of time to pregnancy, associated with elevated serum levels of PBDEs in a group of Californian women.

Assisted reproduction technologies such as *in vitro* fertilization (IVF) provide the opportunity to study stages of reproduction that are otherwise not observable in the general population, such as oocyte quality, fertilization, embryo quality, and implantation. In addition,

biomarkers of environmental exposures, such as PBDEs, may be measured in follicular fluid that is usually collected during IVF and discarded. Previous measurements of contaminants in follicular fluid include PCBs, pesticides, cotinine and cadmium (Younglai et al. 2002). Follicular fluid surrounds the preovulatory oocyte and provides an important microenvironment in which the oocyte develops. Depending on the health endpoint of interest and biological mechanisms involved, the concentration of PBDEs in follicular fluid may be a more biologically relevant measure of exposure to the oocyte than serum. However, serum is the most common matrix in which to measure PBDE concentrations, and it remains unknown if serum PBDE concentrations serve as an adequate estimate of concentrations closer to the target tissue in the case of female reproduction.

In the present study, we measured PBDEs in archived serum and follicular fluid samples from 65 women who participated in a large study of predictors of *in vitro* fertilization (IVF) success, where we found that PCBs and cigarette smoking were associated with IVF implantation success rates (Meeker et al., 2007a, 2011). The objective was to first attempt to quantify PBDE concentrations in follicular fluid, which to our knowledge has not been done previously, then investigate the use of serum as a biomarker of exposure to PBDEs as compared to the potentially more biologically relevant biomarker of follicular fluid (in the case of IVF outcomes). Finally, we conducted an exploratory analysis to investigate the relationship between PBDE exposure and failed embryo implantation. Failed implantation was chosen as the outcome to explore because we had a limited sample size and it is the most common point of failure in IVF cycles.

2. Methods

2.1 Study population

The main study, within which the present sub-study took place, was conducted in two funding phases (1994 - 1998 and 1999 – 2003), and details have been previously described (Meeker et al., 2007a, 2007b). Briefly, women undergoing IVF or intracytoplasmic sperm injection (ICSI) were recruited through three clinics in the Boston area to participate in a study of IVF outcome predictors. Women requiring either donor oocytes or donor semen were excluded. Study protocols were approved by the Human Research Committees at Brigham and Women's Hospital, Harvard School of Public Health, and the University of Michigan. Approximately 65% of those approached agreed to participate in the study.

2.2 Serum and follicular fluid collection

Serum samples were collected within 36 hours of each IVF/ICSI cycle, during the follicular phase immediately prior to human chorionic gonadotropin (HCG) administration. The samples were separated by centrifugation for 5 minutes, aliquoted and stored at -80C until analysis. Oocyte retrieval was performed approximately 36 hours after HCG administration. The follicular fluid was obtained from the largest follicle visualized by ultrasound, and each sample consisted of fluid from only one follicle. After the oocytes were removed, the follicular fluid was centrifuged for 15 minutes, and the supernatant was placed into a clean storage tube, aliquoted and stored at -80C until analysis.

2.3 Measurement of PBDEs

Paired serum and follicular fluid samples from a total of 65 randomly selected women within the larger study were analyzed for PBDEs by the Organic Chemistry Analytical Laboratory, Harvard School of Public Health (Boston, MA). The samples underwent liquid-liquid extraction and silica-gel column chromatography clean up, and the

extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) operated in negative chemical ionization (NCI) mode. Final concentrations were reported after subtracting the concentrations of the analyte measured in the procedural blank associated with the analytic batch. Target analytes were PBDE congeners 28, 47, 99, 100, 153, 154, 183 and 209. Method detection limits (MDL) were determined as three times the standard deviation obtained from the measurement of eight aliquots of bovine serum fortified with target PBDE congeners (spiked at 0.05 ng/g of each analyte, except for BDE 209, which was spiked at 0.5 ng/g). The recoveries of all PBDE congeners ranged from 81% to 105%. The lab has been successfully participating in International Intel-calibration sponsored by AMAP (Arctic Monitoring and Assessment Program), organized by Quebec National Institute of Public Health, Canada (AMAP Ring Test for PCBs, Organochlorine Pesticides and PBDEs in Plasma). The relative percent difference (%RPD) between the laboratory results and the assigned values were <20% for all PBDE congeners, except PBDE 209, which was higher (36%).

Serum total cholesterol and triglycerides were measured enzymatically by the Clinical Laboratory at Children's Hospital (Boston, MA), and total lipids were calculated by Phillips formula (Phillips et al., 1989). It is important to note that the original larger study was designed for analysis of PCBs and IVF outcomes, and therefore there were no precautions taken to prevent PBDE contamination. As a result, there were background concentrations of PBDEs in procedural blanks associated with the samples.

2.4 Data analysis

Data analysis was conducted using SAS software version 9.1 (SAS Institute Inc., Cary, NC). In samples where PBDE concentrations were below the detection limit but a signal

was detected, the concentration estimated by the GC-MS was used in the data analysis. If no signal was detected for a given sample, then a zero concentration was assigned. Calculations involving serum are based on wet weights for consistency with the follicular fluid measurements, although models of failed implantation were also calculated using lipid-adjusted serum. Selected percentiles of PBDE congeners in serum and follicular fluid were tabulated. Kendall's Tau-beta correlation coefficients were calculated to assess the level of agreement between paired serum and follicular fluid PBDE concentrations, and between different PBDE congeners within the same matrix. The ratio of PBDE concentrations in follicular fluid to serum was calculated for each congener in all women who had detectable concentrations in both specimens. Logistic regression, adjusted for age and body mass index (BMI), was used to model the odds of failed embryo implantation associated with concentrations of PBDEs. Other variables that were tested in models for potential confounding include race, cigarette smoking and serum levels of PCBs. This analysis restricted the comparison group to live births, and excluded cases of biochemical pregnancies (positive human chorionic gonadotropin test that did not result in a clinical pregnancy), miscarriages, and ectopic pregnancies to provide a purer comparison and due to low numbers of these endpoints.

3. Results

Table 4.1 provides details of the study population, including IVF outcomes. The mean (SD) age among the women was 36 (3.8) years, and ranged from 27 to 44 years. The mean (SD) BMI was 24(4.4), and ranged from 17 to 43. Most women were Caucasian (84%) and never smoked (53%), and very few women were current smokers (6%). There were 35 (55%)

implantation failures and 18 (28%) live births, for a total sample size of 53 for the logistic regression models for failed implantation.

Table 4.2 presents the distributions and detection rates of PBDE congeners measured in serum and follicular fluid. Lipid-adjusted serum PBDE concentrations are also presented for comparison. In both serum and follicular fluid, BDE 47 had the highest detection rate and mean concentration. Due to relatively high background contamination levels of some PBDE congeners, detection limits are high (0.007-0.112 ng/g), particularly for BDE 209. Because all serum and follicular fluid samples contained levels of BDE 209 below the detection limit, BDE 209 concentrations were not included in statistical analyses. PBDE congeners 47, 99 and 100, main components of the pentaBDE commercial formulation, were correlated with each other within both matrices (Kendall's T_B =0.51-0.69, $p<0.0001$, data not shown). BDE 28, which is present in small amounts in the pentaBDE formulation, showed inconsistent rank correlations with the main pentaBDE congeners in serum (T_B =0.02-0.27, p=0.004-0.8), but were more strongly correlated with the main pentaBDE congeners in follicular fluid (T_B =0.48-0.57, $p<0.0001$, data not shown). BDE 153, which is present in both the pentaBDE and octaBDE formulations, was also moderately correlated with the main pentaBDE congeners in both matrices (T_B =0.26-0.35, $p<0.01$, data not shown). BDE 154, also present in both commercial formulations, was moderately correlated with BDE 100 in serum (T_B =0.21, p=0.02), but more strongly correlated with the main pentaBDE congeners in follicular fluid (T_B =0.35-0.52, $p<0.001$, data not shown). Table 4.3 presents the Kendall's Tau-beta correlations between PBDE concentrations in serum and follicular fluid. There were moderate rank correlations between serum and follicular fluid concentrations of BDE 28, 47, 100 and 154 (T_B =0.29-0.38, $p<0.005$), but BDE 99 and 153 were not correlated between the two matrices (T_B <0.2, $p>0.05$).

Relationships with BDE 183 and BDE 209 were not evaluated due to lower detection rates (serum or follicular fluid detection rate below 25%).

The distributions of the ratios of follicular fluid to serum PBDE concentrations are presented in Table 4.4. Concentrations of PBDEs in follicular fluid were usually lower than PBDE concentrations in serum, although there were several exceptions for all congeners. Additionally, there were notably two outliers where the follicular fluid PBDE concentrations were much higher than the serum concentration for all congeners. There was also a high level of inter-person variability in the ratios for most of the congeners.

Odds ratios calculated from logistic regression models of failed embryo implantation corresponding to all PBDE congeners in follicular fluid and serum are presented in Table 4.5. Women with detectable concentrations of BDE 153 in follicular fluid (39% of samples) had significantly elevated odds of failed implantation compared with women who had non-detectable BDE 153 concentrations in crude models and when adjusting for age and body mass index (adjusted OR = 10.0; 95%CI: 1.9 to 52; p=0.006). There was also evidence for a dose-response trend when women with detectable BDE 153 concentrations in follicular fluid were divided into equal groups (adjusted OR for non-detect, medium, and high BDE 153 groups = 1.0 [reference], 6.7, and 18.7, respectively; p-value for trend = 0.008). Age and BMI were included in the models because both variables may be associated with both PBDE exposure and fertility outcomes. Models used wet weight serum and follicular fluid concentrations of PBDEs, and similar results were found when using lipid-adjusted serum (not shown). The inclusion of ethnicity or smoking status (having ever been a smoker) did not change the model estimates by more than 10 percent and were not included in the final models. Because serum concentrations of polychlorinated biphenyls (PCBs) were found to affect implantation in the main study of IVF outcome predictors

(Meeker et al., 2011), we also tested models when additionally adjusting for PCB concentrations. The inclusion of PCB concentrations did not change the model estimates by more than 10 percent. Additionally, there was no correlation found between PCB and PBDE concentrations in serum or follicular fluid. Therefore, PCB concentrations were not included in the final models.

4. Discussion

To our knowledge this is the first report of PBDE measurement in human follicular fluid. We found increased odds (10-fold) of failed embryo implantation associated with elevated levels of BDE 153 in follicular fluid in a group of women undergoing IVF. Our ability to detect statistically significant associations between other PBDE congeners, in follicular fluid or serum, and failed implantation was limited by relatively high detection limits and a small sample size. Our finding of BDE 153 as the congener with the most influence over implantation may be consistent with the findings in the study by Harley et al. (2010), where BDE 153 was associated with the largest decrease in fecundability odds ratios as compared to BDE 47, 99 and 100. The Harley et al. study (2010) reported that all four of these PBDE congeners were associated with longer time to pregnancy, as assessed by serum PBDE concentrations and interviews of 223 pregnant women.

The PBDE congeners found at the highest concentrations in serum and follicular fluid were BDE 47, 99, 100, 153 and 154. Similarly, in a large US study of human serum, BDE 47, 99, 100 and 153, main components of the pentaBDE commercial mixture, were the congeners found at the highest concentrations (Sjodin et al., 2008b). Because only moderate correlations were observed between PBDE concentrations in serum and follicular fluid, serum concentrations may not be a good indicator of follicular fluid concentrations when studying fertility and early

pregnancy loss in women undergoing IVF. However, our ability to determine a relationship between serum and follicular fluid PBDEs may be hindered by our low detection rates of PBDEs.

The ratios of follicular fluid to serum PBDE concentrations were highly variable between participants. This finding suggests inter-individual differences in exposure or distribution among the women, and that some women may be potentially more susceptible to PBDEs reaching developing follicles. In a previous study (Meeker et al., 2009b) of PCBs in this same population, ratios of follicular fluid PCB concentrations to serum PCB concentrations were less variable (10^{th} percentile=0.2; 90^{th} percentile=0.4) than the PBDE ratios in the present study, which led the authors to conclude that serum measurements of PCBs were reliable measures of exposure to the oocyte. In the present study there were two individual women whose follicular fluid to serum PBDE ratios were high outliers because their follicular fluid PBDE concentrations were consistently higher than their serum concentrations for all the congeners measured. It is possible that these two follicular fluid samples were contaminated. However, relationships between serum and follicular fluid PBDE concentrations did not improve (correlation coefficients changed by less than 5% for most congeners and less than 10% for all congeners) when omitting these outliers. Additionally, the odds ratio of failed implantation for BDE 153 in follicular fluid slightly increased when omitting the two outliers.

The disruption of thyroid hormone homeostasis is a possible mechanism by which PBDEs may influence implantation and fertility. For example, experimental studies have shown that PBDEs may bind to thyroid hormone receptors (Marsh et al., 1998) and PBDE metabolites may displace T4 from binding to transthyretin, the thyroid transport protein (Meerts et al., 2000). Thyroid hormone disruption may play a role in altered follicle formation, as could disruption

along the hypothalamic-pituitary-ovarian axis (Talsness, 2008). For example, several PBDE congeners were found to activate estrogen receptors *in vitro*, and PBDE metabolites were especially potent (Meerts et al., 2001). This same study found that several PBDE congeners, including BDE 153, had antiestrogenic effects. Uterine levels of estrogen and progesterone receptor mRNA were also affected by PBDE exposure, although only BDE 99 was tested (Ceccatelli et al., 2006). Talsness et al. (2005) suggested that disrupted mitochondrial regulation by PBDEs may result in the uncontrolled synthesis of steroid products, subsequent vaginal and uterine abnormalities, and increased resorption rates they reported in rats exposed to PBDEs.

Past studies have assessed PBDE exposure to the developing fetus by measuring PBDE concentrations in human placental tissue (Doucet et al., 2009; Main et al., 2007; Miller et al., 2009). However, to our knowledge, this is the first study to report PBDE concentrations in human follicular fluid and to examine the effects of preconception PBDE exposures on early pregnancy. This study was conducted among a group of women undergoing IVF and may be part of a population more sensitive to factors affecting infertility. Thus, the generalizability of our findings remains unclear. However, PBDE exposures as measured in serum in this group of women are representative of the general population (Sjodin A et al., 2008b), and no evidence exists to date to suggest that these women are more susceptible to potential toxic effects of PBDE exposure than women conceiving naturally. Additionally, an advantage of studying IVF populations is the ability to examine sensitive endpoints that would be very difficult or impossible to study in the general population. In their study on PBDE exposure and reduced fecundability, Harley et al. (2010) point out that, because they studied women who successfully conceived a child naturally, infertile and subfertile women were underrepresented in their study, and PBDE exposure may have an even stronger effect on fertility than they reported.

Implantation failure, which is a common occurrence even among women conceiving naturally (Chard, 1991; Norwitz et al., 2001), occurs prior to the detection of pregnancy, and therefore was not measurable in the pregnant population of women studied by Harley et al. (2010). However, implantation failure may be related to the decreased fecundability found in this population. For this reason, we expect that the study of early pregnancy outcomes, such as the present study of this IVF population, will be useful in elucidating relationships between environmental exposures and fertility for all couples, including those attempting to conceive naturally.

5. Conclusion

We found that PBDEs are detectable in follicular fluid and that levels of BDE 153 in follicular fluid may be associated with failed embryo implantation. Our findings may help explain the previous report of a relationship between PBDE exposure and time to pregnancy, as failed implantation may manifest as reduced fertility and increased time to pregnancy among women attempting to conceive naturally. Further research with a larger sample size is needed to confirm the findings of this pilot study and to explore potential inter-individual differences in PBDE toxicokinetics and susceptibility. Research is also needed to determine the biological mechanisms involved in these relationships.

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Table 4.1. Characteristics of study population

Age, yrs. [median (min,max)]			38 (27,44)
Body mass index [median (min,max)]			23 (17,43)
		n	**(%)**
	Caucasian	54	84
	Other/unspecified	11	16
Smoking status			
	never smoked	34	53
	past smoker	30	47
	current smoker	4	6.3
IVF Outcomes			
Implantation failure		35	55
Live birth		18	28
Chemical pregnancy only		6	9.4
Miscarriage		3	4.7
Ectopic pregnancy		2	3.1

Table 4.2. Distribution of PBDEs (ng/g wet weight) in serum (n=63) and follicular fluid (n=64)

Serum	Detection Limit	Detection Rate	Mean	Selected Percentiles				Maximum
				25th	50th	75th	90th	
BDE28	0.007	43%	0.002	ND	ND	0.003	0.005	0.019
BDE47	0.044	90%	0.037	0.013	0.025	0.043	0.081	0.237
BDE100	0.015	83%	0.008	0.002	0.005	0.009	0.017	0.048
BDE99	0.042	70%	0.010	ND	0.006	0.014	0.029	0.072
BDE154	0.009	86%	0.011	0.005	0.011	0.015	0.023	0.037
BDE153	0.009	44%	0.013	ND	ND	0.007	0.012	0.342
BDE183	0.011	19%	0.002	ND	ND	ND	0.002	0.086
Total PBDEs			0.092	0.038	0.071	0.109	0.189	0.682
Follicular fluid								
BDE28	0.007	39%	0.001	ND	ND	0.001	0.002	0.017
BDE47	0.044	70%	0.026	ND	0.004	0.012	0.054	0.667
BDE100	0.015	38%	0.006	ND	ND	0.002	0.006	0.192
BDE99	0.042	47%	0.014	ND	ND	0.003	0.015	0.248
BDE154	0.009	44%	0.003	ND	ND	0.002	0.004	0.048
BDE153	0.009	39%	0.007	ND	ND	0.002	0.017	0.175
BDE183	0.011	3%	0.000	ND	ND	ND	ND	0.002
Total PBDEs			0.058	0.002	0.008	0.027	0.082	1.186
Lipid-adjusted Serum (ng/g lipid)								
BDE28			0.34	ND	ND	0.55	0.93	3.19
BDE47			7.14	2.27	5.22	9.73	14.53	39.3
BDE100			1.41	0.27	1.00	1.96	3.20	6.80
BDE99			2.10	ND	1.06	3.33	5.17	13.0
BDE154			2.08	0.84	2.21	2.89	4.00	7.11
BDE153			1.88	ND	ND	1.16	2.40	56.8
BDE183			0.37	ND	ND	ND	0.46	15.2
Total PBDEs			15.8	5.50	12.6	20.8	34.1	113

ND = Not detected

Table 4.3. Kendall's Tau-beta correlation coefficients for PBDE concentrations in serum and follicular fluid
(n=62)

	T_B	p-value
BDE 28	0.33	0.002
BDE 47	0.38	<0.0001
BDE 100	0.36	0.0003
BDE 99	0.11	0.26
BDE 154	0.29	0.003
BDE 153	0.15	0.16

Table 4.4. Distribution of follicular fluid:serum ratios

				Selected Percentiles				
	n[a]	Mean	Min	25th	50th	75th	90th	Max
BDE28	14	0.3	0.09	0.2	0.3	0.4	0.4	0.6
BDE47	40	0.8	0.03	0.2	0.3	0.4	0.9	16
BDE100	22	4.0	0.03	0.1	0.3	0.4	1.1	64
BDE99	21	31	0.03	0.2	0.3	0.6	1.6	620
BDE154	25	0.3	0.04	0.1	0.2	0.3	0.6	2.3
BDE153	10	0.6	0.05	0.2	0.2	0.2	0.7	4.6
Total PBDEs	49	0.8	0.01	0.1	0.2	0.3	0.6	18

[a]Ratios only include those for which both serum and follicular fluid had detectable levels of PBDEs.

Table 4.5. Odds ratios for implantation failure associated with elevated (above median, or, if <50% detected, detectable) levels in serum or follicular fluid (n=53)

	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
Serum		
BDE 28	1.2 (0.4, 3.8)	1.2 (0.4,3.9)
BDE 47	1.5 (0.5, 4.7)	1.6 (0.5,5.4)
BDE 100	1.5 (0.5, 4.7)	1.5 (0.5,4.9)
BDE 99	1.5 (0.5, 4.7)	1.7 (0.5,5.6)
BDE 154	2.8 (0.8, 9.4)	3.0 (0.8, 10)
BDE 153	1.0 (0.3, 3.4)	1.2 (0.3,4.4)
Total PBDEs	1.2 (0.4, 3.7)	1.2 (0.4,4.0)
Follicular Fluid		
BDE 28	1.2 (0.4, 3.8)	1.2 (0.4,4.0)
BDE 47	0.8 (0.3, 2.7)	0.9 (0.3,2.7)
BDE 100	0.7 (0.2, 2.3)	0.7 (0.2,2.5)
BDE 99	0.4 (0.1, 1.4)	0.4 (0.1,1.4)
BDE 154	1.9 (0.6, 6.2)	2.1 (0.6,7.4)
BDE 153	5.9 (1.5, 24)	10 (1.9, 51)
Total PBDEs	1.7 (0.5,5.2)	1.8 (0.5,6.0)

[a]Adjusted by age and BMI

CHAPTER V

Conclusion

Summary of the research

The present research investigated exposures and body burdens of BFRs commonly found in the indoor home environment. Serum, follicular fluid and house dust were examined as markers of exposure to BFRs for environmental epidemiology studies. Alterations in serum thyroid and reproductive hormones in men and early pregnancy loss (failed implantation) in women undergoing *in vitro* fertilization (IVF) were investigated in relation to BFR exposure.

The validity of using house dust as an exposure marker was examined in Chapter 2, where house dust concentrations of the major pentaBDE formulation congeners were found to be highly correlated to serum concentrations of the same congeners. This observation supports the argument that dust is a major exposure pathway for PBDEs, and for BDE 47 in particular, which typically represents the majority of the body burden measured in serum. Male and female serum levels of these congeners were also strongly correlated, indicating that adults living in the same household have similar exposures. Because concentrations of the longer half-life congener, BDE 153, were not correlated either between dust and serum or between males and females, it was concluded that there is some other factor (e.g. dietary exposure, individual metabolism or distribution differences) contributing to this discrepancy. This study is the first to provide empirical evidence of the correlation between house dust and serum concentrations of PBDEs. The

three previous studies, which were unable to demonstrate significant correlation, relied on data from European countries where dust concentrations are substantially lower than the US (Fromme et al. 2009; Karlsson et al. 2007; Roosens et al. 2009), indicating that diet is likely the main exposure pathway in Europe.

Chapter 3 utilized the findings of Chapter 2 to inform the methods of PBDE congener groupings for the statistical modeling of PBDE exposure effects on hormone levels in men. Strong correlations between congeners within dust and between certain congeners in dust and serum provided logical evidence for grouping congeners according to the most prevalent congeners in commercial mixtures. The individual congeners were also modeled for comparison, as well as models using data generated from a factor analysis of all congeners detected in dust. Using the factor analysis to generate independent variables representing different weightings of PBDE congeners, effects on each hormone were modeled with all of the factor variables in the same model without the problem of collinearity. This method of data analysis represents a novel look at PBDE congener groupings and how they relate to human health effects. Significant positive associations were found between house dust concentrations of pentaBDEs and serum levels of T4, T3, estradiol, SHBG and prolactin, along with an inverse association with FSH. Positive associations between octaBDE concentrations and serum T4, TSH, LH and testosterone and an inverse association between decaBDE concentrations and testosterone were also found. Additionally, several significant relationships between dust concentrations of alternate BFRs that are replacing PBDEs and hormone levels were found. Despite the limitations in comparing these findings to those of animal or other epidemiological studies that had differences in study design such as specific exposure

(PBDE congener), exposure levels, or exposure timing (e.g. prenatal versus adult), several findings were consistent with the current literature, as discussed in Chapter 3.

Finally, Chapter 4 explored the relationship between serum and ovarian follicular fluid concentrations of PBDEs, a novel human measurement. Because no strong relationship was found between serum and follicular fluid concentrations of PBDEs, it was concluded that serum PBDE concentrations may not be a good estimate of follicular fluid concentrations. The association between PBDE concentrations in each matrix and failed embryo implantation was investigated. Women with detectable levels of BDE 153 in follicular fluid were more likely than those with undetectable levels to have failed embryo implantation following IVF.

Research limitations and further research needs

The present work may be limited in its ability to be generalized beyond the subjects seeking reproductive assistance from an infertility clinic. However, the strength of examining couples attending infertility clinics is the ability to measure fertility markers and early pregnancy outcomes that would not be observable in the general population. The use of pregnancy outcome data from women undergoing IVF was critical in ascertaining sensitive health endpoints such as implantation failure that cannot be assessed in other human populations (i.e. in couples conceiving naturally). This population is also representative of the general US population in terms of BFR exposure. Additionally, there is no reason to suspect that these subjects are more or less susceptible to BFR exposure. Even if this population was somehow more susceptible, the study

would still be of high significance because sensitive populations are often the primary focus of exposure reduction and governmental regulation efforts.

Another limitation is the relatively small number of subjects in each study. However, significant associations between BFR concentrations in house dust and serum, and between BFR exposure and the outcome measures of hormone level alteration and embryo implantation failure were found. Nevertheless, further research with larger sample sizes is warranted to replicate these findings. Another limitation is the large number of exposures, in terms of number of PBDE congeners, which are examined in relation to the large number of hormonal outcomes. As with any small study on multiple relationships, it is possible that some of the associations found are due to chance. Furthermore, endocrine disruption is a complex issue, and more research into the health implications of hormone level alteration is necessary to assess the risks of exposure to endocrine disrupting chemicals such as BFRs. Although there is potential for larger scale investigations on BFR exposure and endocrine disruption, data from the National Health and Nutrition Examination Survey (NHANES) has been limited in the number of hormones that are measured. The year that NHANES began providing serum data on PBDEs (2003) was the same year hormone measurements such as LH and FSH were ceased. Thyroid hormone measurements were also ceased, but have recently been added back into the laboratory profile.

The environmental measures of the present research were limited to dust BFR concentrations. Without other environmental monitoring, such as air sampling, the exposure scenario is incomplete. However, dust is expected to be one of the most, if not the most, important human exposure pathways for BFRs. (Johnson-Restrepo and Kannan

2009; Lorber 2008; Wu et al. 2007). Another potential limitation of the dust measure is that it provides only a cross-sectional view of exposure. Multiple measurements over time for each of the matrices under study would be ideal for a more complete exposure assessment. However, using vacuum bag dust is expected to be a long-term integrative measure of exposure representative of the total or near-total home environment. Thus, for the less persistent PBDE congeners, such as BDE 209 (as well as for alternate BFRs for which biomarkers do not yet exist), dust may in fact be a superior measure of long-term exposure potential in epidemiological studies.

Along with additional studies with larger samples sizes, further research on the transformation of PBDE congeners is needed to better understand the congener patterns in biomarker profiles. Additional studies on dust and biomarker correlations for alternate BFRs are also needed, as well as more research on developing reliable biomarkers of exposure to these compounds with more cost-effective quantification methods. Utilizing techniques that are minimally invasive is one reason to rely on dust as an exposure marker when conducting research, but the cost of analytical procedures is another reason that may prohibit using biomarkers.

There exists some debate in the current literature over which method of dust collection for PBDE measurement is superior for use in exposure assessment and epidemiological studies. There are several different methods of researcher-collected dust collection that occurs at a specific home visit, and vacuum bag dust collection like that which was used in the present research is a participant-collected method. Existing comparisons of these methods did not include biomarkers (Allen et al. 2008), but future

research on dust collection methods of PBDEs should use biomarkers to assess the biological relevance of these measurements.

Because the research presented in Chapter 4 was limited by low detection rates for PBDEs among the samples, it is worth revisiting the hypothesis that serum and follicular fluid concentrations of PBDEs may have a stronger correlation than was observed. A future study designed specifically for PBDE analysis with enhanced sensitivity would potentially allow for a more detailed examination of the effects of various exposure levels and not be limited to a dichotomous view of exposure.

Impact/ Innovation

Because the number of studies on human BFR exposure and health effects is very limited, due primarily to being an emerging research topic, the present research is novel in its scope (number of BFRs investigated, including PBDEs and alternative BFRs) and approach (human health endpoints). This research is the first to study human exposure to several of these compounds. The present work impacts the field of environmental health by improving our understanding of the exposure routes and health risks associated with BFRs and by providing guidance on the use of environmental and biological markers of exposure to environmental epidemiologists. Additionally, because the risks associated with certain BFRs are widely under debate, with legislation under ongoing review, this work will also provide scientific basis for future decisions on the use and limitations of BFRs.

Moving forward

Although certain PBDEs have been banned or discontinued, human exposure may continue for years to come due to their persistence in the environment and the continued use of older products containing PBDEs. Additionally, recycling of materials containing PBDEs may result in continued exposure via new products. For example, polyurethane foam containing PBDEs can be recycled into new carpet padding (Shaw et al. 2010). Electronic waste exported to developing countries has resulted in high body burdens of recycling workers and environmental contamination by BFRs (Wong et al. 2007). We potentially face similar problems from the compounds that are replacing PBDEs in flame resistant products. The overall production of halogenated flame retardants is rapidly increasing in response to increased fire safety legislation (Brown and Cordner 2011; Shaw et al. 2010). However, the public and scientific communities are beginning to question the increased use of flame retardants. A recent report on the identification of various flame retardants in baby products aroused additional public interest (Cressey 2011; Stapleton et al. 2011). Some have pointed out that there are no data to support that flame retardants actually have benefits in some common applications such as furniture and televisions, and suggest that we can reduce the use of toxic or untested flame retardants without compromising fire safety (Shaw et al. 2010). Research by the Consumer Product Safety Commission demonstrated that the addition of flame retardant chemicals to furniture foam did not ensure a reduction in the risk of ignition from small flame sources such as cigarettes, for which the furniture standard TB117 is intended (Medford and Ray 1997). The most effective fire safety strategy has been the reduction of smoking rates (Shaw et al. 2010). Furthermore, the use of fire-safe cigarettes, fire-safe candles, child-resistant lighters, smoke detectors and water sprinkler systems can help

prevent fires regardless of the chemical make-up of indoor furnishings. Additionally, alternative designs that use less flammable materials or green chemistry alternate flame retardants can reduce reliance on halogenated compounds with adverse health and environmental impacts.

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