Fall 2008

Biomonitoring polybrominated diphenyl ethers in lactating women

Rebecca L. Dunn
University of New Hampshire, Durham

BIOMONITORING POLYBROMINATED DIPHENYL ETHERS IN LACTATING WOMEN

BY

REBECCA L. DUNN
B.S. Keene State College, 1993
M.A. Immaculata College, 2002

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Animal & Nutritional Sciences

September, 2008

TABLE OF CONTENTS

DEDICATION

To my husband, Bill Dunn, who provided constant and loving support every step of the way. Thank you. I could not have reached this goal without you.

ACKNOWLEDGEMENTS

The important lessons that I learned throughout my dissertation were that the process required collaboration, posing questions, seeking guidance, and asking for help – lessons that will last a lifetime. I am forever grateful to the numerous people who assisted me over these past 5 ½ years. It has been a tremendous learning experience, and I am appreciative for the opportunity.

I am thankful for the extraordinary mentorship from my advisor, Dr. Gale Carey. I am grateful for the knowledge and experience I have gained by being a member of the Carey laboratory. I am truly appreciative of the countless hours Dr. Carey gave me with her thoughtful review, insightful comments, and meaningful discussions. It has been a pleasure. Thank you.

I am indebted to the members of my dissertation committee: Dr. Bill Condon, Dr. Tony Tagliaferro, Dr. Arnie Schecter, and Dr. Tom Webster. They carefully reviewed my work over the last several years, and provided valuable feedback and guidance along the way.

I would also like to recognize members of my guidance committee: Dr. Eleanor Abrams, Dr. Colette Janson-Sand, Dr. Lee Seidel, and Dr. Tony Tagliaferro. Their insight and suggestions during the early planning phases of my research were very helpful; and they also made my advancement to candidacy a pleasant and positive experience.

I owe Dr. Janice Huwe and her technical support staff, Kristin McDonald, Jean Picard, and Margaret Lorentzsen, an enormous thank you for all of their hard work and expertise with the analytical method.

I was very lucky to have the statistical guidance from Sarah Phillips; she patiently walked me through the data analysis process. Thank you, Sarah, for promptly answering my many, many emails and questions, and giving up too many Sunday mornings to meet with me.

The faculty in the Department of Animal & Nutritional Sciences have had an incredible influence on my academic and professional career. I've encountered many meaningful academic experiences that encouraged me to challenge myself, my expectations, and my knowledge. Special thanks to Dr. Sam Smith and Dr. Paul Tsang for incredible learning experiences in their classrooms; their philosophy on teaching has shaped my pedagogy at Keene State. There are numerous graduate students who've come and gone during my time; each one has made my experience memorable – thank you. I extend my sincere appreciation to everyone in the Department of Animal & Nutritional Sciences who has been a part of my journey.

I owe sincere thanks to Lisa Merrill and Andrea Hoppe for opening up their homes during my "travel years"; this made many days bearable. I also thank them for their friendship, conversation, and overall support.

Shanna Miller, Dr. Kathy Kendall-Tackett, Aviva Meyer, the New Hampshire Breastfeeding Task Force, and focus group participants provided invaluable support, guidance and feedback on the development of the communication pamphlet. Their assistance during the development phase made the pamphlet possible. Thank you.

The Seacoast area lactation consultants provided superb support for my research. Without their efforts and expertise, participant recruitment would have been very challenging. Many thanks to Paula Oliveira, Sue Madden, Jean Loiselle, Jolene Short-Porter, and Heather Hewitt.

Without the support and interest of the participants, my research would not have been possible. It was a great pleasure to work with each participant; I applaud their dedication to breastfeeding, and their contributions to science.

My colleagues at Keene State College have been an incredible support system throughout this entire process. Most notably Dr. Margaret Smith and Dr. Becky Brown offered support, comfort, and laughter. Thank you! I also appreciate the many colleagues who "checked-in" on the status of my work every so often.

My family and friends have been very patient and supportive throughout this entire process – especially during the last six months when I was absent from many, many social gatherings. I appreciate their understanding and encouragement over the years. Many thanks to my sister, Lauren Lachut, who has been my #1 fan.

My husband, Bill Dunn, has been my "rock". He's held down the homestead, kept me focused and nourished, and provided love and support from start to finish. He deserves a medal of honor. Words cannot express how lucky I am to have such a wonderful husband – thank you, Bill.

Support for my research was funded through the following:

New Hampshire Agricultural Experiment Station Grant H-485
University of New Hampshire President's Fund for Excellence
American Dietetic Association Foundation Scholarship

LIST OF TABLES

LIST OF FIGURES

CHAPTER PAGE

CHAPTER II

CHAPTER III

CHAPTER IV

ABSTRACT

BIOMONITORING POLYBROMINATED DIPHENYL ETHERS IN LACTATING WOMEN

by

Rebecca L. Dunn

University of New Hampshire, September 2008

Breast milk is a valuable biological specimen for biomonitoring lipid-soluble polybrominated diphenyl ethers (PBDEs). The goal of this project was to determine the levels of PBDEs in breast milk of lactating women from the Seacoast region of New Hampshire and to examine potential relationships between PBDE levels in breast milk and stage of lactation, maternal characteristics, living environment and dietary intake. Forty women, ages 22 to 40, provided up to three breast milk samples at the end of their first, second and third month of breastfeeding for evaluation of day-to-day and month-to-month variation in PBDE levels. Participants were asked to complete four questionnaires, which provided maternal, living environment, and diet information. The sum of PBDE (∑PBDE) congeners found in breast milk was defined as: BDE-28/33, 47, 85, 99, 100, 153, 154, and 183. The ∑PBDE concentrations in breast milk over the three-month collection period ranged from 6.5 to 166.7 ng/g lipid. The median for the three-month period was 29.7 ng/g. BDE-47 was the predominant congener; however, BDE-153 predominated in 20% of the participants' samples from each month. Day-to-day variation in ∑PBDEs was negligible; there was no significant difference in mean PBDE levels from month-to-month. Regression analyses revealed relationships between log-

transformed PBDE levels in breast milk and questionnaire data. Positive associations were seen between BDE-153 and age ($r = 0.36$ $p = 0.02$), saturated fat consumption ($r = 0.31$ $p = 0.05$), and the home model ($r = 0.51$ $p = 0.004$). There was a negative association between PBDE levels ($\sum$ and BDE-47) and fruit consumption ($r = 0.36$ $p = 0.02$, $\sum$PBDE). Our results indicate that PBDE levels in breast milk from New Hampshire are within the range that has been reported in the U.S., and that levels are stable during the first three-months of lactation. Our findings revealed a higher predominance pattern with BDE-153 compared to other studies, and indicate that PBDE levels are influenced by age, diet, and the home environment.

CHAPTER I

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are synthetic flame retardants that are added to consumer products, such as fabrics, foams, and electronics. Over time, PBDEs can diffuse out of their products, become airborne or enter dust, and disperse into the environment and into humans. Due to their persistent, lipid-soluble characteristics and widespread use in consumer products, PBDEs are bioaccumulating and have been detected in air, dust, mammals, and humans – including human breast milk.

Breast milk is a valuable biological specimen for biomonitoring lipid-soluble PBDEs. The purpose of biomonitoring is to document, update, and expand human exposure information on environmental chemicals (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention [U.S. DHHS, CDC], 2005); breast milk biomonitoring studies can estimate PBDE exposure in both the nursing mother and breastfeeding infant, but can also provide information about sources of PBDE exposure.

In the U.S., a limited number of studies have reported PBDE levels in breast milk, with no documentation to date from the state of New Hampshire. One study in the U.S. has reported rates of PBDE elimination over time in a small sample of nursing mothers from California (Hooper et al., 2007), and one has demonstrated relationships between PBDE levels in breast milk and house dust,

dairy intake, and meat intake (Wu et al., 2007). Given the limited number of studies in the U.S., there is a need to further document PBDE levels in humans, examine PBDE levels in breast milk over time, and further evaluate potential sources of PBDE exposure, all of which contribute to the purpose of biomonitoring.

The goals of *Biomonitoring PBDEs in Lactating Women* were to contribute to the current body of knowledge about PBDE levels in breast milk of lactating women and to examine potential relationships between PBDE levels in breast milk and maternal characteristics, living/occupational environment and dietary intake. This research will provide new knowledge about PBDE levels in the state of New Hampshire. Specific objectives of the research were:

1.) To develop an informative, yet reassuring tool to educate potential subjects about the research being done, body burden, PBDEs, and the importance of breastfeeding.
2.) To determine the levels of PBDEs in breast milk of lactating women from the Seacoast region of New Hampshire.
3.) To evaluate the relationship between PBDE levels in breast milk and stage of lactation.
4.) To evaluate the relationship between PBDE levels in breast milk and maternal characteristics.
5.) To evaluate the relationship between PBDE levels in breast milk and dietary intake.
6.) To evaluate the relationship between PBDE levels in breast milk and maternal environment.

CHAPTER II

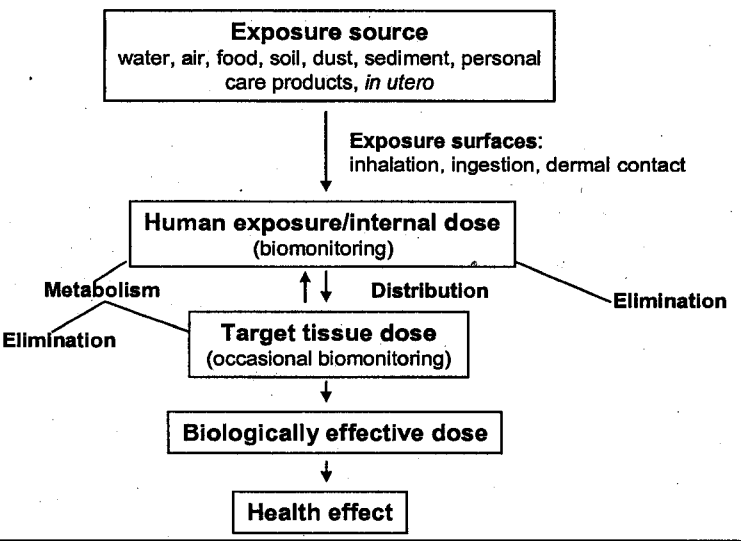
REVIEW OF THE LITERATURE

biomonitoring

Biological monitoring, or biomonitoring is the measurement of environmental chemicals, their metabolites, or their reaction products in the human body, specifically through blood, urine, saliva, breast milk, adipose or other tissue (Needham et al., 2007; U.S. DHHS, CDC, 2005). The CDC (2005) defines an environmental chemical as "a chemical compound or chemical element that is present in air, water, food, soil, dust or other environmental media (e.g., consumer products)" (p.1). The steady increase in the production and use of chemicals in the U.S. since World War II, particularly industrial and agricultural chemicals, has encouraged biomonitoring initiatives worldwide.

The purpose of biomonitoring is to document, update and expand human exposure information on environmental chemicals. Specific uses of biomonitoring data have been identified by the CDC, which are to: a.) determine which chemicals are in the U.S. population and at what levels; b.) determine the prevalence of people with levels of chemicals that are above known toxicity levels; c.) establish reference ranges of chemicals that allows for identification of

a person or group with high exposure; d.) determine if human exposure levels are higher among some groups as compared to others; e.) follow time trends in levels of human exposure; and f.) set research priorities on human health effects (U.S. DHHS, CDC, 2005). Assessing human exposure to environmental chemicals is an important aspect of the exposure-effect continuum (Figure 1) (Needham et al., 2007; Sexton et al., 2004).



Note. Adapted from "Strategic Biomonitoring Initiatives: Moving the Science Forward," by J. Angerer, M.G. Bird, T. Burke, N.G. Doerrer, L.L. Needham, S.H. Robison, L. Sheldon, and H. Zenick, 2006, *Toxicological Sciences, 93*, p. 5. Copyright 2006 by Oxford University Press.

Figure 1. Exposure to health effect continuum.

With more than 80,000 chemicals in use, numerous government-sponsored programs have been initiated in the U.S. to meet public and private demands for biomonitoring including: the National Health and Nutrition Examination Survey, the National Environmental Tracking Program, the National Children's Study, the Agricultural Health Study, the Farm Family Exposure Study, the National Human Exposure Assessment Survey, and the Center for Children's Environmental Health and Disease Prevention Research (Albertini et al., 2006). Biomonitoring has many uses in the area of environmental public health; it seeks to expand the current state of knowledge on assessing human exposure to chemicals (internal dose), exposure sources and pathways, and potential health effects (Figure 1) (Needham et al., 2007).

Assessing Human Exposure

The CDC's National Biomonitoring Program is continuously assessing the U.S. population's exposure to environmental chemicals through the collection of biological samples (blood and urine). The CDC began biomonitoring in 1976 through the second National Health and Nutrition Examination Survey (NHANES II) by measuring blood lead levels in a representative sample of the U.S. population (Sexton et al., 2004). Biomonitoring continued with NHANES III, but data collection was sporadic until 1999 when NHANES became a continuous survey gathering data on a new national sample every two years.

In March 2001, the CDC's Environmental Health Laboratory released the first *National Report on Human Exposure to Environmental Chemicals,* which provided biomonitoring data on 27 chemicals (U.S. DHHS, CDC, 2001). The

report provided exposure levels on 27 environmental chemicals for the U.S. population. Since this time, two additional reports have followed with the most recent released in July 2005, which provided exposure data in a sampling of 2,400 people for 148 environmental chemicals (U.S. DHHS, CDC, 2005).

The groups of environmental chemicals in the third report included the following: metals; tobacco smoke; polycyclic aromatic hydrocarbons; polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and coplanar and mono-ortho-substituted polychlorinated biphenyls; non-dioxin-like polychlorinated biphenyls; phthalates; phytoestrogens; organochlorine pesticides; organophosphate pesticides; herbicides; pyrethroid pesticides; other pesticides; and carbamate insecticides (U.S. DHHS, CDC, 2005). The chemicals for biomonitoring in the first three reports were selected based on scientific data. In upcoming reports, however, chemicals will have been nominated by the public, and prioritized and selected by a panel of experts from the CDC. Recommendations have been set forth by the National Research Council (2006) to include other agencies for chemical selection, such as the Environmental Protection Agency, the National Toxicology Program, the Food and Drug Administration, the U.S. Department of Agriculture and the National Institute of Environmental Health Sciences. With an interagency approach to selecting priority chemicals, this will enhance the overall purpose of biomonitoring.

Assessing Exposure Sources and Surfaces

Needham and colleagues (2005) described exposure as the contact between a source and a target where contact occurs at an exposure surface

during an exposure period. As depicted in Figure 1, an exposure source is water, air, food, soil, dust, sediment, personal care products, and *in utero* exposure. Humans (targets) encounter exposure sources depending on various events in their lifetime (exposure period) which allows for uptake through exposure surfaces (e.g. nasal passages, mouth and skin).

Biomonitoring data can assist in examining overall exposure sources and surfaces; it works in the reverse direction on the exposure-effect continuum by going from human exposure to exposure source (Albertini et al., 2006; Needham et al., 2007). Biomonitoring data that is coupled with other assessment methods, such as questionnaires and personal monitoring can provide information that potentially links internal dose to exposure source.

The collection of serial analytical measurements of exposure sources are necessary for identifying concentrations, variability, and trends of environmental chemicals, which can assist in developing health indices (Bahadori et al., 2007; Needham et al., 2007). For example, the U.S. Environmental Protection Agency's (U.S. EPA) reference dose can provide an estimate of a daily exposure level that is not expected to cause adverse health effects over one's lifetime (Needham et al., 2007). The relationship between one's exposure, the expected internal dose concentration, and the potential health implications are of particular interest to environmental public health scientists.

Assessing Potential Health Effects through Biomonitoring

In the *Third National Report on Human Exposure to Environmental Chemicals*, the CDC (2005) states, "the measurement of an environmental

chemical in a person's blood or urine does not by itself mean that the chemical causes disease" (p. 4). One of the greatest challenges with biomonitoring is evaluating the potential health implications of the data.

The ability to interpret the results of biomonitoring data in the context of a health risk assessment lags behind current analytical methodology (Hays et al., 2007). Human health risk assessment of environmental chemicals can use an interpretative method that compares estimated daily doses to health-based criteria for acceptable, safe, or tolerable intakes. These comparisons can assess whether estimated doses exceed health screening levels. However, biomonitoring data measures environmental chemical concentrations in biological specimens (e.g. blood, urine) rather than estimated intake doses.

Quantitative criteria are needed for interpretation of environmental chemical concentrations in biological specimens and their relevance to human health. In order for these criteria to be developed, advances are necessary in the following areas: toxicologic, epidemiologic and toxicokinetic data, health-based values such as reference doses, health-risk data, and exposure assessment data (National Research Council, 2006). The National Research Council (2006) describes two primary options for interpreting biomonitoring data, which are the descriptive approach and the risk-based approach.

The descriptive approach offers a statistical presentation of the data (National Research Council, 2006). For example, in the *Third National Report on Human Exposure to Environmental Chemicals* (2005), descriptive statistics, such as geometric mean and percentiles, are used to present the data. The use of

percentiles allows for the data to be distributed in reference ranges. Reference ranges offer a point of comparison for individuals and subgroups, and also allows health care professionals to determine high levels of exposure (National Research Council, 2006; U.S. DHHS, CDC, 2005). The descriptive approach is an important step in the interpretation of biomonitoring data; it begins to lay the groundwork for risk-based approaches (National Research Council, 2006).

The risk-based approach is complex, as it uses toxicologic, epidemiologic, or pharmacokinetic modeling information (National Research Council, 2006). These methods evaluate the risk that is associated with the amount of environmental chemical present in the body.

There are several interpretative risk-based options: *biomonitoring-based risk assessment*, *traditional risk assessment*, and *biomonitoring-led risk assessment* (National Research Council, 2006). *Biomonitoring-based risk assessment* uses biomarker-response relationships that have been established in epidemiologic studies; however, there are only a few environmental chemicals that have enough data to use this approach to interpret risk. *Traditional risk assessment* evaluates exposure pathways to estimate the human dose; the dose is then used to calculate health risk as compared to health-based criteria for acceptable, safe, and tolerable intakes. The third option, *biomonitoring-led risk assessment*, uses quantitative data on the absorption, distribution, metabolism, and excretion (pharmacokinetics) of an environmental chemical. With this option, pharmacokinetic modeling techniques are used to convert biomonitoring data into a format, such as a human-exposure dose format, that provides exposure

information in risk assessments. *Biomonitoring-led risk assessment* relies upon an expansive biomonitoring and toxicology database for the environmental chemical of interest; therefore, its uses could be limited depending upon the availability of data for an environmental chemical.

Typically, interpretation of biomonitoring data ends with the observation that the chemical is present in humans (descriptive approach), which becomes the message that is communicated to the public, and can often induce anxiety. The challenge for environmental health scientists is to interpret the health implications of biomonitoring data and appropriately communicate the results to the public (National Research Council, 2006).

Communicating Results of Biomonitoring Studies

Biomonitoring data presents the results of environmental chemical levels in humans; this makes human exposure data personal (National Research Council, 2006). Brody and colleagues (2007) address the ethical challenges that are associated with reporting personal exposure information for environmental chemicals. Deciding whether or not to report individual levels of environmental chemicals requires researchers to consider several principles for protecting human participants: autonomy; beneficence; nonmalfeasance; and justice.

The principles of autonomy and justice support the practice of providing participants with their individual results (Brody et al., 2007). Autonomy is an individual's right to know in order to make decisions based upon the research results while justice emphasizes the provision of information that highlights the benefits and harms as it applies to different groups of people. The principles of

autonomy and justice are typically seen in community-based participatory research and activist exposure studies where participants receive their individual results.

The clinical medicine model emphasizes the principles of beneficence and nonmalfeasance in which participants may not be provided with their individual results (Brody et al., 2007). These principles support the practice of maximizing benefits and minimizing potential harm, particularly when the meaning of results is unclear; and can lead to situations that cause fear, anxiety, and unnecessary interventions.

The challenge with each of these principles is balancing the benefits and harms that are associated with reporting individual exposure levels; this balance should be based on the available research for the environmental chemical. However, regardless of whether individual or group levels are reported, it is important to ensure that communication of results includes proper interpretation and use of biomonitoring data.

The National Research Council (2006) emphasizes early planning as an important component in communicating biomonitoring information. Early planning enables researchers to assess the needs of the study population and to establish partnerships with other constituencies in formulating public health messages. Early planning also encourages researchers to consider both practical and research-based communication recommendations.

Practical recommendations focus on educational aspects, whereas, research-based recommendations promote the advancement of biomonitoring

communication (National Research Council, 2006). Practical biomonitoring communication recommendations highlight the following areas: communication sponsorship, use of consistent terminology and concepts, expansion of biomonitoring education to the public, communication training for organizations and professionals, and public documentation of ways to reduce exposures. Research-based recommendations emphasize the following areas: identification of scientist and non-scientist beliefs about exposures and health effects, assessment of current biomonitoring communication methods and its impact on the public, identification of the public's perception regarding uncertainties in biomonitoring, and identification of the public's beliefs about risk reduction. In addition to these recommendations, Bates and colleagues (2005) further suggest that researchers communicate accessible results of biomonitoring research. For example, researchers should distinguish between statistically significant effects and clinically significant effects. These recommendations are a crucial component in creating and building an infrastructure for communicating results of biomonitoring studies.

If communication is lacking between biomonitoring researchers and the general public, then the results of biomonitoring studies are subject to misinterpretation and misuse, which can lead to public health messages that induce confusion, conflict and anxiety. For example, occasionally, the media will become aware of biomonitoring research, and will provide a condensed, simplified version of the results (Bates et al., 2005). Often, these types of messages present a communication risk, particularly if the biological specimen is

sensationalized with a degree of harm, which can happen when environmental chemicals are detected in breast milk.

breast milk biomonitoring

The use of breast milk as a biological matrix for biomonitoring studies can estimate environmental chemical exposure in both the nursing mother and breastfeeding infant. Determining infant exposure can be done by measuring the concentration of the environmental chemical in breast milk and estimating infant intake of milk (Lovelady et al., 2002).

Breast milk can be a convenient and noninvasive means for estimating environmental chemical exposures as compared to other biological matrices, which may require surgical intervention. (Hooper & She, 2003). However, it is important that sampling does not burden the mother or compromise the infant's nutrition. There are guidelines that should be considered when determining the study design of breast milk biomonitoring programs.

Study Design Considerations

Participant Characteristics. There are a number of influential participant characteristics that can affect environmental chemical concentrations in breast milk samples. Fenton et al. (2005), Lovelady et al. (2002), and the World Health Organization (WHO) (2004; 2007) have described characteristics that should be taken into account when measuring environmental chemicals in breast milk. Fenton and colleagues (2005) list the following characteristics that should be considered: maternal information such as age and body mass index, lifestyle history such as dietary intake, smoking, and medication use, obstetric history,

occupational history, and prior breastfeeding history. The WHO (2004; 2007) provides specific recommendations for participant characteristics in breast milk biomonitoring studies including: first-time mothers, healthy mother and infant, mothers under the age of 30, and primarily or exclusively breastfeeding. The most recent WHO (2007) guidelines recommend that mothers should have resided in the same geographical location for at least the past 10 years, although the residency time frame could pose a challenge for recruiting young mothers who are under the age of 30 into breast milk biomonitoring studies. In addition to considering maternal characteristics for breast milk biomonitoring studies, it is recommended that researchers implement a milk collection protocol.

Breast Milk Expression and Collection. Lovelady and colleagues (2002) recommend collecting and analyzing individual breast milk samples from women rather than pooling samples. Pooling samples is less expensive than analysis of individual samples, but analyzing individual milk samples enables researchers to examine potential associations between participant characteristics and environmental chemical levels. It is also important for researchers to consider individual expression and collection variables that can affect milk composition.

Study design recommendations have suggested hand-expressing samples for breast milk biomonitoring research in order to limit sample contamination from breast pumps. However, recent research by Hooper and colleagues (2007) found that breast pumps can be used for expression of samples as they maintain accuracy of environmental chemical levels when compared to hand-expressed samples. Use of a breast pump is recommended if

researchers wish to receive samples that contain hind milk, which have a higher fat content than foremilk (Lovelady et al., 2002). Obtaining a sample that contains hind milk is useful when analyzing lipid-soluble environmental chemicals and usually requires a complete milk expression from one breast. This method of milk collection enables researchers to estimate exposure of lipid-soluble environmental chemicals in individual infants; however, it is not a typical practice when examining concentrations in the mother because levels are presented on a lipid-adjusted basis. Participant compliance and retention are greater when breast pumps are provided for milk collection, and are especially helpful for women who find manual pumping difficult.

Participant retention can be dependent on timing of sample collection. Lovelady et al. (2002) and the WHO (2007) suggest waiting until week two or three to begin sampling to ensure that lactation has been well established. It is also recommended to collect samples within the first three- months postpartum as early weaning and lower exclusive breastfeeding rates can often be a recruitment and retention factor in breast milk biomonitoring studies, particularly in the U.S. For example, data collected in the U.S. from the National Immunization Survey in 2004, reported that 30.5% of infants were exclusively breastfed at three months, which then decreased to 11.3% at six months of age (U.S. DHHS, CDC, 2007). Therefore, collecting breast milk samples in the first few months postpartum could lead to higher participant retention rates.

Lovelady et al. (2002) suggests collecting samples at different time intervals, such as at month one, two and three, to evaluate for changes in

environmental chemical levels over time. Additionally, the time of day and time elapsed between feedings should be consistent among participants as these variables have been found to contribute to daily variations in the fat content of milk samples, which is an important consideration when assessing exposure of lipid-soluble environmental chemicals in individual infants. Ruel and colleagues (1997) found that the average concentration of fat in a 24-hour pooled sample was 4.2%; the time of day that was in agreement with this percentage was between the hours of 6 a.m. and 8 a.m. Since breast milk composition varies from feed to feed, day to day, and month to month, it is recommended to standardize the sample collection period.

Prior to collecting milk samples, participants should be provided with guidelines for washing the breast and equipped with proper storage containers for their milk samples. Lovelady and colleagues (2002) recommend that mothers wash the breast with a mild, detergent-free soap, and rinse the breast with distilled water to limit potential contamination from creams and ointments. Additionally, it is recommended that participants be provided with suitable storage containers. The U.S. EPA (2003) and the WHO (2007) suggest amber glass bottles that have Teflon fitted lids and have been detergent-water washed, then solvent-rinsed prior to use. The recommendations for containers are necessary as some environmental chemicals are sensitive to ultraviolet light, and if lipid-soluble, may adhere to plastic containers during their handling and storage.

Breast Milk Sample Handling, Storage and Analysis. Once breast milk samples are placed into the appropriate storage container with a participant identification number, they can then be stored in a refrigerator for up to 72 hours (4°C) or placed in the freezer (-20°C) (WHO, 2007). Frozen breast milk samples can be stored at -70° for an indefinite period (Lovelady et al., 2002). Transportation of frozen samples should be on dry ice.

There are several analytical methods for measuring environmental chemicals in breast milk; however, regardless of the selected approach, the process typically consists of the following general steps: 1.) sample preparation, which includes lipid determination and "cleanup"; 2.) sample analysis, which involves chromatographic and detection steps; and 3.) data analysis and evaluation (Needham & Wang, 2002). Selecting an analytical method depends upon the classification of the analytes of interest. Environmental chemicals can be classified as inorganic or organic compounds, and further categorized as volatile or semi-volatile organic environmental chemicals. Regardless of the analytes of interest and the selected analytical approach, there should be methodological goals built into the measurement process that address quality assurance and control to determine specificity, sensitivity, precision, and accuracy of the technique.

Advantages and Disadvantages of Breast Milk Biomonitoring Programs

Breast milk is a valuable matrix for monitoring body burdens of environmental chemicals; however, there are positive and negative aspects when using breast milk to assess human exposure to environmental chemicals (Fenton

et al., 2005; Hooper & She, 2003). Fenton and colleagues (2005) identify the following limitations: 1.) interpretation and communication of study results, which have the potential for affecting breastfeeding rates if inaccurate messages are provided to the public; 2.) recruitment challenges if studies are limited to women who are exclusively breastfeeding; 3.) sampling challenges if analytes of interest have short half-lives; 4.) insufficient data on depuration (elimination kinetics), which limits estimates on infant intake; and 5.) the inability to generalize the results given the selected marker of exposure. Even though there are limitations associated with breast milk biomonitoring studies, several positive aspects have been identified: 1.) the collection of serial samples, which provides information on environmental chemical levels over time; 2.) the use of one biological specimen to assess exposure of the mother and infant; 3.) a better understanding of body burden of breastfed infants; and 4.) the collection of a biological specimen with a high fat content, which is an important consideration for lipid-soluble environmental chemicals – particularly those that have been steadily rising in their production, use, and presence in the environment and in humans, such as synthetic flame retardants.

flame retardants

Every twenty seconds, a fire department responds to a fire somewhere in the U.S. (Karter, 2006). In 2005, fires were responsible for 3,675 civilian-related deaths, 17,925 civilian injuries, and greater than 10 billion dollars in property damage. Since 1977, fire incidence has decreased from 3,264,000 to 1,602,000 due to fire prevention polices that require the presence of flame retardant

chemicals in a variety of different products (Birnbaum & Staskal, 2004; Karter, 2006).

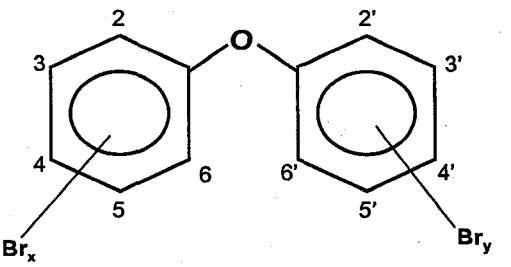
Flame retardants are synthetic chemicals that are added to materials either during or after manufacturing in order to interfere with the combustion process (Bromine Science and Environmental Forum [BSEF], 2000). This interference can occur during heating, decomposition, ignition or flame spread. There are over 175 different types of flame retardants that are divided into four major classes: halogenated organic (usually brominated or chlorinated), phosphorus-containing, nitrogen-containing, and inorganic flame retardants (Birnbaum & Staskal, 2004; BSEF, 2000). The brominated flame retardants (BFRs) are the most widely used due to their low cost and high performance efficiency. Compared to other flame retardants, consumer products use a smaller quantity of BFRs for a higher fire resistance (BSEF, 2000).

Brominated Flame Retardants

There are five major classes of BFRs, including brominated bisphenols, cyclododecanes, phenols, phthalic acid derivatives, and diphenyl ethers (Birnbaum & Staskal, 2004). The most widely used BFRs are tetrabromobisphenol A, hexabromocyclododecane, and three commercial products of polybrominated diphenyl ethers known as decabromodiphenyl ether, octabromodiphenyl ether and pentabromodiphenyl ether (Birnbaum & Staskal, 2004; BSEF, 2000). In 2001, polybrominated diphenyl ethers (PBDEs) represented one of the highest production volumes of BFRs in the U.S. with a total market consumption of 33,100 metric tons (BSEF, 2003).

Polybrominated Diphenyl Ethers

There is the potential for 209 PBDE variants. Each variant can be identified by the number and position of bromine atoms, also known as a PBDE congener (Figure 2) (U.S. EPA, 2006).



Key: PBDE variants: x + y = 1 to 10 bromines

Figure 2. General structure of PBDEs

However, there are fewer PBDE congeners in commercial mixtures than theoretically possible due to their instability and debromination (Birnbaum & Staskal, 2004). Three commercial PBDE products - pentaBDE, octabBDE and decaBDE - contain mixtures of various PBDE congeners, are used in different products, and vary in their market demand (Table 1).

Table 1.

Summary of commercial PBDE products

Commercial PBDE Product	Composition of Mixtures[a]	Uses[b]	Total Demand in U.S.[c] (metric tons)
pentaBDE	24 – 38% tetraBDE 50 – 60% pentaBDE 4 – 8% hexaBDE	Textiles (furniture foam)	7,100
octaBDE	10 – 12% hexaBDE 44% heptaBDE 31 - 35% octaBDE 10 – 11% nonaBDE < 1% decaBDE	Plastics, appliances	1,500
decaBDE	> 97% decaBDE < 3% nonaBDE	Electrical and electronics, textiles	24,500

Note. [a]Adapted from "Environmental Health Criteria 162: Brominated Diphenyl Ethers," by World Health Organization, 1994, *World Health Organization, International Programme on Chemical Safety*. [b]Adapted from "Brominated Flame Retardants: Cause for Concern?," by L.S. Birnbaum and D.F. Staskal, 2004, *Environmental Health Perspectives*, *112,* p. 12. [c]Adapted from "Major Brominated Flame Retardants Volume Estimates, by Bromine Science and Environmental Forum, 2003, *Bromine Science and Environmental Forum*, http://www.bsef.com/docs//BFR_vols_2001.doc.

The total U.S. market demand values for pentaBDE and octaBDE are expected to drop to zero because Great Lakes Chemical Corporation, the sole U.S. manufacturer of pentaBDE and octaBDE, discontinued production of these two commercial products at the end of 2004 (U.S. EPA, 2006).

Even though PBDEs have been in use since the 1970s, they have captured the attention of policymakers, firefighters and the general public, worldwide, over the past 15 years. The European Union adopted legislation in 2002 that prohibited the use of pentaBDE and octaBDE, which took effect in August 2004 (U.S. EPA, 2006). Several states in the U.S. have also adopted bans on the distribution of products that contain pentaBDE and octaBDE, including California, Maine and New York. Although decaBDE has the highest annual global demand at 56,100 metric tons (83% of PBDE demand) (BSEF, 2003), restrictions on its use has been limited. The European Union granted an exemption on prohibiting the use of decaBDE in October 2005 (U.S. EPA, 2006), but Washington state recently became the first in the U.S. to have approved legislation that would ban the use of decaBDE in household goods, which is scheduled to take effect in 2008 (American Chemical Society, 2007). The legislation was supported by Washington state fire chiefs and firefighters. Even though the presence of PBDEs in consumer products such as television sets, carpet padding, mattresses, chair and couch foams, computers, stereos, and hair dryers saves lives each year in the U.S., there are concerns with its widespread use because of increasing environmental and human concentrations.

Bioaccumulation. Due to their persistent, lipid-soluble characteristics and incorporation into a variety of products, PBDEs are bioaccumulating. PBDE bioaccumulation is primarily due to PBDE release from consumer products into the air through normal use, degradation, disposal, and recycling; additional release can also occur during PBDE manufacturing and incorporation into products (Hale et al., 2003). PBDEs are non-chemically bound additives, and may contribute up to 30% by weight of foams, fabrics and plastics (Hale et al., 2003; Hooper & She, 2003). Over time, PBDEs can diffuse out of their products, become airborne or enter dust, and disperse into the environment and into humans.

PBDEs have been detected in air, house dust, sewage sludge, water, sediment, and soil (Allen et al., 2007; de Boer et al., 2003; Hale et al., 2002; Jones-Otazo et al., 2005; Rudel et al., 2003; Wu et al., 2007). It is suggested that ingestion of indoor dust is a major exposure pathway for PBDEs in humans (Allen et al., 2007; Jones-Otazo et al., 2005) and that hand-to-mouth exposure contributes to people's intake of PBDEs (Stapleton et al., 2008).

Studies document PBDE appearance in marine mammals, fish, pet cats, human tissue, human placenta, and human blood samples (Bradman et al., 2007; Dye et al., 2007; Gómara et al., 2007; Guvenius et al., 2003; Johnson-Restrepo et al., 2005; Mazdai et al., 2003; Petreas et al., 2003; Rice et al., 2002; Sandanger et al., 2007; Schecter et al., 2007; Schecter et al., 2005; She et al., 2002; Sjödin et al., 1999; Sjödin et al., 2004; Strandman et al., 2000). Time-trend data from the U.S. indicate increasing PBDE blood concentrations between

1973 and 2003, and between 1985 and 2002 (Schecter et al., 2005; Sjödin et al., 1999).

PBDEs have been detected in various samples of food sources from Japan, Spain & U.S. (Bocio et al., 2003; Huwe & Larsen, 2005; Huwe et al., 2002; Ohta et al., 2002; Schecter et al., 2006; Schecter et al., 2004). Bocio and colleagues (2003) found that foods of animal origin, fish, and fats and oils have higher levels of PBDEs as compared to grains, fruits and vegetables. Higher PBDE levels were detected by Ohta and colleagues (2002) in fish samples as compared to beef and chicken; however, spinach, potato and carrot had higher PBDE concentrations as compared to beef and chicken. Recent work by Schecter et al. (2006) confirmed that the highest PBDE levels in a U.S. market basket survey were detected in fish samples followed by dairy products and meat. PBDEs have also been detected in another biological matrix - studies from Canada, China, Czech Republic, Denmark, Faroe Islands, Finland, Italy, Japan, Poland, Spain, Sweden, Taiwan, Turkey, United Kingdom and U.S. report that PBDEs appear in human breast milk (Akutsu et al., 2003; Bi et al., 2006; Chao et al., 2007; Erdoğrul et al., 2004; Eslami et al., 2006; Fängström et al., 2005; Gómara et al., 2007; Hooper et al., 2007; Ingelido et al., 2007; Jaraczewska et al., 2006; Johnson-Restrepo et al., 2007; Kalantzi et al., 2004; Kazda et al., 2004; Lind et al., 2003; Lunder & Sharp, 2003; Main et al., 2007; Meironyté et al., 1999; Norén & Meironyté, 2000; Ohta et al., 2002; Ryan & Patry, 2000; Ryan et al., 2006; Schecter et al., 2005; Schecter et al., 2003; Schuhmacher et al., 2007; She et al., 2007; Strandman et al., 2000; Wu et al., 2007).

Levels of PBDEs in humans vary geographically, however, the highest PBDE concentrations in humans have been reported in the U.S. (Hites, 2004). With the appearance of PBDEs in the environment and concentrations in humans, this raises concern about the potential human health implications.

Health Implications. With a similar chemical structure to polychiorinated biphenyls (PCBs) and pesticides such as dichlorodiphenyltrichloroethane (DDT), both of which have been banned due to their adverse health outcomes (Hooper & McDonald, 2000), as well as structural similarities to thyroid hormone, research is necessary to understand the human health implications of PBDEs – which are currently unknown.

There are a limited number of epidemiological studies, to date, that have examined PBDE concentrations and human health. In central Taiwan, increased PBDE levels in human breast milk were related to lower birth weight and length, head and chest circumference, and body mass index in newborns (Chao et al., 2007). A recent study by Main and coileagues (2007) revealed that the concentration of PBDEs in breast milk was significantly higher in infant boys with congenital cryptorchidism as compared to controls. A case-control study by Hardell and colleagues (2001) found higher BDE-47 levels in adipose tissue/blood samples from adult Swedish patients with non-Hodgkin's iymphoma when compared to age-matched controls. Furthermore, it was shown that the median titer to Epstein-Barr early antigen was higher among cases than controls, and the highest risk for non-Hodgkin's lymphoma was found with high BDE-47

concentrations and an elevated titer of antibodies to the Epstein-Barr virus antigen.

Research on the biological effects of PBDEs has been conducted mostly in experimental animal models. PBDEs have been shown to cause adverse effects in several biological end-points. The greatest concern for the potential health implications of PBDEs comes from research that has examined developmental neurotoxicity in mice (Birnbaum & Staskal, 2004). Exposure to PBDEs adversely affects spontaneous behavior, and causes learning and memory deficits in mice (Branchi et al., 2002; Eriksson et al., 2001, Viberg et al., 2003; Viberg et al., 2006). The mechanisms for these behavioral and cognitive deficits are not known, but research suggests alterations in cholinergic receptors, arachidonic acid release, and thyroid hormone homeostasis (Birnbaum & Staskal, 2004).

Thyroid hormones influence a variety of physiological processes in the body such as regulating cellular metabolism and its crucial role in the development of the central nervous system. Research has demonstrated that hypothyroidism during pregnancy can adversely affect a child's subsequent performance on neuropsychological tests (Haddow et al., 1999). PBDEs have been shown to disrupt thyroid hormone homeostasis in rats and mice. Studies have shown alterations in thyroid hormone levels, particularly decreased thyroxine levels, after exposure to PBDEs (Fowles et al., 1994; Hallgren et al., 2001; Skarman et al., 2005; Zhou et al., 2001; Zhou et al., 2002). There are several different mechanisms as to how PBDEs interact with thyroid hormone

homeostasis, including alterations in plasma transport of thyroxine, induced hepatic enzyme activities resulting in increased metabolism of thyroxine, and possibiy direct effects on the thyroid gland (Darnerud et al., 2007; Lilienthal et al., 2006; Meerts et al., 2000).

In addition, PBDE-induced imbalances in steroid hormone homeostasis and other steroid-related effects have been documented *in vivo* and *in vitro*. A recent study by Lilienthal and colleagues (2006) documented the effect of BDE-99 on sex steroids, sexual development, and sexually dimorphic behavior in the offspring of treated pregnant rats. Exposure to PBDEs resulted in decreased circulating concentrations of estradiol and testosterone in male offspring, delayed puberty onset in female offspring, a reduction in the number of primordial/primary and secondary ovarian follicles, and an increased sweet preference in male offspring. The increased sweet preference in male offspring was characterized as sexually dimorphic behavior indicating behavioral feminization. Meerts and colleagues (2001) examined the estrogenicity activity of 17 PBDE congeners and 3 hydroxylated PBDEs *in vitro* using an estrogen-responsive luciferase cell line. It was found that cellular exposure to PBDE congeners and hydroxylated PBDEs induced the estrogen receptor signal transduction pathway *in vitro*. The most potent effects were seen with BDE-100, BDE-75, BDE-51, BDE-119 and the T_3-like and T_2-like hydroxylated PBDE compounds. The potential endocrine-disrupting effects of PBDEs could impact growth and development of the central nervous system and reproductive tissues as well as metabolism.

In 2002, a hypothesis was generated on the role of synthetic chemicals in the global obesity epidemic (Baillie-Hamilton, 2002). Hoppe and Carey (2007) pursued this hypothesis with the notion that PBDEs disrupt hormonally-mediated adipose tissue metabolism. After male rats were exposed to pentaBDE daily for 4 weeks, an increase in lipolysis and a decrease in insulin sensitivity was seen, which is consistent with the metabolic effects observed in obese animals. Further research is currently being pursued in this area, so the long-term effects of PBDEs on adipose tissue metabolism and its relationship to the promotion of obesity are not yet clear.

There is limited information as to how PBDE toxicity in animals translates to human health effects. McDonald (2005) recently examined PBDE exposure and risk assessment by comparing PBDE concentrations in humans with concentrations that were associated with developmental neurotoxicity and reproductive effects in rodents. Estimates of human daily PBDE intake was calculated from biomonitoring literature on PBDE concentrations in serum, adipose tissue, and breast milk from women in the U.S. Daily intake data for all human matrices were combined and the 95th percentile was determined, which represented high-end exposures in the U.S. population. The 95th percentile depicted the lipid-normalized PBDE concentrations in humans, which was compared to an estimate of lipid-normalized tissue concentrations in rodents. The rodent-to-human ratio of PBDE tissue concentration was then used as a margin of exposure. The margin of exposure was defined as how many folds higher is the rodent dose that caused no effects from the human dose; the

smaller the distance between doses the greater the health concern. Using this reverse dosimetry pharmacokinetic modeling approach, McDonald (2005) found that there was a small margin of exposure for behavioral and reproductive effects with alterations in male fertility and ovary cells in females. A risk-assessment approach such as this enables researchers to evaluate biomonitoring data from a toxicity standpoint.

Toxicology. The toxicology of the three commercial mixtures (pentaBDE, octaBDE, and decaBDE) and some of the individual PBDE congeners has been reviewed extensively by Darnerud et al. (2001), Gill et al. (2004), and Hardy (2002). The toxicological effects correspond with degree of bromination; the toxicity of the tetra through deca PBDE congeners decreases with increasing levels of bromination.

The toxicological effects of PBDEs have primarily been documented in animal models with almost no available data in humans (Birnbaum & Cohen Hubal, 2006). Overall, the acute toxicity of the commercial products is low when administered through the oral, dermal or pulmonary routes; however, differences exist between the three commercial products and their toxicological effects in repeated dose studies (Darnerud et al., 2001; Gill et al., 2004; Hardy, 2002). For example, oral administration of decaBDE appears to be less acutely toxic as compared to penta and octaBDE. Oral administration of decaBDE to mice and rats at 10% and 5% of their diet for 14 and 90 days, respectively, produced no adverse effects on appearance, behavior, body weight gain, and food consumption (U.S. DHHS, National Toxicology Program, 1986); however, chronic

(2 years) dietary exposure to decaBDE has displayed evidence of carcinogenicity. Oral administration studies have also been conducted in rats with dietary exposure to octaBDE with doses of up to 1000 mg/kg/day (WHO, 1994). These studies have shown no effect on appearance, behavior, or mortality, however, hepatotoxicity was observed. Zhou and colleagues (2001) examined the dose-response relationships of 4-day exposure to the three commercial mixtures on thyroid hormone concentration and hepatic enzyme activities. The octa and pentaBDE formulations caused dose-dependent reductions of thyroxine and inductions of hepatic enzymatic activity.

Out of the three commercial mixtures, penta and octaBDE appear to have the most pronounced dose-dependent toxicity effects in subacute and subchronic animal studies (Darnerud et al., 2001; Gill et al., 2004; Hardy, 2002). The differences in toxicity effects are likely due to variations in absorption, metabolism, and elimination of the individual PBDE congeners from each of the commercial mixtures.

Absorption, Metabolism and Excretion. PBDE toxicokinetics (absorption, elimination, and enzyme induction and metabolism) has been studied primarily in experimental animal models, both *in vitro* and *in vivo*. Oral absorption and metabolism of the individual PBDE congeners varies depending upon the degree of bromination (Gill et al., 2004). Once PBDE exposure has occurred, it's been reported that the higher the degree of bromination, the lower the absorption rate (Birnbaum & Staskal, 2004). Historically, decaBDE has been shown to be poorly absorbed through the oral route due to its high molecular mass (Darnerud et al.,

2001; U.S. DHHS, National Toxicology Program, 1986); however, recent research has demonstrated that decaDBE can be absorbed orally at greater than 10% of the dose in rats (Mörck et al., 2003). Lower brominated congeners, such as BDE-47 (tetra) and BDE-99 (penta), have been shown to have an oral absorption rate of 93% and greater than 50% in mouse and rat models, respectively (Hakk et al., 2002; Örn & Klasson-Wehler, 1998). A higher absorption rate of lower brominated PBDE congeners leads to a greater likelihood for tissue bioaccumulation.

Lipophilic tissues tend to be the preferred sites for PBDE disposition; however, recent work by Inoue and colleagues (2006) evaluated molecular descriptors (e.g. hydrophilicity, molecular size, polar surface area) for PBDEs in human serum and breast milk samples, and found correlations with the milk/serum partition coefficients. The structure-activity relationship suggested that PBDE values in serum and breast milk are related to each other; however, BDE-209 was the predominant congener in serum and BDE-47 was the predominant congener in breast milk.

The congener with the greatest concentration in most biological tissues is BDE-47, followed by BDE-99 or BDE-153 depending on the species (Hakk et al., 2002; Hites, 2004). In 2002, Hakk and colleagues found that BDE-99 had the highest tissue concentrations in adipose, adrenals, gastrointestinal tract, and skin in male rats; however, the distribution pattern of BDE-99 did not consistently correlate with tissue lipid content. Other studies have shown similar results with regard to PBDE disposition and its preference to lipophilic tissues. Örn and

Klasson-Wehler (1998) found that BDE-47 had the highest concentration in adipose tissue, but it was also detected in lung, kidney and brain tissues. Both of these studies also examined routes of elimination. It was found that fecal and urinary excretion were the routes of PBDE elimination, both the parent compounds and their metabolites.

The commercial PBDE mixtures and selected individual congeners have been shown to induce Phase I cytochrome P450 monooxygenase (CYP) enzymes and Phase II conjugation enzymes in the liver, which are responsible for detoxification (Hakk & Letcher, 2003). The penta and octaBDE commercial mixtures have been shown to induce CYP enzymes (CYP1A1, CYP1A2, CYP2B1) in rats as indicated by increased activity of liver microsomes, such as ethoxyresorufin-O-deethylase and pentoxyresorufin-O-deethylase, as well as Phase II induction of uridinediphosphate-glucuronosyltransferase (von Meyerinck et al., 1990; Zhou et al., 2001). When Zhou and colleagues (2001) compared the three PBDE commercial mixtures, they found that decaBDE had no significant effects on the measured Phase 1 and Phase 2 enzymes. At the present time, little is known on PBDE toxicokinetics in humans; however, studies have examined the estimated half-lives of PBDEs.

Estimated Half-Lives. It is known that PBDEs bioaccumulate in humans, but the rate of absorption, the degree of tissue disposition, hepatic enzyme induction, and metabolism are not known. However, PBDE half-lives have been estimated in humans. Thuresson and colleagues (2006) calculated the half-lives for higher brominated PBDEs (deca, nona, octa, and hepta congeners) using

serum data from occupationally exposed workers who were sampled before, during, and after a vacation period. It was found that BDE-209 (deca) had a short half-life of 11 to 18 days, followed by 18 to 39 days for nona congeners, 37 to 91 days for the octa congeners, and 68 to 120 days for BDE-183 (hepta). Therefore, the pattern that was observed by Thuresson et al. (2006) indicated the higher the number of bromine atoms for the PBDE congeners the shorter their half-lives in serum samples. Data has been presented by Geyer and colleagues (2004) on the half-lives of the lower brominated PBDE congeners (tetra, penta, and hexa). Using pharmacokinetic modeling estimates, it was found that the total body half-lives for tetra to hexa congeners were 1.8 to 6.5 years, respectively. These results are consistent with McDonald's (2002) theory that some of the PBDEs have half-lives that are on the order of years. Since PBDEs can be absorbed and deposited in human tissue for extended lengths of time, biomonitoring programs are clearly warranted to assess the extent of exposure.

biomonitoring PBDEs via breast milk

Breast milk is a valuable biological specimen for biomonitoring PBDEs given their lipid-soluble characteristics. PBDEs in breast milk were first reported by Krüger (1988) in German samples, and later by Meironyté et al. (1999) in Swedish samples. PBDE concentrations in Swedish breast milk samples increased from 1972 to 1997, and it was determined that levels were doubling every five years while levels of other environmental chemicals (PCBs and DDT) were decreasing during this same time period (Meironyté et al., 1999; Norén & Meironyté, 2000). Documenting this exponential increase has resulted in PBDE

breast milk biomonitoring initiatives around the globe. These initiatives have focused on: 1.) PBDE levels in breast milk of nursing mothers; 2.) rates of PBDE elimination (depuration) from mothers during lactation; 3.) infant intake of PBDEs; and 4.) potential PBDE sources of exposure.

PBDE Levels in Breast Milk of Nursing Mothers

During the past decade, breast milk biomonitoring has enabled researchers to document and update human exposure information on PBDE levels in nursing mothers (Table 2). Currently, the levels of PBDEs in breast milk samples from the U.S. are 10 to 40 times higher than other regions such as Europe and Japan. The reason for the higher levels of PBDEs in the U.S. is thought to be due to the market demand for these flame retardants as they are incorporated into numerous industrial and household products (Johnson-Restrepo et al., 2007). PBDE concentrations in the U.S. indicate differences in median values depending on the geographical location. For example, median PBDE values are higher from the Pacific Northwest compared to Massachusetts (Table 2) (Johnson-Restrepo et al., 2007; She et al., 2007; Wu et al., 2007). However, one consistent outcome that has been verified in the majority of breast milk biomonitoring studies, to date, is the predominance of the BDE-47 congener.

Rates of PBDE Elimination from Mothers during Lactation

In addition to documenting PBDE levels in breast milk, Hooper and colleagues (2007) recently reported rates of PBDE elimination from nursing mothers; this is the first study to measure PBDE depuration in mothers during lactation. Depuration was examined by measuring PBDE concentrations in serial

Table 2.

Levels of PBDEs in breast milk from around the globe

Location, authors, date of publication	n	PBDE Medians[a] (congener sum)	Ranges[a]	# Congeners in analysis	Dominant Congener	Median BDE-47[a]
U.S., Johnson-Restrepo et al. (2007)	38	19.8	0.06 – 1910	17[c]	BDE-47	7.7
U.S. & British Columbia, She et al. (2007)	40	50	6 – 321	12[c]	BDE-47	27.8
U.S., Wu et al. (2007)	46	30.2	4 – 264	12[c]	BDE-47	13.9
U.S., Schecter et al. (2003; 2005)	59	30.1	6 – 419	13[c]	BDE-47	17.4
Sweden, Lind et al. (2003)	93	3.2	0.9 – 28	5	BDE-47	1.78
Faroe Islands, Fängström et al. (2005)	9	5.8	5 – 13	5	BDE-153	1.3
U.K., Kalantzi et al. (2004)	54	6.3	0.3 – 69	15	BDE-47	2.7
Japan, Eslami et al. (2006)	105	1.3	0.01 – 23	6	BDE-47	0.68
South China, Bi et al. (2006)	27	3.5	1.7 – 7.2	7[c]	BDE-47	1.3
Denmark, Main et al. (2007)	36	3.27[b]	1.09 – 9.07[b]	14	BDE-47	1.05
Finland, Main et al. (2007)	32	3.11[b]	1.04 – 29.17[b]	14	BDE-47	1.24
Spain, Gómara et al. (2007)	22	6.1	1.5 – 63	15[c]	BDE-209	0.37
Spain, Gómara et al. (2007)	30	5.5	0.78 – 34	15[c]	BDE-209	0.22

Note. [a]ng/gram lipid weight; [b]controls (in a case-control study); [c]BDE-209 included in analysis

breast milk samples from two groups (short-term and long-term) of first-time nursing mothers. The short-term group (n = 9) collected seven samples at fixed intervals from weeks 4 to 24. The long-term group (n = 9) collected initial samples 1 to 6 weeks after birth, and final samples 18 to > 85 weeks later. Additionally, two more groups of mothers collected samples 3 to 28 days after birth and 29 to 56 days after birth to examine depuration rates early in lactation.

Patterns of mean PBDE levels in serial breast milk samples were examined over time. The predominant congener was BDE-47 and it correlated with the total sum of PBDEs. Therefore, BDE-47 was used to show depuration rates. Hooper and colleagues (2007) concluded that body burden of PBDEs were lowered by lactation; however, depuration of PBDEs by lactation is slow, averaging 1 to 3% per month or a 12 to 18% decrease after 6 months of breastfeeding. Depuration rates of PBDEs in the earlier stages of lactation were not higher as compared to later in lactation. It was suggested that infant intake of PBDEs for a second child would not be markedly lower as compared to the first child.

Infant Intake of PBDEs

According to Lovelady et al. (2002), breast milk biomonitoring data should not be used to assess infant exposure if only one milk sample has been collected at one time point because environmental chemical concentrations can decrease over the course of lactation. Four breast milk biomonitoring studies have reported the estimated daily intake of PBDEs for infants (Chao et al., 2007; Johnson-Restrepo et al., 2007; Main et al., 2007; Meironyté et al., 1999). Breast

milk biomonitoring studies tend to focus on estimating body burden of the mother, rather than assessing infant exposure, which provides an explanation for the limited number of studies that are available documenting an infant's intake of PBDEs from breast milk.

Meironyté and colleagues (1999) estimated that the average daily intake of PBDEs from pooled breast milk samples for a 5 kg infant increased from 0.3 ng/kg body weight in 1972 to 16.8 ng/kg body weight in 1997. More recently, Main et al. (2007) estimated the median daily intake of PBDEs for a three-month old infant to be 16 ng/kg body weight, which was calculated from one breast milk sample that consisted of several small aliquots collected over successive feedings. Chao and colleagues (2007) reported a daily intake of PBDEs for a breastfed newborn infant to be 20.6 ng/kg body weight; however, it's assumed that only one 25 ml sample was collected from each mother. Based on median PBDE concentrations in breast milk samples from Massachusetts, Johnson-Restrepo et al. (2007), estimated a one-month old infant's intake to be 4 ng/kg body weight for selected congeners (BDE-47, 99, and 153), but it's not known how many samples were collected from each participant.

At this time, additional research is needed to estimate infant intake of PBDEs, specificaliy studies that collect serial breast milk samples over time. Estimating infant intake of PBDEs provides information on an infant's body burden; however, determining infant exposure to environmental chemicals via breastfeeding and the potentiai health implications warrants a longitudinal research design, an assessment of prenatal exposure (usually via cord blood),

and the measurement of environmental chemicals in breast milk at several time points during lactation (Lovelady et al., 2002).

Potential PBDE Sources of Exposure

Breast milk biomonitoring researchers have evaluated PBDE exposure sources through assessment questionnaires (Akutsu et al., 2003; Bi et al., 2006; Chao et al., 2007; Erdoğrul et al., 2004; Eslami et al., 2006; Fängström et al., 2005; Ingelido et al., 2007; Jaraczewska et al., 2006; Kalantzi et al., 2004; Lind et al., 2003; Lunder & Sharp, 2003; Main et al., 2007; Ohta et al., 2002; Schecter et al., 2003; Schuhmacher et al., 2007; She et al., 2007; Wu et al., 2007) and one recent study also analyzed house dust concentrations (Wu et al., 2007). Exposure assessment questionnaires have evaluated the following: a.) dietary intake; b.) personal characteristics (e.g. age, anthropometrics, parity, menstruation); c.) personal habits (e.g. smoking, alcohol consumption); d.) household and work environments (e.g. presence and number of electronics); e.) place of residence (e.g. urban or industrial zones); and/or f.) season in which infant was breastfed. Only a few studies have found significant relationships between PBDE levels in breast milk and exposure sources. The most significant relationships that have been documented are with house dust concentrations, dietary intake and smoking.

Wu and colleagues (2007) found a strong and positive correlation between PBDE concentrations in breast milk and house dust suggesting that the indoor home environment plays an important role in PBDE exposure. Twelve participants out of 40 agreed to dust sampling in this study, and those

participants with high PBDE dust concentrations had PBDE levels in breast milk that were 2.6 times higher as compared to those homes with lower dust concentrations.

In the same study, associations were also found between PBDE concentrations in breast milk and dairy intake as well as total meat consumption (Wu et al., 2007). The strongest association was found with dairy intake, which suggested a 3.6-fold increase in PBDE levels per daily ounce of dairy fat consumed. In addition to dairy intake, total meat consumption was also associated with an increase in PBDE levels per serving. For every 2 to 3 ounces of meat consumed, there was a 1.5-fold increase in PBDE levels. Other dietary factors, such as total fish consumption, were not significantly associated with PBDE levels in breast milk. However, in a small sampling of women from Japan, PBDE concentrations were significantly elevated with a higher frequency of fish consumption (every day) as compared to PBDE concentrations of women with a relatively low fish consumption (1 to 2 days per week) (Ohta et al., 2002). Few other studies have detected an association between PBDE levels in breast milk and dietary factors, but personal habits have indicated a relationship.

Lind and colleagues (2003) found a significant relationship between smoking and PBDE levels in breast milk. Even though the square of the correlation coefficient was low, it increased from 10 to 17% after the addition of smokers into the multiple regression model. Research continues to further examine PBDE exposure sources to better understand what contributes to human exposure.

Advancing Breast Milk Biomonitoring Research

In the U.S., four peer-reviewed research studies have reported PBDE levels in breast milk (Table 2). One study in the U.S. has reported rates of PBDE elimination over time in a small sample of nursing mothers from California (Hooper et al., 2007), and one has demonstrated relationships between PBDE levels in breast milk and house dust, dairy intake, and meat intake (Wu et al., 2007). Given the limited number of PBDE breast milk biomonitoring studies in the U.S. and no studies to date from the state of New Hampshire, there is a need to further document PBDE levels in humans, examine PBDE levels in breast milk over time, and further evaluate potential sources of PBDE exposure, all of which will be addressed in this research.

CHAPTER III

MATERIALS & METHODS

communication pamphlet

One of the primary research objectives of this project was to develop an informative, yet reassuring tool to educate potential subjects about the research. Indeed, the intent of the research was not to undermine breastfeeding and scare mothers away from nursing their infants.

To address this objective, an undergraduate independent study student, who worked with the Carey Laboratory in the spring 2005 semester, assisted with developing the communication pamphlet. The format of the pamphlet contained short sections about the research study, body burden, what PBDEs are and where they come from, why we are measuring PBDEs, and the importance of breastfeeding. The final draft of the communication pamphlet also included a section on "frequently asked questions".

As part of the communication pamphlet development phase, we collaborated with a health promotion advisor from the New Hampshire Department of Health and Human Services (NH DHHS), a faculty member from the Family Research Laboratory at the University of New Hampshire (UNH), the New Hampshire Breastfeeding Task Force and a focus group of women from the community. This collaboration provided us with valuable feedback on the content of the pamphlet, language and our overall research design.

Our first collaborative meeting was in March 2005 with the NH DHHS health promotion advisor and the faculty member from the Family Research Laboratory. Their primary recommendations were to eliminate the technical language in order to decrease the reading level from 12th grade to 8th grade. In addition, they recommended that the pamphlet be visually appealing and be reassigned a less scientific name. The project's secondary name became *Steps to a Healthy Future.*

Our second meeting was in April 2005 with 20 members of the New Hampshire Breastfeeding Task Force. The members of the Task Force are primarily registered nurses and registered dietitians who are lactation specialists. This meeting provided valuable input regarding appropriate breastfeeding language and further elimination of technical wording. During this meeting, members of the Task Force also provided helpful suggestions on our research design including sample collection time, sample size, breastfeeding support to participants and information on personal use vs. rental breast pumps.

Our third meeting was in May 2005 when we conducted a six person, one hour focus group to assess the content of the pamphlet. We recruited women from the UNH campus who were currently breastfeeding or had breastfed their child within the last two years. We provided background information on the study, reviewed each section of the communication pamphlet, and collected verbal and written feedback from the focus group participants. The verbal feedback emphasized refinement of language to further improve the readability of the communication pamphlet. The written feedback was collected by having

participants complete an anonymous 10-question survey (Appendix A) about the content and clarity of the pamphlet at the end of the focus group session. The written feedback directed us to emphasize the health effects of PBDEs, participant time commitment and what will happen with the final results of the study.

The communication pamphlet was completed in August of 2005. The final format was an eight page pamphlet with readability statistics at the 5th grade reading level (Appendix B). Approximately 1500 copies of the pamphlet were distributed to lactation consultants and placed in eight locations around the Seacoast area of New Hampshire (Dover Pediatrics, Exeter Hospital Family Center, Harbour Women's Health, Lamprey Health Care, Portsmouth Regional Hospital, Rochester Women, Infants & Children Clinic, Seacoast Area La Leche League, and Wentworth Douglass Hospital) for subject recruitment.

recruitment

Participants were recruited from the Seacoast region of New Hampshire from November 2005 to July 2006. Recruitment was accomplished by lactation consultants from three area hospitals – Exeter Hospital, Portsmouth Regional Hospital, and Wentworth Douglass Hospital -, distribution of the communication pamphlet, and word of mouth. The study protocol was approved by the UNH Institutional Review Board (IRB # 3433) and approval from each hospital was obtained prior to working with the lactation consultants. I attended monthly breastfeeding classes at each of the hospitals to provide research study

information and the communication pamphlet to pregnant women. The lactation consultants also provided the communication pamphlet to postpartum women prior to their discharge from the hospital. In addition, the pamphlet was distributed to outpatient health care clinics.

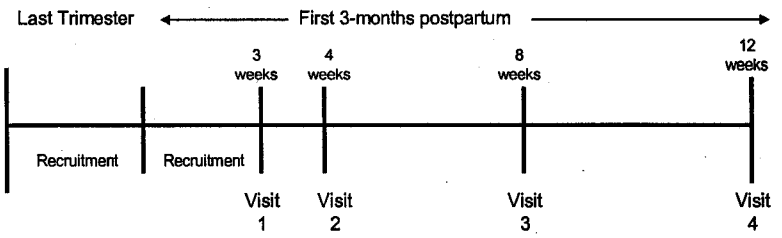
participants

Women, ages to 22 to 40 from the Seacoast region of New Hampshire, who were in the early stages of lactation (less than 2 weeks) or in their last trimester of pregnancy and planned to breastfeed for at least the first three-months postpartum, were eligible for the study. Participant eligibility criteria also included delivering a full-term, healthy infant and planning to provide greater than 80% of breast milk as the primary source of nutrition for the first three-months.

To show our appreciation for participation in the study, enrolled subjects were provided with an Ameda Purely Yours® personal use electric breast pump and kit. Each kit contained one personal use electric breast pump with a dual hygienic milk collection system, six 4-ounce clear, plastic bottles, a power adapter, an insulated tote with cooler pack, and a carry bag. Participants had the opportunity to talk with a professional lactation consultant during their enrollment in the study.

study design, procedures and analytical methods

Participants were asked to visit with researchers on four occasions over a three-month period for a total of approximately four hours (Figure 1). Researchers could meet with participants at their homes or at UNH.



Note:
Visit 1: Study orientation, obtained consent, administered Maternal Characteristics Survey & Food Frequency Questionnaire #1, measured body weight, provided personal use electric breast pump and kit
Visit 2: Sample pick-up (1 or 3 samples), provided Environment Survey
Visit 3: Sample pick-up (1 or 3 samples), retrieved Environment Survey
Visit 4: Sample pick-up (1 or 3 samples), administered Food Frequency Questionnaire #2, measured body weight, study debriefing

Figure 1. Study design from recruitment to visit 4.

The first visit occurred at three weeks postpartum. During the first visit, participants were oriented to the study's purpose and design, completed a food frequency questionnaire and a maternal characteristics survey, were weighed on a Health o meter® mechanical floor-level scale, and signed a UNH IRB subject consent form (Appendix C). Participants received verbal and written instruction on breast milk expression via the personal use electric breast pump, sample collection procedure and sample storage (Appendix D). Participants also received four ounce amber glass storage containers with Teflon fitted lids, a pumping schedule (Appendix E) for the first month collection period and their

personal use breast pump.

The second visit occurred at four weeks postpartum. Participants surrendered the first month's samples, received additional four ounce amber glass storage containers, met with the researchers to answer any questions and set up a pumping schedule for the second month collection period. During the second visit, participants were asked to complete an environmental survey on their own and mail back to UNH in a self-addressed stamped envelope or return it to the researchers at the third visit.

The third visit occurred at eight weeks postpartum. Participants surrendered the second month's samples, provided the completed environmental survey, received additional four ounce amber glass storage containers, met with the researchers to answer any questions and set up the final pumping schedule for the third month collection period. The fourth visit occurred at 12 weeks postpartum, when participants surrendered the third month's samples, had their body weight measured, completed a food frequency questionnaire and received study debriefing.

The first seventeen participants were asked for a maximum of nine breast milk samples during the first three-months of breastfeeding. Each participant provided up to three samples at the end of their first, second, and third month of breastfeeding for evaluation of day-to-day and month-to-month variation in PBDE levels. To evaluate day-to-day variation in PBDE levels, participants provided one sample from three successive mornings at the end of months one, two and

three. Day-to-day variation was analyzed on the first five participants. Month-to-month variation in PBDE levels was evaluated on ail participants by taking one sample at the end of months one, two, and three. The first day sample was taken for month-to-month variation from those participants who provided three samples per month.

Breast Milk Expression and Collection

Participants used the Ameda Purely Yours® personal use electric breast pump to collect their samples. One sample was the equivalent of one complete expression from one breast. The collected volume of breast milk samples differed from participant to participant based upon the mother's milk supply. An emphasis was placed on collecting a complete expression to ensure that hind milk was included in the sample, which enabled us to examine lactation-specific maternal characteristics (e.g. percent lipid content in breast milk) on whole-weight PBDE levels in breast milk Participants were instructed to wash their hands and breast with a mild, detergent-free soap prior to milk collection (Appendix D). In addition, milk expression occurred between the hours of 6 a.m. and 9 a.m. on the determined collection day with a minimum two hour time frame since the previous feeding from that breast; this protocol standardized the sample collection period to reflect the daily average lipid concentration in breast milk. Once milk expression was completed, participants were instructed to detach the kit coilection container from the pump and gently invert the sample three to four times and transfer the milk into a labeled study container, which was the four ounce amber glass storage container that had been detergent-washed

and then rinsed with analytical grade solvents in the laboratory (toluene followed by hexane). Participants completed the label (Appendix F), which included subject number, time of last feeding as well as date and time of milk collection and then placed the sample into the refrigerator.

Breast Milk Sample Handling and Storage

Samples were either picked up by the researchers or transported by the participants to UNH within 72 hours of expression. Samples were transported in a cooler with cooler packs or on ice to keep the samples cold. Upon arrival to UNH, the volume was measured and logged into a participant inventory spreadsheet (Appendix G), each sample was gently inverted three to four times, and an aliquot was transferred into a two ounce amber glass jar with a Teflon fitted lid. All samples were placed into the freezer (-20° C) until shipment.

Frozen samples were shipped on three separate occasions. Each two ounce amber glass jar was wrapped in a plastic storage bag and placed in their original boxes. Boxes were placed in a Styrofoam shipping cooler, packed with dry ice, and shipped overnight to Dr. Janice K. Huwe's Biosciences Research Laboratory of the U.S. Department of Agriculture in Fargo, North Dakota. Upon arrival, samples were placed in the freezer (-65° C) until analysis.

Breast Milk Sample Analysis

Analysis of each breast milk sample was performed using previously published methodology (Huwe & Smith, 2007; Ryan, 1991; U.S. EPA, 2003). Dr. Huwe's laboratory quantified 46 PBDE congeners in 150 breast milk samples by isotope dilution method.

Sample Preparation. Sample preparation required: 1). extraction of fat components and native PBDE analytes from milk samples, 2). determination of fat content in milk samples, and 3). cleanup of the samples via column chromatography.

Prior to the first step (extraction), a mixture of 10 ^{13}C-PBDE recovery standards (BDE-28, 47, 99, 100, 153, 154, 183, 197, 207, and 209) were added to each sample; the addition of the recovery standards quantitates precision and recovery of the analytical method. Selection of the recovery standards was based on the most common congeners that are typically detected in samples and standards represented each homolog group (tri, tetra, penta, hexa, hepta, octa, nona, and deca). All standards were obtained from either Wellington Laboratories (Guelph, Ontario, Canada) or Cambridge Isotope Laboratories (Andover, MA). The following amounts of each standard were added: 2 ng for BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183; 4 ng for BDE-197 and BDE-207; and 20 ng for BDE-209 (J.K. Huwe, personal communication, July 20, 2007).

The purpose of the first step was to liberate the fat components and native PBDE analytes from protein and water soluble substances. This step required approximately 2 hours per set of samples. One set of samples was 3 breast milk samples from a participant and a method blank or method spike. Each breast milk sample (5, 7, or 15 ml) was placed in a Teflon bottle and deproteinated with potassium oxalate (20 mg/g milk) (Ryan, 1991). An equal volume of breast milk to ethanol was used to disrupt the fat globule membranes. The breast milk-

ethanol mixture was extracted with an equal volume of diethyl ether:hexane (2:3, v:v). Once the layers were separated, the upper organic phases were then transferred into large tubes and concentrated on a TurboVap™ concentrator (Zymark Corporation, Hopkinton, MA). The remaining solid and aqueous phases were extracted with a volume of hexane that was equal to the original aliquot volume and centrifuged for 20 minutes. The organic phases were combined with any remaining ether:hexane and further concentrated to small volumes on the TurboVap™; the tube sides were rinsed with hexane while concentrating. Hexane was added to each concentrate; the diluted concentrate was transferred to small Teflon bottles (4 x 10 ml of hexane). The extract was washed with distilled water and dried over 20 grams of anhydrous sodium sulfate.

The second step, determining the percent lipid content of the milk samples gravimetrically, was done by evaporating and weighing aliquots of the extracted samples from step one (10 out of 100 ml). This allows us to report PBDE levels on a lipid-adjusted basis, and as needed, on a whole-weight basis. Using the lipid-adjusted basis allows for normalizing concentrations of PBDEs both within samples from one mother and among samples from many mothers (Needham & Wang, 2002). Additionally, these lipid-adjusted concentrations could be used for infant intake estimates.

Once the lipid weight was determined for each milk sample, the remaining extract was concentrated to 12 ml of hexane, in preparation for the column cleanup procedure. Column cleanup is a critical step in sample preparation as it separates the lipids and native analytes from other lipid soluble components in

the milk sample, such as steroids and vitamins (J.K. Huwe, personal communication, August 1, 2007).

Each sample was chromatographed on disposable triphasic silica gel and basic alumina oxide columns. These columns were used to remove high molecular weight non-polar and polar interferences (U.S. EPA, 2003). All chromatography was performed using an automated Power Prep instrument (Fluid Management Systems, Waltham, MA).

The column chromatography procedure, from the silica column to the alumina oxide column to the collection tube, occurred in one uninterrupted process over a 2 hour period (J.K. Huwe, personal communication, August 1, 2007). The PBDEs were eluted from the silica column on to the alumina column with hexane. The aiumina column was washed with 2% methylene chloride in hexane to remove interfering compounds and then PBDEs were eluted from the alumina oxide column to the collection tube in 125 ml of methlylene chloride/hexane (50/50) and were reduced in volume on the TurboVap™. The small volume (0.2 ml) remaining in the TurboVap™ tube was transferred to an amber vial containing dodecane (15 µl) as a keeper solvent and evaporated by a steady flow of nitrogen. Three ^{13}C-PBDE injection standards (BDE-77, 139 and 205) were added to the final volume (20 µl). The following amounts of each injection standard were added to the final volume: 1.5 ng for BDE-77 and BDE-139, and 3 ng for BDE-205. The injection standards were added prior to high resolution gas chromatography (GC) and provided adequate coverage of the entire GC elution range; the injection standards quantitated the recovery

standards by serving as the internal standard.

Sample Analysis. Samples were analyzed for PBDEs on a Hewlett Packard 5890 GC coupled to a VG Autospec mass spectrometer (Micromass, Beverly, MA). The GC was the analytical instrument for separating the PBDEs. Cool-on-column injection was used to introduce 2 µl of each sample into the GC; this injection system was used to minimize thermal degradation of BDE-209. The sample underwent instantaneous volatilization and was transported by the carrier gas (helium) through the capillary column. Separation of PBDEs was achieved on the capillary column using a 30 m DB-5ms column (0.25 mm internal diameter, 0.25 µm film thickness) with the temperature programmed from 120°C (2 min initial hold) to 300°C at 10°C /min then held for 28 min and the carrier gas flow was pressure programmed from 20 psi (2 min initial hold) to 10 psi at 20 psi/min then to 55 psi at 2 psi/min and held for 15 min (Huwe & Smith, 2007). Isolated PBDEs eluted from the GC column and were then captured by the mass spectrometer.

The mass spectrometer (MS) was run at a resolution of 5000 in the selective ion monitoring mode to detect ion pairs (Huwe & Smith, 2007). Selective ion monitoring was used for its sensitivity with complex samples since only the selected masses were detected and plotted. The MS captured the isolated PBDEs as they eluted off of the GC column and ionized them into electrically charged particles that were transported by an electromagnetic field to the mass analyzer. The mass analyzer then sorted the ions according to their mass-to-charge ratio. The sorted ions were then detected and the signal was

sent to the data system where it was recorded in the format of a mass-to-charge ratio spectrum (McMaster & McMaster, 1998).

For identification and quantitation of PBDEs, the ratio of the paired ions was required to be within 15% of theoretical and the native congener required to co-elute within 1 sec of the ^{13}C-labeled surrogate or within 4 sec of the corresponding standard in the curve when no ^{13}C-labeled surrogate was present (Huwe & Smith, 2007). A six point standard curve was employed to cover the full range of PBDE concentrations.

Limit of Detection. The limit of detection (LOD) is the lowest concentration of a chemical that can be measured by the analytical method (Needham & Wang, 2002). The LODs for each congener were established from method blanks. A method blank or method spike (precision and recovery check) was run every fourth sample. The detection limit was set at three times the standard deviation of the blanks; all values below the detection limit were treated as nondetects (nd) and were set to zero. Data were blank subtracted because method blanks contained detectable amounts of native analytes (J.K. Huwe, personal communication, August 1, 2006).

Quality Assurance/Quality Control. The recoveries of all ^{13}C-surrogates were between 25% and 150%; all native congeners were recovery corrected. Using spiked samples, the reported native analytes showed recovery and precision to be within 20% of the true value. The Huwe laboratory has successfully participated in an international inter-lab comparison study conducted by the Norwegian Public Health and scored within +/- 30% on breast milk

analysis of the major PBDEs (J. K. Huwe, personal communication, August 1, 2006).

Laboratory Techniques. All glassware was washed by hand with detergent and rinsed with tap water, distilled water, and analytical grade solvents prior to use. Sample preparation steps were performed in opaque containers. To minimize exposure to light, samples were either covered or refrigerated between steps.

stability study

There were two unfortunate experiences with some of the breast milk samples during the research study. The first occurred at UNH when the -20°C freezer stopped working at the end of June 2006. The alarm failed to sound and the breast milk samples thawed before the problem was noticed. The second incidence occurred at Dr. Huwe's laboratory when a shipment of samples was accidentally left in the shipping box on dry ice for several days at the beginning of March 2007.

There was a surplus of nine breast milk samples that did not experience either of these incidents. These nine samples underwent a replicated temperature experience (four for the first incident and five for the second) as we wanted confirmation that lipid degradation was not accelerated during the thaw. Data showed that, on average, there was a 4.4% decrease in the percent lipid content of the nine samples, which was indicative of negligible changes. Dr. Huwe then analyzed the PBDE concentrations of three samples (one sample from incident #1 and two samples from incident #2). The average percent

change of the sum of the measured PBDE levels in the three samples was a 15% increase. The congener profiles for the three samples were further examined for differences in their levels; the percent change for the 4 major congeners that represented 73% of the total sum included: a 13% increase in BDE-47, a 17% increase in BDE-153, a 22% increase in BDE-99, and a 38% increase in BDE-100. The stability data is presented in Appendix H. Based on the stability experiment, it was concluded that the temperature incidents had a minimal effect on the lipid content and PBDE concentrations in breast milk.

survey development and administration

A total of four surveys were compieted by the participants during their three-month commitment in the study: A Maternal Characteristics Survey, two Food Frequency Questionnaires, and a Maternal Environment Survey.

Maternal Characteristics Survey

i administered the Maternal Characteristics Survey during visit one (Appendix I). The intent of this survey was to examine potential relationships between PBDE levels in breast milk and personal characteristics such as demographics, measured body weight, calculated body mass index (BMI), breastfeeding history, physical activity level, health history and smoking status. During the development phase of the Maternal Characteristics Survey, feedback was obtained from academic scholars whose expertise was in epidemiology and sociology. Suggestions focused on wording of survey questions to ensure readability and clarity. It was also recommended that we collect demographic information, particularly on education and income. This would permit our

findings to be compared to statewide data from the New Hampshire Department of Health and Human Services (2002).

Food Frequency Questionnaire

The Food Frequency Questionnaire was administered twice during the study, as pregnancy and the postpartum period can cause changes in dietary behavior (Devine et al., 2000; George et al., 2005). Nutrient data was collected using a food frequency questionnaire developed by the Nutrition Assessment Shared Resource (NASR) of Fred Hutchinson Cancer Research Center (FHCRC) (Patterson et al., 1999) (Appendix J). Dietary intake during the last trimester of pregnancy was evaluated at visit one, and dietary intake during the three-month postpartum period was evaluated at visit four. I assisted participants in completing the questionnaire by asking each question and filling out the form while the participant followed along with their own copy. I explained the format of the questionnaire and used food models to assist participants with estimating portion sizes. All completed food frequency questionnaires were sent to FHCRC for a comprehensive analysis of 125 nutrients.

The nutrient data that was extracted from the FHCRC analysis included the following: total calories; grams of carbohydrate, total fiber, water-soluble fiber, insoluble fiber, protein, vegetable protein, animal protein, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, *trans*-fatty acids, and alcohol; and milligrams of cholesterol. In addition, 27 questions were selected to quantify the frequency and amount of meat, fish, egg, dairy product, fruit, vegetable, and fat/oil consumption.

Maternal Environment Survey

A Maternal Environment Survey was completed by each participant on their own time in their home (Appendix K). The intent of the Maternal Environment Survey was to evaluate for associations between PBDE levels in breast milk and living/occupational environment. The survey contained questions on residence history, subject occupation, details of transportation mode, household appliances, furniture, electronics, and other PBDE-containing products. Dr. Tom Webster (personal communication, July 26, 2005) from Boston University School of Public Health shared his survey from the Greater Boston PBDE Body Burden Project, which assisted in developing the content of our Maternal Environment Survey.

A UNH epidemiologist provided guidance during the development phase of the Maternal Environment Survey. The primary suggestion was to validate the survey. it was beyond the scope of this research to implement sensitivity and specificity testing on the survey, but feasible to conduct a pilot-test with a similar population of participants to optimize content, readability and range of responses.

The Maternal Environment Survey underwent a pilot-test with women who were currently lactating or who had breastfed within the past year. A total of 19 surveys were distributed to New Hampshire women during the fall of 2005. Women were asked to complete the Environment Survey in their home, and fill out a feedback questionnaire (Appendix L). Out of 19 surveys, 16 (84%) surveys were completed and returned. Range of responses was reviewed on all completed surveys. This provided valuable insight on the readability, clarity and

interpretation of survey questions. Completed surveys were evaluated for missing data and if information was filled out correctly. A wide range of responses for one question on stereo equipment led us to re-word this question for improved clarity.

In addition to filling out the survey for the pilot-test, women completed a feedback questionnaire in which additional comments on survey questions, length of time to complete the survey and suggestions for improvement were recorded. Comments from participants on the feedback questionnaire provided additional insight on survey questions that required refinement of language and response options. The average length of time to complete the survey was 15 minutes, and participants agreed that completing the survey in the home was helpful. Overall, women found the survey easy to follow with minor suggestions for improvement. The preliminary work for the Maternal Environment Survey provided valuable feedback, and the necessary changes were implemented prior to recruitment.

statistical analysis

PBDE data was log-transformed prior to statistical analyses, and normality of PBDE concentrations was assessed using normal probability plots and Shapiro-Wilk's test. PBDE concentrations in breast milk (monthly means and three-month means) were evaluated for associations with dietary habits (last trimester and postpartum), maternal characteristics, and living/occupational environments. Potential associations were explored using scatter plots, correlation analysis, and backward regression. Differences in dietary intake from

the last trimester and three-month postpartum period were evaluated using paired samples t-test. Differences in mean PBDE values over time and mean percent lipid levels from months one, two, and three were evaluated using repeated-measures analysis of variance. Regression analysis was used to evaluate the relationship between PBDE levels in breast milk and maternal characteristics, living/occupational environments, and dietary intake. Potential confounders were evaluated for associations with exposure variables, and by examining the changes in the β coefficients when excluded variables were added back into the reduced model. If the addition of the excluded variable caused a change in the β coefficient by at least 10%, the variable remained in the model (Friis & Sellers, 2004; Sjödin et al., 2008). Regression coefficients were converted back to the original scale, which provides the fold change (or percent increase or decrease) in breast milk PBDE concentrations per unit of exposure. Statistical analyses were performed using the Statistical Package for Social Science (SPSS) 13.0 version with statistical significance set at 0.05.

CHAPTER IV

MANUSCRIPT 1: BIOMONITORING BREAST MILK POLYBROMINATED DIPHENYL ETHERS AS A FUNCTION OF ENVIRONMENT, DIETARY INTAKE, AND DEMOGRAPHICS OF LACTATING WOMEN IN THE SEACOAST AREA OF NEW HAMPSHIRE

introduction

Biological monitoring, or biomonitoring is the measurement of environmental chemicals, their metabolites, or their reaction products in the human body, specifically through blood, urine, saliva, breast milk, adipose or other tissue (Needham et al., 2007; U.S. DHHS, CDC, 2005). The purpose of biomonitoring is to document, update and expand human exposure information on environmental chemicals. The use of breast milk as a biological matrix for biomonitoring studies can estimate environmental chemical exposure in both the mother and breast feeding infant (Lovelady et al., 2002). Breast milk can be a convenient and noninvasive means for estimating environmental chemical exposures in nursing women as compared to other biological matrices (Hooper & She, 2003); it also enables researchers to collect a biological specimen with a high fat content (Fenton et al., 2005), which is an important consideration for lipid-soluble environmental chemicals – particularly those that have been steadily rising in their production, use, and presence in the environment and in humans, such as synthetic flame retardants.

Flame retardants are added to materials either during or after manufacturing, in order to interfere with the combustion process (Bromine Science and Environmental Forum [BSEF], 2000). This interference can occur during heating, decomposition, ignition or flame spread. Of the four major classes of flame retardants - halogenated organic (usually brominated or chlorinated), phosphorus-containing, nitrogen-containing, and inorganic flame retardants, the brominated flame retardants (BFRs) are the most widely used due to their low cost and high performance efficiency. (Birnbaum & Staskal, 2004; BSEF, 2000). The most widely used BFRs are tetrabromobisphenol A, hexabromo-cyclododecane, and three commercial products of polybrominated diphenyl ethers (PBDEs) known as decabromodiphenyl ether (decaBDE), octabromodiphenyl ether (octaBDE) and pentabromodiphenyl ether (pentaBDE) (Birnbaum & Staskal, 2004; BSEF, 2000). The total U.S. market demand values for pentaBDE and octaBDE are expected to drop to zero because Great Lakes Chemical Corporation, the sole U.S. manufacturer of pentaBDE and octaBDE, discontinued production of these two commercial products at the end of 2004 (U.S. EPA, 2006).

PBDEs are non-chemically bound additives, and may contribute up to 30% by weight of foams, fabrics and plastics (Hale et al., 2003; Hooper & She, 2003). Over time, PBDEs can diffuse out of their products, become airborne or enter dust, and disperse into the environment and into humans. Even though the presence of PBDEs in consumer products such as television sets, carpet padding, mattresses, chair and couch foams, computers, stereos, and hair dryers

saves lives each year in the U.S., there are concerns with its widespread use and increasing environmental and human concentrations. Their persistent, lipid-soluble characteristics permit PBDEs to bioaccumulate as they are released from consumer products into the air through normal use, degradation, disposal, and recycling. Additional release can also occur during PBDE manufacturing and incorporation into products (Hale et al., 2003).

Biomonitoring PBDEs through breast milk has enabled researchers to document levels worldwide (Akutsu et al., 2003; Bi et al., 2006; Chao et al., 2007; Erdoğrul et al., 2004; Eslami et al., 2006; Fängström et al., 2005; Gómara et al., 2007; Ingelido et al., 2007; Jaraczewska et al., 2006; Kalantzi et al., 2004; Kazda et al., 2004; Lind et al., 2003; Main et al., 2007; Meironyté et al., 1999; Norén & Meironyté, 2000; Ohta et al., 2002; Ryan & Patry, 2000; Ryan et al., 2006; Schuhmacher et al., 2007) and in the U.S. (Hooper et al., 2007; Johnson-Restrepo et al., 2007; Lunder & Sharp, 2003; Schecter et al., 2005; Schecter et al., 2003; She et al., 2007; Wu et al., 2007). One study in the U.S. has reported rates of PBDE elimination in a small sample of nursing mothers from California (Hooper et al., 2007). Additionally, breast milk biomonitoring studies provide information about sources of PBDE exposure.

In the U.S. a limited number of studies have demonstrated relationships between PBDE levels in breast milk and house dust, dairy intake, and meat intake (Wu et al., 2007). There is a need to further document PBDE levels in humans, examine PBDE levels in breast milk over time, and further evaluate potential sources of PBDE exposure.

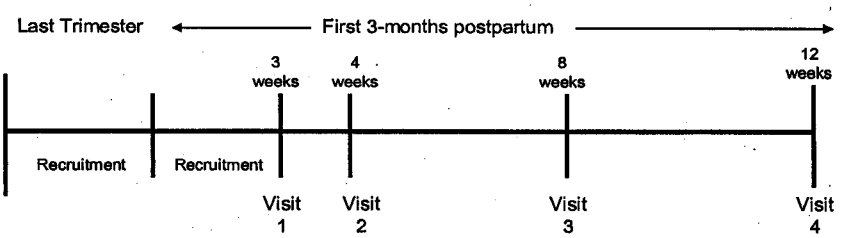
The goal of this research was to determine the levels of PBDEs in breast milk of lactating women from the Seacoast region of New Hampshire and to examine potential relationships between breast milk PBDE levels and stage of lactation, maternal characteristics, living environment and dietary intake.

materials & methods

Participants were recruited from the Seacoast region of New Hampshire from November 2005 to July 2006. Recruitment was accomplished by lactation consultants from three area hospitals, distribution of a communication pamphlet, and word of mouth. Women, ages to 22 to 40 from the Seacoast region of New Hampshire, who were in the early stages of lactation (less than 2 weeks) or in their last trimester of pregnancy and planned to breastfeed for at least the first three-months postpartum, were eligible for the study. Participant eligibility criteria also included delivering a full-term, healthy infant and planning to provide greater than 80% of breast milk as the primary source of nutrition for the first three-months. The study protocol was approved by the University of New Hampshire (UNH) Institutional Review Board (IRB # 3433) and approval from each hospital was obtained prior to working with the lactation consultants. All participants provided informed consent prior to their enrollment into the study.

Study Design

Participants were asked to visit with researchers on four occasions over a three-month period for a total of approximately four hours (Figure 1). Researchers could meet with participants in their homes or at UNH. To show our appreciation for participation in the study, enrolled subjects were provided with an Ameda Purely Yours® personal use electric breast pump and kit. Participants had the opportunity to talk with a professional lactation consultant during their enrollment in the study.



Note:
Visit 1: Study orientation, obtained consent, administered Maternal Characteristics Survey & Food Frequency Questionnaire #1, measured body weight, provided personal use electric breast pump and kit
Visit 2: Sample pick-up (1 or 3 samples), provided Environment Survey
Visit 3: Sample pick-up (1 or 3 samples), retrieved Environment Survey
Visit 4: Sample pick-up (1 or 3 samples), administered Food Frequency Questionnaire #2, measured body weight, study debriefing

Figure 1. Study design from recruitment to visit 4.

Surveys

A total of four surveys were completed by the participants during their three-month commitment in the study: A Maternal Characteristics Survey, two Food Frequency Questionnaires, and a Maternal Environment Survey. All participants were interviewed in person for each survey with the exception of the Maternal Environment Survey. The Maternal Characteristics Survey examined potential relationships between PBDE levels in breast milk and personal characteristics such as demographics, measured body weight, calculated body

mass index (BMI), breastfeeding history, physical activity level, health history and smoking status.

The Food Frequency Questionnaire, developed by the Nutrition Assessment Shared Resource (NASR) of Fred Hutchinson Cancer Research Center (FHCRC) (Patterson et al., 1999) was administered twice during the study – at the beginning to assess dietary intake during the last trimester and at the end to assess dietary intake during the first three-months postpartum. The two time periods were assessed as pregnancy and the postpartum period can cause changes in dietary behavior (Devine et al., 2000; George et al., 2005). We worked with participants by completing the questionnaire with them on both occasions, and showed food models to assist in estimating portion sizes. All completed food frequency questionnaires were sent to FHCRC for a comprehensive analysis of 125 nutrients. The nutrient data that was extracted from the FHCRC analysis included the following: total calories; grams of carbohydrate, total fiber, water-soluble fiber, insoluble fiber, protein, vegetable protein, animal protein, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, *trans*-fatty acids, and alcohol; and milligrams of cholesterol. In addition, 27 questions were selected to quantify the frequency and amount of meat, fish, egg, dairy product, fruit, vegetable, and fat/oil consumption.

The Maternal Environment Survey evaluated associations between PBDE levels in breast milk and living/occupational environment. The survey contained questions on residence history, subject occupation, details of transportation mode, household appliances, furniture, electronics, and other PBDE-containing

products. Prior to recruitment, the Maternal Environment Survey underwent a pilot-test with a similar population of participants to optimize content, readability and range of responses.

Breast Milk Sampling

The first seventeen participants were asked for a maximum of nine breast milk samples during the first three-months of breastfeeding. To evaluate day-to-day variation in PBDE levels, participants provided one sample from three successive mornings at the end of months one, two, and three. Day-to-day variation was analyzed on the first five participants. Month-to-month variation in PBDE levels was evaluated on all participants by taking one sample at the end of months one, two, and three. The first day sample was taken for month-to-month variation from those participants who provided three samples per month.

Participants used the Ameda Purely Yours® personal use electric breast pump to collect their samples. One sample was the equivalent of one complete expression from one breast. The collected volume of breast milk samples differed from participant to participant based upon the mother's milk supply. An emphasis was placed on collecting a complete expression to ensure that hind milk was included in the sample, which enabled us to examine lactation-specific maternal characteristics (e.g. percent lipid content in breast milk) on whole-weight PBDE levels in breast milk. In addition, milk expression occurred between the hours of 6 a.m. and 9 a.m. on the determined collection day with a minimum two hour time frame since the previous feeding from that breast; this protocol standardized the sample collection period to reflect the daily average

lipid concentration in breast milk. Once milk expression was completed, participants were instructed to detach the kit collection container from the pump and gently invert the sample three to four times and transfer the milk into a labeled study container, which was a four ounce amber glass storage container that had been detergent-washed and then rinsed with analytical grade solvents (toluene followed by hexane). Participants labeled the amber glass storage container and placed the sample into the refrigerator.

Samples were either picked up by the researchers or transported to UNH within 72 hours of expression. Samples were transported in a cooler with cooler packs or on ice to keep the samples cold. Upon arrival to UNH, the volume was measured, each sample was gently inverted three to four times, and an aliquot was transferred into a two ounce amber glass jar with a Teflon fitted lid. All samples were placed into the freezer (-20° C) until shipment.

Analytical Methods

Frozen samples were shipped overnight to the Biosciences Research Laboratory of the U.S. Department of Agriculture in Fargo, North Dakota. Upon arrival, samples were placed in the freezer (-65° C) until analysis. Analysis of each breast milk sample was performed using previously published methodology (Huwe & Smith, 2007; Ryan, 1991; U.S. EPA, 2003). Forty-six PBDE congeners in 150 breast milk samples were measured by isotope dilution method.

Prior to extraction, a mixture of 10 ^{13}C-PBDE recovery standards (BDE-28, 47, 99, 100, 153, 154, 183, 197, 207, and 209) were added to each sample. All standards were obtained from either Wellington Laboratories (Guelph, Ontario,

Canada) or Cambridge Isotope Laboratories (Andover, MA). Each breast milk sample (5, 7, or 15 ml) was deproteinated with potassium oxalate (20 mg/g milk). An equal volume of breast milk to ethanol was used to disrupt the fat globule membranes. The breast milk-ethanol mixture was extracted with an equal volume of diethyl ether:hexane (2:3, v:v). Once the layers were separated, the upper organic phases were then transferred into large tubes and concentrated on a TurboVap™ concentrator (Zymark Corporation, Hopkinton, MA). The final extract was washed with distilled water and dried over 20 grams of anhydrous sodium sulfate. After solvent evaporation, the lipid content of the milk samples was determined gravimetrically. Once the lipid weight was determined for each milk sample, the remaining extract was concentrated to 12 ml of hexane, in preparation for the column cleanup procedure.

Each sample was chromatographed on disposable triphasic silica gel and basic alumina oxide columns. All chromatography was performed using an automated Power Prep instrument (Fluid Management Systems, Waltham, MA). The final volume (0.2 ml) was transferred to an amber vial containing dodecane (15 µl) as a keeper solvent and evaporated by a steady flow of nitrogen. Three ^{13}C-PBDE injection standards (BDE-77, 139 and 205) were added to the final volume (20 µl) prior to high resolution gas chromatography (GC).

Samples were analyzed for PBDEs on a Hewlett Packard 5890 GC coupled to a VG Autospec mass spectrometer (Micromass, Beverly, MA). Cool-on-column injection was used to introduce 2 µl of each sample into the GC. Separation of PBDEs was achieved on the capillary column using a 30 m DB-

5ms column (0.25 mm internal diameter, 0.25 μm film thickness) with the temperature programmed from 120°C (2 min initial hold) to 300°C at 10°C /min then held for 28 min and the carrier gas flow was pressure programmed from 20 psi (2 min initial hold) to 10 psi at 20 psi/min then to 55 psi at 2 psi/min and held for 15 min. Isolated PBDEs eluted from the GC column and were then captured by the mass spectrometer. The mass spectrometer (MS) was run at a resolution of 5000 in the selective ion monitoring mode to detect ion pairs (Huwe & Smith, 2007).

For identification and quantitation of PBDEs, the ratio of the paired ions was required to be within 15% of theoretical and the native congener required to co-elute within 1 sec of the ^{13}C-labeled surrogate or within 4 sec of the corresponding standard in the curve when no ^{13}C-labeled surrogate was present (Huwe & Smith, 2007). A six point standard curve was employed to cover the full range of PBDE concentrations.

The limit of detection (LOD) for each congener was established from method blanks. A method blank or method spike (precision and recovery check) was run every fourth sample. The detection limit was set at three times the standard deviation of the blanks; all values below the detection limit were treated as non-detects (nd) and were set to zero. Data were blank subtracted because method blanks contained detectable amounts of native analytes (J.K. Huwe, personal communication, August 1, 2006).

The recoveries of all ^{13}C-surrogates were between 25% and 150%; all native congeners were recovery corrected. Using spiked samples, the reported

native analytes showed recovery and precision to be within 20% of the true value. The Huwe laboratory has successfully participated in an international inter-lab comparison study conducted by the Norwegian Public Health and scored within +/- 30% on breast milk analysis of the major PBDEs (J. K. Huwe, personal communication, August 1, 2006).

All glassware was washed by hand with detergent and rinsed with tap water, distilled water, and analytical grade solvents prior to use. Sample preparation steps were performed in opaque containers. To minimize exposure to light, samples were either covered or refrigerated between steps.

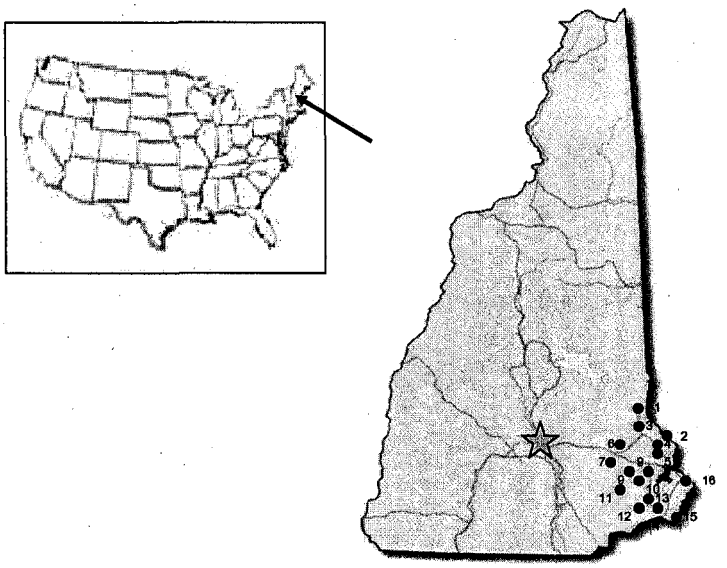
Statistical Analysis

PBDE data was log-transformed prior to statistical analyses, and normality of PBDE concentrations was assessed using normal probability plots and Shapiro-Wilk's test. PBDE concentrations in breast milk (monthly means and three-month means) were evaluated for associations with dietary habits (last trimester and postpartum), maternal characteristics, and living/occupational environments. Potential associations were explored using scatter plots, correlation analysis, and backward regression. Differences in dietary intake from the last trimester and three-month postpartum period were evaluated using paired samples t-test. Differences in mean PBDE values over time and mean percent lipid levels from months one, two, and three were evaluated using repeated-measures analysis of variance. Regression analysis was used to evaluate the relationship between PBDE levels in breast milk and maternal characteristics, living/occupational environments, and dietary intake. Potential confounders were evaluated for associations with exposure variables, and by examining the changes in the β coefficients when excluded variables were added back into the reduced model. If the addition of the excluded variable caused a change in the β coefficient by at least 10%, the variable remained in the model (Friis & Sellers, 2004; Sjödin et al., 2008). Regression coefficients were converted back to the original scale, which provides the fold change (or percent increase or decrease) in breast milk PBDE concentrations per unit of exposure. Statistical analyses were performed using the Statistical Package for Social Science (SPSS) 13.0 version with statistical significance set at 0.05.

results

Demographic Characteristics of Participants

A total of 63 women expressed an interest in the study; however, only 42 women were eligible. Two of the 42 women were unable to participant due to limited milk supply at the start of the study. A total of 40 women completed the study with an average age of 31 years (range 22 to 40). Participants' current residential locations represented 16 different towns from the Seacoast area of New Hampshire (Figure 2). The highest percentage (25%) of participants resided in Dover, New Hampshire.



Note. Town locations on the map are: Milton (1), Somersworth (2), Rochester (3), Rollinsford (4), Dover (5), Barrington (6), Nottingham (7), Madbury (8), Lee (9), Newmarket (10), Epping (11), Exeter (12), Stratham (13), Hampton Falls (14), Seabrook (15), Portsmouth (16).

Figure 2. Breast milk sampling locations in New Hampshire

There was limited diversity among participants: thirty-nine out of 40 participants were Caucasian, and 80% of participants had completed a 4-year college degree or higher (Table 1).

Each participant visited with researchers on four occasions over a three-month period for a total of 160 visits. Participants were provided with the option to conduct visits in their homes or at UNH: a total of 153 visits (96%) were conducted at participant homes and 7 visits were at UNH.

Table 1.

Participant characteristics of study population

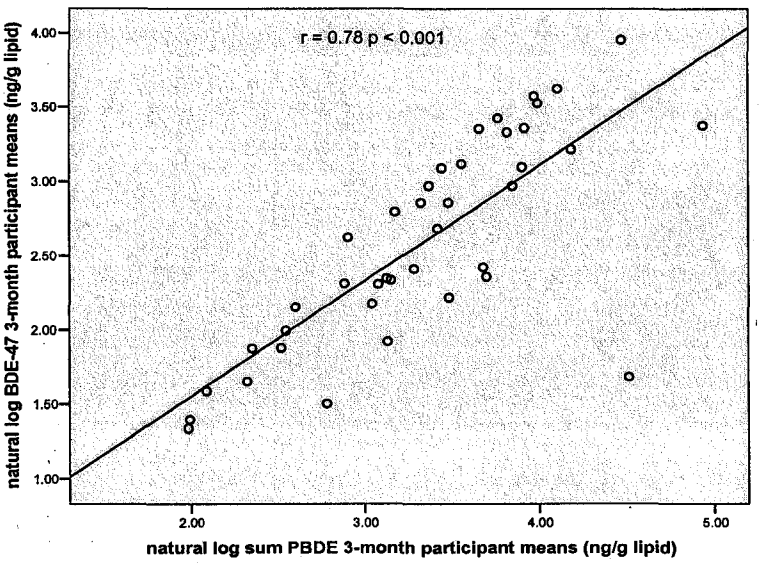
Characteristics	n = 40	
Maternal age, years (mean, SD)	31 ± 4.5	range 22 to 40
Calculated BMI (kg/m^2), start[a] (mean, SD)	27.3 ± 4.1	range 20.8 to 35.9
Calculated BMI (kg/m^2), end[b] (mean, SD)	26.5 ± 4.4	range 19.2 to 36.6
Ethnicity (%)		
Caucasian	97.5	
Asian	2.5	
Level of Education (%)		
Less than high school graduate	0	
High school diploma or GED	2.5	
Some college or technical school	15	
College graduate, 2-year degree	2.5	
College graduate, 4-year degree	42.5	
Masters degree	32.5	
Doctoral degree	5	
Total Household Income (%)		
Less than $20,000	2.5	
$20,000 to $34,999	2.5	
$35,000 to $49,000	2.5	
$50,000 and higher	92.5	
Smoking Status (%)		
Current smoker	0	
Ex-smoker	10	
Lives with smoker	2.5	
First time mothers (%)	77.5	
Parity (years)	1.4	
Exclusively breast feeding, start[c] (%)	87.5	
Exclusively breast feeding, end[d] (%)	80	
Vegetarians[e] (%)	12.5	

Note. [a] Weight measured at the start of the study which was at 2 to 3 weeks postpartum; [b] Weight measured at the end of the study which was at 12 weeks postpartum; [c] Start was 2 to 3 weeks postpartum; [d] End was 12 weeks postpartum; [e] One vegan and 4 semi-vegetarians (2 excluded red meat; 2 excluded red meat and poultry).

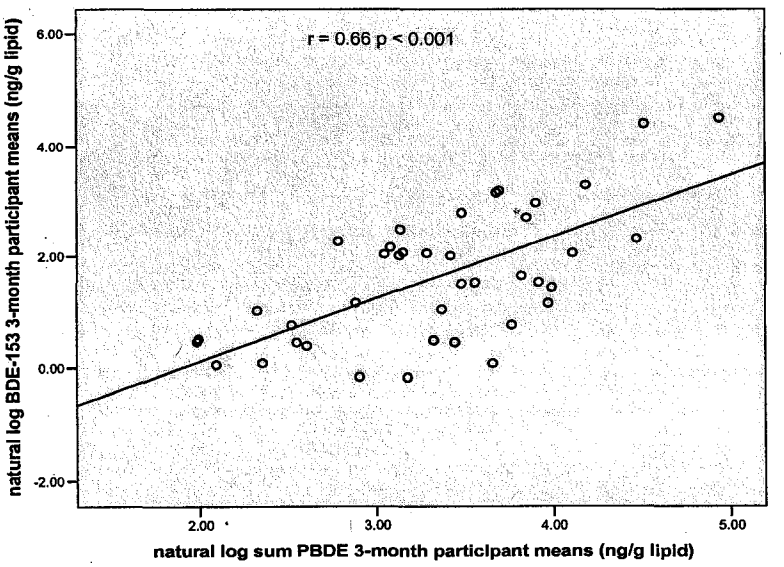
PBDE Levels in Breast Milk

PBDE levels were reported with non-detects = zero. BDE-209 levels were widely variable due to background contamination as detected in method blanks. The detection limit range for BDE-209 was 2.2 to 38.4 ng/g lipid (mean = 12 +/- 9 ng/g lipid). Therefore, BDE-209 was not included in the sum of PBDEs (∑PBDE). The ∑PBDE congeners found in breast milk was defined as: BDE-28/33, 47, 85, 99, 100, 153, 154, and 183. BDE-47 was the predominant congener for each month and correlated with ∑PBDEs ($r = 0.78$ $p < 0.001$). The contribution of BDE-47 to ∑PBDEs increased as the sum increased (Figure 3a). BDE-47 was correlated with BDE-28/33, BDE-85, BDE-99, BDE-100, and BDE-154; however, BDE-47 was not correlated with BDE-153 and BDE-183 (Table 2). BDE-153 was correlated with ∑PBDEs ($r = 0.66$ $p < 0.001$) and BDE-100; the contribution of BDE-153 to the ∑PBDEs increased as the sum increased (Figure 3b). Log-transformed ∑PBDEs, BDE-47, and BDE-153 levels were evaluated over time (stage of lactation) and for associations with maternal characteristics, dietary habits, and living/occupational environments.

(a)



(b)



Figures 3a and 3b: Correlations between breast milk concentrations of the ∑PBDE and BDE-47 (a) and BDE-153 (b)

Table 2.
Spearman correlation coefficients and corresponding *p* values for PBDE congeners

Congener	BDE-28/33 Correlation (*p* value)	BDE-47 Correlation (*p* value)	BDE-85 Correlation (*p* value)	BDE-99 Correlation (*p* value)	BDE-100 Correlation (*p* value)	BDE-153 Correlation (*p* value)	BDE-154 Correlation (*p* value)
BDE-28/33	1	**0.93 (< 0.001)**	**0.44 (0.004)**	**0.78 (< 0.001)**	**0.77 (< 0.001)**	0.18 (0.286)	**0.72 (< 0.001)**
BDE-47		1	**0.53 (< 0.001)**	**0.91 (< 0.001)**	**0.83 (< 0.001)**	0.13 (0.43)	**0.77 (< 0.001)**
BDE-85			1	**0.66 (< 0.001)**	**0.51 (0.001)**	0.038 (0.814)	**0.72 (< 0.001)**
BDE-99				1	**0.71 (< 0.001)**	0.091 (0.576)	**0.79 (< 0.001)**
BDE-100					1	**0.457 (0.003)**	**0.84 (< 0.001)**
BDE-153						1	0.23 (0.162)
BDE-154							1

Note. Correlation analysis was performed on untransformed participant three-month mean PBDE congener values; correlation coefficients and *p* values presented on all congeners with greater than a 60% detection.

Stage of Lactation and Sum of PBDEs: There was no significant difference in mean ∑PBDE levels from months one, two, and three, which was a similar finding to other research (Table 3) (Hooper et al., 2007), thus confirming that PBDE levels in breast milk are stable for the first three-months postpartum. The relationship between ∑PBDE levels and time did not change based on age, BMI, or number of other prior children breastfed. The ∑PBDE concentrations in breast milk over the three-month collection period ranged from 6.5 to 166.7 ng/g lipid. The mean for the three-month period was 35.5 ng/g with a median value of 29.7 ng/g lipid.

Table 3.

∑PBDE levels in breast milk from months one, two, and three

Collection Period n = 40	Minimum ng/g lipid	Maximum ng/g lipid	Mean ng/g lipid	Median ng/g lipid
Month 1	6.8	166.7	37.3[a]	28.6
Month 2	6.6	133.7	35.1[a]	28.1
Month 3	6.5	118.4	34.1[a]	32.8

Note. [a] No significant differences between months 1, 2, and 3 means.

Day-to-day variation in PBDE leveis was analyzed on the first five participants (Figure 4). A total of 45 breast milk samples (9 samples per participant) were analyzed. The variation from day-to-day revealed that 41 out of 45 samples had less than a 20% change in the ∑PBDE concentrations from day one to day two, day two to day three, and day one to day three. The largest variation occurred in only 2 of the 5 samples during month two, day two where the percent change was greater than 40%. Because methodological variation was 20% (J.K. Huwe, personal communication, June 23, 2006), it was concluded that day-to-day variation was minimal. From this point forward, it was decided to only collect and analyze one sample per month from each participant.

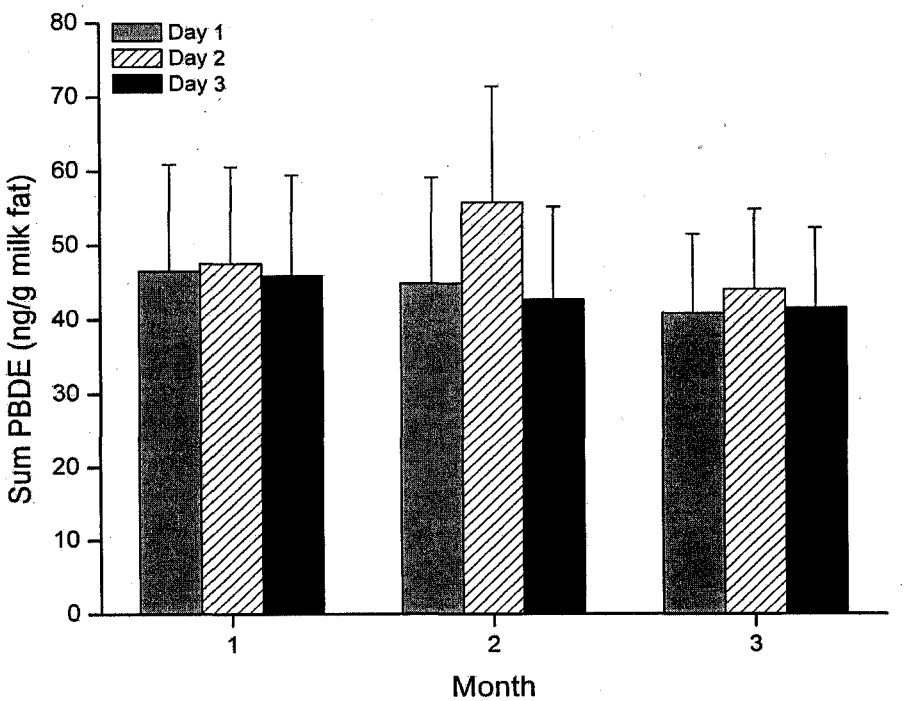


Figure 4. Mean day-to-day variation for months one, two, and three.

Stage of Lactation and Congeners. The median congener pattern of breast milk samples from month one was 47% BDE-47, 17% BDE-153, 9% BDE-100, 8% BDE-99, and 19% other congeners. The average congener pattern from month two was 44% BDE-47, 15% BDE-153, 9% BDE-99, 7% BDE-100, and 25% other congeners. Lastly, the average congener pattern from month three was 38% BDE-47, 14% BDE-153, 8% BDE-99, 6% BDE-100, and 34% other congeners. There were no significant differences in mean BDE-47, BDE-153, or BDE-100 levels from months one, two, and three. The relationship between the congener levels and time did not change based on age, BMI, or number of other prior children breastfed.

BDE-47 was the predominant congener in 80% of the samples, which is similar to previously reported studies. However, BDE-153 was the predominant congener in 20% of the participants' samples from each month. It was also noted that the median BDE-153 contribution to the ∑PBDEs was higher as compared to some of the previously reported breast milk biomonitoring studies in the U.S. (15% vs. 5 to 10% of ∑PBDEs) (Johnson-Restrepo et al., 2007; Schecter et al., 2003; She et al., 2007; Wu et al., 2007). A comparison of the PBDE congener profiles from month-to-month is displayed in Figure 5, and congener levels are provided in Table 4; PBDE levels in individual breast milk samples from each month are reported in Appendix H.

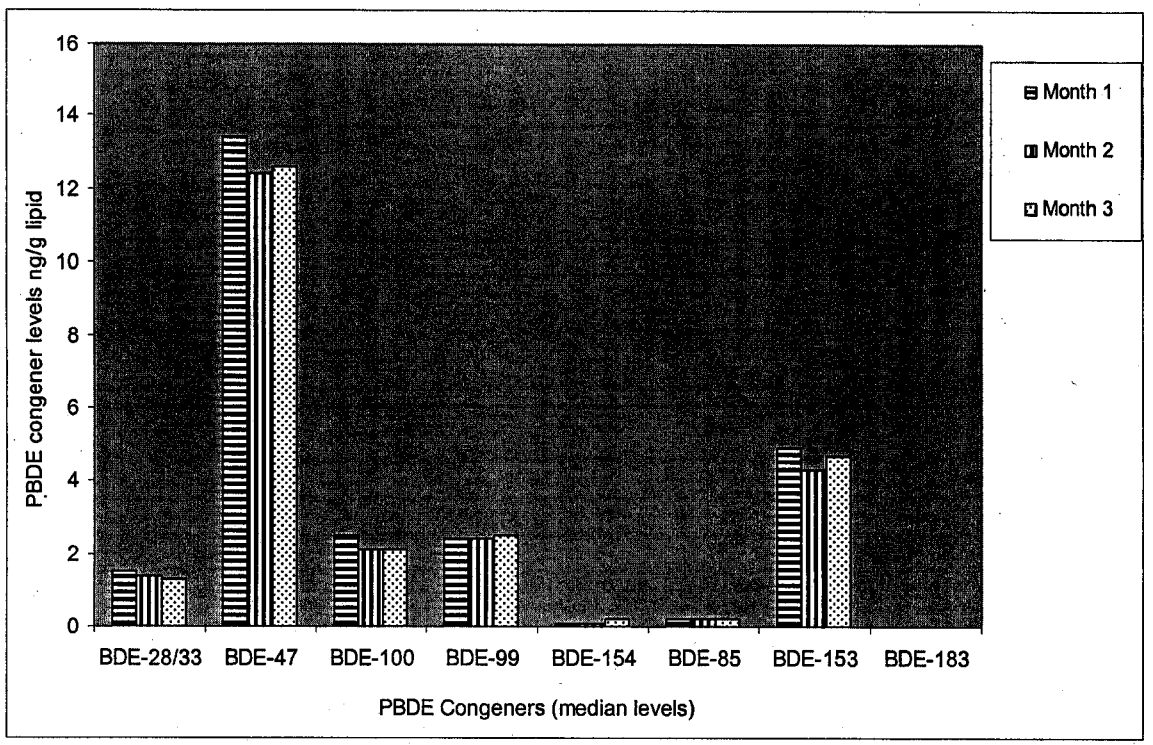


Figure 5. Comparison of PBDE congener patterns in breast milk samples (n = 40, median levels).

Table 4.

Levels of PBDEs – by congener - in breast milk samples (n = 40)

Collection Period and Congener	Median ng/g lipid	Minimum ng/g lipid	Maximum ng/g lipid	% Detection (DL)[a]
Month 1				
BDE-28 & 33	1.5	0.3	5.4	100 (0.06)
BDE-47	13.4	3.7	59.0	100 (0.83)
BDE-85	0.2	< DL	0.8	65 (0.03)
BDE-99	2.4	< DL	6.6	90 (0.65)
BDE-100	2.5	0.4	15.3	100 (0.16)
BDE-153	4.9	0.8	109.6	100 (0.08)
BDE-154	0.1	< DL	0.5	78 (0.07)
BDE-183	< DL	< DL	2.3	33 (0.16)
Month 2				
BDE-28 & 33	1.4	0.4	4.7	100
BDE-47	12.4	3.9	54.1	100
BDE-85	0.2	< DL	2.1	65
BDE-99	2.4	< DL	7.3	88
BDE-100	2.1	0.4	15.7	100
BDE-153	4.3	1.0	83.8	100
BDE-154	0.1	< DL	0.7	73
BDE-183	< DL	< DL	1.3	30
Month 3				
BDE-28 & 33	1.3	0.3	4.3	100
BDE-47	12.6	3.8	43.5	100
BDE-85	0.2	< DL	1.4	55
BDE-99	2.5	< DL	10.2	88
BDE-100	2.1	0.4	12.0	100
BDE-153	4.7	0.8	81.3	100
BDE-154	0.2	< DL	0.5	63
BDE-183	< DL	< DL	0.9	33

Note. < DL = below the detection limit; [a] the average DL values over the sample analysis time frame in ng/g lipid.

PBDE Levels in Breast Milk and Maternal Characteristics

Percent Lipid. The mean lipid content in breast milk for month one was 3.3% (range 1.6 to 5.0%), for month two was 3.3% (range 1.1 to 8.5%), and for month three was 3.0% (range 0.9 to 6.8%). There was no significant difference in mean percent lipid values from months one, two, and three. There was a significant, positive association between ∑PBDE levels (ng/g milk) and percent lipid content in breast milk samples over the three-month period ($r = 0.62$ $p < 0.001$) (Figure 6) with a similar Spearman's correlation coefficient ($r = 0.64$ $p < 0.001$). The significant, positive observation validates reporting PBDE levels on a lipid-adjusted basis.

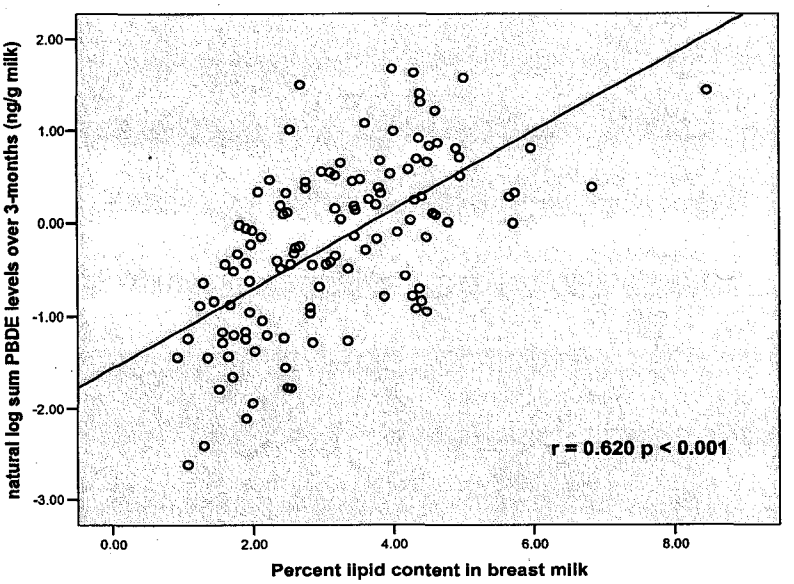


Figure 6. The association between ∑PBDE levels (ng/g milk) and percent lipid content in breast milk.

Maternal Characteristics Survey. Some of the data from the Maternal Characteristics survey indicated limited variability among participants, including education, ethnicity, total household income, and health history information. There were no associations between ∑PBDE levels and age, parity, body mass index, pre-pregnancy weight, number of other children breastfed, physical activity level, weight lost on a diet, vegetarian status, smoking history, or the estimated amount of supplementary formula provided to the infant during the study. Similar findings were also seen with congener (BDE-47 and BDE-153) levels and maternal characteristics, however, an association was seen between BDE-153 and age ($r = 0.36$ $p = 0.02$, participant three-month means). For each unit (year) increase in age, there was a 1.1- fold change (or 10% increase) in BDE-153 (Figure 7). The association between age and BDE-153 weakened after adjusting for change in BMI from the start of the study until the end ($r = 0.36$ $p = 0.07$). Change in BMI was included as a confounding variable based upon its association with age and its effect on the β coefficient (Table 5).

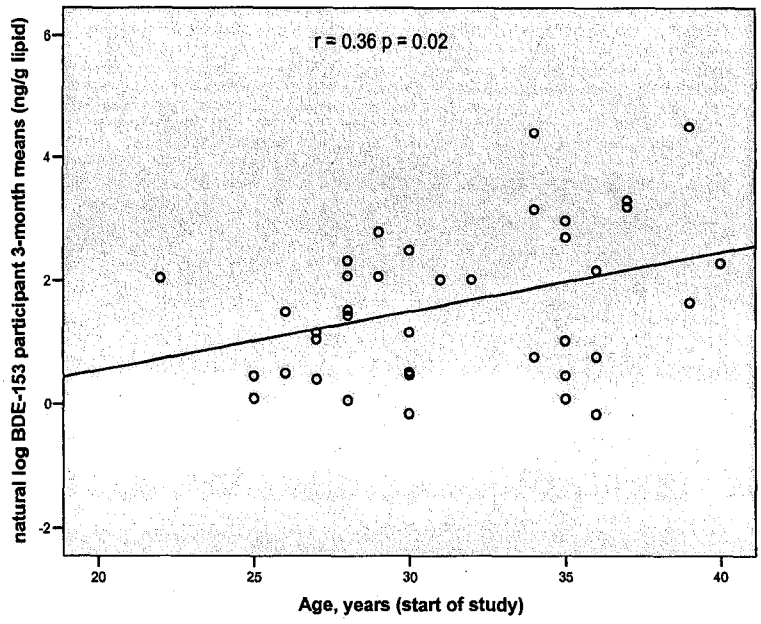


Figure 7. The association between BDE-153 levels in breast milk and age.

Table 5.

The effect of confounding on age

	Age (Exposure)	
Confounder	Spearman's Correlation Coefficient (*p* value)	Adjusted β (95% CI)
Change in BMI from start of study to end	0.45 ($p = 0.004$)	1.2[a] (1.0, 1.2)

Note. [a]Crude β was 1.1 (95% CI = 1.0 – 1.2).

PBDE Levels in Breast Milk and Dietary Intake

The transition from the last trimester of pregnancy to the three-month postpartum period was associated with decreases in mean caloric intakes (2,349 vs. 1,930 calories, $p < 0.001$) as well as decreases in major nutrients of interest, such as grams of carbohydrate (333 vs. 261 grams, $p < 0.001$), grams of fat (84 vs. 71 grams, $p < 0.012$), and grams of protein (85 vs. 71 grams, $p < 0.001$). However, out of the 25 questions selected to quantify meat, fish, egg, dairy product, and fat/oil consumption, only 5 of those questions differed significantly from the last trimester to the three-month postpartum period including: low fat hot dog and sausage consumption (0.6 vs. 3 total servings over three-months, $p < 0.031$); lean lunch meat consumption (14 vs. 22 total servings over three-months, $p < 0.049$); ground meat consumption (16 vs. 12 total servings over three-months, $p < 0.016$); butter/margarine and other fat consumption on starches and vegetables (44 vs. 23 total servings over three-months, $p < 0.009$); and milk consumption (104 vs. 62 total servings over three-months, $p < 0.004$).

There were no significant associations between PBDE levels ($\sum$, BDE-47, and BDE-153) and the majority of nutrients consumed during the last trimester or three-month postpartum period. However, there was a small, but significant association between BDE-153 and saturated fat consumption during the three-month postpartum period. For each unit (gram) increase in daily saturated fat intake, there was a 1.04-fold change (or 4% increase) in BDE-153 levels (monthly means and participant three-month means) ($r = 0.31$ $p = 0.05$, participant three-month means) (Figure 8). The association between saturated

fat intake during the postpartum period and BDE-153 persisted after adjusting for total meat consumption during the postpartum period (r = 0.41 p = 0.03, participant three-month means) (Table 6). Total meat consumption during the postpartum period was included as a confounding variable based upon its association with saturated fat and its effect on the β coefficient (Table 7). There was no association between saturated fat consumption and BDE-153 during the last trimester.

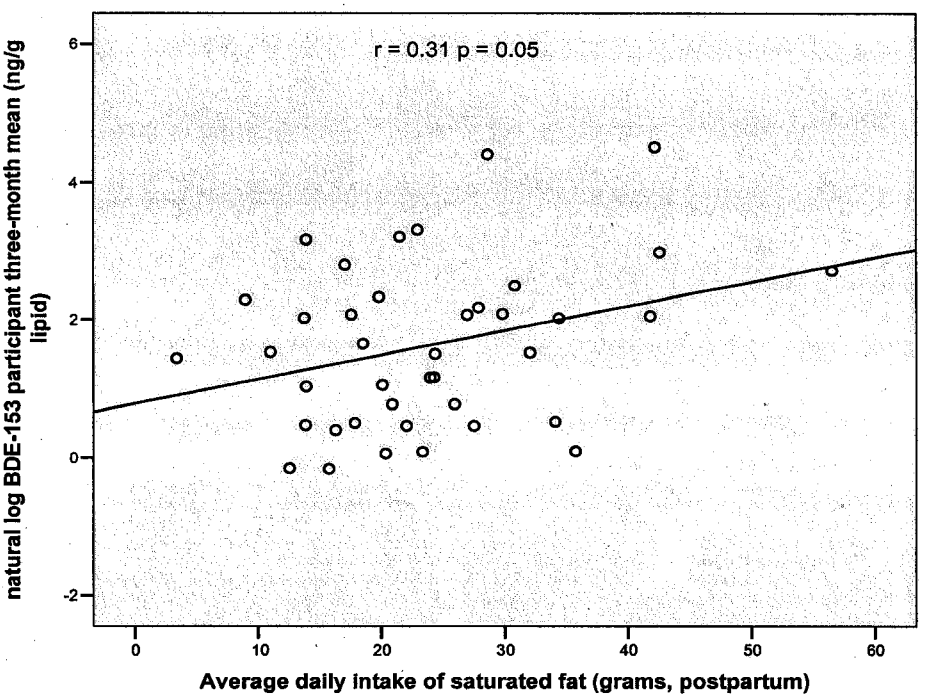


Figure 8. The association between BDE-153 levels in breast milk and consumption of saturated fat during the three-month postpartum period.

Table 6.

Association between BDE-153 and saturated fat consumption during the three-month postpartum period

	Transformed β[a]	*p* value	Adjusted β[b]	*p* value[b]
BDE-153 and saturated fat consumption	1.04	0.05	1.1	0.03

Note. [a] The β coefficients provide the fold change in BDE-153 concentrations in breast milk per unit of exposure (grams); [b] Adjusted for total meat intake in the postpartum period.

Table 7.

The effect of confounding on saturated fat consumption

	Saturated Fat Consumption (Exposure)	
Confounder	Spearman's Correlation Coefficient (*p* value)	Adjusted β (95% CI)
Total meat consumption during the postpartum period	0.41 (*p* = 0.009)	1.1[a] (1.0, 1.1)

Note. [a] Crude β was 1.04 (95% CI = 1.0 – 1.1).

The frequency of meat, fish, dairy product, egg, fat/oil, and vegetable consumption during the last trimester and three-month postpartum period was not associated with PBDE levels (∑, BDE-47, and BDE-153). However, the strongest association between ∑PBDE levels and diet was seen with total fruit consumption in the last trimester of pregnancy (Figure 9). For each unit (serving) increase in fruit, there was a 0.87-fold change (or 13% decrease) in ∑PBDE levels (monthly means and participant three-month means) ($r = 0.36$ $p = 0.02$, participant three-month means). The association persisted between fruit consumption during the last trimester and ∑PBDE levels after adjusting for total calorie and carbohydrate consumption during the last trimester ($r = 0.56$ $p = 0.02$, participant three-month means) (Table 8). Calorie and carbohydrate consumption during the last trimester were included as confounding variables based upon their association with fruit intake and their effect on the β coefficient (Table 9). Similar findings were also seen with BDE-47 and fruit consumption in the last trimester (monthly means and participant three-month means) (Table 8). The association was not seen with total fruit consumption in the three-month postpartum period.

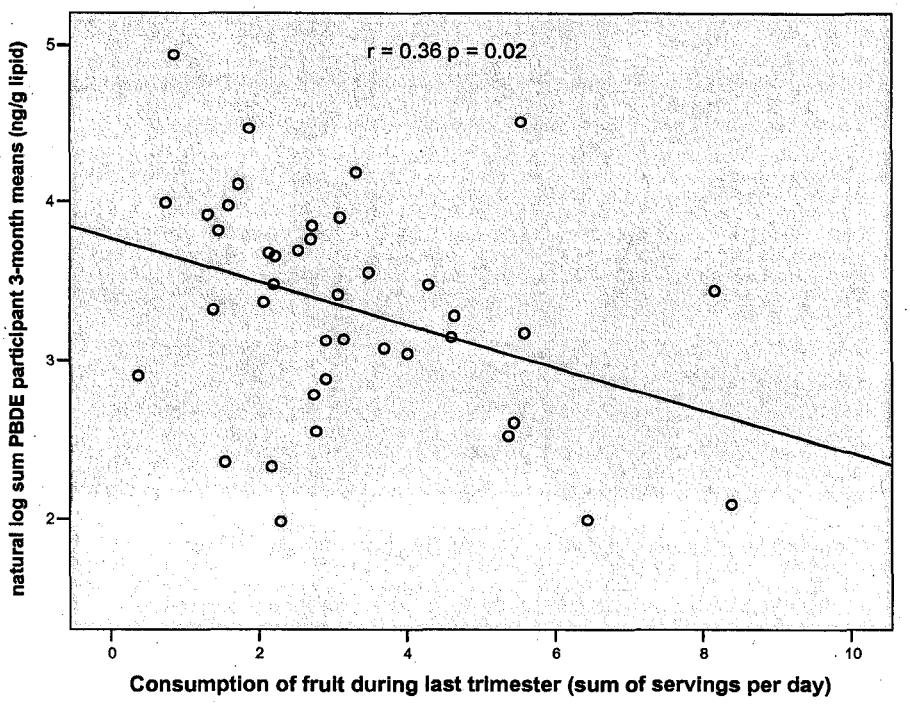


Figure 9. The association between ∑PBDE levels in breast milk and consumption of fruit during the last trimester.

Table 8.

Association between PBDEs and fruit consumption during the last trimester

	Transformed β[a]	*p* value	Adjusted β[b]	*p* value[b]
∑PBDEs and fruit consumption	0.87	0.02	0.80	0.02
BDE-47 and fruit consumption	0.86	0.01	0.80	0.02

Note. [a] The β coefficients provide the fold change in PBDE concentrations in breast milk per unit of exposure (servings); [b] Adjusted for calorie and carbohydrate consumption during the last trimester.

Table 9.

The effect of confounding on fruit consumption

Confounders	Fruit Consumption (Exposure) Spearman's Correlation Coefficient (p value)	Adjusted β (95% CI)
Calorie consumption during the last trimester	0.48 ($p = 0.002$)	0.81[a] (0.71, 0.93)
Carbohydrate consumption during the last trimester	0.56 ($p < 0.001$)	0.79[a] (0.70, 0.92)

Note. [a] Crude β was 0.87 (95% CI = 0.78 – 0.98).

PBDE Levels in Breast Milk and Maternal Environment

There were no associations between ∑PBDE levels in breast milk and geographical location (e.g. town vs. city), subject occupation, transportation (e.g. year automobile was purchased, hours per week in the car), and other possible PBDE exposures (e.g. hobbies that involved using fabric). Usage of household appliances and electronic equipment was not associated with ∑PBDE levels in breast milk, nor was their year of purchase. The sum of cushioned furniture in the home was not associated with ∑PBDE levels in breast milk, nor was the year of purchase. Similar findings were also seen with congener (BDE-47 and BDE-153) levels and living/occupational characteristics; however, an association was seen between BDE-153 and the home model ($r = 0.51$ $p = 0.004$) (Table 10), which included the total number of rooms in the home with curtains/valences and total number of rooms in the home with carpeting. To reach the final home

model, backward regression was used with a threshold of $p < 0.05$ for keeping a variable in the model. The association persisted between the home model and BDE-153 after adjusting for total rooms in the home and total months at current residence ($r = 0.53$ $p = 0.02$, participant three-month means). Total rooms in the home and months at current residence were included as confounding variables based upon their association with the home model and their effect on the β coefficient (Table 11).

Table 10.

Association between BDE-153 and the home model

Home Model	Transformed β[a]	*p* value	Adjusted β[d]	*p* value[d]
Total number of rooms with curtains/valences	1.24[b]	0.002	1.21	0.007
Total number of rooms with carpeting	0.86[c]	0.056	0.86	0.08

Note. [a] The β coefficients provide the fold change in BDE-153 concentrations in breast milk per unit of exposure (rooms); [b] indicates a fold change or 24% increase; [c] indicates a fold change or 14% decrease; [d]Adjusted for total rooms in the home and total months at current residence.

Table 11.

The effect of confounding on the home model

Confounders	**Spearman's Correlation Coefficient** (*p* value)		**Adjusted β** (95% CI)	
	Curtains	Carpet	Curtains[a]	Carpet[b]
Total rooms in home	0.29 ($p = 0.13$)	0.21 ($p = 0.19$)	1.22 (1.08, 1.41)	0.84 (0.72, 1.0)
Total months at residence	0.28 ($p = 0.07$)	-0.25 ($p = 0.11$)	1.21 (1.07, 1.40)	0.88 (0.74, 1.0)

Note. [a] Crude β was 1.24 (95% CI = 1.1 – 1.4).
[b] Crude β was 0.86 (95% CI = 0.74 – 1.0)

discussion

This was the first study to document PBDE levels in breast milk of women from the state of New Hampshire. Our study had three main findings which were: 1.) BDE-153 and its congener pattern; 2.) BDE-153 and its relationship to age, the home, and saturated fat; and 3.) PBDE levels (∑ and BDE-47) and its relationship to fruit consumption.

BDE-153: Congener Patterns

Even though BDE-47 was the predominant congener in most of our participants' breast milk samples, a predominance of BDE-153 was observed in 20% of the study population – the highest percentage in the U.S. compared to 7% (Wu et al., 2007), 8% (She et al., 2007), and 10.5% (Sjödin et al., 2008). Two European studies that have documented BDE-153 as the predominant congener in breast milk samples report high seafood consumption in their

subjects (Fängström et al., 2005; Ingelido et al., 2007); however, an association between BDE-153 and seafood was not seen with these studies. It's noted that the congener profile for seafood items in these European regions do not reflect BDE-153 as the predominant congener, which is a similar observation by Ohta and colleagues (2002) from Japan. The source of BDE-153 is uncertain at this time given the lack of association with seafood.

The predominant congener pattern (BDE-47 followed by BDE-153) from our research is similar to the recently reported National Health and Nutrition Examination Survey (NHANES) data on serum concentrations of PBDEs (Sjödin et al., 2008) and breast milk biomonitoring studies from Japan, East coast of the U.S., Pacific Northwest of the U.S. and Canada, Poland, South China, Taiwan, and United Kingdom (Bi et al., 2006; Chao et al., 2007; Kalantzi et al., 2004; Kazda et al., 2006; Ohta et al., 2002; She et al., 2007; Wu et al., 2007). Other breast milk biomonitoring studies have reported predominant congener patterns of BDE-47 followed by BDE-99 or BDE-100 (Eslami et al., 2006; Johnson-Restrepo et al., 2007; Kazda et al., 2004; Schecter et al., 2003). Earlier work from Sweden documents BDE-47 as the predominant congener followed by BDE-99 and BDE-153 (Meironyté et al., 1999). However, in 1972, only two PBDE congeners were detected in breast milk samples from Sweden (BDE-47 and BDE-153).

Correlation analysis revealed that BDE-153 had no correlation to BDE-47; however, as the ∑PBDE increased in breast milk so did each of the congeners (Figures 3a and 3b). BDE-153 is considered to be a minor component of the

pentaBDE and octaBDE commercial products (La Guardia et al., 2006), however, its estimated half-life is three times longer than that of the BDE-47 congener (6.5 years vs. 1.8 years, respectively) (Geyer et al., 2004), and it's suggested that over time, BDE-153 will become the predominant congener in the body (Sandanger et al., 2007). The apparent increase in the predominance of BDE-153 in breast milk samples from New Hampshire is suggestive of a shift in congener patterns, which could be related to when our samples were collected (2005-2006) relative to other studies (e.g. 2004-2005) (Wu et al., 2007).

BDE-153 and Age

Our finding of an association between age and BDE-153 levels is the first breast milk biomonitoring study to document this positive relationship in 22 to 40 year old women, which may be an indicator of the long half-life of this congener paired with the physiological demands of lactation on the body. However, we noted that the association between age and BDE-153 weakened when we accounted for changes in body composition due to weight changes. Sjödin and colleagues (2008) recently reported a linear decrease and positive quadratic trend with age and serum concentrations of BDE-153 with the highest levels in the 12 to 19 year old group. These findings are not necessarily comparable to ours as Sjödin et al. (2008) looked at age groups from 12 to 19, 20 to 39, 40 to 59, and 60 + years. Sjödin et al. (2008) did not stratify across their age groups, such as the 20 to 39 year olds, which was the total range for our study, and the NHANES population included both males and females.

PBDE Levels and Exposure Sources

We found a statistically significant association between the home model and BDE-153. To our knowledge, this is the first study to document a relationship between BDE-153 levels in breast milk and living environment. It is unclear from our results whether or not the household items themselves (e.g. curtains/valences) or the dust they collect are the sources of the exposure, or if there was an association between household dust concentrations and PBDE sources (e.g. furniture, curtains, electronics) as recently described by Allen et al. (2008). We were not able to make this distinction because we did not sample house dust from the homes of our participants nor did we measure bromine concentrations in consumer products.

We noted several trends with some of our living environment data. For example, we found that those who used their microwave less than 6 times per week had lower ∑PBDE levels as compared to those who used it greater than one time per day ($p = 0.086$). We also observed lower ∑PBDE levels in those with newer (2000 to present) all-in-one stereo equipment compared to older models (prior to 1999) ($p = 0.086$). Those with newer cushioned desk chairs (2000 to present) had lower ∑PBDE levels compared to those with older cushioned desk chairs (prior to 1999) (n = 18, n = 9, respectively, $p = 0.053$).

We were surprised with the limited findings between PBDE levels and dietary intake given the recent work by Wu and colleagues (2007) who reported positive associations between PBDE levels in breast milk and the consumption of dairy products and meat pre-pregnancy. Our limited findings may be attributed to

the study's small sample size and our choice of timing for collecting dietary information (two 3-month periods). Perhaps if we expanded our time frame for assessing dietary intake (e.g. throughout the entire pregnancy), we may have encountered different results, especially since the half-lives of many congeners are on the magnitude of years. However, we did find a small, but statistically significant, positive association between BDE-153 levels and saturated fat intake during the three-month postpartum period. Of interest, this finding was not seen during the last trimester. The significant finding during the postpartum period may reflect the metabolic demands that are induced on the body with the process of lactation. BDE-153 is present in foods that contain saturated fat (e.g. meat, meat fat, dairy) based on the findings from market basket surveys in the U.S. (Huwe & Larsen, 2005; Schecter et al., 2004), however, it's not a predominant congener in these food samples.

To our knowledge, this is the first study to find a statistically significant, negative relationship with food consumption and PBDE levels in breast milk. The association between fruit consumption and PBDE ($\sum$ and BDE-47) levels during the last trimester suggests that fruit may contribute less to PBDE body burden. Our finding with fruit consumption corresponds with vegan trends that were observed by Schecter et al. (2006a) where blood levels of PBDEs were lower in those participants who had longer time periods without consuming foods of animal origin. However, we were surprised with our lack of findings between vegetable consumption and PBDE levels.

Estimated Infant Intake of PBDEs from Breast Milk

In addition to determining levels of PBDEs in breast milk samples from lactating women, we were able to estimate infant exposure to PBDEs. We estimated infant intake of PBDEs using previously published guidelines (Lovelady et al., 2002); however, we did not collect information from our participants on infant weights and daily breast milk intakes. We estimated the infant weight based upon the 50^{th} percentile weight for age clinical growth charts using 4.3 kg for month one, 4.9 kg for month two, and 5.8 kg for month three (U.S. DHHS, CDC, 2001). The majority of participants in our study were exclusively breastfeeding their infants, so we assumed that the daily consumption of milk was 800 ml (Dewey et al., 1991; Kent et al., 2006).

The estimated median daily intake of PBDEs for an infant from the Seacoast region of New Hampshire at one month of age was 197 ng/kg body weight, month two was 140 ng/kg body weight, and month three was 105 ng/kg body weight. The estimated daily PBDE intake for infants from New Hampshire is higher compared to breastfed newborns from Taiwan (20.6 ng/kg body weight), one-month olds from Western Massachusetts (4 ng/kg body weight for three selected congeners), and three-month olds from Finland and Denmark (16 ng/kg body weight) (Chao et al., 2007; Johnson-Restrepo et al., 2007; Main et al., 2007). However, the estimated daily intake for PBDEs is higher in Texas (306.6 ng/kg body weight) and Canada (280 ng/kg body weight) compared to New Hampshire (Jones-Otazo et al., 2005; Schecter et al., 2006b). At this time, it is not clear as to what the implications are of estimated infant intakes of PBDEs; as

noted by Jones-Otazo et al. (2005), average intakes are below the chronic oral reference dose (RfD) that has been established by the U.S. Environmental Protection Agency (1990a; 1990b; 1995) for penta (RfD = 0.002 mg/kg body weight/day), octa (RfD = 0.003 mg/kg body weight/day), and deca (RfD = 0.01 mg/kg body weight/day).

Comparing and Contrasting Breast Milk Biomonitoring Studies from the U.S. & British Columbia

PBDE levels among studies of breast milk in the U.S. and British Columbia differ in ranges and median values with our study exhibiting the lowest maximum PBDE level (Table 12) and only one participant who had levels that were greater than 100 ng/g lipid. However, our median ∑PBDE and BDE-47 levels are similar to levels reported by Wu and colleagues (2007) from the Boston, Massachusetts area; it's noted that our median BDE-47 levels are lower compared to the NHANES data (Sjödin et al., 2008). Our study had the lowest number of congeners represented in ∑PBDEs, our sum did not include BDE-209, and our percent detection was lower for some of the major congeners (BDE-85, BDE-154, and BDE-183) compared to Wu et al. (2007) all of which could have contributed to our lower maximum levels. When comparing the median value of the predominant congener (BDE-47) in each of the breast milk biomonitoring studies, the lowest median value was from samples collected in the Western Massachusetts area (Johnson-Restrepo et al., 2007).

A common finding among studies in the U.S. as well as worldwide (Akutsu et al., 2003; Bi et al., 2006; Chao et al., 2007; Erdoğrul et al., 2004; Eslami et al.,

Table 12.

Levels of PBDEs in breast milk from the U.S. and British Columbia

Location, Authors, date of publication	n	PBDE Medians, congener sum (Range)[a, b]	# Congeners in sum	Median BDE-47 (Range)[a]	Median BDE-153 (Range)[a]
Massachusetts, Johnson-Restrepo et al. (2007)	38	19.8 (0.06 – 1910)	17[c]	7.7 (0.84 – 1100)	1.1 (0.04 – 82.3)
Pacific Northwest & British Columbia She et al. (2007)	40	50 (6.3 – 321)	12[c]	27.8 (2.6 – 201)	4.8 (0.84 – 169)
Massachusetts, Wu et al. (2007)	46	30.2 (4.2 – 263.5)	12[c]	13.9 (2 – 126.6)	3.0 (0.4 – 91.7)
Texas, Schecter et al. (2003; 2005)	59	30.1 (6.2 – 418.8)	13[c]	17.4 (2.9 – 271.5)	2.1 (0.4 – 21.8)
U.S., various states Lunder & Sharp, (2003)	20	58 (9 – 1078)	35[c]	24.8 (5.5 – 589)	10.1 (1.1 – 122)
New Hampshire, Dunn et al. (in progress)	40	29.7 (6.4 – 166.7)	8	12.6 (3.7 – 59)	4.6 (0.8 – 109.6)

Note. [a] ng/g lipid; [b] BDE-47 was the predominant congener in each study; [c]BDE-209 included in analysis

2006; Ingelido et al., 2007; Jaraczewska et al., 2006; Kalantzi et al., 2004; Kazda et al., 2004; Lind et al., 2003; Main et al., 2007; Meironyté et al., 1999; Norén & Meironyté, 2000; Ohta et al., 2002; Ryan & Patry, 2000), is the predominance of the BDE-47 congener in breast milk samples. The pentaBDE commercial mixture contained 24 to 38% of BDE-47. PentaBDE was primarily used to flame retard polyurethane foams up until its voluntary phase out at the end of 2004 (Hale et al., 2003; WHO, 1994) and BDE-47 was considered a major contributor to the commercial mixture (Birnbaum & Staskal, 2004). With its persistent and high lipophilic characteristics as well as its distribution in U.S. homes (Stapleton et al., 2005; Wu et al., 2007), BDE-47 bioaccumulates in humans (Hale et al., 2003), which would provide an explanation for its predominance in breast milk samples.

It can be challenging to compare and contrast the results of breast milk biomonitoring studies because differences exist among study designs and congener analyses. As noted in Table 12, ∑PBDEs is defined from 8 to 35 congeners depending on the study. If assessing whether or not labs are comparable, the percent detection is a useful measure, but not always available from published studies. In addition, breast milk biomonitoring research in the U.S. and British Columbia exhibit similarities and differences in their participant profiles and study designs (Table 13), which couid potentially influence PBDE levels in breast milk.

Guidelines have been established by the WHO (2007) that recommends recruiting first-time mothers for biomonitoring studies as previous elimination of

Table 13.

Study design characteristics of breast milk biomonitoring research in the U.S. and British Columbia

Location, authors, date of publication	First-time mothers	Hand or pump expression	Storage containers used	Number of samples collected	Week(s) of collection[c]	Time of day	Amount collected
Massachusetts, Johnson-Restrepo et al. (2007)	No	n/a[a]	Glass	1	n/a[a]	n/a[a]	n/a[a]
Pacific Northwest & British Columbia She et al. (2007)	Yes	Hand	Amber or foil-wrapped glass	2	2 to 8 weeks	n/a[a]	100 ml
Massachusetts, Wu et al. (2007)	Yes	Pump	Glass (foil-wrapped)[b]	1	2 to 8 weeks	n/a[a]	50 ml
Texas, Schecter et al. (2003)	Yes	Both	Glass	1	2 to 109 weeks	n/a[a]	30 ml
U.S., 14 different states Lunder & Sharp, (2003)	Yes	Both	n/a[a]	1	1 to 13 weeks	n/a[a]	n/a[a]
New Hampshire, *Dunn et al. (in progress)*	No	Pump	Amber glass	3	4, 8, 12 weeks	a.m.[d]	Complete expression

Note: [a] n/a indicates that information was not available from the published study; [b] Dr. Tom Webster, personal communication, June 24, 2008; [c] indicates time frame in the postpartum period in which the sample(s) were collected; [d] indicates samples were collected in the morning hours between 6 a.m. and 9 a.m.

environmental chemicals through lactation would not have occurred. Most participants enrolled in our study were first-time mothers (n = 31), but 21.5% (n = 9) had delivered and/or nursed one or more previous children. We did not find a relationship between parity and PBDE concentrations nor did we see an association with prior breastfeeding history that would indicate lower PBDE levels.

Study design recommendations have suggested hand-expressing samples for breast milk biomonitoring research in order to limit sample contamination from breast pumps. However, Hooper et al. (2007) and Main et al. (2007) documented that breast pump use is an acceptable method for milk collection when analyzing samples for PBDEs, therefore methods of milk collection (hand vs. pump expression) should not influence PBDE levels. It is important to ensure that breast milk samples are not exposed to ultraviolet light or stored in plastic containers; amber glass storage containers can meet both of these specifications to minimize ultraviolet light exposure and limit adherence of lipid-soluble PBDEs to the storage container.

Lovelady and colleagues (2002) suggest collecting serial samples rather than one sample of breast milk to account for changes in environmental chemicals over time. Our results indicate no significant difference in mean PBDE levels from months one, two, and three, which is a similar finding to Hooper et al. (2007) in which the 0-28 days after birth group was not different from the 29 to 56 days after birth group. Additionally, over the long-term, Hooper and colleagues (2007) found that depuration of PBDEs by lactation is slow in primiparae,

averaging 1 to 3% per month or a 12 to 18% decrease after six months. Consequently, it appears that one breast milk sample would estimate PBDE levels.

There appears to be a gap in the biomonitoring literature that evaluates if the time of day and/or quantity of milk collected (e.g. complete expression vs. foremilk expression) influences lipid-adjusted PBDE levels in breast milk. It's noted that collection amounts vary from study to study and most likely collection times (Table 13); however, median ∑PBDE and BDE-47 levels are similar between Boston, Massachusetts (Wu et al., 2007) and our work (Table 12) suggesting that a standardized timing protocol for milk collection and a complete expression may not be necessary. This gap in knowledge could be answered in future breast milk biomonitoring research of PBDEs.

Strengths and Limitations of Our Research

There were several strengths to our study. First, the collection of serial breast milk samples enabled us to evaluate changes in PBDE levels over time. Our results further contribute to a small body of evidence that minimal changes exist in PBDE levels in breast milk over the first three-months postpartum. An additional strength to our study was a standardized milk collection protocol and the collection of a complete expression, which provided information on these parameters and their influence on lipid-adjusted PBDE levels in breast milk.

We also viewed our survey work as strengths to our study because this evaluated potential exposure sources. To our knowledge, this is the first study to inquire about year of purchase on items in the mother's environment that are part

of her day-to-day living, such as electronics, appliances, and cushioned furniture. We also standardized lifestyle and living environment questions from the Greater Boston PBDE Body Burden Project (Wu et al., 2007). Although we had a different population in our study, we had similar findings for questions such as smoking, occupational, and hobby exposures. We attempted to reduce measurement error by pilot testing the Maternal Environment Survey prior to its implementation.

The use of a validated food frequency questionnaire was a strength to our study; we were confident with the accuracy of the instrument used to assess dietary intake based upon its validation for the Women's Health Initiative (Patterson et al., 1999). In addition, we obtained a comprehensive analysis of 125 nutrients, and quantified the frequency and amount of food consumption for 27 selected questions, which provided a substantial amount of dietary information - an important aspect given that diet is considered to be a source of exposure.

In general, collecting dietary information can pose a challenge in obtaining accurate information due to prevarication bias, the reliance on memory, and the estimation of portion sizes. As noted by Willet (1990), food frequency questionnaires have their drawbacks including restrictions in collecting information with fixed food lists, which we encountered as we were unable to distinguish between kinds of dairy fat consumed since some participants included multiple types. Since Wu and colleagues (2007) found an association between

∑PBDE levels in breast milk and consumption of dairy fat, this would have been an important aspect to evaluate from our data.

Our findings are limited due to the small sample size and our specific population of nursing mothers, therefore our results may not be applicable to the general population. As noted from previous research, infants ingest more PBDEs compared to adults (Jones-Otazo et al., 2005; Schecter et al., 2006), and males are 2.1 times more likely to be above the 95th percentile for BDE-153 as compared to females (Sjödin et al., 2008).

Conclusions and Future Directions for Breast Milk Biomonitoring of PBDEs

This was the first study in the state of New Hampshire to document and evaluate PBDE levels in nursing mothers over time. Our results indicate that PBDE levels from New Hampshire are within the range that has been reported in the U.S., and that levels are stable during the first three-months of lactation. Our findings revealed a higher predominance pattern with BDE-153 compared to other studies, which may be reflective of its long half-life. Our data indicates that PBDE levels are influenced by age, diet, and the home environment. Our diet and home environment findings contribute to the limited body of knowledge on exposure sources of PBDEs. The negative association between fruit consumption and PBDE (∑ and BDE-47) levels during the last trimester was surprising, and suggests that fruit may contribute less to PBDE levels in the body.

In the future, it will be important to document time-trends of PBDEs given the recent phase-out of pentaBDE and octaBDE commericial products and to

further evaluate geographical trends in the U.S. Lastly, further research should assess for potential health consequences associated with PBDE body burden and the nursing infant, with a focus on the long half-life congener BDE-153.

references

Akutsu, K., Kitagawa, M., Nakazawa, H., Makino, T., Iwazaki, K., Oda, H., et al. (2003). Time-trend (1973-2000) of polybrominated diphenyl ethers in Japanese mother's milk. *Chemosphere, 53*, 645-654.

Allen, J.G., McClean M.D., Stapleton, H.M., & Webster, T.F. (2008). Linking PBDEs in house dust to consumer products using X-ray fluorescence. *Environmental Science & Technology, 42,* 4222-4228.

Bi, X., Weiyue, Q., Sheng, G., Zhang, W., Bixian, M., Chen, D. et al. (2006). Polybrominated diphenyl ethers in South China maternal and fetal blood and breast milk. *Environmental Pollution, 144*, 1024-1030.

Birnbaum, L.S., & Staskal, D.F. (2004). Brominated flame retardants: Cause for concern? *Environmental Health Perspectives, 112*, 9-17.

Bromine Science and Environmental Forum. (2000). *An introduction to brominated flame retardants.* Retrieved June 15, 2007, from http://www.ebfrip/org/download/weeeqa.pdf.

Chao, H.R., Wang, S.L., Lee, W.J., Wang, Y.F., & Päpke, O. (2007). Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. *Environment International, 33*, 239-245.

Devine, C.M., Bove, C.F., & Olson, C.M. (2000). Continuity and change in women's weight orientations and lifestyle practices through pregnancy and the postpartum period: The influence of life course trajectories and transitional events. *Social Science Medicine, 50*, 567-582.

Dewey, K.G., Heinig, J., Nommsen, L.A., & Lonnerdal, B. (1991). Maternal versus infant factors related to breast milk intake and residual milk volume: The DARLING study. *Pediatrics, 87,* 829-837.

Erdoğrul, O., Covaci, A., Kurtul, N., & Schepens, P. (2004). Levels of organohalogenated persistent pollutants in human milk from Kahramanmaraş region, Turkey. *Environment International, 30*, 659-666.

Eslami, B., Koizumi, A., Ohta, S., Inoue, K., Aozasa, O., & Harada, K., et al. (2006). Large-scale evaluation of the current level of polybrominated diphenyl ethers (PBDEs) in breast milk from 13 regions of Japan. *Chemosphere, 63*, 554-561.

Fängström, B., Strid, A., Grandjean, P., Weihe, P. & Bergman, Å. (2005). A retrospective study of PBDEs and PCBs in human milk from the Faroe Islands. *Environmental Health: A global access science source, 4* (12), 1-9. Retrieved August 7, 2006, from http://www.ehjournal.net/content/4/1/12.

Fenton, S.E., Condon, M., Ettinger, A.S., LaKind, J.S., Mason, A., McDiarmid, M., et al. (2005). Collection and use of exposure data from human milk biomonitoring in the United States. *Journal of Toxicology and Environmental Health, Part A, 68,* 1691-1712.

Friis, R. H. & Sellers, T.A. (2004). *Epidemiology for public health practice* (3rd ed.). Sudbury, Massachusetts: Jones and Bartlett Publishers.

George, G.C., Hanss-Nuss, H., Milani, T.J., & Freeland-Graves, J.H. (2005). Food choices of low-income women during pregnancy & postpartum. *Journal of the American Dietetic Association, 105,* 899-907.

Geyer, H.J., Schramm, K.W., Darnerud, P.O., Aune, M., Feicht, E. A., Fried, K.W., et al. (2004). Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. *Organohalogen Compounds, 66,* 3867-3872.

Gómara, B., Herrero, L., Ramos, J.J., Mateo, J.R., Fernández, M.A., García, J.F., et al. (2007). Distribution of polybrominated diphenyl ethers in human, umbilical cord serum, paternal serum, maternal serum, placentas, and breast milk from Madrid population, Spain. *Environmental Science & Technology, 41,* 6961-6968.

Hale, R.C., Alaee, M., Manchester-Neesvig, J.B., Stapleton, H.M., & Ikonomou, M.G. (2003). Polybrominated diphenyl ether flame retardants in the North American environment. *Environment International, 29,* 771-779.

Hooper, K. & She, J. (2003). Lessons from the polybrominated diphenyl ethers (PBDEs): Precautionary principle, primary prevention, and the value of community-based body-burden monitoring using breast milk. *Environmental Health Perspectives, 111*, 109-114.

Hooper, K., She, J., Sharp, M., Chow, J., Jewell, N., Gephart, R., et al. (2007). Depuration of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from California first-time mothers (Primiparae). *Environmental Health Perspectives, 115,* 1271-1275.

Huwe, J.K. & Larsen, G.L. (2005). Polychlorinated dioxins, furans, and biphenyls, and polybrominated diphenyl ethers in a U.S. meat market basket and estimates of dietary intake. *Environmental Science & Technology, 39,* 5606-5611.

Huwe, J.K., & Smith, D.J. (2007). Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male Sprague-Dawley rate following dietary exposure. *Environmental Science & Technology, 41,* 2371-2377.

Ingelido, A.M., Ballard, T., Dellatte, E., di Domenico, A., Ferri, F., Fulgenzi, A.R., et al. (2007). Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk from Italian women living in Rome and Venice. *Chemosphere, 67,* S301-S306.

Jaraczewska, K., Lulek, J., Covaci, A., Voorspoels, S., Kaluba-Skotarczak, A., Drews, K., et al. (2006). Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominated diphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland. *Science of the Total Environment, 372,* 20-31.

Johnson-Restrepo, B., Addink, R., Wong, C., Arcaro, K., & Kannan, K. (2007). Polybrominated diphenyl ethers and organochlorine pesticides in human breast milk from Massachusetts, USA. *Journal of Environmental Monitoring, 9,* 1205-1212.

Jones-Otazo, H.A., Clarke, J.P., Diamond, M.R., Archbold, J.A., Ferguson, G., Harner, T., et al. (2005). Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. *Environmental Science & Technology, 39,* 5121-5130.

Kalantzi, O. I., Martin, F. L., Thomas, G. O., Alcock, R. E., Tang, H. R., Drury, S. C., et al. (2004). Different levels of polybrominated diphenyi ethers (PBDEs) and chlorinated compounds in breast milk from two U.K. regions. *Environmental Health Perspectives, 112,* 1085-1091.

Kazda, R., Hajšlová, J., Poustka, J., & Čajka, T. (2004). Determination of polybrominated diphenyl ethers in human milk samples in the Czech Republic. *Analytica Chimica Acta, 520,* 237-243.

Kent, J.C., Mitoulas, L.R., Cregan, M.D., Ramsay, D.T., Doherty, D.A., & Hartmann, P.E. (2006). Volume and frequency of breastfeedings and fat content of breast milk throughout the day. *Pediatrics, 117,* e387-e395.

La Guardia, M.J., Haie, R.C., & Harvey, E. (2006). Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environmental Science & Technology, 40,* 6247-6254.

Lind, Y., Darnerud, P. O., Atuma, S., Aune, M., Becker, W., Bjerselius, R., et al. (2003). Polybrominated diphenyl ethers in breast milk from Uppsala County, Sweden. *Environmental Research, 93,* 186-194.

Lovelady, C.A., Dewey, K.G., Picciano, M.F., & Dermer, A. (2002). Guidelines for collection of human milk samples for monitoring and research of environmental chemicals. *Journal of Toxicology and Environmental Health, Part A, 65,* 1881-1891.

Lunder, S. & Sharp, R. (2003). *Mothers' milk: Record levels of toxic fire retardants found in American mothers' breast milk.* Retrieved September 24, 2003, from http://www.ewg.org.

Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J. et al., (2007). Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. *Environmental Health Perspectives. 115,* 1519-1526.

Meironyté, D., Norén, K., & Bergman, A. (1999). Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. *Journal of Toxicology and Environmental Health, Part A, 58,* 329-341.

Needham, L.L., Calafat, A.M., & Barr, D.B. (2007). Uses and issues of biomonitoring. *International Journal of Hygiene & Environmental Health, 210,* 229-238.

Norén, K. & Meironyté, D. (2000) Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere, 40,* 1111-1123.

Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Shimidzu, Y., et al. (2002). Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats, and levels in human milk of nursing women in Japan. *Chemosphere, 46,* 689-696.

Patterson, R.E., Kristal, A.R., Fels Tinker, L., Carter, R.A., Bolton, M.P., & Agurs-Collins, T. (1999). Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Annals of Epidemiology, 9,* 178-187.

Ryan, J.J. (1991). Method 2: Extraction of human and cows' milk samples for the determination of polychlorinated dibenzodioxins and dibenzofurans. In C. Rappe, H.R. Buser, B. Dodet & I.K. O'Neill (Eds.), *Environmental carcinogens methods of analysis & exposure measurement: Polychlorinated dioxins and dibenzofurans* (pp. 205-209). Lyon, France: International Agency for Research on Cancer.

Ryan, J. J. & Patry, B. (2000). Determination of brominated diphenyl ethers (BDEs) and levels in Canadian human milks. *Organohalogen Compounds, 47,* 57-60.

Ryan, J.J., Wainman, B.C., Schecter, A., Moisey, J., Kosarac, I., & Sun, W.F. (2006). Trends of the brominated flame retardants, PBDEs and HBCD, in humans milks from North America. *Organohalogen Compounds, 68,* 778-781.

Sandanger, T.M., Sinotte, M., Dumas, P., Marchand, M., Sandau, C.D., Pereg, D., et al. (2007). Plasma concentrations of selected organobromine compounds and polychlorinated biphenyls in postmenopausal women of Quebec, Canada. *Environmental Health Perspectives, 115,* 1429-1434.

Schecter, A., Harris, T.R., Päpke, O., Tung, K.C., & Musumba, A. (2006a). Polybrominated diphenyl ether (PBDE) levels in the blood of pure vegetarians. *Toxicological & Environmental Chemistry, 88,* 107-112.

Schecter, A., Päpke, O., Harris, T.R., Tung, K.C., Musumba, A., Olson, J., et al. (2006b). Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S. food and estimated PBDE dietary intake by age and sex. *Environmental Health Perspectives, 114,* 1515-1520.

Schecter, A., Päpke, O., Tung, K.C., Joseph, J., Harris, T.R., Dahlgren, J. (2005). Polybrominated diphenyl ether flame retardants in the U.S. population: Current levels, temporal trends, and comparison with dioxins, dibenzofurans, and polychlorinated biphenyls. *Journal of Occupational & Environmental Medicine, 47,* 199-211.

Schecter, A., Päpke, O., Tung, K.C., Staskal, D., & Birnbaum, L. (2004). Polybrominated diphenyl ethers contamination of United States food. *Environmental Science & Technology, 38,* 5306-5311.

Schecter, A., Pavuk, M., Päpke, O., Ryan, J.J., Birnbaum, L., & Rosen, R. (2003). Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environmental Health Perspectives, 111,* 1723-1729.

Schuhmacher, M., Kiviranta, H., Vartiainen, T., & Domingo, J.L. (2007). Concentrations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk of women from Catalonia, Spain. *Chemosphere, 67,* S295-S300.

She, J., Holden, A., Sharp, M., Tanner, M., Williams-Derry, C., & Hooper, K. (2007). Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from the Pacific Northwest. *Chemosphere, 67,* S307-S317.

Sjödin, A., Wong, L., Jones, R.S., Park, A., Zhang, Y., Hodge, C. et al. (2008). Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. *Environmental Science & Technology, 42,* 1377-1384.

Stapleton, H.M., Dodder, N.G., Offenberg, J.H., Schantz, M.M., & Wise, S. (2005). Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environmental Science & Technology, 39,* 925-931.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2001). *Clinical growth charts*. Retrieved May 26, 2008, fromhttp://www.cdc.gov/nchs/about/major/nhanes/growthcharts/clinical_charts.htm.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2005). *Third national report on human exposure to environmental chemicals* (NCEH Publication No. 05-0570). Atlanta, GA: National Center for Environmental Health.

U.S. Environmental Protection Agency. (2003). *Draft method 1614: Brominated diphenyl ethers in water, soil, sediment, and tissue by HRGC/HRMS.* Retrieved April 20, 2005, from http://www.accustandard.com/asi/epa_downloads.php3.

U.S. Environmental Protection Agency. (2006). *Polybrominated diphenyl ethers project plan*. Retrieved July 9, 2007, from http://www.epa.gov/oppt/pbde/pubs/ proj-plan32906a.pdf.

U.S. Environmental Protection Agency, Integrated Risk Information System. (1990a). *Pentabromodiphenyl ether.* Retrieved June 8, 2008, from http://www.epa.gov/ncea/iris/subst/0184.htm.

U.S. Environmental Protection Agency, Integrated Risk Information System. (1990b). *Octabromodiphenyl ether.* Retrieved June 8, 2008, from http://www.epa.gov/ncea/iris/subst/0180.htm.

U.S. Environmental Protection Agency, Integrated Risk Information System. (1995). *2,2',3,3',4,4',5,5',6,6' - decabromodiphenyl ether (BDE-209).* Retrieved June 8, 2008, from http://www.epa.gov/ncea/iris/subst/0035.htm.

Willet, W. (1990). *Nutritional epidemiology.* New York: Oxford University Press.

World Health Organization. (1994). *Environmental health criteria 162: Brominated diphenyl ethers.* Retrieved September 10, 2007, from http://www.inchem.org/documents/ehc/ehc162.htm#PartNumber:2.

World Health Organization. (2007). *Fourth WHO-coordinated survey of human milk for persistent organic pollutants in cooperation with UNEP: Guidelines for developing a national protocol.* Retrieved October 22, 2007, from http://www.who.int/foodsafety/chem/POPprotocol.pdf.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., et al. (2007). Human exposure to PBDEs: Associations of PBDE body burdens with food consumption and house dust concentrations. *Environmental Science & Technology, 41,* 1584-1589.

CHAPTER V

MANUSCRIPT 2: DEVELOPING A BIOMONITORING COMMUNICATION PAMPHLET FOR POTENTIAL PARTICIPANTS IN A BREAST MILK BIOMONITORING STUDY

introduction

The National Research Council (2006) emphasizes early planning as an important component in communicating biomonitoring information. Early planning enables researchers to assess the needs of the study population and to establish partnerships with other constituencies in formulating public health messages. Early planning also encourages researchers to consider both practical and research-based communication recommendations.

Practical recommendations focus on educational aspects, whereas, research-based recommendations promote the advancement of biomonitoring communication (National Research Council, 2006). Practical biomonitoring communication recommendations highlight the following areas: communication sponsorship, use of consistent terminology and concepts, expansion of biomonitoring education to the public on how to interpret biomonitoring results, communication training for organizations and professionals, and public documentation of ways to reduce exposures. Research-based recommendations emphasize the following areas: identification of scientist and non-scientist beliefs about exposures and health effects, assessment of current biomonitoring

communication methods and its impact on the public, identification of the public's perception regarding uncertainties in biomonitoring, and identification of the public's beliefs about risk reduction. Employing aspects of these communication recommendations would enhance the efforts of biomonitoring research.

If communication is lacking between biomonitoring researchers and the general public, then the results of biomonitoring studies are subject to misinterpretation and misuse, which can lead to public health messages that induce confusion, conflict and anxiety. For example, occasionally, the media will become aware of biomonitoring research, and will provide a condensed, simplified version of the results (Bates et al., 2005). Often, these types of messages present a communication risk, particularly if the biological specimen is sensationalized with a degree of harm, which can happen when environmental chemicals are detected in breast milk.

Breast milk is a valuable matrix for monitoring body burdens of environmental chemicals; however, there are positive and negative aspects when using breast milk to assess human exposure to environmental chemicals (Fenton et al., 2005; Hooper & She, 2003). Fenton and colleagues (2005) identify limitations with using breast milk in biomonitoring studies as the interpretation and communication of study results have the potential for affecting breastfeeding rates if inaccurate messages are provided to the public. Even though there are limitations associated with breast milk biomonitoring studies, positive aspects have been identified including the collection of a biological specimen with a high fat content. This positive aspect is an important consideration for lipid-soluble

environmental chemicals – particularly those that have been steadily rising in their production, use, and presence in the environment and in humans including synthetic flame retardants such polybrominated diphenyl ethers (PBDEs).

PBDEs in breast milk were first reported by Krüger (1988) in German samples, and later by Meironyté et al. (1999) in Swedish samples. PBDE concentrations in Swedish breast milk samples increased from 1972 to 1997, and it was determined that levels were doubling every five years while levels of other environmental chemicals (PCBs and DDT) were decreasing during this same time period (Meironyté et al., 1999; Norén & Meironyté, 2000). Documenting this exponential increase in PBDEs has resulted in breast milk biomonitoring initiatives around the globe, but with limited consideration to practical and research-based communication recommendations. One of the primary research objectives of *Biomonitoring PBDEs in Lactating Women* was to develop an informative, yet reassuring communication pamphlet to educate potential subjects about the research without scaring mothers away from nursing their infants.

the process

To address this objective, we drafted a communication pamphlet. The format of the pamphlet contained short sections about the research study, body burden, what PBDEs are and where they come from, why we are measuring PBDEs, what we hope to learn from the study, and the importance of breastfeeding. The final draft of the communication pamphlet also included a section on "frequently asked questions".

As part of the communication pamphlet development phase, we collaborated with a health promotion advisor from the New Hampshire Department of Health and Human Services (DHHS), a faculty member from the Family Research Laboratory at UNH, the New Hampshire Breastfeeding Task Force and a focus group of women from the community. This collaboration provided us with valuable feedback on the content and language of the pamphlet.

Our first collaborative meeting was in March 2005 with the DHHS health promotion advisor and the faculty member from the Family Research Laboratory. Their primary recommendations were to eliminate the technical language in order to decrease the reading level from 12th grade to 8th grade. In addition, they recommended that the pamphlet be visually appealing and be reassigned a less scientific name. The project's secondary name became *Steps to a Healthy Future.*

Our second meeting was in April 2005 with 20 members of the New Hampshire Breastfeeding Task Force. The members of the Task Force are primarily registered nurses and registered dietitians who are lactation specialists. This meeting provided valuable input regarding appropriate breastfeeding language and further elimination of technical wording. An added advantage of this meeting was that members of the Task Force provided helpful suggestions on our biomonitoring research design including sample collection time, sample size, breastfeeding support to participants and information on personal use vs. rental breast pumps.

Our third meeting was in May 2005 when we conducted a six person, one hour focus group to assess the content of the pamphlet. We recruited women from the UNH campus who were currently breastfeeding or had breastfed their child within the last two years. We provided background information on the study, reviewed each section of the communication pamphlet, and collected verbal and written feedback from the focus group participants. The verbal feedback emphasized refinement of language to further improve the readability of the communication pamphlet. The written feedback was collected by having participants complete an anonymous 10-question survey about the content and clarity of the pamphlet at the end of the focus group session. The written feedback directed us to emphasize the heaith effects of PBDEs, participant time commitment and what will happen with the final results of the study.

The communication pamphlet was completed in August of 2005. The final format was an eight page pamphlet with readability statistics at the 5th grade reading level. Approximately 1500 copies of the pamphlet were distributed to lactation consultants and placed in eight locations around the Seacoast area of New Hampshire (Dover Pediatrics, Exeter Hospital Family Center, Harbour Women's Health, Lamprey Health Care, Portsmouth Regional Hospital, Rochester Women, Infants & Children Clinic, Seacoast Area La Leche League, and Wentworth Douglass Hospital) for subject recruitment.

Participants were recruited from the Seacoast region of New Hampshire from November 2005 to July 2006. Recruitment was accomplished by lactation consultants from three area hospitals – Exeter Hospital, Portsmouth Regional

Hospital, and Wentworth Douglass Hospital -, distribution of the communication pamphlet, and word of mouth. Approval from each hospital was obtained prior to working with the lactation consultants. Monthly breastfeeding classes were attended by the researchers at each of the hospitals to provide study information and the communication pamphlet to pregnant women. The lactation consultants provided the communication pamphlet to postpartum women prior to their discharge from the hospital. In addition, the pamphlet was distributed to outpatient health care clinics.

the outcome and conclusions

All 40 participants in the *Biomonitoring PBDEs in Lactating Women* research study received a copy of the communication pamphlet. Thirty-eight percent of the participants received the pamphlet from a lactation consultant; twenty-eight percent of the participants received the pamphlet at breastfeeding classes; twenty-three percent through a friend/colleague; and thirteen percent through picking up the pamphlet at area hospitals or outpatient health care clinics.

The communication pamphlet assisted us in conveying information pertinent to our study to potential participants. We discovered that our process, after the publication of *Human Biomonitoring for Environmental Chemicals* (National Research Council, 2006), employed some of the principles of practical and research-based communication recommendations. For example, our pamphlet discussed the uncertainties that exist between PBDE levels in humans and health effects. Additionally, we included in the communication pamphlet our

approach on how we would disseminate the results of our study. We decided to use a clinical medicine model approach, which emphasizes minimizing potential harm from reporting uncertain information (Brody et al., 2007). We informed participants that they would not receive individual results as there is not enough information to interpret the meaning of PBDE levels in humans.

It is noted that practical and research-based recommendations emphasized by the National Research Council (2006) primarily focus on communicating and interpreting the results for the public. However, our approach to the communication pamphlet focused on providing information to potential participants prior to their commitment to our biomonitoring study. In addition, we focused on fostering partnerships with organizations and professionals (e.g. New Hampshire Breastfeeding Task Force and lactation consultants) to provide them with an understanding of biomonitoring and our research prior to recruiting participants into the study.

For future biomonitoring research, we would utilize this process again. However, we would assess the effectiveness of our communication pamphlet with participants who enrolled in the study through either a questionnaire or focus group. We would further implement additional practical and research-based communication recommendations in an effort to further advance the work of biomonitoring research.

references

Bates, M.N., Hamilton, J.W., LaKind, J.S., Langenberg, P., O'Malley, M., & Snodgrass, W. (2005). Workgroup report: Biomonitoring study design, interpretation, and communication – lessons learned and path forward. *Environmental Health Perspectives, 113,* 1615-1621.

Brody, J.G., Morello-Frosch, R., Brown, P., Rudel, R.A., Altman, R.G., Frye, M., et al. (2007). Is it safe?: New ethics for reporting personal exposures to environmental chemicals. *American Journal of Public Health, 97,* 1547-1554.

Fenton, S.E., Condon, M., Ettinger, A.S., LaKind, J.S., Mason, A., McDiarmid, M., et al. (2005). Collection and use of exposure data from human milk biomonitoring in the United States. *Journal of Toxicology and Environmental Health, Part A, 68,* 1691-1712.

Hooper, K. & She, J. (2003). Lessons from the polybrominated diphenyl ethers (PBDEs): Precautionary principle, primary prevention, and the value of community-based body-burden monitoring using breast milk. *Environmental Health Perspectives, 111*, 109-114.

Krüger, C. (1988). Polybromierte biphenyle und polybromierte biphenylether – Nachweis und Bestimmung in ausgewählten Lebensmittel. Thesis, Westfälischen Wilhelms Universität zu Münster, Germany.

Meironyté, D., Norén, K., & Bergman, A. (1999). Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. *Journal of Toxicology and Environmental Health, Part A, 58,* 329-341.

National Research Council. (2006). *Human biomonitoring for environmental chemicals*. Washington DC: The National Academies Press.

Norén, K. & Meironyté, D. (2000) Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere, 40*, 1111-1123.

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

This was the first study in the state of New Hampshire to document and evaluate PBDE levels in nursing mothers over time. Our results indicate that PBDE levels from New Hampshire are within the range that has been reported in the U.S., and that levels are stable during the first three-months of lactation. Our findings revealed a higher predominance pattern with BDE-153 compared to other studies, which may be reflective of its long half-life. Our data indicates that PBDE levels are influenced by age, diet, and the home environment. Our diet and home environment findings contribute to the limited body of knowledge on exposure sources of PBDEs.

We found that our communication pamphlet assisted us in conveying information pertinent to our study to potential participants. For future biomonitoring research, we would utilize this process again. However, we would assess the effectiveness of our communication pamphlet with participants who enrolled in the study through either a questionnaire or focus group.

In the future, it will be important to document time-trends of PBDEs given the recent phase-out of pentaBDE and octaBDE commericial products and to further evaluate geographical trends in the U.S. Lastly, further research should assess for potential health consequences associated with PBDE body burden and the nursing infant, with a focus on the long half-life congener BDE-153.

LIST OF COMPLETE REFERENCES

Akutsu, K., Kitagawa, M., Nakazawa, H., Makino, T., Iwazaki, K., Oda, H., et al. (2003). Time-trend (1973-2000) of polybrominated diphenyl ethers in Japanese mother's milk. *Chemosphere, 53*, 645-654.

Albertini, R., Bird, M., Doerrer, N., Needham, L., Robison, S., Sheldon, L., et al. (2006). The use of biomonitoring data in exposure and human health risk assessments. *Environmental Health Perspectives, 114*, 1755-1762.

Allen, J.G., McLean, M.D., Stapleton, H.M., Nelson, J.W., & Webster, T.F. (2007). Personal exposure to polybrominated diphenyl ethers (PBDEs) in residential indoor air. *Environmental Science & Technology, 41*, 4574-4579.

Alien, J.G., McClean M.D., Stapieton, H.M., & Webster, T.F. (2008). Linking PBDEs in house dust to consumer products using X-ray fluorescence. *Environmental Science & Technology, 42,* 4222-4228.

American Chemicai Society. (2007). *Washington state passes PBDE ban.* Retrieved May 28, 2007, from http://pubs.acs.org/subscribe/journals/esthag-w/ 2007/apr/policy/nl_PBDEs.html.

Angerer, J., Bird, M.G., Burke, T.A., Doerrer, N.G., Needham, L., Robison, S.H., et al. (2006). Strategic biomonitoring initiatives: Moving the science forward. *Toxicological Sciences, 93*, 3-10.

Bahadori, T., Phillips, R.D., Money, C.D., Quackenboss, J.J., Cleweli, H.J., Bus, J.S., et al. (2007). Making sense of human biomonitoring data: Findings and recommendations of a workshop. *Journal of Exposure Science & Environmental Epidemiology, 17,* 308-313.

Baiilie-Hamiltion, P.F. (2002). Chemical toxins: A hypothesis to explain the global obesity epidemic. *Journal of Alternative and Complementary Medicine, 8,* 185-192.

Bates, M.N., Hamilton, J.W., LaKind, J.S., Langenberg, P., O'Malley, M., & Snodgrass, W. (2005). Workgroup report: Biomonitoring study design, interpretation, and communication – lessons learned and path forward. *Environmental Health Perspectives, 113,* 1615-1621.

Bi, X., Weiyue, Q., Sheng, G., Zhang, W., Bixian, M., Chen, D. et al. (2006). Polybrominated diphenyl ethers in South China maternal and fetal blood and breast milk. *Environmental Pollution, 144*, 1024-1030.

Birnbaum, L.S. & Cohen Hubal, E.A. (2006). Polybrominated diphenyl ethers: A case study for using biomonitoring data to address risk assessment questions. *Environmental Health Perspectives, 114,* 1770-1775.

Birnbaum, L.S., & Staskal, D.F. (2004). Brominated flame retardants: Cause for concern? *Environmental Health Perspectives, 112*, 9-17.

Bocio, A., Llobet, J.M., Domingo, J.L., Corbella, J., Teixidó, A., & Casas, C. (2003). Polybrominated diphenyl ethers (PBDEs) in foodstuffs: Human exposure through the diet. *Journal of Agricultural & Food Chemistry, 51*, 3191-3195.

Bradman, A., Fenster, L., Sjödin, A., Jones, R.S., Patterson, D.G., & Eskenazi, B. (2007). Polybrominated diphenyl ether levels in the blood of pregnant women living in an agricultural community in California. *Environmental Health Perspectives, 115,* 71-74.

Branchi, I., Alleva, E., & Costa L. G., (2002). Effects of perinatal exposure to polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioral development. *Neurotoxicology, 23*, 375-384.

Brody, J.G., Morello-Frosch, R., Brown, P., Rudel, R.A., Altman, R.G., Frye, M., et al. (2007). Is it safe?: New ethics for reporting personal exposures to environmental chemicals. *American Journal of Public Health, 97,* 1547-1554.

Bromine Science and Environmental Forum. (2000). *An introduction to brominated flame retardants.* Retrieved June 15, 2007, from http://www.ebfrip/org/download/weeeqa.pdf.

Bromine Science and Environmental Forum. (2003). *Major brominated flame retardants volume estimates.* Retrieved August 14, 2007, from http://www.bsef.com/docs/BFR_vols_2001.doc.

Chao, H.R., Wang, S.L., Lee, W.J., Wang, Y.F., & Päpke, O. (2007). Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. *Environment International, 33*, 239-245.

Darnerud, P.O., Aune, M., Larsson, L., & Hallgren, S. (2007). Plasma PBDE and thyroxine levels in rats exposed to Bromkal or BDE-47. *Chemosphere, 67,* S386-S392.

Darnerud, P.O., Eriksen, G.S., Jóhannesson, T., Larsen, P.B., & Viluksela, M. (2001). Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environmental Health Perspectives, 109,* 49-68.

de Boer, J., Wester, P.G., van der Horst, A., & Leonards, P.E.G. (2003). Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from the Netherlands. *Environmental Pollution, 122,* 63-74.

Devine, C.M., Bove, C.F., & Olson, C.M. (2000). Continuity and change in women's weight orientations and lifestyle practices through pregnancy and the postpartum period: The influence of life course trajectories and transitional events. *Social Science Medicine, 50,* 567-582.

Dewey, K.G., Heinig, J., Nommsen, L.A., & Lonnerdal, B. (1991). Maternal versus infant factors related to breast milk intake and residual milk volume: The DARLING study. *Pediatrics, 87,* 829-837.

Dye, J.A., Venier, M., Zhu, L., Ward, C.R., Hites, R.A., & Birnbaum, L.S. (2007). Elevated PBDE levels in pet cats: Sentinels for humans? *Environmental Science & Technology, 41,* 6350-6356.

Erdoğrul, O., Covaci, A., Kurtul, N., & Schepens, P. (2004). Levels of organohalogenated persistent pollutants in human milk from Kahramanmaraş region, Turkey. *Environment International, 30,* 659-666.

Eriksson, P., Jakobsson, E. & Fredriksson, A. (2001). Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environmental Health Perspectives, 109,* 903-908.

Eslami, B., Koizumi, A., Ohta, S., Inoue, K., Aozasa, O., & Harada, K., et al. (2006). Large-scale evaluation of the current level of polybrominated diphenyl ethers (PBDEs) in breast milk from 13 regions of Japan. *Chemosphere, 63,* 554-561

Fängström, B., Strid, A., Grandjean, P., Weihe, P. & Bergman, Å. (2005). A retrospective study of PBDEs and PCBs in human milk from the Faroe Islands. *Environmental Health: A global access science source, 4* (12), 1-9. Retrieved August 7, 2006, from http://www.ehjournal.net/content/4/1/12.

Fenton, S.E., Condon, M., Ettinger, A.S., LaKind, J.S., Mason, A., McDiarmid, M., et al. (2005). Collection and use of exposure data from human milk biomonitoring in the United States. *Journal of Toxicology and Environmental Health, Part A, 68,* 1691-1712.

Fowles, J.R., Fairbrother, A., Baccher-Steppan, L., & Kerkvliet, N.I. (1994). Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. *Toxicology, 86*, 49-61.

Friis, R. H. & Sellers, T.A. (2004). *Epidemiology for public health practice* (3rd ed.). Sudbury, Massachusetts: Jones and Bartlett Publishers.

George, G.C., Hanss-Nuss, H., Milani, T.J., & Freeland-Graves, J.H. (2005). Food choices of low-income women during pregnancy & postpartum. *Journal of the American Dietetic Association, 105,* 899-907.

Geyer, H.J., Schramm, K.W., Darnerud, P.O., Aune, M., Feicht, E. A., Fried, K.W., et al. (2004). Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. *Organohalogen Compounds, 66,* 3867-3872.

Gill, U., Chu, I., Ryan, J.J., & Feeley, M. (2004). Polybrominated diphenyl ethers: Human tissue levels and toxicology. *Reviews of Environmental Contaminants & Toxicology, 183,* 55-97.

Gómara, B., Herrero, L., Ramos, J.J., Mateo, J.R., Fernández, M.A., García, J.F., et al. (2007). Distribution of polybrominated diphenyl ethers in human, umbilical cord serum, paternal serum, maternal serum, placentas, and breast milk from Madrid population, Spain. *Environmental Science & Technology, 41,* 6961-6968.

Guvenius, D. M., Aronsson, A., Ekman-Ordeberg, G, Bergman, A., & Norén, K. (2003). Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentochlorophenol. *Environmental Health Perspectives, 111,* 1235-1241.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., et al. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsycholgical development of the child. *New England Journal of Medicine, 341*, 549-555.

Hakk, H., Larsen, G., & Klasson-Wehler, E., (2002). Tissue disposition, excretion and metabolism of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) in the male Sprague-Dawley rat. *Xenobiotica, 32,* 369-382.

Hakk, H. & Letcher, R.J. (2003). Metabolism in the toxicokinetics and fate of brominated flame retardants – a review. *Environment International, 29,* 801-828.

Hale, R.C., Alaee, M., Manchester-Neesvig, J.B., Stapleton, H.M., & Ikonomou, M.G. (2003). Polybrominated diphenyl ether flame retardants in the North American environment. *Environment International, 29,* 771-779.

Hale, R.C., La Guardia, M.J., Harvey, E., & Mainor, T.M. (2002). Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment. *Chemosphere, 46,* 729-735.

Hallgren, S., Sinjari, T., Håkansson, H., & Darnerud, P.O. (2001). Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid and vitamin A levels in rats and mice. *Archives in Toxicology, 75,* 200-208.

Hardell, L., Eriksson, M., Lindström, G., Van Bavel, B., Linde, A., Carlberg, M., et al. (2001). Case-control study on concentrations of organohalogen compounds and titers of antibodies to Epstein-Barr virus antigens in the etiology of non-Hodgkin's lymphoma. *Leukemia & Lymphoma, 42,* 619-629.

Hardy, M.L. (2002). The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants. *Chemosphere, 46,* 757-777.

Hays, S.M., Becker, R.A., Leung, H.W., Aylward, L.L., & Pyatt, D.W. (2007). Biomonitoring equivalents: A screening approach for interpreting biomonitoring results from a public health risk perspective. *Regulatory Toxicology & Pharmacology, 47,* 96-109.

Hites, R.A. (2004). Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environmental Science & Technology,* 38, 945-956.

Hooper, K. & McDonald, T.A. (2000). The PBDEs: An emerging environmental challenge and another reason for breast-milk monitoring programs. *Environmental Health Perspectives, 108,* 387-392.

Hooper, K. & She, J. (2003). Lessons from the polybrominated diphenyl ethers (PBDEs): Precautionary principle, primary prevention, and the value of community-based body-burden monitoring using breast milk. *Environmental Health Perspectives, 111,* 109-114.

Hooper, K., She, J., Sharp, M., Chow, J., Jewell, N., Gephart, R., et al. (2007). Depuration of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from California first-time mothers (Primiparae). *Environmental Health Perspectives, 115,* 1271-1275.

Hoppe, A.C. & Carey, G.B. (2007). Polybrominated diphenyl ethers (PBDEs) as endocrine disruptors of adipocyte metabolism. *Obesity, 15,* 2942-2950.

Huwe, J.K. & Larsen, G.L. (2005). Polychlorinated dioxins, furans, and biphenyls, and polybrominated diphenyl ethers in a U.S. meat market basket and estimates of dietary intake. *Environmental Science & Technology, 39,* 5606-5611.

Huwe, J.K., Lorentzsen, M., Thuresson, K., & Bergman, A. (2002). Analysis of mono- and deca-brominated diphenyl ethers in chickens at the part per billion level. *Chemosphere, 46,* 635-640.

Huwe, J.K., & Smith, D.J. (2007). Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male Sprague-Dawley rate following dietary exposure. *Environmental Science & Technology, 41,* 2371-2377.

Ingelido, A.M., Ballard, T., Dellatte, E., di Domenico, A., Ferri, F., Fulgenzi, A.R., et al. (2007). Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk from Italian women living in Rome and Venice. *Chemosphere, 67,* S301-S306.

Inoue, K., Harada, K., Takenaka, K., Uehara, S., Kono, M., Shimizu, T., et al. (2006). Levels and concentration ratios of polychlorinated biphenyls and polybrominated diphenyl ethers in serum and breast milk in Japanese mothers. *Environmental Health Perspectives, 114,* 1179-1185.

Jaraczewska, K., Lulek, J., Covaci, A., Voorspoels, S., Kaluba-Skotarczak, A., Drews, K., et al. (2006). Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominated diphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland. *Science of the Total Environment, 372,* 20-31.

Johnson-Restrepo, B., Addink, R., Wong, C., Arcaro, K., & Kannan, K. (2007). Polybrominated diphenyl ethers and organochlorine pesticides in human breast milk from Massachusetts, USA. *Journal of Environmental Monitoring, 9,* 1205-1212.

Johnson-Restrepo, B., Kannan, K., Rapaport, D.P., & Rodan, B.D. (2005). Polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue from New York. *Environmental Science & Technology, 39,* 5177-5182.

Jones-Otazo, H.A., Clarke, J.P., Diamond, M.R., Archbold, J.A., Ferguson, G., Harner, T., et al. (2005). Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. *Environmental Science & Technology, 39,* 5121-5130.

Kalantzi, O. I., Martin, F. L., Thomas, G. O., Alcock, R. E., Tang, H. R., Drury, S., et al. (2004). Different levels of polybrominated diphenyl ethers (PBDEs) and chlorinated compounds in breast milk from two U.K. regions. *Environmental Health Perspectives, 112,* 1085-1091.

Karter, M.J. (2006). *Fire loss in the United States during 2005.* Retrieved August 13, 2007 from http://www.nfpa.org/assets/files/PDF/ OS.fireloss.pdf.

Kazda, R., Hajšlová, J., Poustka, J., & Čajka, T. (2004). Determination of polybrominated diphenyl ethers in human milk samples in the Czech Republic. *Analytica Chimica Acta, 520,* 237-243.

Kent, J.C., Mitoulas, L.R., Cregan, M.D., Ramsay, D.T., Doherty, D.A., & Hartmann, P.E. (2006). Volume and frequency of breastfeedings and fat content of breast milk throughout the day. *Pediatrics, 117,* e387-e395.

Krüger, C. (1988). Polybromierte biphenyle und polybromierte biphenylether – Nachweis und Bestimmung in ausgewählten Lebensmittel. Thesis, Westfälischen Wilhelms Universität zu Münster, Germany.

La Guardia, M.J., Hale, R.C., & Harvey, E. (2006). Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environmental Science & Technology, 40,* 6247-6254.

Lilienthal, H., Hack, A., Roth-Härer, A., Wichert Grande S., & Talsness, C.E. (2006). Effects of developmental exposure to 2,2',4,4',5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. *Environmental Health Perspectives, 114,* 194-201.

Lind, Y., Darnerud, P. O., Atuma, S., Aune, M., Becker, W., Bjerselius, R., et al. (2003). Polybrominated diphenyl ethers in breast milk from Uppsala County, Sweden. *Environmental Research, 93,* 186-194.

Lunder, S. & Sharp, R. (2003). *Mothers' milk: Record levels of toxic fire retardants found in American mothers' breast milk.* Retrieved September 24, 2003, from http://www.ewg.org.

Lovelady, C.A., Dewey, K.G., Picciano, M.F., & Dermer, A. (2002). Guidelines for collection of human milk samples for monitoring and research of environmental chemicals. *Journal of Toxicology and Environmental Health, Part A, 65,* 1881-1891.

Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J. et al., (2007). Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. *Environmental Health Perspectives. 115,* 1519-1526.

Mazdai, A., Dodder, N.G., Abernathy, M.P., Hites, R.A., & Bigsby, R.M. (2003). Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environmental Health Perspectives, 111*, 1249-1252.

McDonald, T.A. (2002). A perspective on the potential health risks of PBDEs. *Chemosphere, 46,* 745-755.

McDonald, T.A. (2005). Polybrominated diphenylether levels among United States residents: Daily intake and risk of harm to the developing brain and reproductive organs. *Integrated Environmental Assessment and Management, 1*, 343-354.

McMaster, M., & McMaster, C. (1998). *GC/MS: A practical user's guide.* New York: Wiley-VCH.

Meerts, I.A.T.M., Letcher, R.J., Hoving, S., Marsh, G., Bergman, A., Lemmen, J,G., et al. (2001). In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environmental Health Perspectives, 109,* 399-407.

Meerts, I.A.T.M., van Zanden, J.J., Luijks, E.A.C., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., et al. (2000). Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin *in vitro. Toxicological Sciences, 56,* 95-104.

Meironyté, D., Norén, K., & Bergman, A. (1999). Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. *Journal of Toxicology and Environmental Health, Part A, 58,* 329-341.

Mörck, A., Hakk, H., Örn, U., & Klasson Wehler, E. (2003). Decabromodiphenyl ether in the rat: Absorption, distribution, metabolism, and excretion. *Drug Metabolism & Distribution, 31,* 900-907.

National Research Council. (2006). *Human biomonitoring for environmental chemicals.* Washington DC: The National Academies Press.

Needham, L.L., Calafat, A.M., & Barr, D.B. (2007). Uses and issues of biomonitoring. *International Journal of Hygiene & Environmental Health, 210,* 229-238.

Needham, L.L., Özkaynak, H., Whyatt, R.M., Barr, D.B., Wang, R.Y., Naeher, L., et al. (2005). Exposure assessment in the National Children's Study: Introduction. *Environmental Health Perspectives, 113*, 1076-1082.

Needham, L.L. & Wang, R.Y. (2002). Analytic considerations for measuring environmental chemicals in breast milk. *Environmental Health Perspectives, 110,* A317-A324.

New Hampshire Department of Health and Human Services. (2002). *Findings from the behavioral risk factor surveillance system in New Hampshire.* Retrieved April 29, 2005, from http://www.dhhs.state.nh.us/DHHS/HSDM/behavioral-risk.htm.

Norén, K. & Meironyté, D. (2000) Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere, 40*, 1111-1123.

Ohta, S., Ishizuka, D., Nishimura, H., Nakao, T., Aozasa, O., Shimidzu, Y., et al. (2002). Comparison of polybrominated diphenyl ethers in fish, vegetables, and meats, and levels in human milk of nursing women in Japan. *Chemosphere, 46,* 689-696.

Örn, U. & Klasson-Wehler, E. (1998). Metabolism of 2,2',4,4'-tetrabromodiphenyl ether in rat and mouse. *Xenobiotica, 28,* 199-211.

Patterson, R.E., Kristal, A.R., Fels Tinker, L., Carter, R.A., Bolton, M.P., & Agurs-Collins, T. (1999). Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Annals of Epidemiology, 9*, 178-187.

Petreas, M., She, J., Brown, F.R., Winkler, J., Windham, G., Rogers, E., et al. (2003). High body burdens of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in California women. *Environmental Health Perspectives, 111,* 1175-1179.

Rice, C.P., Chernyak, S.M., Begnoche, L., Quintal, R., & Hickey, J. (2002). Comparisons of PBDE composition and concentration in fish collected from the Detroit River, MI and Des Plaines River, IL. *Chemosphere, 49*, 731-737.

Rudel, R.A., Camann, J.D., Spengler, J.D., Korn, L.R., & Brody, J.G. (2003). Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environmental Science & Technology, 37,* 4543-4553.

Ruel, M.T., Dewey, K.G., Martinez, C., Flores, R., & Brown, K.H. (1997). Validation of single day-time samples of human milk to estimate the 24-hour concentration of lipids in urban Guatemalan mothers. *American Journal of Clinical Nutrition, 65,* 439-444.

Ryan, J.J. (1991). Method 2: Extraction of human and cows' milk samples for the determination of polychlorinated dibenzodioxins and dibenzofurans. In C. Rappe, H.R. Buser, B. Dodet & I.K. O'Neill (Eds.), *Environmental carcinogens methods of analysis & exposure measurement: Polychlorinated dioxins and dibenzofurans* (pp. 205-209). Lyon, France: International Agency for Research on Cancer.

Ryan, J. J. & Patry, B. (2000). Determination of brominated diphenyl ethers (BDEs) and levels in Canadian human milks. *Organohalogen Compounds, 47,* 57-60.

Ryan, J.J., Wainman, B.C., Schecter, A., Moisey, J., Kosarac, I., & Sun, W.F. (2006). Trends of the brominated flame retardants, PBDEs and HBCD, in humans milks from North America. *Organohalogen Compounds, 68,* 778-781.

Sandanger, T.M., Sinotte, M., Dumas, P., Marchand, M., Sandau, C.D., Pereg, D., et al. (2007). Plasma concentrations of selected organobromine compounds and polychlorinated biphenyls in postmenopausal women of Quebec, Canada. *Environmental Health Perspectives, 115,* 1429-1434.

Schecter, A., Harris, T.R., Päpke, O., Tung, K.C., & Musumba, A. (2006a). Polybrominated diphenyl ether (PBDE) levels in the blood of pure vegetarians. *Toxicological & Environmental Chemistry, 88,* 107-112.

Schecter, A., Johnson-Welch, S., Tung, K.C., Harris,T.R., Päpke, O., & Rosen, R. (2007). Polybrominated diphenyl ether (PBDE) levels in livers of U.S. human fetuses and newborns. *Journal of Toxicology & Environmental Health, Part A, 70,* 1-6.

Schecter, A., Päpke, O., Harris, T.R., Tung, K.C., Musumba, A., Olson, J., et al. (2006b). Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S. food and estimated PBDE dietary intake by age and sex. *Environmental Health Perspectives, 114,* 1515-1520.

Schecter, A., Päpke, O., Tung, K.C., Joseph, J., Harris, T.R., Dahlgren, J. (2005). Polybrominated diphenyl ether flame retardants in the U.S. population: Current levels, temporal trends, and comparison with dioxins, dibenzofurans, and polychlorinated biphenyls. *Journal of Occupational & Environmental Medicine, 47,* 199-211.

Schecter, A., Päpke, O., Tung, K.C., Staskal, D., & Birnbaum, L. (2004). Polybrominated diphenyl ethers contamination of United States food. *Environmental Science & Technology, 38,* 5306-5311.

Schecter, A., Pavuk, M., Päpke, O., Ryan, J.J., Birnbaum, L., & Rosen, R. (2003). Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environmental Health Perspectives, 111,* 1723-1729.

Schuhmacher, M., Kiviranta, H., Vartiainen, T., & Domingo, J.L. (2007). Concentrations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk of women from Catalonia, Spain. *Chemosphere, 67,* S295-S300.

Sexton, K., Needham, L.L., & Pirkle, J.L. (2004, January-February). Human biomonitoring of environmental chemicals. *American Scientist, 92,* 38-45.

She, J., Holden, A., Sharp, M., Tanner, M., Williams-Derry, C., & Hooper, K. (2007). Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from the Pacific Northwest. *Chemosphere, 67,* S307-S317.

She, J., Petreas, M., Winkler, J., Visita, P., McKinney, M., & Kopec, D. (2002). PBDEs in the San Francisco bay area: Measurements in harbor seal blubber and human breast adipose tissue. *Chemosphere, 46,* 697-707.

Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E., & Bergman, A. (1999). Flame retardant exposure: Polybrominated diphenyl ethers in blood from Swedish workers. *Environmental Health Perspectives, 107,* 643-648.

Sjödin, A., Jones, R.S., Focant, J.F., Lapeza, C., Wang, R.Y., McGahee, E.E., et al. (2004). Retrospective time-trend study of polybrominated diphenyl ether and polybrominated and polychlorinated biphenyl levels in human serum from the United States. *Environmental Health Perspectives, 112,* 654-658.

Sjödin, A., Wong, L., Jones, R.S., Park, A., Zhang, Y., Hodge, C. et al. (2008). Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. *Environmental Science & Technology, 42,* 1377-1384.

Skarman, E., Darnerud, P.O., Öhrvik, H., & Oskarsson, A. (2005). Reduced thyroxine levels in mice perinatally exposed to polybrominated diphenyl ethers. *Environemental Toxicology & Pharmacology, 19,* 273-281.

Strandman, T., Koistinen, J., & Vartiainen, T. (2000). Polybrominated diphenyl ethers (PBDEs) in placenta and human milk. *Organohalogen Compounds, 47,* 61-64.

Stapleton, H.M., Dodder, N.G., Offenberg, J.H., Schantz, M.M., & Wise, S. (2005). Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environmental Science & Technology, 39,* 925-931.

Stapleton, H.M., Kelly, S.M., Allen, J.G., McClean, M.D., & Webster, T.F. (2008). Measurement of polybrominated diphenyl ethers on hand wipes: Estimating exposure from hand-to-mouth contact. *Environmental Science & Technology, 42,* 3329-3334.

Thuresson, K., Höglund, P., Hagmar, L., Sjödin, A., Bergman, A., & Jakobsson, K. (2006). Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. *Environmental Health Perspectives, 114,* 176-181.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2001). *Clinical growth charts.* Retrieved May 26, 2008, from http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/clinical_charts.htm.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2001). *National report on human exposure to environmental chemicals* (NCEH Publication No. 01-0379). Atlanta, GA: National Center for Environmental Health.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2005). *Third national report on human exposure to environmental chemicals* (NCEH Publication No. 05-0570). Atlanta, GA: National Center for Environmental Health.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (2007). *Breastfeeding practices: Results from the National Immunization Survey.* Retrieved October 22, 2007, from http://www.cdc.gov/breastfeeding/data/NIS_data/data_2004.htm.

U.S. Department of Health and Human Services, National Toxicology Program (1986). Toxicology and carcinogenesis studies of decabromodiphenyl oxide in F344/N rats and B6C3F$_1$ mice (feed studies). Retrieved October 11, 2007, from http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr309.pdf.

U.S. Environmental Protection Agency. (2003). *Draft method 1614: Brominated diphenyl ethers in water, soil, sediment, and tissue by HRGC/HRMS.* Retrieved April 20, 2005, from http://www.accustandard.com/asi/epa_downloads.php3.

U.S. Environmental Protection Agency. (2006). *Polybrominated diphenyl ethers project plan.* Retrieved July 9, 2007, from http://www.epa.gov/oppt/pbde/pubs/ proj-plan32906a.pdf.

U.S. Environmental Protection Agency, Integrated Risk Information System. (1990a). *Pentabromodiphenyl ether.* Retrieved June 8, 2008, from http://www.epa.gov/ncea/iris/subst/0184.htm.

U.S. Environmental Protection Agency, Integrated Risk Information System. (1990b). *Octabromodiphenyl ether.* Retrieved June 8, 2008, from http://www.epa.gov/ncea/iris/subst/0180.htm.

U.S. Environmental Protection Agency, Integrated Risk Information System. (1995). *2,2',3,3',4,4',5,5',6,6' - decabromodiphenyl ether (BDE-209).* Retrieved June 8, 2008, from http://www.epa.gov/ncea/iris/subst/0035.htm.

Viberg, H., Fredriksson, A., & Eriksson, P. (2003). Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behavior, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicology & Applied Pharmacology, 192,* 95-106.

Viberg, H., Johansson, N., Fredriksson, A., Eriksson, J., Marsh, G., & Eriksson, P. (2006). Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. *Toxicological Sciences, 92,* 211-218.

von Meyerinck, L., Hufnagel, B., Schmoldt, A., & Benthe, H.F. (1990). Induction of rat liver microsomal cytochrome P-450 by the pentabromo diphenyl ether Bromkal 70 and half-lives of its components in the adipose tissue. *Toxicology, 61,* 259-274.

Willet, W. (1990). *Nutritional epidemiology.* New York: Oxford University Press.

World Health Organization. (1994). *Environmental health criteria 162: Brominated diphenyl ethers.* Retrieved September 10, 2007, from http://www.inchem.org/documents/ehc/ehc162.htm#PartNumber:2.

World Health Organization. (2004). *Fourth WHO-coordinated survey of human milk for persistent organic pollutants: A protocol for collection, handlIng, and analysis of samples at the country level.* Retrieved October 10, 2005, from http://www.euro.who.int/foodsafety/chemical/20050923_1.

World Health Organization. (2007). *Fourth WHO-coordinated survey of human milk for persistent organic pollutants in cooperation with UNEP: Guidelines for developing a national protocol.* Retrieved October 22, 2007, from http://www.who.int/foodsafety/chem/POPprotocol.pdf.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., et al. (2007). Human exposure to PBDEs: Associations of PBDE body burdens with food consumption and house dust concentrations. *Environmental Science & Technology, 41,* 1584-1589.

Zhou, T., Ross, D. G., DeVito, M. J., & Crofton, K. M. (2001). Effects of short-term *in vivo* exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzymes activities in weanling rats. *Toxicological Sciences, 61,* 76-82.

Zhou, T., Taylor, M.M., DeVito, M.J., & Crofton, K.M. (2002). Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicological Sciences, 66,* 105-116.

APPENDICES

APPENDIX A

FCOUS GROUP SURVEY

UNIVERSITY of NEW HAMPSHIRE

Biomonitoring PBDEs in Lactating Women
Focus Group Questionnaire for Educational Tooi

1. After hearing the script, do you feel the importance of breast feeding is sufficiently emphasized?

   Yes No

   If no, do you have suggestions for improving this area?

2. If you were a potential participant, do you feel the information on human research is helpful?

   Yes No

   If no, do you have suggestions for improving this area?

3. Do you understand the term "body burden"?

   Yes No

   If no, what would help in clarifying the term?

4. Do you understand why we've chosen breast milk as our "window" into the human body?

   Yes No

   If no, what would help in clarifying this area?

5. Can you identify where PBDEs come from?

   Yes No

6. Can you identify the potential health effects of PBDEs?

   Yes No

7. Do you feel there is enough information on the following areas in regard to each participant's obligation to the study:

   a. Time commitment

      Yes No

   b. The number of breast milk samples that will be provided

      Yes No

   c. Subject compensation

      Yes No

8. If you were a participant in this study and PBDEs were detected in your breast milk, would you stop breast feeding?

   Yes No

   How would you feel?

   What questions would you have?

9. Do you understand what will happen with the results of the study?

   Yes No

10. Do you have suggestions for the visual component of the educational tool?

    Yes No

    If yes, please describe.

APPENDIX B

COMMUNICATION PAMPHLET

Steps to a Healthy Future

Planning to breast feed?

Would you like to participate in a research study?

Read on....

Overview:

YOU ARE ELIGIBLE IF:

- You are pregnant and plan to breast feed.

OR

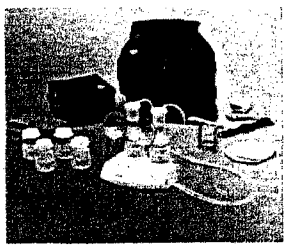
- You are a new mother who has been breast feeding for less than 2 weeks.

YOUR ROLE WOULD BE:

- To provide us with up to 9 breast milk samples over 3 months.
- To meet with us on 4 occasions (at UNH or at your home).
- To complete diet and environment surveys.

OUR GIFT TO YOU!

- A ***Purely Yours*** personal breast pump.

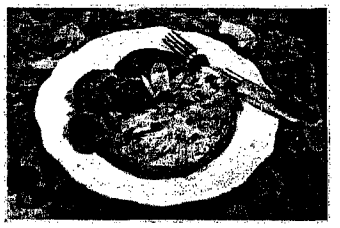


Our Goal....

To learn more about environmental chemicals that build up in the body also known as "body burden."

How does "body burden" happen?

We are exposed to chemicals each day, and some are stored in our bodies. We may breathe in environmental chemicals, eat them in food, or even absorb them through our skin.



What chemicals are we interested in?

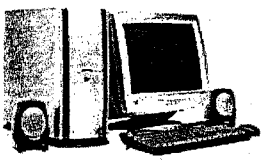
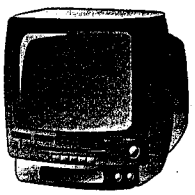
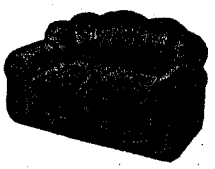
Since the 1970's, a family of chemicals that has increased dramatically in our environment is **polybrominated diphenyl ethers**, also known as **PBDEs.**

What are PBDEs?

- Chemical flame retardants. They keep things from burning.

Where are PBDEs found?

- PBDEs are in products like couches, stereos, carpets, television sets, and computers.

Why measure PBDEs?

- Research using laboratory animals suggests that high levels of PBDEs in animals can interfere with thyroid hormone, learning, memory, and behavior.

Can these same health effects happen in people?

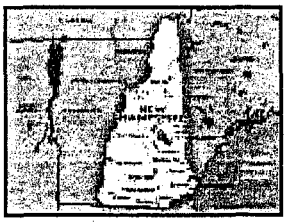
- We don't know. A first step is to measure PBDEs in people.

How will we measure PBDEs?

- We can measure PBDEs in blood or breast milk. But, breast milk is a better window into the human body. **Why?**
- PBDEs collect in body fat, and breast milk contains fat.

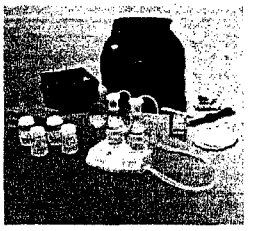
What we will learn:

- This study will help us to learn about PBDEs in the Seacoast region of New Hampshire.
- We hope to find out if there are relationships between PBDEs, diet and environment.



To thank you.....

Each participant will receive a **Purely Yours** personal breast pump.



Breast milk is the most nourishing food you can give your baby.

Whether or not you decide to participate in the ***Steps to a Healthy Future*** study, know that you are making the right choice in breastfeeding your baby.

If you would like more information on the ***Steps to a Healthy Future*** research study, please contact:

Gale Carey or Rebecca Dunn
at the *University of New Hampshire,*
603-862-2550

UNIVERSITY of NEW HAMPSHIRE

Frequently Asked Questions

1. Will my participation interfere with breast feeding my baby?
No

2. How many breast milk samples will I provide?
A maximum of 9 samples

3. What is the size of each sample?
Each sample will be one complete expression from one breast.

4. Will I receive instructions on how to collect and store breast milk samples?
Yes, this will happen on your first visit with us.

5. What if I have difficulty breast feeding?
Breast feeding support is available from a professional lactation consultant.

6. What if I have to stop breast feeding?
If you stop breast feeding due to unforeseen circumstances, just let us know. You are free to leave the study at any point.

7. What will happen to the results of the study?
The anonymous results will be published in a scientific journal.

8. Will I receive my PBDE levels?
No, individual levels will not be provided as there is not enough information to know what the levels mean. But, you will receive a summary of the findings and the published report.

APPENDIX C

CONSENT FORM

UNIVERSITY of NEW HAMPSHIRE

INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS IN RESEARCH
INFORMED CONSENT FOR PARTICIPANTS:
STEPS TO A HEALTHY FUTURE

Purpose: The purpose of this study is to collect information on levels of polybrominated diphenyl ethers (PBDEs) in breast milk, and to determine if there is a relationship between PBDE level, maternal characteristics and the environment.

Description: You are being asked to participate in a 3-month study through the University of New Hampshire. We are in need of 100 lactating women, ages 22 to 40, who are in the early stages of breastfeeding (less than 2 weeks) or in the last trimester of pregnancy and plan on breastfeeding. We are asking for a maximum of 9 breast milk samples during the first three months of breast feeding. One sample is the equivalent of one complete expression from one breast. You will provide up to 3 samples at the end of the first, second, and third month of breast feeding.

You are being asked to completely express milk from one breast in the morning on 3 successive days, once per month for three months. You are being asked to gently mix the milk and transfer your breast milk sample to a storage container and place it in the refrigerator.

You are being asked to visit with the University of New Hampshire researchers on 4 occasions over a 3-month period for a total of approximately 6 hours. On the first visit, you will be oriented to the study's purpose and design, asked to complete a food frequency questionnaire and a maternal characteristics survey and sign the subject consent form. You will receive instruction on breast milk expression via a personal use breast pump, sample collection procedure and sample storage. You will be given a kit containing written instructions, milk storage containers, and a milk transport cold box (if needed).

The second visit will occur after 1 month. You will surrender your first month's samples (up to 3 samples) and meet with the researchers to answer any questions. You will be asked to complete an environmental survey. The third visit will occur after the second month. You will surrender the second month's samples (up to 3 samples) and meet with the researchers to answer any questions. The fourth visit will occur at the end of the study, when you will surrender the third month's samples (up to 3 samples), complete a food frequency questionnaire and receive study debriefing.

To show our appreciation for your time, you will receive a personal use breast pump and pumping kit during your first visit with the researchers. In addition, you will also be provided with breast feeding support from a professional lactation consultant.

We will share our results with the scientific community; the route by which scientists communicate with their peers and the larger community. We will provide you with the same published information. After we have published our results, you may hear about the study on television or in the newspaper.

To ensure confidentiality of subject information, all data will be stored in a locked room within the graduate student office (418 Kendall Hall) at the University of New Hampshire. Data will be coded to insure your privacy. You will be assigned a number, which will be used on all the records of data collected and no names will be disclosed.

Potential Risks: Risks to you could be psychological harm. Although we will release only cumulative data on all subjects, not individual data on each subject (as there is no context in which to evaluate this data), you may feel anxious knowing that your breast milk contains PBDEs. In order to address this possibility, you will be asked to view our education tool at the outset of the study.

Although unlikely, some slight breast discomfort may occur due to the use of the breast pump.

1. You understand that the use of human subjects in this project has been approved by the University of New Hampshire Institutional Review Board for the Protection of Human Subjects In Research.

2. You understand the purpose of this research project, the procedures to be followed, the expected duration of your participation, and have viewed the educational tool.

3. You have received a description of any reasonable foreseeable risks or discomforts associated with being a subject in this research, have had them explained to you, and understand them.

4. The investigator seeks to maintain the confidentiality of all data and records associated with your participation in this research. You should understand, however, there are rare instances when the investigator is required to share personally-identifiable information (e.g., according to policy, contract, and regulation). For example, in response to a complaint about the research, officials at the University of New Hampshire, designees of the sponsor(s), and/or regulatory and oversight government agencies may access research data. You also should understand that the investigator is required by law to report certain information to government and/or law enforcement officials (e.g., child abuse, threatened violence against self or others, communicable diseases).

5. As a participant, you have the right to revoke your consent to use personal health information. If you chose to do so, contact the Project Director, Rebecca Dunn at 603-862-2550. There is the potential risk that personal health Information provided to others has the potential risk of being re-disclosed.

6. You understand that your consent to participate in this research is entirely voluntary, and that your refusal to participate will involve no prejudice, penalty or loss of benefits to which you would otherwise be entitled.

7. You further understand that if you consent to participate, you may discontinue your participation at any time without prejudice, penalty, or loss of benefits to which you would otherwise be entitled.

8. You confirm that no coercion of any kind was used in seeking your participation in this research project.

9. In the unlikely event that physical injury should occur from participating in this project, medical treatment should be sought from your own health care provider. No compensation for injury is available. If you are a UNH student and have a paid health fee, you may be seen at the UNH Health Services.

10. Information on University policy and procedure for research involving human subjects can be obtained from Julie Simpson at the Human Subjects Review Board, Office of Sponsored Research at 862-2003. Please call Dr. Gale Carey or Rebecca Dunn at the numbers below if you have questions regarding the procedures or any other aspect of this study. The *Steps to a Healthy Future* research study will expire in summer 2008 when data collection, data analysis and reporting of results is completed.

Gale B. Carey, Ph.D.
603-862-4628

Rebecca L. Dunn, M.A., R.D., L.D.
603-862-2550

I have read the following information and have had all questions concerning this study answered to my satisfaction. The information obtained will be treated as privileged and confidential and will not be released without my consent. However, this information may be used for statistical or scientific purposes with my right of privacy retained. I understand and agree that in the event of injury, which I may sustain as a result of my participation, no financial compensation is available but medical treatments within the limits of treatment normally offered by the University of New Hampshire Health Center will be available. I understand that I will be assessed a fee for any medical services.

Subject Signature	Witness Signature	Date

APPENDIX D

COLLECTION INSTRUCTIONS

STEPS TO A HEALTHY FUTURE

Collection Instructions for:_____

- Wash your hands and breast with a mild, detergent-free soap, such as Ivory soap.
  - ***Please note,*** *if possible, avoid using creams and ointments on the breasts during milk collection periods.*
- Milk collection should occur between the hours of **6 AM and 9 AM**.
  - ***Please note****: at least two hours of time should have elapsed since the previous feeding from that breast.*
- Using the *Purely Yours* breast pump, completely express milk from one breast into a collection container.
- Detach container from pump and cover with screw cap. Invert 3 to 4 times, and then transfer the milk into the labeled "study" container.
- Complete the label on the "study" container (date, time, etc.).
- Place the "study" container in the refrigerator.
- The remainder of the milk in the collection container can be fed back to your baby or stored.
- Repeat this procedure for three days in a row at the end of months 1, 2, & 3 of lactation.

APPENDIX E

COLLECTION SCHEDULE

Collection Schedule – Steps to a Healthy Future

To be completed by researchers and participant

Month	Date	Day of Week	Sample Pick-up/Delivery Date
One			
One			
One			
Two			
Two			
Two			
Three			
Three			
Three			

APPENDIX F

PARTICIPANT LABEL

Subject #:_____

Date Pumped:_____Time Pumped:_____

Time of Last Feeding:_____

APPENDIX G

PARTICIPANT INVENTORY SPEADSHEET

	Participant ID:				
	Date Pumped/Amount	**Date Collected**	**Date Frozen/Amount**	**Pumped b/w 6-9 am**	**2 hours after last feed**
Month 1 Samples					
Sample 1 - 4 ounces					
Sample 2 - 4 ounces					
Sample 3 - 4 ounces					
Sample 1 - 2 ounce aliquot					
Sample 2 - 2 ounce aliquot					
Sample 3 - 2 ounce aliquot					
Month 2 Samples					
Sample 1 - 4 ounces					
Sample 2 - 4 ounces					
Sample 3 - 4 ounces					
Sample 1 - 2 ounce aliquot					
Sample 2 - 2 ounce aliquot					
Sample 3 - 2 ounce aliquot					
Month 3 Samples					
Sample 1 - 4 ounces					
Sample 2 - 4 ounces					
Sample 3 - 4 ounces					
Sample 1 - 2 ounce aliquot					
Sample 2 - 2 ounce aliquot					
Sample 3 - 2 ounce aliquot					
# of samples to North Dakota					
Date Sent					

APPENDIX H

PBDE LEVELS IN INDIVIDUAL BREAST MILK SAMPLES

Appendix H: PBDE levels in breast milk samples from month one										
	Percent Lipid	BDE28/33	BDE47	BDE85	BDE99	BDE100	BDE153	BDE154	BDE183	∑PBDE
Month 1 Subject #										
Month 1 101-05	3.7	2.9	19.2	0.3	4.4	3.6	4.6	0.3	0.1	35.4
Month 1 102-05	4.5	2.6	22.7	0.2	2.8	2.1	20.4	0.1	0.04	50.9
Month 1 103-05	5.0	5.4	59.0	0.9	4.6	15.3	10.4	0.6	0.1	96.3
Month 1 104-05	5.0	2.6	29.4	0.2	3.6	2.8	2.2	0.2	0.1	41.0
Month 1 105-05	2.5	0.5	4.4	0.1	0.9	0.8	1.8	ND	0.1	8.6
Month 1 106-06	3.8	1.8	24.4	0.4	3.7	2.7	5.2	0.2	ND	38.4
Month 1 107-06	3.4	0.3	4.9	0.1	1.2	0.8	1.1	0.1	ND	8.4
Month 1 108-06	2.9	0.6	5.1	ND	0.7	0.6	2.7	ND	ND	9.7
Month 1 109-06	3.4	1.3	13.7	0.1	1.4	0.8	0.8	0.1	ND	18.2
Month 1 110-06	2.8	2.1	33.7	0.5	6.6	4.5	9.0	0.3	0.21	56.7
Month 1 111-06	4.2	1.7	26.1	0.5	4.1	4.9	4.5	0.3	ND	42.1
Month 1 112-06	2.8	0.4	3.7	ND	ND	0.4	9.9	ND	ND	14.3
Month 1 113-06	2.1	2.9	27.1	ND	3.8	2.7	3.9	ND	ND	40.5
Month 1 114-06	2.5	0.6	3.7	ND	0.6	0.4	1.5	ND	ND	6.8
Month 1 115-06	2.7	3.4	35.5	ND	5.0	12.8	109.6	0.5	ND	166.7
Month 1 116-06	4.4	1.6	14.3	0.1	2.6	3.6	7.4	0.1	ND	29.9
Month 1 117-06	2.5	0.6	6.2	ND	1.5	1.8	97.7	ND	ND	107.7
Month 1 119-06	4.6	2.6	16.7	ND	2.2	2.0	0.7	0.1	ND	24.3
Month 1 120-06	4.3	1.6	14.9	0.2	2.9	2.7	1.6	0.1	0.1	24.1
Month 1 121-06	4.3	1.7	13.0	ND	1.1	2.6	27.3	0.1	0.1	45.9
Month 1 122-06	1.6	0.5	7.7	ND	ND	1.6	2.4	ND	2.3	14.6
Month 1 123-06	2.1	1.8	25.3	0.3	3.6	7.6	28.4	0.4	ND	67.3
Month 1 124-06	4.3	0.7	5.9	0.2	0.8	0.8	1.6	0.03	0.7	10.7
Month 1 125-06	1.7	1.2	11.4	ND	1.4	2.3	8.7	ND	ND	25.0
Month 1 126-06	3.0	0.9	8.2	ND	ND	0.8	6.9	ND	0.2	17.1
Month 1 127-06	3.2	1.0	8.1	ND	ND	2.9	9.9	0.1	0.1	22.1
Month 1 129-06	2.5	3.3	34.9	ND	3.0	9.5	4.7	0.2	ND	55.7
Month 1 130-06	2.0	2.2	19.1	ND	2.5	1.6	1.7	ND	0.3	27.5
Month 1 132-06	4.4	2.2	38.9	0.6	5.7	6.2	3.2	0.4	ND	57.2
Month 1 133-06	1.6	1.1	11.4	0.2	1.7	2.1	23.6	ND	ND	40.1
Month 1 134-06	2.5	1.4	11.5	0.2	1.4	2.4	8.2	0.1	ND	25.3
Month 1 135-06	4.5	0.8	5.1	0.1	0.7	0.8	1.1	0.1	ND	8.7
Month 1 136-06	3.5	2.9	33.7	0.4	3.5	3.3	1.3	0.2	ND	45.2
Month 1 137-06	4.3	1.1	9.6	0.1	0.8	4.5	13.5	0.1	ND	29.6
Month 1 138-06	3.1	3.5	31.5	0.8	5.2	8.8	5.2	0.4	ND	55.5
Month 1 139-06	2.5	4.5	19.4	0.3	2.6	4.3	13.7	0.2	ND	45.0
Month 1 141-06	4.8	0.8	9.6	0.1	1.5	1.8	7.1	0.1	ND	21.1
Month 1 142-06	4.6	1.4	9.9	0.2	1.8	3.0	7.2	0.1	ND	23.6
Month 1 143-06	4.2	0.9	8.8	0.1	1.1	1.2	1.5	0.1	ND	13.7
Month 1 144-06	2.0	1.0	11.0	0.2	2.2	1.8	3.5	0.1	ND	19.7
Minimum	1.6	0.3	3.7	< DL	< DL	0,4	0.8	< DL	< DL	6.8
Maximum	5.0	5.4	59.0	0.9	6.6	15.3	109.6	0.5	2.3	166.7
Median	3.3	1.5	13.4	0.2	2.4	2.5	4.9	0.1	0.1	28.6
Mean	3.3	1.8	17.5	0.3	2.6	3.4	11.9	0.2	0.4	37.3
Note. PBDE levels are reported with non-detects (ND) = 0.										

Appendix H: PBDE levels in breast milk samples from month two											
	Percent Lipid	Lipid[a]	BDE28/33	BDE47	BDE85	BDE99	BDE100	BDE153	BDE154	BDE183	∑PBDE
Month 2 Subject #											
Month 2 101-05	2.6		3.0	15.4	0.2	2.3	2.7	4.0	0.3	ND	27.8
Month 2 102-05	4.6		2.7	22.9	0.2	3.1	2.1	20.0	0.1	0.02	51.0
Month 2 103-05	4.4		4.7	54.1	0.8	4.4	15.7	11.6	0.7	0.1	92.2
Month 2 104-05	4.9		2.9	32.6	0.3	4.1	3.2	2.1	0.2	ND	45.4
Month 2 105-05	2.5		0.4	3.9	0.1	ND	0.7	1.6	ND	ND	6.6
Month 2 106-06	3.3		1.6	21.0	0.2	2.8	2.2	4.1	0.1	ND	32.0
Month 2 107-06	2.4		0.4	7.6	0.1	1.9	0.9	1.0	0.1	ND	11.9
Month 2 108-06	4.4		0.5	5.0	ND	0.8	0.7	2.7	ND	ND	9.8
Month 2 109-06	4.5		1.3	14.5	0.1	1.4	0.8	1.0	ND	ND	19.1
Month 2 110-06	3.0	3.0	2.2	35.8	ND	7.3	4.2	7.9	0.3	0.4	58.1
Stability 110-06[b]			2.4	37.4	ND	9.8	5.4	8.7	0.3	0.2	64.2
Month 2 111-06	4.0		1.7	26.6	ND	4.1	5.0	5.2	0.3	ND	43.0
Month 2 112-06	1.9		0.4	4.3	ND	ND	0.5	11.1	ND	ND	16.4
Month 2 113-06	2.6		2.1	19.5	ND	2.5	2.0	3.1	ND	ND	29.2
Month 2 114-06	1.3		0.8	4.0	ND	ND	0.4	1.7	ND	ND	6.9
Month 2 115-06	4.0		2.7	29.6	ND	6.6	10.4	83.8	0.6	ND	133.7
Month 2 116-06	2.4		1.6	13.8	0.2	2.4	3.3	6.9	0.1	ND	28.3
Month 2 117-06	4.4		0.5	5.0	ND	1.2	1.3	76.1	0.1	ND	84.1
Month 2 119-06	3.5		2.4	17.4	ND	2.3	2.0	0.9	0.1	ND	25.2
Month 2 120-06	1.4		1.8	19.2	0.3	3.9	3.1	1.5	0.2	ND	30.0
Month 2 121-06	3.2		1.5	11.1	ND	ND	2.3	21.4	0.1	0.3	36.7
Month 2 122-06	1.5		0.5	6.2	ND	ND	1.2	2.0	ND	1.1	11.0
Month 2 123-06	2.3		1.9	27.0	0.3	4.3	7.8	29.1	0.4	ND	70.7
Month 2 124-06	1.9		0.9	9.0	0.2	1.8	1.1	1.5	0.1	0.7	15.2
Month 2 125-06	2.9		1.3	10.4	ND	1.4	2.1	7.0	0.1	0.2	22.4
Month 2 126-06	4.1		1.1	10.1	ND	1.6	1.1	8.3	0.1	0.2	22.4
Month 2 127-06	1.1		1.0	7.3	ND	ND	3.7	15.1	ND	ND	27.0
Month 2 129-06	1.8		3.3	33.7	2.1	2.7	8.5	3.9	ND	ND	54.1
Month 2 130-06	2.4		2.0	18.0	0.2	2.6	1.5	1.1	0.1	ND	25.5
Month 2 132-06	8.5		1.9	33.3	0.5	4.9	5.5	3.1	0.3	ND	49.5
Month 2 133-06	1.8		0.9	10.4	0.3	2.0	2.1	24.6	ND	ND	40.2
Month 2 134-06	5.7		1.3	10.2	ND	1.0	2.3	8.3	0.1	ND	23.3
Month 2 135-06	4.3		0.8	5.5	0.1	0.6	0.8	1.1	0.1	0.3	9.2
Month 2 136-06	6.0	5.2	2.3	27.2	0.3	2.5	2.7	1.1	0.1	1.3	37.5
Month 2 137-06	3.5		1.1	9.1	ND	0.8	4.9	16.8	0.1	0.3	33.1
Month 2 138-06	2.4		3.3	29.0	0.7	4.3	7.9	4.6	0.3	ND	50.2
Month 2 139-06	1.9		4.4	20.2	0.3	3.2	4.8	16.7	0.3	ND	49.8
Month 2 141-06	3.1		0.7	8.6	0.1	1.5	1.9	8.2	0.1	ND	21.2
Month 2 142-06	5.7		1.4	9.3	0.2	1.6	3.1	8.3	0.1	ND	24.0
Month 2 143-06	2.2		0.9	8.6	0.1	1.2	1.3	1.6	ND	ND	13.6
Month 2 144-06	2.1		0.9	9.6	0.1	1.5	1.4	2.9	ND	ND	16.5
Minimum	1.1		0.4	3.9	< DL	< DL	0.4	1.0	< DL	< DL	6.6
Maximum	8.5		4.7	54.1	2.1	7.3	15.7	83.8	0.7	1.3	133.7
Median	2.9		1.4	12.4	0.2	2.4	2.1	4.3	0.1	0.3	28.1
Mean	3.3		1.7	16.6	0.3	2.7	3.2	10.8	0.2	0.4	35.1

Note. PBDE levels are reported with non-detects (ND) = 0; [a] Data represents the percent lipid content from the stability study;
[b] Data in the rectangular box represents the congener profiles of the sample from the stability study.

Appendix H: PBDE levels in breast milk samples from month three											
	Percent Lipid	Lipid[a]	BDE28/33	BDE47	BDE85	BDE99	BDE100	BDE153	BDE154	BDE183	∑PBDE
Month 3 Subject #											
Month 3 101-05	1.9		2.9	17.8	0.3	4.5	3.4	4.9	0.4	0.05	34.1
Month 3 102-05	2.0		2.6	20.9	0.1	2.4	1.8	18.5	0.1	ND	46.4
Month 3 103-05	4.6		3.9	43.5	0.7	3.6	12.0	8.7	0.5	0.10	73.2
Month 3 104-05	4.5		2.6	30.3	0.2	4.5	3.0	2.2	0.2	ND	42.9
Month 3 105-05	1.1		0.4	3.8	0.1	ND	0.7	1.7	ND	ND	6.8
Month 3 106-06	1.7		1.5	22.5	ND	3.6	2.4	4.4	0.2	ND	34.7
Month 3 107-06	4.4		0.4	7.1	0.1	1.5	0.8	1.2	0.1	ND	11.3
Month 3 108-06	1.7		0.5	5.6	ND	1.3	0.8	2.9	ND	ND	11.2
Month 3 109-06	5.7		1.2	13.2	ND	1.3	0.8	0.8	0.1	ND	17.4
Month 3 110-06	4.0	3.5	2.3	42.9	ND	10.2	4.7	6.9	0.3	0.2	67.4
Stability 110-06[b]			3.0	49.5	0.7	13.7	6.3	9.4	0.4	0.3	83.3
Month 3 111-06	3.8		1.9	31.3	ND	5.8	5.9	5.9	0.4	0.10	51.3
Month 3 112-06	1.6		0.3	5.5	0.2	2.5	0.8	8.5	ND	ND	17.8
Month 3 113-06	1.7		1.2	11.9	ND	1.6	1.1	1.6	ND	ND	17.5
Month 3 114-06	2.0	2.0	0.7	3.8	ND	0.8	0.4	1.6	ND	ND	7.2
Stability 114-06[b]			0.8	4.5	0.1	0.8	0.6	1.7	ND	ND	8.5
Month 3 115-06	4.3		2.2	22.7	ND	3.3	8.6	81.3	0.3	ND	118.4
Month 3 116-06	1.2		1.8	16.0	0.2	3.1	3.8	8.3	ND	ND	33.2
Month 3 117-06	3.6		0.5	5.1	ND	1.0	1.3	73.7	0.1	ND	81.6
Month 3 119-06	3.8	3.3	2.1	15.1	ND	2.2	1.9	0.9	0.1	ND	22.3
Month 3 120-06	2.7	3.1	1.8	18.3	0.2	3.7	3.1	1.8	0.2	ND	29.1
Month 3 121-06	3.8		1.1	9.8	ND	ND	2.3	22.1	ND	0.66	36.0
Month 3 122-06	3.9		0.5	5.7	0.4	1.0	1.2	2.1	ND	0.9	11.8
Month 3 123-06	3.3		1.6	22.5	0.2	3.2	6.5	24.2	0.4	ND	58.6
Month 3 124-06	2.0		0.7	7.2	ND	1.2	0.8	1.6	0.1	0.7	12.5
Month 3 125-06	3.1		1.2	9.6	ND	1.3	2.0	6.8	0.1	0.1	21.1
Month 3 126-06	0.9		1.4	11.9	ND	ND	0.6	11.1	ND	0.8	25.7
Month 3 127-06	1.6		0.7	5.3	ND	ND	2.6	11.3	ND	ND	19.8
Month 3 129-06	2.8	2.8	3.3	33.2	1.4	2.5	8.1	4.1	0.2	ND	52.8
Month 3 130-06	1.3		3.1	28.8	ND	3.8	2.2	2.0	ND	0.9	40.8
Month 3 132-06	3.2		2.0	34.5	0.6	5.8	5.9	3.3	0.4	ND	52.5
Month 3 133-06	1.8		1.0	9.9	0.2	1.7	2.0	25.5	ND	ND	40.2
Month 3 134-06	6.8		1.2	9.4	ND	1.1	2.1	7.5	0.1	0.1	21.4
Month 3 135-06	1.9		0.6	4.0	0.1	ND	0.7	1.0	ND	ND	6.5
Month 3 136-06	5.0	3.6	2.1	25.0	0.3	2.5	2.4	0.9	0.1	0.1	33.4
Month 3 137-06	3.5	3.7	1.0	8.9	ND	1.0	4.8	18.8	0.1	ND	34.6
Month 3 138-06	2.4		2.9	25.8	0.6	4.0	7.1	4.1	0.3	ND	44.9
Month 3 139-06	3.4		4.3	19.0	0.3	2.8	4.3	14.8	0.2	ND	45.8
Month 3 141-06	3.6		0.7	8.2	0.1	1.7	1.9	7.9	0.1	ND	20.8
Month 3 142-06	3.8		1.5	14.3	0.3	4.3	3.6	8.2	0.2	ND	32.3
Month 3 143-06	2.8		0.8	8.4	0.1	1.4	1.3	1.5	ND	ND	13.5
Month 3 144-06	1.4		0.9	9.7	0.2	1.9	1.5	3.2	ND	ND	17.4
Minimum	0.9		0.3	3.8	< DL	< DL	0.4	0.8	< DL	< DL	6.5
Maximum	6.8		4.3	43.5	1.4	10.2	12.0	81.3	0.5	0.90	118.4
Median	2.9		1.3	12.6	0.2	2.5	2.1	4.7	0.2	0.14	32.8
Mean	3.0		1.6	16.2	0.3	2.8	3.0	10.4	0.2	0.39	34.1
Note. PBDE levels are reported with non-detects (ND) = 0; [a] Data represents the percent lipid content from the stability study;											
[b] Data in the rectangular boxes represents the congener profiles of those samples from the stability study.											

APPENDIX I

MATERNAL CHARACTERISTICS SURVEY

UNIVERSITY of NEW HAMPSHIRE

STEPS TO A HEALTHY FUTURE
Survey #1: Maternal Characteristics

Subject #_____

Today's Date_____

NAME:_____

ANTHROPOMETRIC MEASUREMENTS:

	WEIGHT (pounds) (measured)	**HEIGHT (ft., inches) (reported)**	**BMI Wt (kg)/ Ht m^2 (calculated)**	**PRE-PREGNANCY WEIGHT (reported)**
Start of study (date:_____)				
End of study (date:_____)				

Would you like a free telephone consultation with a lactation specialist to answer any questions you might have on breast feeding and breast pump use?

Yes No

1. Is this your first child?	1. Yes No If no, how many other children have you delivered?
2. Is this your first time breast feeding?	2. Yes No If no, how many other children have you breast fed? 1 2 3 4 5 Other _____ How long did you breast feed your 1st child _____, 2nd child _____, 3rd child _____, 4th child _____, 5th child _____. Please indicate days, months, or years.
3. Is your infant receiving only breast milk?	3. Yes No If no, what supplementary feeding(s) are you using? Formula Water Juice Solid Foods Other_____ How much per day of each are you supplementing with: _____ _____
4. Please indicate your ethnicity.	4. White, non-Hispanic ☐ Hispanic or Latino ☐ Black or African American ☐ American Indian ☐ Alaskan Native ☐ Asian, Native Hawaiian, or Pacific Islander ☐ Other ☐ Please specify:_____

5. Please indicate your level of education.	5. Less than high school graduate ☐ High school diploma or GED ☐ Some college or technical school ☐ College graduate, 2-year degree ☐ College graduate, 4-year degree ☐ Masters degree ☐ Doctoral degree ☐
6. Please indicate your total household income.	6. Less than $20,000 ☐ $20,000 to $34,999 ☐ $35,000 to $49,000 ☐ $50,000 and higher ☐
7. Do you engage in physical activity?	7. Yes No If yes, what category best describes you: ☐ Any leisure time physical activity ☐ Regular and sustained physical activity for 30 minutes or more 5 times per week ☐ Regular and vigorous activity that involves large muscle groups for 20 minutes or more three times per week

HEALTH HISTORY:

Have you been diagnosed by a health care provider with any of the following:

Diabetes	Yes	No	If yes: Type 1 Type 2 Gestational
Asthma	Yes	No	
High Blood Pressure	Yes	No	If yes, current blood pressure:_____
Heart Disease	Yes	No	
High Cholesterol	Yes	No	
Digestive Disorders	Yes	No	Is yes, please indicate which disorder(s) by circling the following:

Crohn's Disease Ulcerative Colitis
Lactose Intolerant Irritable Bowel
Reflux (Heartburn)

Do you have other health issues?

Yes No If yes, please explain: _____

_____

Are you currently taking any medications or dietary supplements?

Yes No If yes, please list:_____

_____

Are you a vegetarian? Yes No If yes, do you exclude (circle all that apply):

Red meat Dairy Products Eggs

Fish Poultry

Have you gone on a diet to lose weight in the past three years? Yes No

If yes, how much weight did you lose? _____

Before you became pregnant, did you smoke cigarettes? Yes No

a. If yes, how many cigarettes on average did you smoke each day? _____

b. For how many years had you been smoking? _____

Do you currently smoke? Yes No

a. If yes, how many cigarettes per day? _____

Do you currently live with or share an office with a smoker? Yes No

a. If yes, how many hours per day are you at home or in the office with a smoker? _____

Is there anything you want to explain regarding your answers?

_____

_____

_____

_____

_____

APPENDIX J

FOOD FREQUENCY QUESTIONNAIRE

A sample of the Food Frequency Questionnaire can be viewed at:

http://www.fhcrc.org/science/shared_resources/nutrition/

Contact information:
Fred Hutchinson Cancer Research Center
1100 Fairview Ave. N. M1-B831
Seattle, WA 98109

Email: ffq@fhcrc.org
Tel.: 1-800-460-7270
Fax: 206-667-7864

APPENDIX K

MATERNAL ENVIRONMENT SURVEY

UNIVERSITY of NEW HAMPSHIRE

STEPS TO A HEALTHY FUTURE
Survey #3: Maternal Environment

Subject #_____
Today's Date_____

RESIDENTIAL INFORMATION
SECTION A

1. What town do you reside in? _____

   a. How long have you been at your current residence? _____

   b. Prior to this residence, where did you live and for how long?

   _____

2. In the past three years, have you lived within a 10 minute walk of an industrial complex? (By industrial complex, we mean an area with several manufacturing facilities)

   Yes No

   If yes, what types of industries were located in that complex?

   _____

   _____

3. In the past three years, have you ever lived within a 10 minute walk of any of the following:

   Yes No

   If yes, please check all that apply.

_____ a. incinerator

_____ b. garbage processing facility/recycling center

_____ c. landfill

_____ d. sewage treatment plant

4. Have you ever been in a house, office, or car fire?

   Yes No

5. On average, how many hours per day are you at home, including sleeping hours? (Please circle one)
   a. Less than 9 hours per day
   b. 10 to 12 hours per day
   c. 13 to 15 hours per day
   d. greater than 15 hours per day

6. Are you a stay at home mom? Yes No

   i. If yes, how long have you been a stay at home mom?

   _____

   (If greater than 1 year skip SECTION B)

OCCUPATIONAL INFORMATION - SECTION B

1. Are you currently working? Yes No
2. Are you on maternity leave from a full-time or part-time job? Yes No

If you answered yes to question 1 or 2, please complete questions a. through k.

a. Where do you work? _____

b. What is your occupation? _____

c. How long have you been at this job? _____

d. What town do you work in? _____

e. How many days per week do you work? _____

f. How long do you work each day? _____

g. What kind of work do you do there? _____

h. Does this job sometimes involve the manufacturing or processing of fabrics, plastic, or foam? Yes No

i. Does this job sometimes involve working on a computer or copy machine?
   Yes No

j. Is there computer equipment, a fax machine, or a copy machine in your office?
   Yes No

k. Do you sit on a foam or padded chair? Yes No

TRANSPORTATION INFORMATION
SECTION C

1. Do you drive your own car? Yes No
   If yes, answer 1a to c. If no, skip to 1d.

   a. What is the year of the car you most often drive? _____

   b. Does your car have ripped seats or exposed foam? Yes No

   c. How many hours per week do you spend in the car? _____

   d. Do you regularly (more than once per week on average) drive or ride in a car other than your own? Yes No

   If yes, answer 1e to g, If no, skip to question 2.

   e. What is the year of this car? _____

   f. Does this car have ripped seats or exposed foam? Yes No

   g. How many hours per week do you spend in that car? _____

2. Do you regularly (once or more per week on average) take the bus or commuter rail?

   Yes No

HOUSEHOLD INFORMATION
SECTION D

1. How many rooms are in your home (including bathrooms): _____

2. Do you have the following products in your home:

   a.Curtains/Valences Yes No

      i. If yes, how many rooms have curtains? _____

   b.Wall to wall carpeting Yes No

   *(**Note:** We don't mean small area rugs, but carpeting that covers most of the floor and is installed with padding underneath)*

      i. If yes, how many rooms have wall to wall carpeting? _____

   c. Hardwood flooring Yes No

      i. If yes, how many rooms have hardwood flooring?
      _____

d. Coffee maker Yes No

i. If yes, how often is the coffee maker used?
   1. less than 1 day per week
   2. 1 to 3 days per week
   3. 3 to 5 days per week
   4. 5 to 7 days per week
   5. greater than once per day

ii. When was your coffee maker purchased?
   1. 2000 to present
   2. 1995 to 1999
   3. 1990 to 1994
   4. 1985 to 1989
   5. Prior to 1984

e. Microwave Yes No

i. If yes, how often is the microwave used?
   1. less than 4 times per week
   2. 5 to 6 times per week
   3. 1 to 2 times per day
   4. greater than 3 times per day

ii. When was your microwave purchased?
   1. 2000 to present
   2. 1995 to 1999
   3. 1990 to 1994
   4. 1985 to 1989
   5. Prior to 1984

f. Toaster Oven Yes No

i. If yes, how often is the toaster oven used?
   1. less than 4 times per week
   2. 5 to 6 times per week
   3. 1 to 2 times per day
   4. greater than 3 times per day

ii. When was your toaster oven purchased?
   1. 2000 to present
   2. 1995 to 1999
   3. 1990 to 1994
   4. 1985 to 1989
   5. Prior to 1984

g. Hair dryer Yes No

i. If yes, how often is the hair dryer used?
   1. less than 1 day per week
   2. 1 to 3 days per week
   3. 3 to 5 days per week
   4. 5 to 7 days per week
   5. greater than once per day

ii. When was your hair dryer purchased?
   1. 2000 to present
   2. 1995 to 1999
   3. 1990 to 1994
   4. 1985 to 1989
   5. Prior to 1984

h. Television set Yes No

i. If yes, how many television sets do you have?
_____

ii. When did you purchase each of your television sets? Place a check mark in the appropriate box for each television set.

	TV Set #1	TV Set #2	TV Set #3	TV Set #4	TV Set #5	TV Set #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

iii. How often is any television on each day?
   1. less than 1 hour per day
   2. 1 to 2 hours per day
   3. 2 to 3 hours per day
   4. 3 to 4 hours per day
   5. greater than 4 hours per day

i. Computer Yes No

i. If yes, how many computers do you have? _____
[One computer is the equivalent of 1 laptop or 1 desktop (monitor & tower)]

ii. When did you purchase each of your computers? Place a check mark in the appropriate box for each computer.

	Computer #1	Computer #2	Computer #3	Computer #4	Computer #5	Computer #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

iii. How often is any computer left on each day?

1. less than 1 hour per day
2. 1 to 3 hours per day
3. 3 to 5 hours per day
4. greater than 5 hours per day

j. Computer Printer Yes No

i. If yes, how many printers do you have?

_____

ii. When did you purchase each of your printers? Place a check mark in the appropriate box for each printer.

	Printer #1	Printer #2	Printer #3	Printer #4	Printer #5	Printer #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

iii. How often is any printer left on each day?
1. less than 1 hour per day
2. 1 to 3 hours per day
3. 3 to 5 hours per day
4. greater than 5 hours per day

k. Stereo Yes No

i. If yes, which of the following do you have for a stereo (**indicate how many of each**):
1. All-in-one stereo (i.e. "boom box") _____
2. Receiver _____
3. CD player _____
4. Cassette player _____
5. Speakers _____
6. Other (_____) _____

ii. When did you purchase your stereo equipment? Place a check mark in the appropriate box for each piece of stereo equipment.

	All in One	Receiver	CD Player	Cassette	Speakers	Other
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

iii. How often is any stereo left on each day?
1. less than 1 hour per day
2. 1 to 3 hours per day
3. 3 to 5 hours per day
4. greater than 5 hours per day

l. Bed Mattress (that you sleep on) Yes No

i. If yes, when was your bed mattress purchased?
1. 2000 to present
2. 1995 to 1999
3. 1990 to 1994
4. 1985 to 1989
5. Prior to 1984

m. What is inside the bed pillows you currently use? Is it:

1. foam
2. down
3. both foam and down
4. not certain
5. other (please specify: _____)

n. In the past three years, have you had any furniture in your house such as a sofa or chair that had crumbling foam?

Yes No

o. Do you have **couches** with cushions/padding? Yes No

i. If yes, how many couches do you have with cushions/padding?
_____

ii. When did you purchase each of your cushioned/padded couches? Place a check mark in the appropriate box for each couch.

	Couch #1	Couch #2	Couch #3	Couch #4	Couch #5	Couch #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

p. Do you have **living room/family room chairs** with cushions/padding?

Yes No

i. If yes, how many living room/family room chairs do you have with cushions/padding? _____

ii. When did you purchase each of your cushioned/padded living room/family room chairs? Place a check mark in the appropriate box for each chair.

(see next page)

	Chair #1	Chair #2	Chair #3	Chair #4	Chair #5	Chair #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

q. Do you have **rocking chairs** with cushions/padding? Yes No

i. If yes, how many rocking chairs do you have with cushions/padding?

_____

ii. When did you purchase each of your cushioned/padded rocking chairs? Place a check mark in the appropriate box for each rocking chair.

	Chair #1	Chair #2	Chair #3	Chair #4	Chair #5	Chair #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

r. Do you have **kitchen chairs** with cushions/padding? Yes No

i. If yes, how many kitchen chairs do you have with cushions/padding?

_____

ii. When did you purchase each of your cushioned/padded kitchen chairs? Place a check mark in the appropriate box for each kitchen chair.

(see next page)

	Chair #1	Chair #2	Chair #3	Chair #4	Chair #5	Chair #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

s. Do you have **dining room chairs** with cushions/padding? Yes No

i. If yes, how many dining room chairs do you have with cushions/padding? _____

ii. When did you purchase each of your cushioned/padded dining chairs? Place a check mark in the appropriate box for each dining room chair.

	Chair #1	Chair #2	Chair #3	Chair #4	Chair #5	Chair #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

t. Do you have **desk chairs** with cushions/padding? Yes No

i. If yes, how many desk chairs do you have with cushions/padding?
_____

ii. When did you purchase each of your cushioned/padded desk chairs? Place a check mark in the appropriate box for each desk chair.
(see next page)

	Chair #1	Chair #2	Chair #3	Chair #4	Chair #5	Chair #6
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

u. Do you have **other furniture** with cushions/padding? Yes No

i. If yes, please indicate what these furniture pieces are?

_____

_____

_____

ii. When did you purchase each of these pieces? Place a check mark in the appropriate box for each piece.

	Other #1 _____	Other #2 _____	Other #3 _____	Other #4 _____	Other #5 _____	Other #6 _____
2000 to present						
1995 to 1999						
1990 to 1994						
1985 to 1989						
Prior to 1984						

HOBBIES/CRAFT/HOME IMPROVEMENT INFORMATION
SECTION E

This section of questions is about hobbies, crafts, or work around the house that you might do when you're not at work.

1. Do you have any hobbies like arts and crafts or model building or do you do any home improvement work that involve working with plastic, foam, or fabric?

   Yes No

   If yes:

   a. **In what way do you work with plastic? Check all that apply.**

   ____ Cutting/Shredding

   ____ Sanding

   ____ Burning

   ____ Melting

   ____ Molding

   ____ Other (Please specify:_____)

   b. **In what way do you work with foam? Check all that apply.**

   ____Cutting/Shredding

   ____Burning

   ____Molding

   ____Other (Please specify:_____)

   c. **In what way do you work with fabric? Check all that apply.**

   ____ Sewing clothing

   ____ Furniture repair/reupholstering

   ____ Sewing other household items such as curtains or linens

   ____ Other (Please specify:_____)

Is there anything you want to explain regarding your answers?

APPENDIX K

FEEDBACK QUESTIONNAIRE

STEPS TO A HEALTHY FUTURE
We would love your feedback!

Feedback Questionnaire for Maternal Environment Survey

1. How long did the Maternal Environment Survey take to complete?

2. Did you have a hard time answering some of the questions?
   If yes, please indicate section and question number and WHY.

3. Were any questions confusing?
   If yes, please indicate section and question number and WHY.

4. Did you find it helpful to complete the survey in your home?

5. Do you have any suggestions for improving the survey?

APPENDIX M

INSTITUTIONAL REVIEW BOARD APPROVAL LETTERS

UNIVERSITY of NEW HAMPSHIRE

April 18, 2005

Dunn, Rebecca
Animal & Nutritional Sciences, Kendall Hall
219 Brickyard Road
Nelson, NH 03457

IRB #: 3433
Study: Biomonitoring Polybrominated Diphenyl Ethers (PBDEs) in Lactating Women
Approval Date: 04/18/2005

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved the protocol for your study as Expedited as described in Title 45, Code of Federal Regulations (CFR), Part 46, Subsection 110.

Approval is granted to conduct your study as described in your protocol for one year from the approval date above. At the end of the approval period, you will be asked to submit a report with regard to the involvement of human subjects in this study. If your study is still active, you may request an extension of IRB approval.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the attached document, *Responsibilities of Directors of Research Studies Involving Human Subjects.* (This document is also available at http://www.unh.edu/osr/compliance/IRB.html.) Please read this document carefully before commencing your work involving human subjects.

If you have questions or concerns about your study or this approval, please feel free to contact me at 603-862-2003 or Julie.simpson@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

Julie F. Simpson
Manager

cc: File
Gale Carey

UNIVERSITY of NEW HAMPSHIRE

September 16, 2005

Rebecca Dunn
Animal & Nutritional Sciences, Kendall Hall
219 Brickyard Road
Nelson, NH 03457

IRB #: 3433
Study: Biomonitoring Polybrominated Diphenyl Ethers (PBDEs) in Lactating Women
Approval Expiration Date: 04/18/2006 **Modification Approval Date:** 09/13/2005
Modification: Changes to study as per letter to IRB dated August 26, 2005

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved your modification to this study, as indicated above. Further changes in your study must be submitted to the IRB for review and approval prior to implementation.

Approval for this protocol expires on the date indicated above. At the end of the approval period you will be asked to submit a report with regard to the involvement of human subjects in this study. If your study is still active, you may request an extension of IRB approval.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the document, *Responsibilities of Directors of Research Studies Involving Human Subjects.* This document is available at http://www.unh.edu/osr/compliance/irb.html or from me.

If you have questions or concerns about your study or this approval, please feel free to contact me at 603-862-2003 or Julie.simpson@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

Julie F. Simpson
Manager

cc: File
Gale Carey