Integrating psychosocial stress into children's molecular epidemiology research: An investigation of flame retardants, telomeres and neuroendocrine development

Whitney J. Cowell

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2018

ABSTRACT

Integrating psychosocial stress into children's molecular epidemiology research: An investigation of flame retardants, telomeres and neuroendocrine development

Whitney J. Cowell

Background & Objectives: This dissertation is comprised of two independent projects that seek to answer the research questions outlined in Aims 1 and 2. The first project is focused on measuring exposure to polybrominated diphenyl ethers (PBDE) throughout the early lifecourse, as well as investigating how exposure at different developmental periods relates to neuroendocrine endpoints. PBDEs are flame retardant chemicals that were used extensively in furniture and furnishings sold throughout the United States until their phase-out in 2004. Human exposure occurs primarily through incidental ingestion of PBDE-contaminated dust present in the indoor environment. The second project aimed to characterize telomere dynamics in maternal-child pairs and to evaluate associations between telomere dynamics and indicators of stress and stressful conditions. Telomeres are non-coding nucleotide repeats located at chromosome ends; they serve several functions, such as buffering against loss of important protein coding DNA regions during cell division. Both projects are focused on exposure-response relationships during early life and a central theme throughout this dissertation relates to the intersection of date, time and age in longitudinal cohort studies. Finally, the third aim seeks to integrate findings from Projects I and II and is focused on investigating whether telomere dynamics can be used as a biological indictor of stress in epidemiological research examining associations between low-level environmental chemical exposures and neurodevelopmental endpoints.

Methods: Both projects were conducted using data and samples collected as part of the Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns study. In Project I, PBDEs were measured by the Centers for Disease Control and Prevention (CDC) using gas chromatography-mass spectrometry (GC-MS) in plasma samples collected repeatedly between birth and age 9 years. We examined determinants of 1) prenatal exposure to PBDEs (Chapter 2), and 2) trajectories of PBDE exposure over childhood, which we estimated using latent class growth analysis (LCGA) (Chapter 3). We also examined PBDE trajectories in relation to performance on tests of visual, verbal and working memory among early adolescents (Chapter 4) and investigated associations between prenatal exposure to PBDEs and thyroid hormone parameters, which were measured by radioimmunoassay in plasma samples collected at multiple ages (Chapter 5). In Project II, we used monochrome multiplex quantitative polymerase chain reaction (MMqPCR) to measure relative leukocyte telomere length (rLTL) in samples collected from mothers and newborns (umbilical cord blood) at the child's delivery and from children at ages 2, 3, 5, 7 and 9-years (Chapter 6). We aimed to characterize rLTL dynamics over early life, examine the correlation between paired maternal-newborn rLTL, and examine associations between rLTL with measures of financial strain, perceived stress and maternal distress.

Results: In Project I, we detected PBDEs in over 80% of cord blood samples and in multivariable models, sociodemographic and lifestyle factors explained 12% of cord blood PBDE variability. The largest determinant of exposure was ethnicity, with Dominican newborns having lower exposure compared to African American newborns, likely due to the reduced amount of time Dominican mothers had spent in the United States when they gave birth to the

study child. Across postnatal life (2000 to 2013), PBDE concentrations in child blood decreased by approximately 12% per year, suggesting that exposure has continually declined since the PBDE phase-out in 2004. Trajectory analyses revealed several unique patterns of PBDE exposure over the early lifecourse, with the majority of children characterized by exposure that was persistently low or that peaked during toddler years. Smaller groups of children were characterized by exposure that was highest during the prenatal period and decreased after birth or by a pattern of high exposure during toddler years that remained elevated into middle childhood. We identified several important predictors of childhood PBDE exposure patterns, including modifiable factors, such as cleaning behaviors. In relation to neurodevelopmental outcomes, we found that children with sustained high exposure to PBDEs scored approximately 5-8 points lower on tests of visual memory. Associations between prenatal exposure and working memory significantly varied by sex, with inverse associations (approximately 8 points lower) observed only among girls. Children with PBDE plasma concentrations that peaked during toddler years performed better on verbal domains, however, these associations were significant only among children breastfed for more than 12 weeks. Finally, in relation to thyroid hormone levels, children with BDE-47 concentrations in the third and fourth quartiles of the exposure distribution (versus first quartile) had significantly lower TSH and free T_4 levels, respectively. We did not detect associations between BDE-47 and total T_4 levels; likewise, we did not detect associations between other pentaBDE congeners and any thyroid parameter.

In Project II, we found that maternal-newborn rLTL in paired samples was moderately correlated and that maternal rLTL at delivery explained 8% of the variability (R^2) in newborn rLTL. In relation to measures of hardship, perceived stress and demoralization, we found an inverse, albeit

not statistically significant, association between maternal perceived stress and newborn rLTL. We did not detect an association with maternal rLTL, nor did we detect associations between material hardship or demoralization and maternal or newborn rLTL. When examining rLTL in child blood samples collected between birth and age 9 years, we observed a U-shaped pattern characterized by rapid shortening of rLTL between birth and 2 years, followed by gradual lengthening between ages 3 and 9 years. It remains unresolved whether this pattern reflects a true biological phenomenon or if it is an artifact of measurement error introduced by analytic or pre-analytic conditions.

Conclusions: Despite the phase-out of PBDEs in 2004, exposure among children residing in New York City remained nearly ubiquitous through 2013, however, concentrations did decline over time. Our finding of several PBDE trajectories suggests that, despite the relatively long half-lives of PBDEs, a single measure may not accurately reflect exposure throughout childhood. Our findings of reduced scores on tests of working and visual memory during the prenatal and postnatal periods, respectively, support a growing body of literature linking early life PBDE exposure to disrupted neurodevelopment. The results of our analysis examining thyroid hormone disruption during childhood revealed a pattern consistent with hypothalamic or pituitary-level disruption during prenatal programming of the thyroid regulatory system. This is the first study to examine prenatal PBDE exposure in relation to childhood thyroid hormone levels, therefore, it is important that this finding is replicated by future research. Our finding of an inverse association between newborn rLTL and maternal perceived stress is consistent with results from previous research and suggests that the developing fetus may be sensitive to maternal stress perception during pregnancy, however, additional research is needed to more fully understand

the mechanisms through which this transmission occurs. Our finding of increasing telomere length between toddler years and middle childhood is unexpected and raises questions about the suitability of the qPCR assay for analyzing telomere length in archived samples. Additional analyses are needed to determine whether the observed patterns reflect true biological changes or relate to measurement error introduced during sample processing, storage or analysis. Given these outstanding issues, we were ultimately unable to draw conclusions about the usefulness of telomere dynamics as a stress-sensitive biomarker.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Introduction

Chapter 1

Chapter 2

Chapter 3

Chapter 4

LIST OF ABBREVIATIONS

ACI	Attention Concentration Index
BIC	Bayesian Information Criterion
CAL-117	California Technical Bulletin-117
CCCEH	Columbia Center for Children's Environmental Health
CDC	Centers for Disease Control and Prevention
CMS	Children's Memory Scales
CPF	Chlorpyrifos
CV	Coefficient of Variation
DAG	Directed Acyclic Graph
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
ETS	Environmental tobacco smoke
GBTM	Group-based trajectory modeling
GCMS	Gas chromatography mass spectrometry
GEE	Generalized estimating equation
HOME	Home Observation for Measurement of the Environment
HSC	Hematopoietic stem cell
IQ	Intelligence quotient
LCGA	Latent class growth analysis
LOD	Limit of detection
MMqPCR	Monochrome multiplex quantitative polymerase chain reaction
NIEHS	National Institute of Environmental Health Science
NYC	New York City
Pb	Lead
PBB	Polybrominated biphenyl
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PERI-D	Psychiatric Epidemiology Research Instrument- Demoralization scale
POP	Persistent organic pollutant
PSS-4	Perceived Stress Scale, 4 item version
QFISH	Quantitative Fluorescent in situ hybridization
qPCR	Quantitative polymerase chain reaction
rLTL	Relative leukocyte telomere length
RCMAS	Revised Children's Manifest Anxiety Scale
RNA	Ribonucleic acid
ROS	Reactive oxygen species

STELA	Single Telomere Length Analysis
T3	Triiodothyronine
T4	Thyroxine
TC	Total cholesterol
TG	Total triglycerides
TL	Total lipid
TONI	Test of nonverbal intelligence
TRF	Terminal restriction fragment
TRH	Thyroid releasing hormone
TSH	Thyroid stimulating hormone
UNEP	United national environmental program
VeMI	Verbal memory index
ViMI	Visual memory index
WBC	White blood cell
WISC-IV	Wechsler intelligence scales for children, 4th edition

ACKNOWLEDGEMENTS

Over the past 5 years many people have not only provided support, insight and warmth, but have challenged me to think critically, to always remember the bigger picture, and to appreciate the hidden art of data analysis and presentation. I am especially grateful to Julie Herbstman, who has been an inspiration, mentor and friend; she has always supported my ideas and encouraged my interests regardless of the direct relevance to her work. My time as a doctoral student was undoubtedly enriched by the opportunity to train under her mentorship.

Robin Whyatt, an early mentor and committee member, nurtured my interests in combined exposures to chemical and non-chemical stressors and was instrumental in forming my dissertation committee. The other members of my committee: Virginia Rauh, Regina Santella, Rosalind Wright and Andrea Baccarelli, reviewed drafts of this work and provided helpful feedback and ideas throughout the dissertation research and writing process.

I am grateful to have had the opportunity to work with an amazing dataset generated by the outstanding and dedicated team of research workers, scientists, and academics at the Columbia Center for Children's Environmental Health (CCCEH). A special thank you to Lehyla Calero -- who encouraged and made possible my participation in all aspects of the research process, Darrell Holmes – who always had the answer to my wide-ranging questions relating to the birth cohort, Amy Margolis – who has an expansive understanding of child development and guided my decision to focus on children's memory outcomes, Shuang Wang and Ya Wang -- who provided statistical oversight and support throughout Project I of this dissertation, and Frederica Perera who has, despite the ever changing funding landscape, succeeded in keeping the Mothers

and Newborns birth cohort alive over the past 21 years. The breadth and depth of data that has been collected on these mothers and children is truly remarkable and this dissertation would not exist without it. I would also like to thank the mothers and children (and now adolescents!) who have participated in the cohort over the past 21 years.

I owe a special thank you to the CCCEH Molecular Epidemiology Laboratory: Deliang Tang-- who guided the work on telomeres and helped trouble shoot all aspects of this research, Jie Yu-- who spent countless hours optimizing the PCR protocol, patiently taught me the ins and outs of pipetting, and persisted with this project after nearly a year of failed trials, and Leena Qu who has recently spent a great deal of time helping to sort through archived laboratory data in our attempt to understand some of the less intuitive results of this work.

Likewise, I am grateful to have had the opportunity to work with Andreas Sjodin at the CDC who in addition to analyzing the PBDE concentrations investigated here, read many drafts of this work and encouraged my somewhat nuanced thinking related to biomarker measures.

Graduate school was undoubtedly made more enjoyable by the ambitious and generous group of peers I have had the privilege to learn and grow with; the high level of support among students is a good reminder of our common goal to promote well-being. A special thank you to Eliza Little, who helped me stay grounded throughout this process, and to my pseudo-cohort -- Ashlinn Quinn, Caitlin Howe, and Tiffany Sanchez – who adopted me into their circle early in the program and eased some of the isolating experiences often ascribed to graduate school.

My decision to pursue a PhD was cultivated by Wendy Heiger-Bernays, my MPH advisor at Boston University and fostered by Susan Korrick, who acted more as a mentor than an employer during the two years I worked on her New Bedford-based birth cohort. I would not have matriculated in this program without their early support and encouragement.

Lastly, a special thank you to my parents who have supported my interests and decision to enter the field of public health [and to my mom for illustrating the "Hierarchy of Hematopoiesis" presented in Figure 2 of the Introduction!]. To my brother Mackenzie who is curious about the world and shaped my interests in science and health from an early age and my sister Katherine who wants the best for everyone and reminds me to stop and laugh every once in a while.

A theme throughout this dissertation relates to the growth and development that inextricably occurs over time and as I reflect on my work over the past 5 years, I realize Columbia has provided a setting that has enabled a great deal of personal growth and change, for which I am deeply grateful.

PREFACE

Experimental research provides convincing evidence that stress may modify susceptibility to developmental neurotoxicants through multiple biological mechanisms (Hougaard and Hansen 2007). Findings from studies spanning species and life stages support a model in which chronic exposure to stress leads to disrupted internal equilibriums, thereby enhancing normal age-related 'wear and tear' on the body and ultimately augmenting an individual's vulnerability to concurrent or subsequent external exposures (Bellinger 2000; McEwen and Tucker 2011; Weiss and Bellinger 2006; Wright 2009). A decade ago, the Environmental Protection Agency (EPA) began emphasizing the importance of integrating susceptibility into standard risk assessment approaches (EPA 2007) and recently the National Institute of Environmental Health Science (NIEHS) identified the interaction between chemical and non-chemical stressors as a priority area in the field of environmental health (NIEHS 2017). Despite these calls to action, the majority of environmental epidemiology research continues to investigate main effects associated with individual chemical exposures, likely due in part to the challenges associated with identifying and measuring the salient aspects of our social environment. For example, while the exposure sciences have excelled at developing biomarkers for measuring cellular and systemic indicators of exposure to environmental chemicals, few biological markers of the cumulative physiological effects of stress on the body exist. Over the past decade, research has emerged suggesting that telomeres, which are non-coding nucleotide repeats that cap chromosome ends, may serve as an indicator of cellular 'wear and tear', potentially providing a tangible and objective measure for integrating stress into environmental epidemiology research. Importantly, the majority of current research investigating associations between stress – or stress correlates (i.e. financial strain) and telomere dynamics is limited by cross-sectional study

designs, small sample sizes and investigation of adult populations (Coimbra et al. 2017; Hanssen et al. 2017; Li et al. 2017; Oliveira et al. 2016; Ridout et al. 2017; Shalev 2012). The overarching goal of the research presented in this dissertation was to determine whether early life telomere dynamics can serve as a cellular indicator of cumulative 'wear and tear' and, in turn, susceptibility to the adverse effects of concurrent or subsequent exposure to developmental neurotoxicants.

Chapter 1 reviews recent research and putative mechanisms underlying the interaction between psychosocial stressors and environmental neurotoxicants and serves as a 'proof of concept' that supports the overarching aim of this dissertation. The remaining chapters present the results of original research, which was conducted in the form of two independent projects (Project I and II) and is presented as several self-contained papers. The first four studies (Project I), make up Chapters 2-5, and are focused on evaluating time-trends, developmental patterns and determinants of polybrominated diphenyl ether (PBDE) exposure, as well as assessing PBDEs in relation to thyroid hormone parameters and neurocognitive outcomes (Aim 1). In the United States, PBDEs were used as flame retardants in household consumer products manufactured between 1975 and 2013 (Abbasi et al. 2015), resulting in nearly ubiquitus human exposure (Lorber 2008). PBDEs structurally resemble several legacy pollutants with known human toxicity, including polychlorinated biphenyls (PCBs), and have been classified as developmental neurotoxicants and endocrine disrupting chemicals (Linares et al. 2015). Project II is presented in Chapter 6 and further discussed in the concluding chapter on future directions. This work is focused on characterizing telomere dynamics over the early lifecourse, as well as investigating

associations between several dimensions of stress and telomere length measured in maternal and cord blood samples collected at delivery (Aim 2).

The introduction provides an overview of the study population, presents a brief review of published literature, highlights key methodological issues relevant to Projects I and II, and presents a Statement of Hypotheses. Detailed background and methodological information, as well as findings specific to each of the self-contained studies is provided within each chapter. In addition to the self-contained studies, challenges associated with completing Aim 3, which sought to integrate Projects I and II by examining whether associations between PBDE exposure and neuroendocrine outcomes varied by telomere length, is presented in a concluding sect

STATEMENT OF HYPOTHESES

Aim 1. Estimate trajectories of early life PBDE exposure and assess how exposure relates to thyroid hormone levels and child memory outcomes.

- Hypothesis 1.1: PBDEs will be detectable in the majority of samples and exposure will peak during early childhood.
- Hypothesis 1.2: Higher prenatal and postnatal PBDE exposure will be associated with lower memory scores among children.
- Hypothesis 1.3: Controlling for postnatal PBDE exposure, prenatal PBDE exposure will be associated with decreased T_4 and increased TSH levels.

Aim 2. Characterize the association between objective, perceptive and emotional dimensions of stress on telomere dynamics during early life.

- Hypothesis 2.1: Telomere length at birth and rate of erosion will vary among children.
- Hypothesis 2.2: Variation in telomere length at birth will be associated with maternal experiences of stress and stressful conditions reported during pregnancy; greater stress will be associated with shorter telomere length at birth.
- Hypothesis 2.3: Variation in rate of telomere erosion during childhood will be associated with maternal and child experiences of stress and stressful conditions experienced during early life; greater stress will be associated with accelerated erosion during childhood.

Aim 3. Determine if early life telomere dynamics can serve as an indicator of susceptibility to the adverse effects of concurrent or subsequent neurotoxicant exposure.

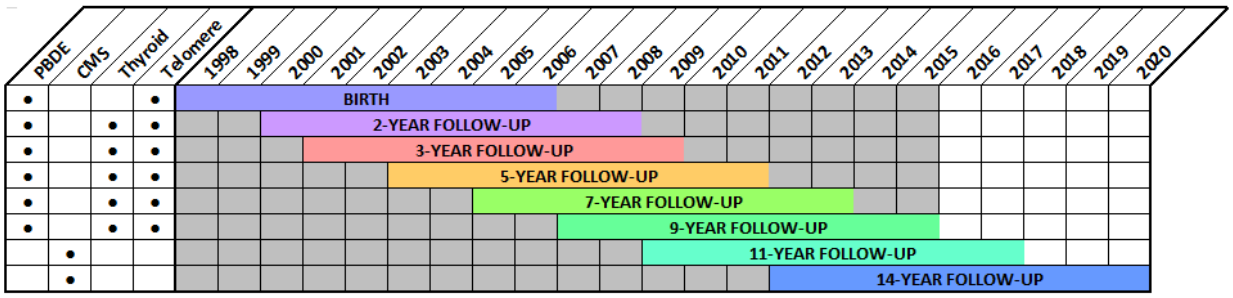
- Hypothesis 3.1: The effect of PBDEs on cognitive outcomes will be greatest among children experiencing the greatest early life stress, indicated by shorter telomere length.

INTRODUCTION

OVERVIEW OF THE STUDY POPULATION

The research presented in this dissertation was conducted using samples and data collected from 727 maternal-child pairs enrolled in the Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns birth cohort. Healthy, pregnant women were recruited from two prenatal clinics in Northern Manhattan between 1998 and 2006. The cohort has been followed since 1998 by a team of research workers trained in administering questionnaires, conducting neurodevelopmental assessments and collecting biological samples. As illustrated by Figure 1, this dissertation includes data and samples collected during pregnancy, at birth, and at ages 2, 3, 5, 7, 9, 11 and 14 year visits; given the multi-year enrollment design, the year of study visit ranges from 1998 to 2015.

Figure 1. Overview of the CCCEH data and samples analyzed in this dissertation.



Legend: CMS: Children's Memory Scales (standardized neurodevelopment test)

At the time of enrollment, the study population was 35% African American and 65% Dominican. On average, mothers were 25 years old, 36% had less than a high school education, and approximately 60% reported an annual household income less than or equal to $20,000. Additional details describing the study population are provided within each chapter.

BACKGROUND FOR PROJECT I: PBDE EXPOSURE OVER THE EARLY LIFECOURSE IN RELATION TO NEUROENDOCRINE OUTCOMES

History of PBDE use in the United States

In 1970, approximately 37% of adults smoked cigarettes and household fires attributable to ignition of upholstered furniture from improperly extinguished cigarettes was cited as the leading cause of fire-related deaths in the United States (Callahan et al. 2012). In response to these statistics, the state of California initiated legislation requiring that companies manufacture self-extinguishing cigarettes. In turn, the tobacco industry lobbied against the proposed legislation, while simultaneously promoting the passage of fire safety standards that would require the use of flame retardant chemicals in household consumer products (Callahan et al. 2012). In 1975, five years after the initial fire-safe cigarette proposal, California passed Technical Bulletin 117 (Cal-117), which required all components of upholstered furniture to comply with an 'open flame' test before entering state commerce (Cal-117 1975). It was effectively impossible to pass this standard without the use of added flame retardant chemicals, and because the majority of furniture in the United States is sold at the national (versus state) level, Cal-117 became a *de facto* national fire safety standard.

Overview of PBDE properties, sources, exposure pathways & health outcomes

PBDEs were the primary flame retardant chemical used to meet Cal-117. This class of chemicals consists of 209 organohalogenated congeners that vary in the number and position of bromine atoms around a diphenyl ether backbone (Bergman et al. 2012). Commercially, PBDEs were marketed as three technical mixtures referred to as pentaBDE, octaBDE and decaBDE, each of which contains fewer than 10 congeners (EPA 2010). This dissertation focuses on BDE-47,

BDE-99, BDE-100, and BDE-153, which are the predominant congeners in the pentaBDE formulation (Talsness 2008). Figure 2 presents the chemical structures of these four congeners.

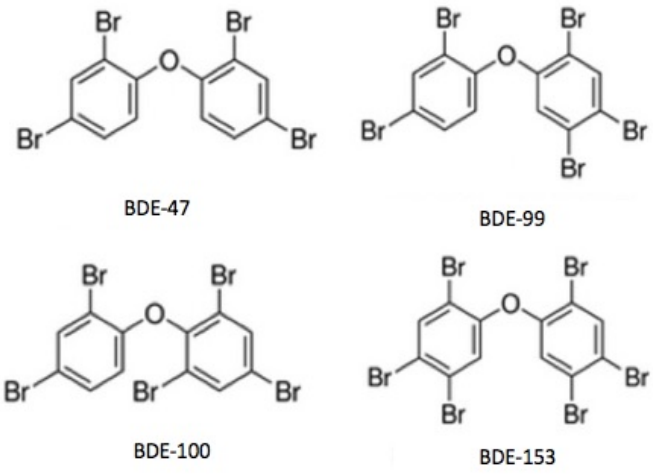


Figure 2: Chemical structures of four PBDE congeners of interest

Extensive background information on PBDE sources, exposure pathways, body burdens, and health endpoints is presented in Chapters 2-5. Briefly, pentaBDE was primarily applied to furniture and other furnishings containing polyurethane foam, with an estimated 46,000 tons used in North America between 1975 and 2004 (Abbasi et al. 2015). Human exposure occurs primarily through incidental ingestion of household dust.

Rodent studies indicate that when PBDEs are administered by gavage, 70-85% [pentaBDE congeners] is absorbed and distributed widely throughout the body (ATSDR 2017). Owing to their lipophilic properties, PBDEs accumulate in adipose tissue (ATSDR 2017), cross the placenta (Dassanayake et al. 2009) and partition into breast milk (Fang et al. 2015). PentaBDE congeners are biotransformed into several mono- and di-hydroxylated metabolites, likely through the catalytic action of CYP2B6 (Erratico et al. 2013; Feo et al. 2013). In adults, PBDE

elimination half-lives are estimated to range from 1.6 years (BDE-99) to 6.5 years (BDE-153) (Geyer et al. 2004), however, little is known about the kinetics of PBDE uptake, body burden, or elimination in children (Gyalpo et al. 2015).

Animal and human research provides evidence that prenatal and childhood exposure to PBDEs is associated with multiple health effects (Linares et al. 2015), including disrupted neurocognitive development (Roth and Wilks 2014). A recent systematic review and meta-analysis concluded that there is sufficient evidence of moderate quality to support an association between developmental (in-utero, perinatal, childhood) exposure to PBDEs and impaired intellectual functioning. Specifically, the authors estimated a 10-fold increase in exposure to be associated with a loss of 3.7 intelligence quotient (IQ) points (Lam et al. 2017). Likewise, studies conducted in rodents provide evidence of an inverse associations between PBDE exposure and performance on tests of memory and learning (Driscoll et al. 2009; Dufault et al. 2005; Viberg et al. 2003; Viberg et al. 2006).

As illustrated by Figure 3, PBDEs and their hydroxylated metabolites structurally resemble the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), which play critical roles during brain development (Mary and Zoeller 2010).

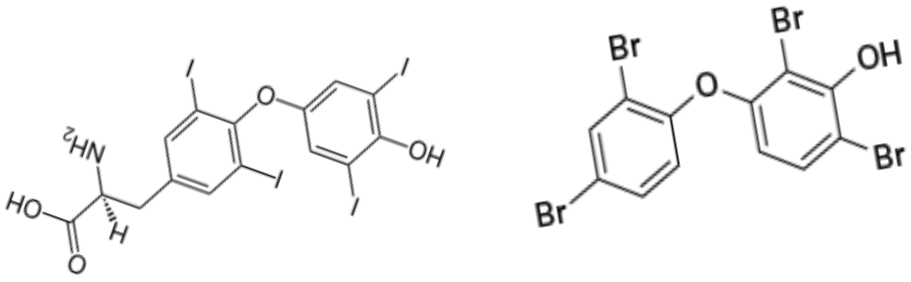


Figure 3. Comparison of the structures of T_4 (left) and hydroxylated BDE-47 (right)

Inadequate T_4 during the first trimester, when the developing fetus is completely dependent on maternal thyroid hormones (Williams 2008), is associated with impaired visual processing and memory deficits (Zoeller and Rovet 2004). Likewise, deficiency during later pregnancy and infancy is associated with impaired language skills and verbal memory (Zoeller and Rovet 2004). The structural similarity between PBDE metabolites and thyroid hormones has prompted researchers to investigate whether thyroid hormone disruption serves as a mechanism through which PBDEs exert their neurotoxic effects. Experimental research has consistently documented an inverse association between prenatal exposure to pentaBDE congeners and T_4 levels in fetal and adult rats (Costa et al. 2014) and lambs (Abdelouahab et al. 2009), however, results from prospective birth cohorts have been inconsistent. In the largest human study (n=380), cord blood PBDE concentrations were associated with decreased total T_3 and T_4, but increased free (not protein bound) T_3 and T_4 (Abdelouahab et al. 2013). Smaller studies have detected a mix of results including decreased total T_4 and TSH (Chevrier et al. 2010; Vuong et al. 2015), increased T_4 and TSH (Stapleton et al. 2011), increased T_4 and decreased TSH (Vuong et al. 2015), and decreased TSH with no change in T_4 (Herbstman et al. 2008). Importantly, due to normal fluctuations in thyroid hormones that occur during pregnancy and parturition, a major limitation of these studies is the measurement of thyroid hormone levels in blood collected during these periods.

Putative mechanisms underlying the findings observed in animal studies include PBDE-induced upregulation of T_4 metabolism and excretion, interference with thyroid transport systems, and interaction with thyroid receptors (Costa et al. 2014). Additionally, fetal exposure to inappropriately high or low thyroid hormone levels has been shown to alter prenatal

programming of the hypothalamic-pituitary-thyroid (HPT) axis, which regulates circulating thyroid hormone levels through a negative feedback mechanism (Fisher and Klein 1981). Therefore, it is possible that direct fetal exposure to PBDEs, or fetal exposure to PBDE-related reductions in maternal thyroid hormone levels, may disrupt prenatal setting of this regulatory system (Cavaliere et al. 1985).

Owing to mounting health concerns, as well as evidence indicating PBDEs are resistant to environmental degradation and capable of long-range transport in the environment (Carlsson et al. 2011), pentaBDE was phased-out of United States commerce in 2004 and added to the Stockholm Convention's list of persistent organic pollutants (POPs) in 2009 (UNEP 2012). Most recently, California amended its flame retardant standard from an open flame test to a smolder test, which can be passed without the use of added flame retardant chemicals (Cal-117 2013). Despite these regulatory changes, human exposure is expected to continue for decades due to the continued release of PBDEs from household products that are infrequently replaced.

Methods of PBDE measurement

Given the relatively long half-lives of PBDEs (1.5-6.5 years), serum and plasma PBDE concentrations are an established and reliable biomarker of exposure (EPA 2010). Blood PBDE concentrations are typically measured by gas chromatography isotope dilution high-resolution mass spectrometry; the Centers for Disease Control and Prevention's (CDC) Persistent Organic Pollutants (POP) laboratory, which analyzed all samples in this dissertation, has extensive experience performing this protocol.

PBDEs partition into blood lipids, which vary postprandially. The majority of large epidemiologic studies, especially those involving pregnant women and children, are not able to collect fasting blood samples for feasibility reasons. As an alternative, variation in blood lipid levels is typically controlled for by standardization (i.e. ng PBDE/g lipid) or lipid adjustment. A recent study that used directed acyclic graph (DAG) theory to investigate bias introduced by various lipid control approaches concluded that lipid standardization or standardization plus adjustment performed better than adjustment alone (O'Brien et al. 2016). Based on these findings, we report PBDE concentrations on a lipid-standardized basis throughout this dissertation and perform sensitivity analyses adjusting for lipid concentrations as a covariate when relevant.

Due to the large amount of blood required to measure phospholipids and free cholesterol, it is common for researchers studying POPs to estimate total blood lipid (TL) levels from measured concentrations of total cholesterol (TC) and total triglycerides (TG) using a standard summation equation ($TL_{adult} = 2.27 \times TC + TG + 0.623$, in grams/liter) (Phillips et al. 1989). This equation was derived from measures of lipid components in serum samples collected from adult men (n=81) living in the 1960s. In addition to changes in population blood lipid levels over the past 50 years, the unique lipid profile of cord blood (Dyerberg et al. 1974) raises questions about the suitability of this equation for lipid-standardizing POPs measured in cord blood. To investigate the degree of error introduced by this formula, we worked with the CDC to derive a new, cord blood specific equation ($TL_{cord}=2.66 \times TC + TG + 0.268$) based on measurement of all four lipid components in n=100 cord plasma samples collected from a cohort of healthy infants (cohort described: (Herbstman et al. 2010)). We further validated this equation using measures of all four

lipid components in cord plasma samples collected from 40 infants enrolled in a second cohort of healthy infants (cohort described: (Cowell et al. 2017)). We determined that the bias introduced by estimating total cord blood lipids based on the original adult formula was 9% compared to <1% from the new, cord blood-specific formula (Sjodin A, 2016, unpublished data). Given these findings, we estimate total cord blood lipids using the new formula in all analyses presented in this dissertation.

BACKGROUND FOR PROJECT II: CHARACTERIZATION OF TELOMERE DYNAMICS OVER THE EARLY LIFECOURSE AND ASSOCIATIONS WITH MATERIAL HARDSHIP, MATERNAL STRESS AND PSYCHOLOGICAL DISTRESS

Telomere structure and function

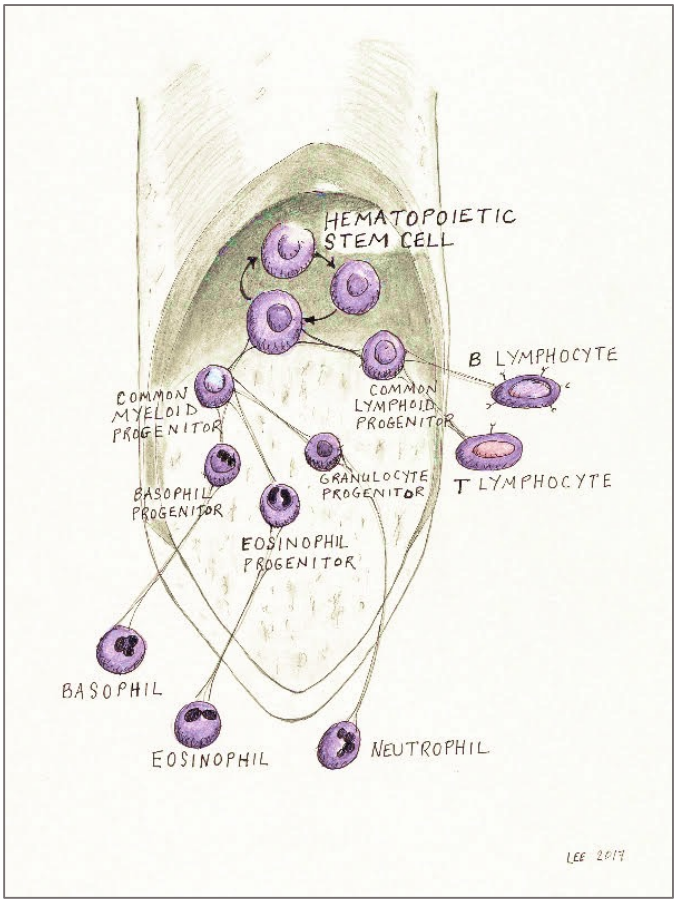
The body consists of both non-renewable (i.e. brain, heart, skeletal muscle) organs comprised of differentiated, post-mitotic cells largely incapable of cellular division and renewable (i.e. skin, intestine, hematopoietic) systems made up of stem and progenitor cells that proliferate throughout the lifespan (Aubert and Lansdorp 2008). In dividing cells, polymerase is unable to replace the terminal RNA primer on the lagging DNA strand; as a solution to this "end replication problem", telomerase, a highly conserved RNA template-containing reverse transcriptase, binds to and extends the lagging strand to completion (Bonetti et al. 2013). In humans, telomerase expression is highest in stem cells, lower in progenitor cells, and largely absent in somatic cells. In dividing cells expressing no or low levels of telomerase, chromosome erosion (~50-200 base pairs) occurs with each cell division (Harley et al. 1990). To prevent loss of protein coding regions, terminal chromosome ends consist of repeating T_2AG_3 sequences, known as telomeres (Moyzis et al. 1988). In addition to acting as a buffer, telomeres serve as a substrate for telomerase binding, provide chromosomal stability, prevent chromosome ends from appearing as double strand breaks, and reduce fusing and breakage during mitosis (Zhao 2014).

Telomere length across the lifespan

Circulating leukocytes are replenished from progenitor cells, which are derived from a pool of pluripotent hematopoietic stem cells (HSCs) in the bone marrow (Werner et al. 2015). HSCs are defined by the ability to differentiate into all functional blood cells and the capacity to self-

renew, which is estimated to occur approximately 100 times during the human lifespan (Seita and Weissman 2010). In order to maintain the approximately ~4.10^{15} blood cells produced over a lifetime, hematopoiesis follows a hierarchical pattern of division in which multipotency is progressively restricted (Nordfjall et al. 2009). As illustrated by Figure 4, HSCs can 1) self-renew, 2) give rise to a common myeloid progenitor, or 3) give rise to a common lymphoid progenitor. In turn, myeloid progenitors give rise to granulocytes (primarily neutrophils, eosinophils, basophils) and lymphoid progenitors give rise to lymphocytes (primarily B cells and T cells). Collectively, these cells are referred to as leukocytes or white blood cells (WBCs) (Werner et al. 2015).

Figure 4. Hierarchy of Hematopoiesis



To better understand the organization and turnover of HSCs, in the late 1990s a group of researchers began investigating changes in leukocyte telomere length over the lifecourse. In WBCs collected from 508 individuals between birth and age 90 years, granulocyte and lymphocyte telomere lengths decreased rapidly in the first 6 and 18 months of life, respectively, after which a more gradual decline occurred (Rufer et al. 1999). This biphasic pattern of telomere shortening has been replicated by several studies, including an analysis of newborn-parent-grandparent families, which found that differences in telomere length were significantly greater between newborns and parents compared to parents and grandparents (Frenck et al. 1998). While few studies have directly investigated telomere length in human HSCs, mounting evidence suggests this biphasic trajectory of telomere erosion reflects a large-scale expansion of self-renewing stem cells soon after birth, which creates a pool of progenitor cells that undergo progressive telomere shortening throughout the remainder of life (Aubert and Lansdorp 2008).

Telomere length and health

A recent meta-analysis of 19,713 individuals enrolled in six cohorts estimated that telomere length heritability is approximately 70% (Broer et al. 2013). Additionally, research indicates that by early adulthood most individuals display a fixed ranking and tracking of telomere length, suggesting that length at birth and erosion during childhood largely explain inter-individual variation observed in adults (Hjelmborg et al. 2015). Several large cohort studies have demonstrated that after controlling for age, shorter telomere length is associated with elevated risk of multiple age-related diseases, including heart disease, diabetes, dementia, osteoporosis, and mortality (D'Mello et al. 2015; Fitzpatrick et al. 2011; Honig et al. 2012; Mons et al. 2017; Valdes et al. 2007). Since the prescient observations of Hans Selye ("every organism pays for its

survival after a stressful situation by becoming a little older") (Selye 1956), extensive research has linked stressful life events with accelerated aging (summarized by: McEwen 1998). These findings, in combination with evidence documenting associations between telomeres and diseases of aging, as well as research emphasizing the importance of early life in setting lifelong telomere dynamics, raise questions about the impact of stress and adversities experienced during early life on telomere length.

Evidence linking telomere length and stress

Over the past two years (2015-2017), research investigating associations between childhood stress and telomere length has expanded rapidly. Coimbra et al. systematically reviewed 11 studies examining telomere length in relation to adversity (violence, family disruption, institutionalization, maternal depression and low socioeconomic status) among children between the ages of 3 and 15 years (Coimbra et al. 2017). The authors concluded that across these domains, children experiencing greater adversity experience accelerated telomere erosion. Notably, only one of the 11 reviewed studies was longitudinal by design (Shalev et al. 2013), raising questions about the appropriateness of their conclusion relating to change (shortening) over time. In a similar review, Naess et al. examined results from 26 studies investigating the association between childhood stress and telomere length, which was largely measured during adulthood. Studies were grouped by whether they measured 1) maternal stress during pregnancy, 2) parental caregiving ability, 3) childhood psychosocial stress (abuse, neglect, loss), or 4) childhood socioeconomic status (Naess and Kirkengen 2015). The majority of studies indicated an inverse correlation between stress and telomere length, but associations with socioeconomic status were less robust and too few studies examined maternal stress during pregnancy for the

authors to draw conclusions relating to gestational stress exposure. Similarly, Oliveira systematically reviewed papers examining the association between chronic social stress and telomere length across the lifecourse (Oliveira et al. 2016). The authors defined chronic stress as "stressful situations that originate from one's social environment", which they grouped into three 'social processes': 1) caregiving of disruptive children or disabled elderly, 2) poverty, and 3) exposure to violence. Based on results extracted from 18 studies (16 cross sectional and 2 longitudinal), the authors concluded that chronic social stress during early or adult life is associated with telomere shortening across each of these 'social processes' (Oliveira et al. 2016).

Hanssen et al performed a meta-analysis of the association between the degree of psychosocial stress (neglect, violence, trauma, abuse, institutional care, adversity, life stress and separation) experienced during childhood and telomere length among 16,238 participants from 27 samples. The authors found a significant association between higher childhood stress and shorter telomere length measured at a mean (across studies) age of 42 years (Hanssen et al. 2017). Similarly, Li et al. performed a meta-analysis investigating childhood trauma (physical abuse, sexual abuse, emotional abuse, physical neglect, emotional neglect, and general trauma) in relation to accelerated telomere erosion in adulthood among 30,909 participants from 26 studies. The authors detected small inverse effects between specific traumas (i.e. abuse or neglect) and telomere length, but larger inverse associations between "general trauma" and telomere length (Li et al. 2017). Lastly, Ridout et al. performed a similar meta-analysis investigating associations between early adversity (abuse, neglect, socioeconomic status, and other adverse experiences) and telomere length among 30,773 individuals enrolled in 41 studies (Ridout et al. 2017). The authors concluded that early adversity is inversely related to telomere length and that both the

type and timing of adversity significantly impacted associations with telomere length. Notably, many of the studies examined in these various review and meta-analyses overlapped.

Despite the clear importance of early life in setting lifelong telomere dynamics, few longitudinal studies have investigated change in telomere length across childhood. Indeed, a defining theme across these six systematic reviews and meta-analyses was the consistent call for future longitudinal research investigating within child change in telomere length over time.

Longitudinal research investigating stress in relation to childhood telomere attrition

Of the studies discussed by the above systematic reviews and meta-analyses, only two examined repeated measures of telomere length. Humphreys et al. examined the association between institutional care in Romania and buccal cell telomere shortening among 79 children (n=247 observations) between the ages of 6 and 15 years (Humphreys et al. 2016). For each child, telomere length was measured in 2-4 repeatedly collected samples. In adjusted models, the authors found that children who had ever been institutionalized showed accelerated telomere attrition (β= -0.12, n= 50) compared to children who had never been institutionalized (β= -0.06, n=29). In a study of 236 children residing in the United Kingdom, Shalev et al. found that exposure to two or more types of violence (maternal domestic violence, bullying victimization and physical maltreatment, n=39) was associated with significantly more telomere erosion between the ages of 5 and 10 years in adjusted models (β= -0.05, p=0.02) (Shalev et al. 2013).

Putative mechanisms underlying associations between stress and telomeres

DNA damage attributable to elevated levels of oxidative stress is the best supported mechanism linking psychosocial stress and adversity with shortened telomere length (Bauer et al. 2009; Haussmann and Heidinger 2015). Reactive oxygen species (ROS) (i.e. superoxide, hydroxyl radicals, hydrogen peroxide) are generated through endogenous (i.e. mitochondrial electron transport chain) and exogenous (i.e. cigarette smoking) processes (Newsholme et al. 2016). Research in animal models (Colaianna et al. 2013) and humans (Epel et al. 2004) has demonstrated that chronic stress leads to elevated ROS production, likely partially through stress hormone (i.e. cortisol)-mediated pathways (Aschbacher et al. 2014).

Oxidative stress has been shown to accelerate telomere attrition. For example, in cell culture studies using human fibroblasts, a continuous and exponential correlation between increasing oxidative stress levels and rate of telomere shortening was observed (Richter and von Zglinicki 2007). Several aspects of telomere structure heighten its vulnerability to oxidative damage. For example, the high number of guanines, which are more readily oxidized compared to other base pairs, leads to formation of 7,8-dihydro-8-oxoguanine (8-oxo-dG) lesions (Houben et al. 2008). Further, telomeres end in a single stranded 3' overhang, which is stabilized by folding back on itself; it is hypothesized that this 'T-loop' structure may obstruct DNA repair enzyme access, leading to unrepaired single-strand breaks (Bonetti et al. 2013). In turn, DNA breaks can impede replication by polymerase or may result in incorporation of an incorrect base at the corresponding site on the undamaged strand (von Zglinicki 2000, 2002). Further, research has demonstrated that telomerase can directly mis-incorporate 8-oxo-dG during telomere elongation, which terminates the enzyme's activity (von Zglinicki 2002). Interestingly, telomerase is not

impeded by terminal 8-oxo-dG lesions, but rather, research suggests that under certain conditions (i.e. in the presence of G-quadruplexes) these lesions may enhance the ability of telomerase to add nucleotides by improving telomere accessibility (Opresko 2008). Importantly, levels of oxidative stress studied *in vitro* are typically higher than those occurring in the body and therefore results from basic research examining oxidative stress in relation to *in vivo* telomere shortening in humans ultimately remains inconclusive (von Zglinicki 2002).

Methods of telomere measurement

Several molecular biology techniques have been developed to measure telomere length, including cell and chromosome-specific approaches (i.e. Single Telomere Length Analysis (STELA) and quantitative fluorescence *in situ* hybridization (Q-FISH)). However, based on methodological requirements (i.e. fresh blood) these approaches are typically used only in the clinical management of telomere-based diseases and are largely considered unsuitable for research involving a large number of participants (Montpetit et al. 2014).

The majority of research studies use either terminal restriction fragment (TRF) or quantitative polymerase chain reaction (qPCR) assays, each of which has unique requirements, advantages and limitations. Briefly, when performing the TRF method, DNA is digested using restriction enzymes that preserve telomeric regions. Intact telomeres from all chromosomes are then run on a gel and visualized using southern blotting. The size of the fragments, which reflects the average telomere length across all chromosomes and cells, is determined by comparison to a DNA ladder of known length (Kimura et al. 2010). Advantages of this method include the ability to measure absolute telomere length in kilobases; however, the procedure is labor intensive,

requires a large amount of DNA (micrograms), and is highly sensitive to DNA degradation. Additionally, a region of sub-telomeric DNA is measured, which cannot be distinguished from the telomeric region, potentially leading to measurement error (Montpetit et al. 2014).

Historically, qPCR was not used to measure telomeres because the primers for the repeating T_2AG_3 sequence had a propensity to form dimers. However, this problem was resolved in 2002 with the development of specially designed primers that bind to G/C-rich telomeric segments, but are mismatched at the other bases (Cawthon 2002). In traditional qPCR, telomere length is quantified by measuring the telomere amplicon (T) to a single-copy gene (S) amplicon in separate wells and a T/S ratio is calculated that reflects the average telomere length across all chromosomes in all cells sampled. In 2009, a monochrome multiplex qPCR (MMqPCR) telomere assay was developed, which allows the quantity of T and S to be measured in the same well, thereby eliminating variation introduced from differences in the quantity of starting DNA pipetted into the well (Cawthon 2009). Advantages of qPCR include the capacity to analyze a large number of samples relatively quickly and the small quantity of DNA required (nanograms); however, it is limited in that it provides a measure of relative (i.e. ranking across samples) versus absolute length (i.e. base pairs).

A large international collaboration between 10 laboratories that each investigated telomere length in a set of identical samples concluded that both TRF/southern blotting and qPCR assays generally perform well for measuring telomere length, but that the degree of technical variation precludes pooling data across studies produced by either technique (Martin-Ruiz et al. 2015).

REFERENCES

Abbasi G, Buser AM, Soehl A, Murray MW, Diamond ML. 2015. Stocks and flows of pbdes in products from use to waste in the u.S. And canada from 1970 to 2020. Environmental science & technology 49:1521-1528.

Abdelouahab N, Suvorov A, Pasquier JC, Langlois MF, Praud JP, Takser L. 2009. Thyroid disruption by low-dose bde-47 in prenatally exposed lambs. Neonatology 96:120-124.

Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. 2013. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. American journal of epidemiology 178:701-713.

Aschbacher K, Kornfeld S, Picard M, Puterman E, Havel PJ, Stanhope K, et al. 2014. Chronic stress increases vulnerability to diet-related abdominal fat, oxidative stress, and metabolic risk. Psychoneuroendocrinology 46:14-22.

ATSDR. 2017. Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers (pbdes). Atlanta, GA.

Aubert G, Lansdorp PM. 2008. Telomeres and aging. Physiol Rev 88:557-579.

Bauer ME, Jeckel CM, Luz C. 2009. The role of stress factors during aging of the immune system. Annals of the New York Academy of Sciences 1153:139-152.

Bellinger DC. 2000. Effect modification in epidemiologic studies of low-level neurotoxicant exposures and health outcomes. Neurotoxicology and teratology 22:133-140.

Bergman A, Ryden A, Law RJ, de Boer J, Covaci A, Alaee M, et al. 2012. A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environment international 49:57-82.

Bonetti D, Martina M, Falcettoni M, Longhese MP. 2013. Telomere-end processing: Mechanisms and regulation. Chromosoma.

Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, et al. 2013. Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. Eur J Hum Genet 21:1163-1168.

Cal-117. 1975. Requirements, test procedure and apparatus for testing the flame retardance of resilient filling materials used in upholstered furniture (technical bulletin 117).

Cal-117. 2013. Technical bulletin 117-2013.

Callahan P, Roe S, Hawthorne M. 2012. Playing with fire. Chicago Tribune (Chicago, IL).

Carlsson P, Herzke D, Wedborg M, Gabrielsen GW. 2011. Environmental pollutants in the swedish marine ecosystem, with special emphasis on polybrominated diphenyl ethers (pbde). Chemosphere 82:1286-1292.

Cavaliere H, Medeiros-Neto GA, Rosner W, Kourides IA. 1985. Persistent pituitary resistance to thyroid hormone in congenital versus later-onset hypothyroidism. J Endocrinol Invest 8:527-532.

Cawthon RM. 2002. Telomere measurement by quantitative pcr. Nucleic acids research 30:e47.

Cawthon RM. 2009. Telomere length measurement by a novel monochrome multiplex quantitative pcr method. Nucleic acids research 37:e21.

Chevrier J, Harley KG, Bradman A, Gharbi M, Sjodin A, Eskenazi B. 2010. Polybrominated diphenyl ether (pbde) flame retardants and thyroid hormone during pregnancy. Environmental health perspectives 118:1444-1449.

Coimbra BM, Carvalho CM, Moretti PN, Mello MF, Belangero SI. 2017. Stress-related telomere length in children: A systematic review. J Psychiatr Res 92:47-54.

Colaianna M, Schiavone S, Zotti M, Tucci P, Morgese MG, Backdahl L, et al. 2013. Neuroendocrine profile in a rat model of psychosocial stress: Relation to oxidative stress. Antioxid Redox Signal 18:1385-1399.

Costa LG, de Laat R, Tagliaferri S, Pellacani C. 2014. A mechanistic view of polybrominated diphenyl ether (pbde) developmental neurotoxicity. Toxicology letters 230:282-294.

Cowell WJ, Stapleton HM, Holmes D, Calero L, Tobon C, Perzanowski M, et al. 2017. Prevalence of historical and replacement brominated flame retardant chemicals in new york city homes. Emerging Contaminant 3:32-39.

Cunningham JM, Johnson RA, Litzelman K, Skinner HG, Seo S, Engelman CD, et al. 2013. Telomere length varies by DNA extraction method: Implications for epidemiologic research. Cancer Epidemiol Biomarkers Prev 22:2047-2054.

D'Mello MJ, Ross SA, Briel M, Anand SS, Gerstein H, Pare G. 2015. Association between shortened leukocyte telomere length and cardiometabolic outcomes: Systematic review and meta-analysis. Circ Cardiovasc Genet 8:82-90.

Dagnall CL, Hicks B, Teshome K, Hutchinson AA, Gadalla SM, Khincha PP, et al. 2017. Effect of pre-analytic variables on the reproducibility of qpcr relative telomere length measurement. PloS one 12:e0184098.

Dassanayake RM, Wei H, Chen RC, Li A. 2009. Optimization of the matrix solid phase dispersion extraction procedure for the analysis of polybrominated diphenyl ethers in human placenta. Analytical chemistry 81:9795-9801.

Driscoll LL, Gibson AM, Hieb A. 2009. Chronic postnatal de-71 exposure: Effects on learning, attention and thyroxine levels. Neurotoxicology and teratology 31:76-84.

Dufault C, Poles G, Driscoll LL. 2005. Brief postnatal pbde exposure alters learning and the cholinergic modulation of attention in rats. Toxicological sciences : an official journal of the Society of Toxicology 88:172-180.

Dyerberg J, Hjorne N, Nymand G, Olsen JS. 1974. Reference values for cord blood lipid and lipoprotein concentrations. Acta Paediatr Scand 63:431-436.

EPA. 2007. Concepts, methods and data sources for cumulative health risk assessment of multiple chemicals, exposures and effects: A resources doccument. Cincinnati, OH:Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment.

EPA. 2010. An exposure assessment of polybrominated diphenyl ethers. EPA/600/R-08/086F. Washington, DC:U.S. Environmental Protection Agency.

Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, et al. 2004. Accelerated telomere shortening in response to life stress. Proceedings of the National Academy of Sciences of the United States of America 101:17312-17315.

Erratico CA, Szeitz A, Bandiera SM. 2013. Biotransformation of 2,2',4,4'-tetrabromodiphenyl ether (bde-47) by human liver microsomes: Identification of cytochrome p450 2b6 as the major enzyme involved. Chem Res Toxicol 26:721-731.

Fang J, Nyberg E, Winnberg U, Bignert A, Bergman A. 2015. Spatial and temporal trends of the stockholm convention pops in mothers' milk -- a global review. Environ Sci Pollut Res Int 22:8989-9041.

Feo ML, Gross MS, McGarrigle BP, Eljarrat E, Barcelo D, Aga DS, et al. 2013. Biotransformation of bde-47 to potentially toxic metabolites is predominantly mediated by human cyp2b6. Environmental health perspectives 121:440-446.

Fisher DA, Klein AH. 1981. Thyroid development and disorders of thyroid function in the newborn. The New England journal of medicine 304:702-712.

Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS, et al. 2011. Leukocyte telomere length and mortality in the cardiovascular health study. J Gerontol A Biol Sci Med Sci 66:421-429.

Frenck RW, Jr., Blackburn EH, Shannon KM. 1998. The rate of telomere sequence loss in human leukocytes varies with age. Proceedings of the National Academy of Sciences of the United States of America 95:5607-5610.

Geyer H, Schramm K, Darnerud P, Aune M, Feicht E, Fried K. 2004. Terminal elimination half-lives of the brominated flame retardants tbbpa, hbcd, and lower brominated pbdes in humans. Organohalogen Comp 66:5.

Gyalpo T, Toms LM, Mueller JF, Harden FA, Scheringer M, Hungerbuhler K. 2015. Insights into pbde uptake, body burden, and elimination gained from australian age-concentration trends observed shortly after peak exposure. Environmental health perspectives 123:978-984.

Hanssen LM, Schutte NS, Malouff JM, Epel ES. 2017. The relationship between childhood psychosocial stressor level and telomere length: A meta-analysis. Health Psychol Res 5:6378.

Harley CB, Futcher AB, Greider CW. 1990. Telomeres shorten during ageing of human fibroblasts. Nature 345:458-460.

Haussmann MF, Heidinger BJ. 2015. Telomere dynamics may link stress exposure and ageing across generations. Biol Lett 11.

Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, et al. 2008. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (pcb) and polybrominated diphenyl ether (pbde) and neonatal thyroid hormone levels. Environmental health perspectives 116:1376-1382.

Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. 2010. Prenatal exposure to pbdes and neurodevelopment. Environmental health perspectives 118:712-719.

Hjelmborg JB, Dalgard C, Moller S, Steenstrup T, Kimura M, Christensen K, et al. 2015. The heritability of leucocyte telomere length dynamics. J Med Genet 52:297-302.

Honig LS, Kang MS, Schupf N, Lee JH, Mayeux R. 2012. Association of shorter leukocyte telomere repeat length with dementia and mortality. Arch Neurol 69:1332-1339.

Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ. 2008. Telomere length assessment: Biomarker of chronic oxidative stress? Free Radic Biol Med 44:235-246.

Hougaard KS, Hansen AM. 2007. Enhancement of developmental toxicity effects of chemicals by gestational stress. A review. Neurotoxicology and teratology 29:425-445.

Humphreys KL, Esteves K, Zeanah CH, Fox NA, Nelson CA, 3rd, Drury SS. 2016. Accelerated telomere shortening: Tracking the lasting impact of early institutional care at the cellular level. Psychiatry Res 246:95-100.

Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, Cao X, et al. 2010. Measurement of telomere length by the southern blot analysis of terminal restriction fragment lengths. Nat Protoc 5:1596-1607.

Lam J, Lanphear BP, Bellinger DC, Axelrad DA, McPartland J, Sutton P, et al. 2017. Developmental pbde exposure and iq/adhd in childhood: A systematic review and meta-analysis. Environmental health perspectives 125.

Li Z, He Y, Wang D, Tang J, Chen X. 2017. Association between childhood trauma and accelerated telomere erosion in adulthood: A meta-analytic study. J Psychiatr Res 93:64-71.

Linares V, Belles M, Domingo JL. 2015. Human exposure to pbde and critical evaluation of health hazards. Arch Toxicol 89:335-356.

Lorber M. 2008. Exposure of americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol 18:2-19.

Martin-Ruiz CM, Baird D, Roger L, Boukamp P, Krunic D, Cawthon R, et al. 2015. Reproducibility of telomere length assessment--an international collaborative study. Int J Epidemiol 44:1749-1754.

Mary EG, Zoeller RT. 2010. Thyroid hormones?Impact on the developing brain: Possible mechanisms of neurotoxicity. In: Neurotoxicology:Informa Healthcare, 79-111.

McEwen BS. 1998. Stress, adaptation, and disease. Allostasis and allostatic load. Annals of the New York Academy of Sciences 840:33-44.

McEwen BS, Tucker P. 2011. Critical biological pathways for chronic psychosocial stress and research opportunities to advance the consideration of stress in chemical risk assessment. Am J Public Health 101 Suppl 1:S131-139.

Mons U, Muezzinler A, Schottker B, Dieffenbach AK, Butterbach K, Schick M, et al. 2017. Leukocyte telomere length and all-cause, cardiovascular disease, and cancer mortality: Results from individual-participant-data meta-analysis of 2 large prospective cohort studies. American journal of epidemiology 185:1317-1326.

Montpetit AJ, Alhareeri AA, Montpetit M, Starkweather AR, Elmore LW, Filler K, et al. 2014. Telomere length: A review of methods for measurement. Nurs Res 63:289-299.

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, et al. 1988. A highly conserved repetitive DNA sequence, (ttaggg)n, present at the telomeres of human chromosomes. Proceedings of the National Academy of Sciences of the United States of America 85:6622-6626.

Naess AB, Kirkengen AL. 2015. Is childhood stress associated with shorter telomeres? Tidsskr Nor Laegeforen 135:1356-1360.

Nagin D. 2005. Group-based modeling of development. Cambridge, Massachusetts,:Harvard University Press.

Nagin DS. 2014. Group-based trajectory modeling: An overview. Ann Nutr Metab 65:205-210.

Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI, Jr. 2016. Molecular mechanisms of ros production and oxidative stress in diabetes. Biochem J 473:4527-4550.

NIEHS. 2017. Exposure biology and the exposome: Goal 4- combined environmental exposures. Available: https://www.niehs.nih.gov/about/strategicplan/implementation/goal4/index.cfm 2014].

Nordfjall K, Svenson U, Norrback KF, Adolfsson R, Lenner P, Roos G. 2009. The individual blood cell telomere attrition rate is telomere length dependent. PLoS Genet 5:e1000375.

O'Brien KM, Upson K, Cook NR, Weinberg CR. 2016. Environmental chemicals in urine and blood: Improving methods for creatinine and lipid adjustment. Environmental health perspectives 124:220-227.

Oliveira BS, Zunzunegui MV, Quinlan J, Fahmi H, Tu MT, Guerra RO. 2016. Systematic review of the association between chronic social stress and telomere length: A life course perspective. Ageing Res Rev 26:37-52.

Opresko PL. 2008. Telomere resque and preservation--roles for the werner syndrome protein and other recq helicases. Mechanisms of ageing and development 129:79-90.

Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL. 1989. Chlorinated hydrocarbon levels in human serum: Effects of fasting and feeding. Arch Environ Contam Toxicol 18:495-500.

Raschenberger J, Lamina C, Haun M, Kollerits B, Coassin S, Boes E, et al. 2016. Influence of DNA extraction methods on relative telomere length measurements and its impact on epidemiological studies. Sci Rep 6:25398.

Reichert S, Froy H, Boner W, Burg TM, Daunt F, Gillespie R, et al. 2017. Telomere length measurement by qpcr in birds is affected by storage method of blood samples. Oecologia 184:341-350.

Richter T, von Zglinicki T. 2007. A continuous correlation between oxidative stress and telomere shortening in fibroblasts. Exp Gerontol 42:1039-1042.

Ridout KK, Levandowski M, Ridout SJ, Gantz L, Goonan K, Palermo D, et al. 2017. Early life adversity and telomere length: A meta-analysis. Molecular psychiatry.

Roth N, Wilks MF. 2014. Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: A systematic review of the epidemiological literature using a quality assessment scheme. Toxicology letters 230:271-281.

Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, et al. 1999. Telomere fluorescence measurements in granulocytes and t lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory t cells in early childhood. J Exp Med 190:157-167.

Seita J, Weissman IL. 2010. Hematopoietic stem cell: Self-renewal versus differentiation. Wiley Interdiscip Rev Syst Biol Med 2:640-653.

Selye H. 1956. The stress of life. New York,:McGraw-Hill.

Shalev I. 2012. Early life stress and telomere length: Investigating the connection and possible mechanisms: A critical survey of the evidence base, research methodology and basic biology. BioEssays : news and reviews in molecular, cellular and developmental biology 34:943-952.

Shalev I, Moffitt TE, Sugden K, Williams B, Houts RM, Danese A, et al. 2013. Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: A longitudinal study. Molecular psychiatry 18:576-581.

Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. 2011. Associations between polybrominated diphenyl ether (pbde) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. Environmental health perspectives 119:1454-1459.

Talsness CE. 2008. Overview of toxicological aspects of polybrominated diphenyl ethers: A flame-retardant additive in several consumer products. Environmental research 108:158-167.

Tolios A, Teupser D, Holdt LM. 2015. Preanalytical conditions and DNA isolation methods affect telomere length quantification in whole blood. PloS one 10:e0143889.

UNEP. 2012. Listing of pops under the stockholm convention. Available: http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx [accessed 20 February 2015 2015].

Valdes AM, Richards JB, Gardner JP, Swaminathan R, Kimura M, Xiaobin L, et al. 2007. Telomere length in leukocytes correlates with bone mineral density and is shorter in women with osteoporosis. Osteoporos Int 18:1203-1210.

Viberg H, Fredriksson A, Eriksson P. 2003. Neonatal exposure to polybrominated diphenyl ether (pbde 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. Toxicol Appl Pharmacol 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 : an official journal of the Society of Toxicology 92:211-218.

von Zglinicki T. 2000. Role of oxidative stress in telomere length regulation and replicative senescence. Annals of the New York Academy of Sciences 908:99-110.

von Zglinicki T. 2002. Oxidative stress shortens telomeres. Trends Biochem Sci 27:339-344.

Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, et al. 2015. Maternal polybrominated diphenyl ether (pbde) exposure and thyroid hormones in maternal and cord sera: The home study, cincinnati, USA. Environmental health perspectives 123:1079-1085.

Weiss B, Bellinger DC. 2006. Social ecology of children's vulnerability to environmental pollutants. Environmental health perspectives 114:1479-1485.

Werner B, Beier F, Hummel S, Balabanov S, Lassay L, Orlikowsky T, et al. 2015. Reconstructing the in vivo dynamics of hematopoietic stem cells from telomere length distributions. Elife 4.

Williams GR. 2008. Neurodevelopmental and neurophysiological actions of thyroid hormone. Journal of neuroendocrinology 20:784-794.

Wright RJ. 2009. Moving towards making social toxins mainstream in children's environmental health. Current opinion in pediatrics 21:222-229.

Zhao Z, Pan X, Liu L, Liu N. 2014. Telomere length maintenance, shortening, and lengthening. J Cell Physiol 229:1323-1329.

Zoeller RT, Rovet J. 2004. Timing of thyroid hormone action in the developing brain: Clinical observations and experimental findings. Journal of neuroendocrinology 16:809-818.

CHAPTER 1: Sex-specific Effects of Combined Exposure to Chemical and Non-Chemical Stressors on Neuroendocrine Development: A Review of Recent Findings and Putative Mechanisms

Whitney J. Cowell[1,2], Rosalind J. Wright[3,4]

[1]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA. [2]Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, NY, USA. [3]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [4]Institute for Exposomic Research, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Corresponding author: Whitney J. Cowell, Columbia Center for Children's Environmental Health, 12th Floor, Mailman School of Public Health, 722 West 168th St, New York, NY 10032 USA. Telephone: (212) 304-7273. Fax: (212) 544-1943. E-mail: wc2449@cumc.columbia.edu

Acknowledgments
During her training and preparation of this manuscript, WJC was supported by the National Institute Environmental Health Science grants T32 ES023772 and T32 ES007322 and Environmental Protection Agency STAR Fellowship: FP-91779001.

Disclaimers
This publication was developed under STAR Fellowship Assistance Agreement no. FP-91779001 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by EPA. The views expressed in this publication are solely those of the authors.

Keywords: sexually dimorphic, neurodevelopment, stress, chemical, neurotoxicant, prenatal

Publication status: https://www.ncbi.nlm.nih.gov/pubmed/29027649
Cowell, WJ, Wright, RJ. Sex-Specific Effects of Combined Exposure to Chemical and Non-chemical Stressors on Neuroendocrine Development: a Review of Recent Findings and Putative Mechanisms. Curr Environ Health Rep. 2017. PMID: 29027649

Abstract

Purpose of review: Environmental toxicants and psychosocial stressors share many biological substrates and influence overlapping physiological pathways. Increasing evidence indicates stress-induced changes to the maternal milieu may prime rapidly developing physiological systems for disruption by concurrent or subsequent exposure to environmental chemicals. In this review, we highlight putative mechanisms underlying sex-specific susceptibility of the developing neuroendocrine system to the joint effects of stress or stress correlates and environmental toxicants (bisphenol A, alcohol, phthalates, lead, chlorpyrifos and traffic-related air pollution).

Recent findings: We provide evidence indicating that concurrent or tandem exposure to chemical and non-chemical stressors during windows of rapid development is associated with sex-specific synergistic, potentiated and reversed effects on several neuroendocrine endpoints related to hypothalamic-pituitary-adrenal axis function, sex steroid levels, neurotransmitter circuits and innate immune function. We additionally identify gaps, such as the role that the endocrine-active placenta plays, in our understanding of these complex interactions. Finally, we discuss future research needs, including the investigation of non-hormonal biomarkers of stress.

Summary: We demonstrate multiple physiologic systems are impacted by joint exposure to chemical and non-chemical stressors differentially among males and females. Collectively, the results highlight the importance of evaluating sex-specific endpoints when investigating the neuroendocrine system and underscore the need to examine exposure to chemical toxicants within the context of the social environment.

Introduction

For decades, the fields of psychology and child development have embraced the concept that an "umbilical transference" [1] occurs during prenatal life, in which the developing fetus is not only susceptible to risks conferred by physical and chemical exposures, but is sensitive to the vicissitudes of maternal psychological state and affect. Indeed, in the 90 years since Freud inferred the importance of the maternal-fetal bond ("[there is] much more continuity between intra-uterine life and earliest infancy than the impressive caesura of birth would have us believe" [2]), maternal emotional state and hormonal fluctuations have been associated with spontaneous abortion, fetal distress, premature labor and other pregnancy complications [3-5].

More recently, the fields of toxicology and environmental epidemiology have begun to adopt these principles and move towards an 'exposome' approach, in which an individual's cumulative "internal chemical environment" is thought to reflect exposure from both exogenous sources (i.e. environmental toxicants) and endogenous processes (i.e. stress-induced hormonal changes) [6]. From a health perspective, embracing the exposome is critical as environmental toxicants and psychosocial stressors share many biological substrates and increasing evidence indicates that stress-induced changes to the maternal milieu may prime rapidly developing physiological systems for disruption by concurrent or subsequent exposure to environmental chemicals and vice versa [7, 8].

In this review, we summarize four putative mechanisms underlying sex-specific susceptibility of the developing neuroendocrine system to the joint effects of psychosocial stress and environmental toxicants (Figure 1). We focus on this system given the sex bias of many

neurocognitive and behavioral disorders [9] and evidence demonstrating stress and an array of toxicants independently disrupt neurodevelopmental trajectories and alter programing of the fetal brain, including organization of sexually dimorphic regions [10-12]. We support each mechanism with examples from animal research, and when available, we discuss parallel epidemiologic findings. We draw from studies examining six well-established developmental neurotoxicants, including: bisphenol A (BPA), alcohol, phthalates, lead, chlorpyrifos and traffic-related air pollution. An exhaustive review of sex-specific neurodevelopmental effects associated with isolated exposure to psychosocial stress and these toxicants is beyond the scope of this paper, however, when available we point readers to previously published review articles. Finally, we identify gaps in our current understanding of these complex interactions and discuss future research needs.

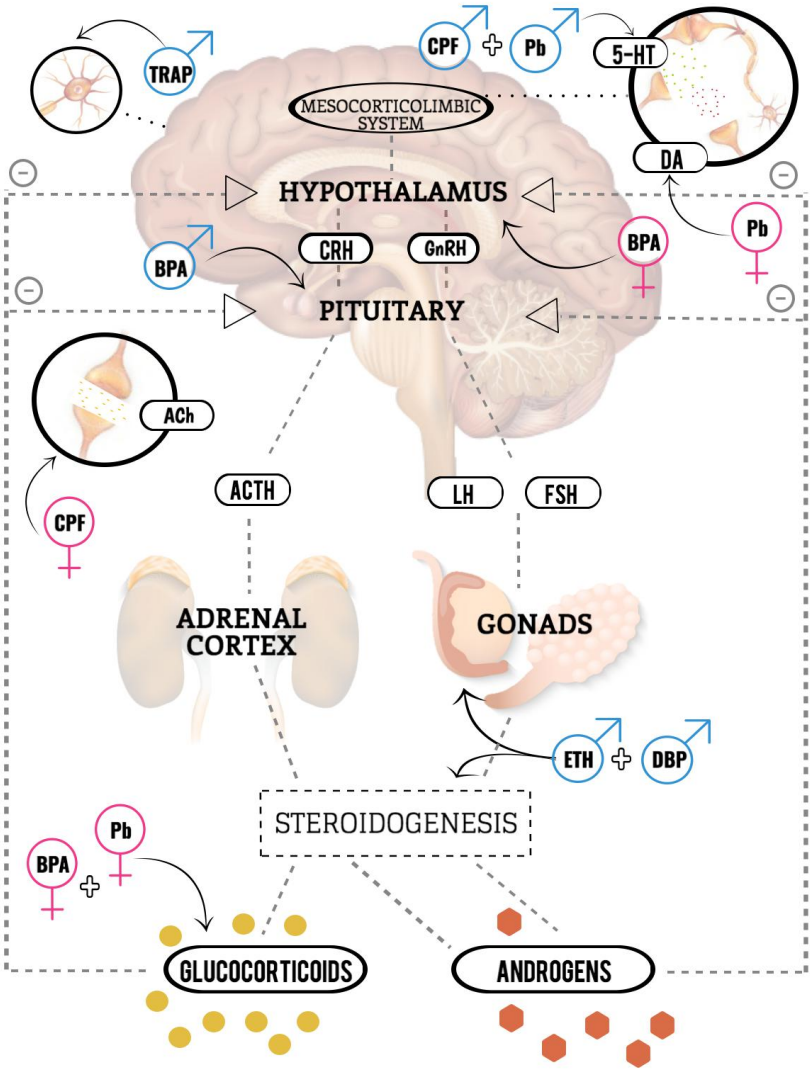
Background

Measures of Stress

Stress is a complex, multilevel construct characterized by cognitive appraisal of potentially stressful stimuli and consequent physiological reactions at both the cellular and emotional levels. Variation in stress responses reflects features of the stimuli (i.e. context, intensity, chronicity) and individual (i.e. sex, life stage, appraisal, coping capacity), thus requiring a diverse set of instruments and protocols for measurement. In epidemiologic research, measures of physical strain (e.g., malnutrition, sleep deprivation) and socioeconomic correlates of stress (e.g., education, resource accessibility), as well as scales of negative life events, stress perception, and negative affect are often used [13]. In experimental animal studies, common protocols include physical restraint, forced swimming or restricted access to bedding material. Additionally, both

animal and human studies frequently measure biomarkers of stress, such as glucocorticoids (i.e. cortisol in humans or corticosterone in rodents), under basal conditions and/or following exposure to stressful stimuli. Similarly, researchers can evaluate the timing and intensity of stress responses by examining physiologic and molecular changes following administration of synthetic glucocorticoids.

Figure 1. Conceptual model illustrating sex-specific neuroendocrine targets of chemical and non-chemical stressors.



Legend: All toxicant associations reflect interactions with stress; however, stress is not visually depicted. Abbreviations: 5-HT: serotonin; ACh: acetylcholine; ACTH: adrenocorticotropic hormone; BPA: bisphenol A, CRH: corticotropin releasing hormone; CPF: chlorpyrifos; DA: dopamine; DBP: dibutyl phthalate; ETH: alcohol; FSH: follicular stimulating hormone; GnRH: gonadotropin releasing hormone; LH: luteinizing hormone; Pb: lead; TRAP: traffic related air pollution.

Mechanism 1: Disrupted HPA Axis Function

Circulating glucocorticoid levels are maintained by the Hypothalamic-Pituitary-Adrenal Axis (HPA), which regulates physiological responses to potentially stressful stimuli [14]. The HPA axis includes the hypothalamus and pituitary gland located in the brain, and the adrenal glands, which are situated above the kidneys. In response to signals from brain nuclei involved in emotion-regulation, hypothalamic neuroendocrine cells produce corticotropin releasing hormone (CRH), which triggers secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. ACTH signals the adrenal cortex to synthesize and release cholesterol, which undergoes steroidogenic conversion to glucocorticoids, mineralocorticoids, and to a lesser extent androgens (i.e. testosterone). Glucocorticoid receptors located on the hypothalamus and pituitary detect circulating levels and terminate axis activity though a feedback inhibition mechanism.

Bisphenol-A (BPA)

BPA is an endocrine disrupting chemical associated with sex-specific neurobehavioral problems in children [15]. It has been widely used in food cans, plastic bottles and other consumer products leading to nearly ubiquitous human exposure [15]. *In silica* research indicates BPA is a glucocorticoid receptor agonist [16], and it has been associated with altered HPA axis function in juvenile trout [17] and pregnant women [18]. During pregnancy, surges in glucocorticoid levels are essential for normal maturation of several organ systems, however, elevated glucocorticoids at incorrect developmental stages have been shown to disrupt fetal programming of the HPA axis [19, 20].

Based on these factors, Pantagiotidou et al investigated sex-specific effects of perinatal exposure to BPA on HPA axis responsiveness during adolescence using a murine model [21]. Among female rats, BPA was associated with altered basal corticosterone (increased) and hypothalamic glucocorticoid receptor (decreased) levels. In response to acute stress (forced swimming), BPA-exposed females exhibited anxious coping behaviors and a dampened corticosterone response with failed downregulation of hypothalamic glucocorticoid receptor expression. In contrast, BPA-exposed males did not show altered basal HPA axis function, however, they failed to upregulate pituitary CRH receptor 1 expression in response to acute-stress (Table 1). Taken together, these findings suggest prenatal BPA exposure may program a hyperactive HPA axis with impaired negative feedback responsiveness to circulating corticosterone levels among females and a dampened stress response among males. Notably, HPA axis hyperactivity has been associated with anxiety and depression-like behaviors in rats [22] and diagnosis of anxiety and major depressive disorder in children and adolescents [23]. These findings suggest sex-specific reprogramming of the HPA axis by *in utero* exposure to BPA may permanently alter individual responses to stressful stimuli and contribute to life-long neuropsychological problems.

Mechanism 2: Altered Sex Steroid Levels

Similar to adrenal steroidogenesis, the gonads produce sex steroids under the control of the Hypothalamic-Pituitary-Gonadal (HPG) axis. Precisely timed surges of gonadal and adrenal sex steroids are critical for sexually-dimorphic differentiation, including de-feminization and masculinization of the male brain [24, 25, 9].

Alcohol

Increasing evidence indicates stressful stimuli [26] and environmental toxicants [27] disrupt the fetal HPG axis. Given these findings, Ward et al. conducted a series of rodent studies to examine the effects of combined exposure to prenatal stress (restraint) and alcohol on fetal testosterone patterns and later sexual behavior [28]. Male rats born to dams exposed to alcohol or stress during pregnancy were characterized by a testosterone surge (timing and duration) similar to that of controls, however, at each gestational day studied levels of testosterone were elevated in the alcohol-only group and depressed in the stress-only group [28]. Among males in the combined exposure group, the fetal testosterone surge was significantly delayed and abbreviated ($p=0.02$) compared to the unexposed and single-exposed groups [28]. As adults, males in the combined exposure group displayed feminized sexual behavior (e.g., lordosis) and reduced incidence of copulation with estrous females (Table 1) [29, 30]. Notably, in typically developing rats, the fetal testosterone surge corresponds with development of the sexually dimorphic nucleus in the hypothalamic preoptic area (SDN-POA), which plays important roles in controlling expression of sexual behaviors [26]. These findings suggest that combined exposure to alcohol and stress may desynchronize the temporal overlap between the fetal testosterone surge and development of the SDN-POA, resulting in feminization of the male brain.

Phthalates

Consistent with the effects of alcohol, exposure to phthalates during the fetal masculinization window has been shown to alter rat gonadal steroidogenesis [31-33] and disrupt development of the male reproductive tract. In humans, prenatal exposure to phthalates has been associated with altered sex steroid levels [34-36] and de-masculinized phenotypes among boys, including reduced anogenital distance [37]. In turn, anogenital distance is considered a sensitive marker of

androgen activity in early gestation [38] and correlates with sex-specific neurobehaviors [39]. Dibutyl phthalate (DBP) is a plasticizer that has been used extensively in toys and personal care products [40]. Given nearly ubiquitous human exposure to both DBP and stressful stimuli, Drake et al used a rat model to investigate the effects of concurrent exposure to DBP and the synthetic glucocorticoid dexamethasone [41]. Compared to unexposed controls, male offspring prenatally exposed DBP had lower fetal intra-testicular testosterone levels, reduced expression of key genes (*StAR, CYP11a1*) involved in gonadal steroidogenesis, and several anatomical malformations, including shortened anogenital distance. Exposure to dexamethasone alone showed no effects on male reproductive endpoints, however, when combined with DBP it enhanced the severity or incidence of most DBP-induced effects. Furthermore, combined exposure revealed effects on anogenital distance at lower doses of DBP (Table 1).

Diethylhexyl phthalate (DEHP) is a second anti-androgenic phthalate that is used to enhance flexibility of plastic-based consumer products [42]. Using data from a multi-center birth cohort, Barrett et al investigated prenatal exposure to stress and DEHP among a sample of infants (n=137 boys, n=136 girls) [43]. Stress was measured using a scale of stressful life events administered to couples during pregnancy and DEHP metabolites were measured in a maternal spot urine sample. Consistent with previous findings [44], stressful life events and DEHP exposure were independently associated with reduced anogenital distance among boys, albeit the association with stressful life events was not statistically significant [43]. Interestingly, when stratified by stress (<4 vs. 4+ stressful life events), DEHP metabolites were associated with reduced anogenital distance only among boys in the low stress group. These findings suggest that among males, stress in combination with DEHP exposure may interact such that their joint

exposure protects against the anti-androgenic effects conferred by each in isolation. Among girls, prenatal stress was associated with significantly longer anogenital distance, indicative of a masculinizing phenotype, however, no interaction with DEHP metabolites was observed. Importantly, given the relatively small size of the study sample, it is important that these findings be replicated by future studies.

Collectively, these results provide evidence that in rats, exposure to stress during pregnancy may augment the effects of concurrent exposure to environmental chemicals on male reproductive track outcomes and development of sexually-dimorphic brain regions, however, more research is needed to translate these findings to humans.

Mechanism 3: Changes to Neurotransmitter Systems

The brain is organized into complex neural networks that rely on the action of neurotransmitter-specific synapses for communication. Several of these circuits, such as the mesocorticolimbic dopamine circuit, also innervate key regulatory systems, including the HPA axis [45]. The mesocorticolimbic neurotransmitter system projects from the ventral tegmental area to the nucleus accumbens, striatum, cortex and limbic centers; its coordinated function is important in motivation, memory, and positive reinforcement of emotion-related behaviors [46].

Lead (Pb)

Prenatal exposure to Pb and stress have each been shown to independently act on the mesocorticolimbic circuit [47, 48], leading Cory-Slechta et al to investigate the effects of combined exposure in a series of rat studies. The researchers found that among adult female rats

prenatally exposed to acute stress (restraint) and Pb, but neither in isolation, frontal cortex dopamine levels were reduced and circulating corticosterone levels were elevated [49]. Females in the combined exposure group also exhibited learning deficits [50] and increased rates of stress-induced food reward responding, an indicator of impulsive choice behavior [48]. Parallel findings were not observed among males. In a subsequent study, the research group found indications of a trend towards disrupted mesocorticolimbic serotonin function and altered delay discounting behavioral performance among males exposed to Pb and stress, however, a statistically significant interaction was not observed [51]. Serotonin is a critical mediator of dopamine function [52], is important to HPA axis programming [53], and has been associated with sex-specific impulsive choice behavior in rats [54] and humans [55]. While substantial evidence indicates that stress and Pb act at the intersection of the mesocorticolimbic dopamine circuit and HPA axis, future research is needed to understand whether serotonin modulates this interaction.

Chlorpyrifos

Chlorpyrifos is a widely used agricultural pesticide [56] that disrupts neurotransmitter circuits by inhibiting acetylcholinesterase, the enzyme responsible for breaking down acetylcholine at neuromuscular junctions. Unsurprisingly, human exposure results in similar effects on acetylcholine systems [57], as well as disruption of serotonin circuits [58, 59]. Among children, prenatal exposure has been associated with tremor [60] and lower scores on measures of cognitive and neurobehavioral development [61, 62]. Glucocorticoids also target cholinergic [63] and serotonergic [64] circuits and recent findings suggest prenatal exposure to glucocorticoids may prime these neurotransmitter systems for enhanced disruption by subsequent chlorpyrifos

exposure. Using a murine model, Slotkin et al examined several neurochemical and behavioral endpoints in offspring exposed to the synthetic glucocorticoid dexamethasone and/or chlorpyrifos during the prenatal and neonatal periods, respectively [65-67]. Among males, each exposure was associated with reduced presynaptic acetylcholine activity and combined exposure demonstrated additive effects on this endpoint. Conversely, among females, dexamethasone and chlorpyrifos were each associated with increased presynaptic activity, whereas tandem exposure was associated with significantly reduced presynaptic activity (indexed by decreased choline transport protein binding and reduced choline acetyltransferase activity) and lost postsynaptic reactivity (indexed by decreased postsynaptic receptor binding) [66]. Tandem exposure was also associated with enhanced deficiencies in serotonin turnover, an indicator of pre-synaptic impulse activity, and attenuated upregulation of both serotonin receptor and transport protein expression [65]. Notably, serotonin effects were stronger among male pups, who additionally exhibited significantly greater hyperactivity compared to males in the control and single-exposure groups (Table 1) [67]. These findings suggest prenatal exposure to elevated glucocorticoids may enhance vulnerability of cholinergic (girls) and serotonergic (boys) systems to disruption by postnatal chlorpyrifos exposure.

Mechanism 4: Immune Dysregulation

Exposure to stress during fetal and neonatal life has been shown to alter fetal immune pathways [68, 69, 70], which play important roles in development of sexually dimorphic brain regions. For example, research conducted in male rodents suggests that increased estradiol levels resulting from aromatization of testosterone upregulates several microglial immune response genes resulting in the release of inflammatory signaling molecules (e.g. cytokines, prostaglandins) [71].

In turn, elevated prostaglandin E_2 triggers a signaling cascade that ultimately augments dendritic spine density in the medial preoptic area, which play a central role in the ability of males to detect olfactory cues from sexually receptive females (71). This complex molecular pathway has been substantiated by experimental studies demonstrating male copulatory behavior during adulthood is completely suppressed among rats subjected to microglial ablation during the neonatal period (72). Additional pathways through which the immune system influences programming of the developing brain have been reviewed by Bilbo et al. [73].

Traffic-Related Air Pollution

Prenatal exposure to traffic-related air pollution is associated with sex-specific disruption of brain development, neurocognitive and behavioral endpoints, and innate immune function [74]. The effects of combined exposure to stress and diesel exhaust particles on immune markers was recently studied using a murine model. Bolton et al found that male rat pups born to dams exposed to diesel exhaust and subjected to nest restriction during pregnancy had significantly elevated expression of microglial toll-like receptor-4 (TLR4) and its downstream effector caspase-1. TLR4 is an innate immune receptor responsive to both exogenous and endogenous danger-associated molecular patterns [75]. As adults, male pups in the combined exposure group also exhibited a greater pro-inflammatory bias (pro-inflammatory IL-1β /anti-inflammatory IL-10 ratio) in microglial derived cytokine levels compared to females. Subsequent analyses demonstrated these sex-specific molecular changes extended to cognitive and behavioral impairments, with males displaying significant hippocampal-dependent memory deficiency and increased anxiety-like behavior compared to males in the control and single-exposure groups (Table 1) [75]. These findings suggest maternal stress-induced changes in TLR4 signaling may

enhance the effects of diesel exhaust exposure through microglia-mediated inflammation pathways within the fetal rat brain.

Using data from a Boston, MA-based birth cohort, we previously investigated relationships between maternal report of negative life events during pregnancy and prenatal exposure to black carbon, a measure of traffic-related air pollution, on memory and learning domains among 6-year old children (n=145 boys, n=113 girls) [76]. Consistent with Bolton et al, we found that high exposure to black carbon was associated with significant deficits in attention-concentration scores only among boys born to mothers with high negative life event scores. While we did not study immune pathways, previous research in children has demonstrated stress [77] and black carbon [78] are independently associated with significant increases in pro-inflammatory IL-1β levels, supporting the role of the immune system as a potential mediator of our observed findings.

Table 1. Summary of effects from combined exposure to stress and several developmental toxicants on neuroendocrine and immune endpoints in rodents.

Toxicant	Stressor	Male	Female	Ref
BPA (prenatal)	Forced swimming (adolescence)	Failed upregulation of pituitary corticotropin releasing hormone receptor 1	↑ anxious coping behavior, ↓ corticosterone response; failed down regulation of hypothalamic glucocorticoid receptor expression	[21]
Alcohol (prenatal)	Restraint (prenatal)	Delayed & abbreviated fetal testosterone surge, feminized sexual behavior	Not studied	[28, 29]
DBP (prenatal)	Dexamethasone (prenatal)	↓ fetal intra-testicular testosterone levels, ↓ expression of gonadal steroidogenesis genes, ↑ severity & incidence of reproductive organ malformations	Not studied	[41]
Pb (prenatal)	Restraint (prenatal)	Disrupted mesocorticolimbic serotonin function and altered delay discounting behavioral performance (trend only, not significant)	↓ frontal cortex dopamine levels; ↑ circulating corticosterone levels, ↑ learning deficits; ↑ impulsive choice behavior	[48, 49, 50, 51]
Chlorpyrifos (neonatal)	Dexamethasone (prenatal)	↓ serotonin turnover, ↓ upregulation of serotonin receptor & transport protein expression, ↑ hyperactivity	↓ choline transport protein binding, ↓ choline acetyltransferase activity, ↓ postsynaptic receptor binding	[65, 66, 67]
Diesel exhaust	Nest restriction (prenatal)	↑ expression of microglial toll-like receptor-4 and caspase-1, ↑ pro-inflammatory bias, ↑ anxiety	No interaction observed	[75]

BPA: bisphenol a; DBP: dibutyl phthalate; Pb: lead

Summary of Mechanisms

Collectively, the studies reviewed here demonstrate that psychosocial stress and chemical toxicants interact to disrupt several physiological systems important to neurodevelopment and that these interactions vary by sex. However, given the limited research on these complex interactions, especially among humans, consistent patterns of disruption across chemicals, stressors and physiological systems remain poorly understood.

Future Research Needs

Role of the Placenta

While the studies summarized here focus directly on the fetal and maternal systems, emerging research indicates the placenta also plays a critical role in shaping sex-specific neurodevelopment [79]. Despite its design as a constitutional barrier between the mother and fetus, the placenta is penetrated by several neurodevelopmental toxicants and recent research indicates it is sensitive to changes in maternal state [79-81]. In mice, exposure to stressful stimuli during pregnancy has been shown to downregulate placental expression of O-linked-N-glycosyl transferase (*OGT*), an x-linked gene involved in regulating epigenetic modification of a global repressive histone mark (H3K27me3) [82, 83]. Notably, OGT escapes x-inactivation, leading to basal levels that are twice as low in males compared to females [82, 83]. The additional stress-induced decrease in OGT may result in an activated state among males via reduced transcriptional repression, ultimately rendering males more sensitive to concurrent or subsequent insults. Moreover, prenatal exposure to stress has been shown to significantly reduce associations between OGT and the 17-beta-hyroxysteroid dehydrogenase-3 (*HSD17β3*) gene locus in male placentas. This decrease results in a corresponding reduction in placental expression of

HSD17β3, which is responsible for converting androstenedione to testosterone [82]. As expected, prenatally stressed male mice present with increased androstenedione and decreased testosterone, as well as a dysmasculanized behavioral phenotype characterized by stress responses, cognitive function, and spatial strategies more similar to control females than control males. Despite these findings, no studies have investigated the sex-specific combined effects of stress and neurotoxicants on placental structure or function. Future research at the intersection of the maternal-placental-fetal unit is needed to more fully understand how gestational perturbations affect placental function, including altered gene expression patterns.

Improved Biomarkers of Stress

The majority of biomarkers currently used as indicators of stress are hormones (i.e. cortisol), which typically fluctuate in response to acute stress [84], may not accurately reflect maternal stress responses during pregnancy due to altered endocrine system homeostasis [85], and are affected by variation in several enzymes, including placental *HSD11β2*, which converts cortisol to inactive cortisone [86]. Recently, telomeres have been identified as a potential biomarker of cumulative wear and tear on the body [87, 88]. Telomeres are repetitive, non-coding T_2AG_3 sequences located at terminal chromosome ends. During cell division, chromosomes erode owing to limitations of DNA replication machinery, thus telomeres serve a self-sacrificing role against damage and degradation of protein coding regions [89]. Several recent epidemiological studies have demonstrated associations between early life social disadvantage and shorter telomere length [90-93], however, these studies are largely limited by cross-sectional designs, small sample sizes, and retrospective reporting of childhood experiences. Future research investigating telomere dynamics during pregnancy and throughout early life is needed to

understand whether telomeres can serve as a stress-sensitive biomarker during periods of rapid growth and development.

Expanded Research in Humans

As is evident from our focus on animal research, epidemiologic research investigating sex-specific neuroendocrine effects of developmental exposure to chemical and non-chemical stressors is limited. While animal research provides a unique opportunity to experimentally manipulate and control study conditions, substantial anatomical, functional, developmental and behavioral differences between species limit the generalizability of results to humans. For example, rat adrenal glands to not produce androgens, which are important contributors to sexually dimorphic differentiation of the brain in humans [94]. Likewise, the masculinization programming window is only 3-5 days in rats compared to 4-6 weeks in humans, potentially providing a greater time-opportunity for physiological disruption or recovery [41]. More observational epidemiology research investigating stress-chemical-sex interactions is needed to confirm the biological mechanisms elucidated by animal research.

Investigation of Other Systems and Mechanisms

The reviewed studies do not encompass all psychosocial stressors, toxicants, physiological systems or biological mechanisms known to influence brain development, but rather were selected to highlight enhanced disruption of pathways central to sex-specific programming. Moreover, while reviewed separately, these pathways overlap extensively and should be conceptualized as contributing to a single integrated system. For example, the immune system plays an important role in regulating levels of stress-sensitive neurotransmitters [95], in turn,

neurotransmitters influence sex steroid levels via modulation of the HPG axis at the level of the hypothalamus [96]. Moreover, the extent to which stress may act to increase exposure at the biological level is poorly understood. For example, rats exposed to stress have increased brain Pb [51] and decreased blood alcohol [97] levels compared to non-stressed animals exposed to the same dose of chemical. These results suggest that rather than directly acting on overlapping biological substrates, stress may alter blood brain barrier permeability, alcohol metabolism, and/or other pathways involved in toxicant distribution and excretion. Expanded research on the integration of these systems, as well as studies on other biological systems (i.e. autonomic) and cellular mechanisms (i.e. oxidative stress) sensitive to stress and chemical toxicants is needed to more fully understand the pathways through which these exposures interact.

Implications for Health Disparities

Members of low-income, minority communities experience more frequent negative life events, such as witnessing violence and suffering discrimination, which in turn may activate stress responses and precipitate feelings of anxiety, exclusion, and anger [98-102]. Likewise, the multiple challenges associated with financial strain, such as inadequate housing conditions, inability to afford food, and reduced access to health care, can tax individual coping strategies and lead to heightened emotional distress [103, 104]. In addition, parents facing multiple adversities are less likely to spend time at home (i.e. due to working multiple jobs with longer commutes and unfavorable shifts [105]) and are more likely to employ controlling, restrictive and punitive parenting strategies, often as an adaptive and protective response to neighborhood crime or other dangerous circumstances [104].

As described extensively by the environmental justice literature, minority and socioeconomically

disadvantaged individuals not only experience greater levels of psychosocial stress, but often bear a disproportionate burden of environmental risk [106-108]. For example, nationwide statistics indicate members of racial and ethnic minorities are more likely to live within 150 meters of a major U.S. highway, putting these groups at increased risk for exposure to traffic-related air pollution [109]. Likewise, exposure to Pb has historically been higher among black [110] and low-income [111] children, who disproportionately live in urban neighborhoods with older housing that may contain deteriorating lead-based paint.

The socioeconomic and environmental challenges faced by these populations likely contribute to the persistence of health disparities across ethnic and economic groups in the United States [109]. For example, the rate of premature birth is significantly higher among black (17%) compared to white (11%) infants [109]. Premature birth is estimated to account for one third of all infant deaths and is associated with numerous childhood and later life disorders, including neurocognitive and behavioral problems [112]. Notably, black children (8.4%) and those living below the federal poverty level (11%) are more likely to be diagnosed with a learning disability or attention deficit hyperactivity disorder compared to white children (7.5%) and those living at or above 200% of the poverty level (5.8%), respectively [113]. Likewise, infants born to mothers with less than a high school education, an indicator of socioeconomic status, are nearly twice as likely to die in the first year of life and approximately six times more likely to be rated in poor or fair health during childhood [114]. Similar trends have been documented for other indicators of socioeconomic status (i.e. family structure) and childhood morbidities (i.e. asthma) [113].

Conclusions

Health inequalities, including disparities in neurocognitive and behavioral outcomes, persist across ethnic and economic groups in the United States. As demonstrated here, multiple sexually dimorphic biological systems involved in programming the developing brain are susceptible to enhanced disruption by concurrent or consecutive exposure to psychosocial stress and chemical toxicants, which often co-occur among minority and socioeconomically disadvantaged communities. The reviewed studies highlight the importance of examining exposure to chemical toxicants within the context of the social environment, as well as the need to consider the influence of sex when investigating neuroendocrine and immune endpoints. Despite challenges associated with investigating these relationships among humans, such as the large sample size requirements needed to investigate 3-way interactions in observational studies and ethical considerations associated with randomized controlled trials, future research focused on studying these interactions among human populations is critically needed.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance

1. Culbert-Koehn J. Don't get stuck in the mother: regression in analysis. Journal of Analytical Psychology. 1997;42:99-104.

2. Freud S. Inhibitions, Symptoms and Anxiety. London: Hogarth Press; 1926.

3. Mitrani JL. Toward an understanding of unmentalized experience. Psychoanal Q. 1995;64(1):68-112.

4. Beydoun H, Saftlas AF. Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. Paediatr Perinat Epidemiol. 2008;22(5):438-66.

5. Savitz D, Dunkel-Schetter C. Preterm Birth: Causes, Consequences and Prevention. In: Behrman R, AS B, editors. Behavioral and psychosocial contributors to preterm birth. Washington DC: National Academy Press; 2006. p. 87-123.

6. Rappaport SM. Implications of the exposome for exposure science. J Expo Sci Environ Epidemiol. 2011;21(1):5-9.

7. Weiss B, Bellinger DC. Social ecology of children's vulnerability to environmental pollutants. Environ Health Perspect. 2006;114(10):1479-85.

8. Wright RJ. Moving towards making social toxins mainstream in children's environmental health. Curr Opin Pediatr. 2009;21(2):222-9.

9. Pfaff DW, Christen Y. Multiple origins of sex differences in brain neuroendocrine functions and their pathologies. Research and perspectives in endocrine interactions. Heidelberg; New York: Springer; 2013.

10. •Antonelli MC, Pallares ME, Ceccatelli S, Spulber S. Long-term consequences of prenatal stress and neurotoxicants exposure on neurodevelopment. Prog Neurobiol. 2016.
The authors provide a detailed summary of neurodevelopmental consequences of exposure to gestational stress and environmental toxicants, including sex-specific effects, and the overlapping pathways they each target.

11. Graignic-Philippe R, Dayan J, Chokron S, Jacquet AY, Tordjman S. Effects of prenatal stress on fetal and child development: a critical literature review. Neurosci Biobehav Rev. 2014;43:137-62.

12. Jurewicz J, Polanska K, Hanke W. Exposure to widespread environmental toxicants and children's cognitive development and behavioral problems. Int J Occup Med Environ Health. 2013;26(2):185-204.

13. Cohen S, Kessler R, Underwood Gorden L. Measuring Stress: A guide for health and social scientists. New York: Oxford University Press; 1995.

14. The Handbook of Stress Science. New York: Springer Publishing Company; 2010.

15. Mustieles V, Perez-Lobato R, Olea N, Fernandez MF. Bisphenol A: Human exposure and neurobehavior. Neurotoxicology. 2015;49:174-84.

16. Prasanth GK, Divya LM, Sadasivan C. Bisphenol-A can bind to human glucocorticoid receptor as an agonist: an in silico study. J Appl Toxicol. 2010;30(8):769-74.

17. Aluru N, Leatherland JF, Vijayan MM. Bisphenol A in oocytes leads to growth suppression and altered stress performance in juvenile rainbow trout. PLoS One. 2010;5(5):e10741.

18. Giesbrecht GF, Liu J, Ejaredar M, Dewey D, Letourneau N, Campbell T et al. Urinary bisphenol A is associated with dysregulation of HPA-axis function in pregnant women: Findings from the APrON cohort study. Environ Res. 2016;151:689-97.

19. Moisiadis VG, Matthews SG. Glucocorticoids and fetal programming part 2: Mechanisms. Nat Rev Endocrinol. 2014;10(7):403-11.

20. Tegethoff M, Pryce C, Meinlschmidt G. Effects of intrauterine exposure to synthetic glucocorticoids on fetal, newborn, and infant hypothalamic-pituitary-adrenal axis function in humans: a systematic review. Endocr Rev. 2009;30(7):753-89.

21. Panagiotidou E, Zerva S, Mitsiou DJ, Alexis MN, Kitraki E. Perinatal exposure to low-dose bisphenol A affects the neuroendocrine stress response in rats. J Endocrinol. 2014;220(3):207-18.

22. Chen F, Zhou L, Bai Y, Zhou R, Chen L. Hypothalamic-pituitary-adrenal axis hyperactivity accounts for anxiety- and depression-like behaviors in rats perinatally exposed to bisphenol A. J Biomed Res. 2015;29(3):250-8.

23. Steingard R, Biederman J, Keenan K, Moore C. Comorbidity in the interpretation of dexamethasone suppression test results in children: a review and report. Biol Psychiatry. 1990;28(3):193-202.

24. Corbier P, Roffi J, Rhoda J. Female sexual behavior in male rats: effect of hour of castration at birth. Physiol Behav. 1983;30(4):613-6.

25. Hoepfner BA, Ward IL. Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. Behav Neurosci. 1988;102(1):61-5.

26. Shansky RM. Sex differences in the central nervous system. Neuroscience Net Reference Book Series Book 4. Boston: Elsevier Academic Press; 2016.

27. Hampl R, Kubatova J, Starka L. Steroids and endocrine disruptors--History, recent state of art and open questions. J Steroid Biochem Mol Biol. 2016;155(Pt B):217-23.

28. Ward IL, Ward OB, Affuso JD, Long WD, 3rd, French JA, Hendricks SE. Fetal testosterone surge: specific modulations induced in male rats by maternal stress and/or alcohol consumption. Horm Behav. 2003;43(5):531-9.

29. Ward OB, Ward IL, Denning JH, Hendricks SE, French JA. Hormonal mechanisms underlying aberrant sexual differentiation in male rats prenatally exposed to alcohol, stress, or both. Arch Sex Behav. 2002;31(1):9-16.

30. Ward IL, Bennett AL, Ward OB, Hendricks SE, French JA. Androgen threshold to activate copulation differs in male rats prenatally exposed to alcohol, stress, or both factors. Horm Behav. 1999;36(2):129-40.

31. Supornsilchai V, Soder O, Svechnikov K. Stimulation of the pituitary-adrenal axis and of adrenocortical steroidogenesis ex vivo by administration of di-2-ethylhexyl phthalate to prepubertal male rats. J Endocrinol. 2007;192(1):33-9.

32. Sekaran S, Jagadeesan A. In utero exposure to phthalate downregulates critical genes in Leydig cells of F1 male progeny. J Cell Biochem. 2015;116(7):1466-77.

33. Akingbemi BT, Youker RT, Sottas CM, Ge R, Katz E, Klinefelter GR et al. Modulation of rat Leydig cell steroidogenic function by di(2-ethylhexyl)phthalate. Biol Reprod. 2001;65(4):1252-9.

34. Araki A, Mitsui T, Goudarzi H, Nakajima T, Miyashita C, Itoh S et al. Prenatal di(2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study. Sci Total Environ. 2017;581-582:297-304.

35. Ferguson KK, Peterson KE, Lee JM, Mercado-Garcia A, Blank-Goldenberg C, Tellez-Rojo MM et al. Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. Reprod Toxicol. 2014;47:70-6.

36. Watkins DJ, Tellez-Rojo MM, Ferguson KK, Lee JM, Solano-Gonzalez M, Blank-Goldenberg C et al. In utero and peripubertal exposure to phthalates and BPA in relation to female sexual maturation. Environ Res. 2014;134:233-41.

37. Swan SH, Sathyanarayana S, Barrett ES, Janssen S, Liu F, Nguyen RH et al. First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod. 2015;30(4):963-72.

38. Thankamony A, Pasterski V, Ong KK, Acerini CL, Hughes IA. Anogenital distance as a marker of androgen exposure in humans. Andrology. 2016;4(4):616-25.

39. Pasterski V, Acerini CL, Dunger DB, Ong KK, Hughes IA, Thankamony A et al. Postnatal penile growth concurrent with mini-puberty predicts later sex-typed play behavior: Evidence for neurobehavioral effects of the postnatal androgen surge in typically developing boys. Horm Behav. 2015;69:98-105.

40. Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006;29(1):134-9.

41. Drake AJ, van den Driesche S, Scott HM, Hutchison GR, Seckl JR, Sharpe RM. Glucocorticoids amplify dibutyl phthalate-induced disruption of testosterone production and male reproductive development. Endocrinology. 2009;150(11):5055-64.

42. Zarean M, Keikha M, Poursafa P, Khalighinejad P, Amin M, Kelishadi R. A systematic review on the adverse health effects of di-2-ethylhexyl phthalate. Environ Sci Pollut Res Int. 2016;23(24):24642-93.

43. Barrett ES, Parlett LE, Sathyanarayana S, Redmon JB, Nguyen RH, Swan SH. Prenatal Stress as a Modifier of Associations between Phthalate Exposure and Reproductive Development: results from a Multicentre Pregnancy Cohort Study. Paediatr Perinat Epidemiol. 2016;30(2):105-14.

44. Barrett ES, Parlett LE, Sathyanarayana S, Liu F, Redmon JB, Wang C et al. Prenatal exposure to stressful life events is associated with masculinized anogenital distance (AGD) in female infants. Physiol Behav. 2013;114-115:14-20.

45. Sullivan RM, Dufresne MM. Mesocortical dopamine and HPA axis regulation: role of laterality and early environment. Brain Res. 2006;1076(1):49-59.

46. Strominger N, Demarest R, Laemle L. Noback's Human Nervous System. New York: Springer Science+Business Media; 2012.

47. Rossi-George A, Virgolini MB, Weston D, Thiruchelvam M, Cory-Slechta DA. Interactions of lifetime lead exposure and stress: behavioral, neurochemical and HPA axis effects. Neurotoxicology. 2011;32(1):83-99.

48. Virgolini MB, Rossi-George A, Lisek R, Weston DD, Thiruchelvam M, Cory-Slechta DA. CNS effects of developmental Pb exposure are enhanced by combined maternal and offspring stress. Neurotoxicology. 2008;29(5):812-27.

49. Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. Maternal stress modulates the effects of developmental lead exposure. Environ Health Perspect. 2004;112(6):717-30.

50. Cory-Slechta DA, Stern S, Weston D, Allen JL, Liu S. Enhanced learning deficits in female rats following lifetime pb exposure combined with prenatal stress. Toxicol Sci. 2010;117(2):427-38.

51. Weston HI, Weston DD, Allen JL, Cory-Slechta DA. Sex-dependent impacts of low-level lead exposure and prenatal stress on impulsive choice behavior and associated biochemical and neurochemical manifestations. Neurotoxicology. 2014;44:169-83.

52. Hensler JG, Artigas F, Bortolozzi A, Daws LC, De Deurwaerdere P, Milan L et al. Catecholamine/Serotonin interactions: systems thinking for brain function and disease. Adv Pharmacol. 2013;68:167-97.

53. Kapoor A, Dunn E, Kostaki A, Andrews MH, Matthews SG. Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. J Physiol. 2006;572(Pt 1):31-44.

54. Baarendse PJ, Vanderschuren LJ. Dissociable effects of monoamine reuptake inhibitors on distinct forms of impulsive behavior in rats. Psychopharmacology (Berl). 2012;219(2):313-26.

55. Walderhaug E, Magnusson A, Neumeister A, Lappalainen J, Lunde H, Refsum H et al. Interactive effects of sex and 5-HTTLPR on mood and impulsivity during tryptophan depletion in healthy people. Biol Psychiatry. 2007;62(6):593-9.

56. Chlorpyrifos. U.S. Environmental Protection Agency. Available: https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos

57. Prueitt RL, Goodman JE, Bailey LA, Rhomberg LR. Hypothesis-based weight-of-evidence evaluation of the neurodevelopmental effects of chlorpyrifos. Crit Rev Toxicol. 2011;41(10):822-903.

58. Slotkin TA, Seidler FJ. The alterations in CNS serotonergic mechanisms caused by neonatal chlorpyrifos exposure are permanent. Brain Res Dev Brain Res. 2005;158(1-2):115-9.

59. Aldridge JE, Seidler FJ, Meyer A, Thillai I, Slotkin TA. Serotonergic systems targeted by developmental exposure to chlorpyrifos: effects during different critical periods. Environ Health Perspect. 2003;111(14):1736-43.

60. Rauh VA, Garcia WE, Whyatt RM, Horton MK, Barr DB, Louis ED. Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. Neurotoxicology. 2015;51:80-6.

61. Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics. 2006;118(6):e1845-59.

62. Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect. 2011;119(8):1189-95.

63. Emgard M, Paradisi M, Pirondi S, Fernandez M, Giardino L, Calza L. Prenatal glucocorticoid exposure affects learning and vulnerability of cholinergic neurons. Neurobiol Aging. 2007;28(1):112-21.

64. St-Pierre J, Laurent L, King S, Vaillancourt C. Effects of prenatal maternal stress on serotonin and fetal development. Placenta. 2016;48 Suppl 1:S66-S71.

65. Slotkin TA, Card J, Seidler FJ. Prenatal dexamethasone, as used in preterm labor, worsens the impact of postnatal chlorpyrifos exposure on serotonergic pathways. Brain Res Bull. 2014;100:44-54.

66. Slotkin TA, Card J, Infante A, Seidler FJ. Prenatal dexamethasone augments the sex-selective developmental neurotoxicity of chlorpyrifos: implications for vulnerability after pharmacotherapy for preterm labor. Neurotoxicol Teratol. 2013;37:1-12.

67. Levin ED, Cauley M, Johnson JE, Cooper EM, Stapleton HM, Ferguson PL et al. Prenatal dexamethasone augments the neurobehavioral teratology of chlorpyrifos: significance for maternal stress and preterm labor. Neurotoxicol Teratol. 2014;41:35-42.

68. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain Behav Immun. 2007;21(3):343-50.

69. Cryan JF, Dinan TG. Unraveling the longstanding scars of early neurodevelopmental stress. Biol Psychiatry. 2013;74(11):788-9.

70. Johnson, FK, Kaffman A. Early life stress perturbs the function of microglia in the developing rodent brain: new insights and future directions. Brain, Behav and Immun. 2017.

71. McCarthy M, Nugent BM, Lenz KM. Neuroimmunology and neuroepigenetics in the establishment of sex differences in the brain. Nature Reviews Neuroscience. 2017;18:471-84.

72. VanRyzin JW, Yu SJ, Perez-Pouchoulen M, McCarthy MM. Temporary depletion of microglia during the early postnatal period induces lasting sex-dependent and sex-independent effects on behavior in rats. eNeuro. 2016;3(6).

73. Bilbo SD, Schwarz JM. The immune system and developmental programming of brain and behavior. Front Neuroendocrinol. 2012;33(3):267-86.

74. •Costa LG, Cole TB, Coburn J, Chang YC, Dao K, Roque PJ. Neurotoxicity of traffic-related air pollution. Neurotoxicology. 2017;59:133-9.
This recent review summarizes results from toxicologic and epidemiologic research investigating neurodevelopmental and neurodegeneraive effects of exposure to air pollution. The paper highlights the need for future research investigating sex differences and gene-environment interactions.

75. Bolton JL, Huff NC, Smith SH, Mason SN, Foster WM, Auten RL et al. Maternal stress and effects of prenatal air pollution on offspring mental health outcomes in mice. Environ Health Perspect. 2013;121(9):1075-82.

76. Cowell WJ, Bellinger DC, Coull BA, Gennings C, Wright RO, Wright RJ. Associations between Prenatal Exposure to Black Carbon and Memory Domains in Urban Children: Modification by Sex and Prenatal Stress. PLoS One. 2015;10(11):e0142492.

77. Tyrka AR, Parade SH, Valentine TR, Eslinger NM, Seifer R. Adversity in preschool-aged children: Effects on salivary interleukin-1beta. Dev Psychopathol. 2015;27(2):567-76.

78. De Prins S, Dons E, Van Poppel M, Int Panis L, Van de Mieroop E, Nelen V et al. Airway oxidative stress and inflammation markers in exhaled breath from children are linked with exposure to black carbon. Environ Int. 2014;73:440-6.

79. Bale TL. Sex differences in prenatal epigenetic programming of stress pathways. Stress. 2011;14(4):348-56.

80. Howerton CL, Bale TL. Prenatal programing: at the intersection of maternal stress and immune activation. Horm Behav. 2012;62(3):237-42.

81. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. Endocr Rev. 2006;27(2):141-69.

82. •Howerton CL, Bale TL. Targeted placental deletion of OGT recapitulates the prenatal stress phenotype including hypothalamic mitochondrial dysfunction. Proc Natl Acad Sci U S A. 2014;111(26):9639-44.
This important paper demonstrates that knocking out a specific placental gene results in mice pups characterized by stress respones similar to those of mice exposed to stress during gestation. The paper provides convincing evidence that in mice, levels of this protein (OGT) may serve as a placental biomarker of stress.

83. Howerton CL, Morgan CP, Fischer DB, Bale TL. O-GlcNAc transferase (OGT) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. Proc Natl Acad Sci U S A. 2013;110(13):5169-74.

84. Smith MN, Griffith WC, Beresford SA, Vredevoogd M, Vigoren EM, Faustman EM. Using a biokinetic model to quantify and optimize cortisol measurements for acute and chronic environmental stress exposure during pregnancy. J Expo Sci Environ Epidemiol. 2014;24(5):510-6.

85. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis--2012 Curt Richter Award Winner. Psychoneuroendocrinology. 2013;38(1):1-11.

86. Benediktsson R, Calder AA, Edwards CR, Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf). 1997;46(2):161-6.

87. Mitchell C, Hobcraft J, McLanahan SS, Siegel SR, Berg A, Brooks-Gunn J et al. Social disadvantage, genetic sensitivity, and children's telomere length. Proc Natl Acad Sci U S A. 2014;111(16):5944-9.

88. •Shalev I. Early life stress and telomere length: investigating the connection and possible mechanisms: a critical survey of the evidence base, research methodology and basic biology. BioEssays : news and reviews in molecular, cellular and developmental biology. 2012;34(11):943-52.
This thourough review paper summarizes the evidence linking early life stress with telomere dynamics and also provides a clear and concise review of methodoligical issues and limitations relating to telomere measurement (i.e. relating to assay choice, cell type, measurement error).

89. Bonetti D, Martina M, Falcettoni M, Longhese MP. Telomere-end processing: mechanisms and regulation. Chromosoma. 2013.

90. Entringer S, Epel ES, Kumsta R, Lin J, Hellhammer DH, Blackburn EH et al. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proc Natl Acad Sci U S A. 2011;108(33):E513-8.

91. Entringer S, Epel ES, Lin J, Buss C, Shahbaba B, Blackburn EH et al. Maternal psychosocial stress during pregnancy is associated with newborn leukocyte telomere length. Am J Obstet Gynecol. 2013;208(2):134 e1-7.

92. Kiecolt-Glaser JK, Gouin JP, Weng NP, Malarkey WB, Beversdorf DQ, Glaser R. Childhood adversity heightens the impact of later-life caregiving stress on telomere length and inflammation. Psychosom Med. 2011;73(1):16-22.

93. Tyrka AR, Price LH, Kao HT, Porton B, Marsella SA, Carpenter LL. Childhood maltreatment and telomere shortening: preliminary support for an effect of early stress on cellular aging. Biol Psychiatry. 2010;67(6):531-4.

94. Moisiadis VG, Matthews SG. Glucocorticoids and fetal programming part 1: Outcomes. Nat Rev Endocrinol. 2014;10(7):391-402.

95. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008;9(1):46-56.

96. Watanabe M, Fukuda A, Nabekura J. The role of GABA in the regulation of GnRH neurons. Front Neurosci. 2014;8:387.

97. Ward IL, Ward OB, French JA, Hendricks SE, Mehan D, Winn RJ. Prenatal alcohol and stress interact to attenuate ejaculatory behavior, but not serum testosterone or LH in adult male rats. Behav Neurosci. 1996;110(6):1469-77.

98. King K, Ogle C. Negative life events vary by neighborhood and mediate the relation between neighborhood context and psychological well-being. PLoS One. 2014;9(4):e93539.

99. Myers HF. Ethnicity- and socio-economic status-related stresses in context: an integrative review and conceptual model. J Behav Med. 2009;32(1):9-19.

100. Wade R, Jr., Cronholm PF, Fein JA, Forke CM, Davis MB, Harkins-Schwarz M et al. Household and community-level Adverse Childhood Experiences and adult health outcomes in a diverse urban population. Child Abuse Negl. 2016;52:135-45.

101. Williams DR, Neighbors HW, Jackson JS. Racial/ethnic discrimination and health: findings from community studies. Am J Public Health. 2003;93(2):200-8.

102. Wilson WC, Rosenthal BS. The relationship between exposure to community violence and psychological distress among adolescents: a meta-analysis. Violence Vict. 2003;18(3):335-52.

103. Becerra BJ, Sis-Medina RC, Reyes A, Becerra MB. Association Between Food Insecurity and Serious Psychological Distress Among Hispanic Adults Living in Poverty. Prev Chronic Dis. 2015;12:E206.

104. Chen E, Miller GE. Socioeconomic status and health: mediating and moderating factors. Annu Rev Clin Psychol. 2013;9:723-49. doi:10.1146/annurev-clinpsy-050212-185634.

105. Bradley RH, Corwyn RF, McAdoo HP, Coll CG. The home environments of children in the United States part I: variations by age, ethnicity, and poverty status. Child Dev. 2001;72(6):1844-67.

106. •Corburn J. Concepts for Studying Urban Environmental Justice. Curr Environ Health Rep. 2017;4(1):61-7.
This review paper summarizes conceptual thinking and current research frameworks related to environmental justice in the United States with a focus on urban neighborhoods.

107. Cushing L, Morello-Frosch R, Wander M, Pastor M. The haves, the have-nots, and the health of everyone: the relationship between social inequality and environmental quality. Annu Rev Public Health. 2015;36:193-209.

108. Vrijheid M, Martinez D, Aguilera I, Ballester F, Basterrechea M, Esplugues A et al. Socioeconomic status and exposure to multiple environmental pollutants during pregnancy: evidence for environmental inequity? Journal of epidemiology and community health. 2012;66(2):106-13.

109. CDC. Centers for Disease Control and Prevention, Health Disparities & Ineqalities Report - United States. Morbidity & Mortality Weekly Report (MMWR) Supplement. 2013;62:1-187.

110. White BM, Bonilha HS, Ellis C, Jr. Racial/Ethnic Differences in Childhood Blood Lead Levels Among Children <72 Months of Age in the United States: a Systematic Review of the Literature. J Racial Ethn Health Disparities. 2016;3(1):145-53.

111. Moody HA, Darden JT, Pigozzi BW. The Relationship of Neighborhood Socioeconomic Differences and Racial Residential Segregation to Childhood Blood Lead Levels in Metropolitan Detroit. J Urban Health. 2016;93(5):820-39.

112. McCormick MC, Litt JS, Smith VC, Zupancic JA. Prematurity: an overview and public health implications. Annu Rev Public Health. 2011;32:367-79.

113. CDC. Centers for Disease Control and Prevention. National Center for Health Statistics, National Health Interview Survey: Summary Health Statistics. 2015. Available: https://www.cdc.gov/nchs/nhis/shs/tables.htm

114. Braveman PA, Egerter SA, Mockenhaupt RE. Broadening the focus: the need to address the social determinants of health. Am J Prev Med. 2011;40:S4-18.

CHAPTER 2: Determinants of prenatal exposure to polybrominated diphenyl ethers (PBDEs) among urban, minority infants born between 1998-2006

Whitney J. Cowell[a,b], Andreas Sjödin[c], Richard Jones[c], Ya Wang[a,d], Shuang Wang[a,d], Julie B. Herbstman[a,b]

[a]Columbia Center for Children's Environmental Health, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032 USA

[b]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York, 10032, United States

[c]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30329 USA

[d]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, New York, 10032, United States

Address correspondence to: Julie Herbstman, Columbia Center for Children's Environmental Health, 12th Fl, Mailman School of Public Health, 722 West 168th St, New York, NY 10032 USA. Telephone: (212) 304-7273. E-mail: jh2678@cumc.columbia.edu

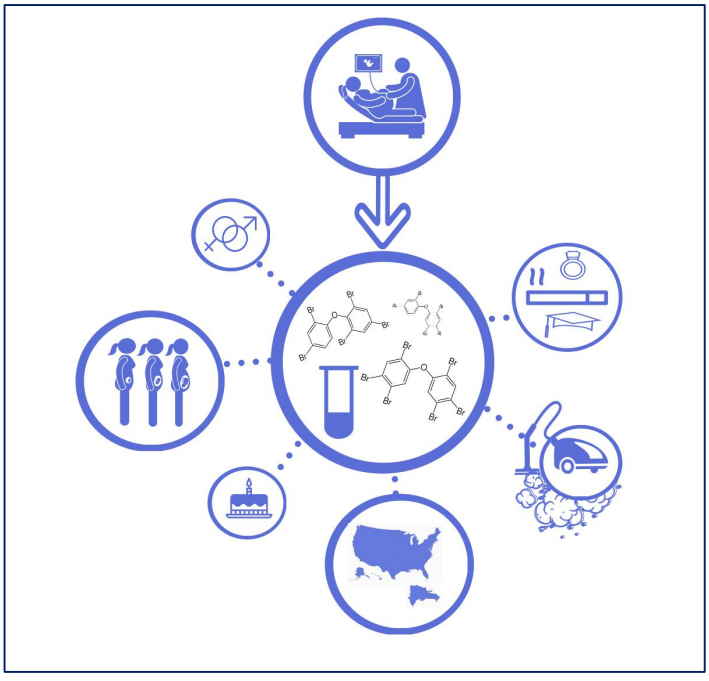
Funding: This research was supported by: the Passport, Forsythia, and Fine Foundations and NIH R01 ES021806. During preparation of this manuscript, WJC was supported by NIH T32 ES023772, NIH T32 ES007322 and EPA FP-91779001.

Keywords: PBDE, flame retardant, prenatal, exposure

Publication status: Accepted at Environmental Pollution

Abstract

Polybrominated diphenyl ethers **(**PBDEs) are environmentally persistent chemicals that structurally resemble legacy pollutants, such as polychlorinated biphenyls (PCBs). PBDEs were added to consumer products for over 30 years, before being phased out due to evidence of toxicity. We examined temporal changes in prenatal exposure to PBDEs, as well as other sources of variation in cord blood concentration among 327 minority infants born in New York City between 1998 and 2006. We used linear regression to examine changes in concentrations over time and in relation to lifestyle characteristics collected during pregnancy. We detected BDE-47 in 80% of samples with a geometric mean concentration of 14.1 ng/g lipid. Ethnicity was the major determinant of PBDE exposure; African American infants had 58% higher geometric mean cord plasma concentrations of BDE-47 ($p<0.01$) compared to Dominican infants. Notably, African American mothers were more likely to be born in the United States, which itself was associated with 40% ($p<0.01$) higher concentrations. We observed small decreases in PBDE concentrations by date of birth and no difference before and after their phase-out in 2004. Final multivariable models explained 8-12% of variability in PBDE concentration, depending on the congener. Our finding that prenatal exposure to PBDEs decreased only modestly between 1998 and 2006 is consistent with the lipophilic properties of PBDEs and their ongoing release from existing consumer products.



Introduction

Following a 1975 fire-safety law passed by the state of California, companies across the United States began adding polybrominated diphenyl ethers (PBDEs) to couches, chairs and other upholstered products, including those designed for infants and children (1, 2). Industry has primarily relied on three commercial formulations, which each consist of several PBDE congeners that vary in the number and location of bromine atoms around a diphenyl ether backbone (3). Over time, PBDEs migrate away from consumer products and enter house dust (4). In the United States, human exposure occurs primarily through incidental ingestion of dust, with consumption of meat, fish and dairy products considered secondary sources (5). PBDEs are classified as persistent organic pollutants (POPs). Structurally, they resemble several organohalogenated pollutants with known human toxicity, including polybrominated biphenyls (PBBs) and polychlorinated biphenyls (PCBs) (3). PBDEs are persistent, bioaccumulative and capable of long-range transport once released into the environment (6). Due to their lipophilic properties, PBDEs bioaccumulate in adipose tissue, cross the placenta, and partition into breastmilk (4, 5). As previously reviewed (7, 8), research examining human exposure has documented associations between PBDEs and endocrine disruption, reproductive problems and neurodevelopmental deficits. Owing to these health and environmental concerns, the three major commercial formulations were phased out of production in the United States between 2004 (PentaBDE, OctaBDE) and 2013 (DecaBDE) (9, 10). Despite these phase-outs, existing consumer products that are infrequently replaced, such as upholstered furniture, continue to release PBDEs. In this study, we examined time trends and other potential determinants of prenatal exposure to PBDEs, including ethnicity, in a low-income, minority cohort of infants born between 1998 and 2006.

Methods

Study population

We conducted this study among a subset of 727 participants enrolled in the Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns birth cohort, which was designed to examine sub-clinical health effects associated with prenatal exposure to several environmental chemicals. Women with a singleton pregnancy were recruited from two prenatal clinics in Northern Manhattan between 1998 and 2006. Women were excluded if they were younger than 18 or older than 35 years, started prenatal care after the 20th week of pregnancy, were active smokers, had a history of drug abuse, or had diabetes, hypertension or known HIV infection (11). All study protocols were approved by the Institutional Review Board of Columbia University; it was determined that the Centers for Disease Control and Prevention were not engaged in human subjects' research. Before each study visit, mothers were informed about all study procedures and provided written informed consent to participate.

Data collection

At the prenatal visit, trained research workers conducted structured interviews to ascertain information on demographic and several lifestyle factors, including material hardship and environmental tobacco smoke exposure. We defined material hardship as the inability to afford food, clothing or housing, and assessed environmental tobacco smoke (ETS) exposure by maternal report of smokers in the home in combination with cord blood cotinine concentrations as previously described (12). We assessed home cleaning habits by asking question about the

frequency of vacuuming and mopping. After birth, research workers abstracted data related to the pregnancy, delivery, and newborn from hospital medical records.

Umbilical cord blood collection and PBDE analysis

At delivery, umbilical cord blood was collected by study staff and transported to the CCCEH laboratory where samples were processed and stored in multiple aliquots at -70°C. Scientists at the CDC measured 11 PBDE congeners (BDE-17, -28, -47, -66, -85, -99, -100, -153, -154, -183, and 209) in stored umbilical cord plasma (13, 14). Briefly, after fortification with internal standards, plasma samples were extracted using a Gilson 215 liquid handler (Gilson Inc., Middleton, WI) and lipids were removed on a Rapid Trace modular SPE work station (Biotage, Uppsala, Sweden). Final analytic concentrations were determined by gas chromatography isotope dilution high-resolution mass spectrometry on a DFS instrument (ThermoFisher, Bremen, Germany). Blanks (N=3) were processed with every 30 samples and the median blank value was subtracted from the final result. Serum lipids (total cholesterol and triglycerides) were measured using commercially available test kits (Roche Diagnostics, Indianapolis, IN) and total blood lipids were estimated using a recently developed cord blood-specific formula [total cord blood lipids = 2.657 × total cord blood cholesterol + cord blood triglycerides + 0.268, in g lipids/L plasma] (A Sjodin, unpublished data, November 2016].

PBDE predictors

We reviewed published literature to identify potential determinants of PBDE exposure related to: 1) the mother (ethnicity, country of birth, residential history, age at delivery, education, relationship status, parity, time since previous pregnancy, and employment during pregnancy); 2)

the newborn (sex, birthweight, gestational age); and 3) the household (material hardship, cleaning habits, environmental tobacco smoke) for which we had collected information during the prenatal period.

Statistical analysis

We focused our analysis on BDEs-47 (detection frequency: 80%), -99 (50%), -100 (42%) and -153 (38%), which were the most frequently detected congeners. We natural-log transformed concentrations to better approximate a normal distribution and examined each congener as a continuous, lipid-adjusted variable. We imputed concentrations below the limit of detection (LOD) using a distribution-based multiple imputation approach (R function available in Supplemental Information Table 1 and LODs available in Supplemental Information Table 2). Specifically, to account for each sample's unique LOD, which is proportional to the available plasma volume and lipid content, we randomly drew a value from a congener-specific distribution of detected data for which the LOD was less than or equal to that of the non-detected concentration. We repeated this imputation procedure 10 times and pooled parameter estimates for all subsequent analyses using the Multiple Imputation by Chained Equations (MICE) R package.

We examined descriptive statistics for each potential determinant identified from our review of the literature. To evaluate whether children without a measure of PBDEs were different from those with a measure of PBDEs, we conducted student's t, Wilcoxon-Mann-Whitney, and Pearson's chi-square tests to identify differences in predictors among children included versus excluded for not having a measure of PBDEs.

We used linear regression to model the association between continuous date of birth (examined in units of days and years) and natural-log transformed PBDE concentrations (ng/g lipid). In addition to time, we examined effect estimates and p-values from models between PBDE concentrations and other potential predictors, which we included in congener-specific multivariable models if the univariate p-value was less than 0.1. We visualized our findings by plotting geometric mean PBDE concentrations within levels of each predictor, which we dichotomized at the median if the variable was not already categorical.

A woman's ancestry, geography, language, religion, lifestyle and cultural traditions shape her ethnicity. In turn, these factors may influence individual determinants of PBDE exposure. For example, PBDEs sorb to dust particles and ethnicity may influence home cleaning habits. Therefore, to better understand the impact of ethnicity on exposure, we examined potential determinants of cord blood PBDE concentration among African American and Dominican participants in separate models. These models included the same predictors examined in unstratified models. We further examined demographic and lifestyle differences between African American and Dominican participants using student's t-tests and Pearson's chi-square tests for categorical and continuous variables, respectively.

Finally, several characteristics that have been previously associated with PBDE concentrations, such as maternal age (15), may affect cord blood lipid levels (16). Therefore, we used linear regression to model the association between each potential determinant and total cord blood lipid levels (mg lipid/dL blood). We performed all analyses using RStudio (v0.99.891) or SAS (v9.4).

Results

Study population

Of the 727 children fully enrolled in the Mothers and Newborns cohort, 577 (79%) had a cord blood sample collected at delivery. We quantified PBDEs in umbilical cord plasma from 327 of these 577 infants (57%), which comprised all available stored samples. Table 1 presents demographic and lifestyle characteristics of the study sample. All maternal-child pairs are African American (n=121) or Dominican (n=206) and at delivery all mothers resided in Northern Manhattan or the South Bronx. Among the African American mothers, 96% were born in the United States, compared to only 22% of Dominican mothers; of mothers born outside of the United States, 86% were born in the Dominican Republic. At delivery, 35% of mothers had less than a high school education and 39% reported experiencing material hardship. On average, infants with a measure of PBDEs were born earlier during the enrollment period ($p<0.01$), weighed more at birth ($p<0.01$) and were more often born to a nulliparous mother ($p<0.01$) compared to infants without a measure of PBDEs. We detected no other significant differences between mother-child pairs included in the present analysis and those excluded. Among participants with a PBDE measure, we found several significant differences by ethnicity. On average, African American mothers were younger ($p=0.01$), had a higher pre-pregnancy body mass index ($p<0.01$), gained less weight during pregnancy ($p=0.04$), delivered lower birthweight babies ($p<0.01$) with shorter gestations ($p<0.01$), were less frequently in a stable relationship while pregnant ($p<0.01$) and were enrolled earlier during the study period ($p<0.01$). With regard to the home environment, African American mothers were less likely to experience material hardship ($p<0.01$) or use a wet mop for cleaning ($p<0.01$), but more likely to use a damp mop ($p<0.01$), vacuum ($p<0.01$) and be exposed to environmental tobacco smoke ($p<0.01$).

Table 1 - Demographic and Lifestyle Characteristics of Study Participants with a Measure of PBDEs Enrolled between 1998 and 2006 in a New York City-Based Birth Cohort			
N (%) or Mean±SD	All (n=327)[a]	African American (n=121)	Dominican (n=206)
Maternal characteristics at delivery			
African American	121 (37)	(100)	
Dominican	206 (63)		206 (100)
Born in the United States*	161 (49)	116 (96)	45 (22)
Age (years)*	25.1±4.9	24.2±4.9	25.6±4.8
Less than high school education	114 (35)	43 (36)	71 (34)
Married or in a stable relationship*	81 (25)	16 (13)	65 (22)
Office work during pregnancy	36 (11)	13 (11)	23 (11)
Pre-pregnancy body mass index*	26.0±6.1	27.3±6.7	25.2±5.6
Weight gain during pregnancy (kg)*	17.1±6.8	16.1±6.6	17.8±6.9
Nulliparous	166 (51)	58 (48)	108 (52)
Years since previous pregnancy[b]	2.2±2.3	2.3±2.2	2.1±2.3
Child characteristics			
Male	149 (46)	51 (42)	98 (48)
Gestational age (months)*	39.4±1.3	39.1±1.4	39.5±1.2
Birth weight (kg)*	3.4±0.4	3.4±0.5	3.5±0.4
Birth year: 1998-2000*	176 (54)	81 (67)	95 (46)
Birth year: 2001-2003*	88 (27)	24 (20)	64 (31)
Birth year: 2004-2006*	63 (19)	16 (13)	47 (23)
Household characteristics			
At least one material hardship*	127 (39)	35 (29)	92 (45)
Tobacco smoke exposure in home*	115 (35)	56 (46)	59 (29)
Ever vacuum to clean floors*	56 (18)	32 (27)	24 (12)
Ever damp mop to clean floors*	183 (57)	81 (68)	102 (51)
Ever wet mop to clean floors*	189 (59)	57 (48)	132 (66)

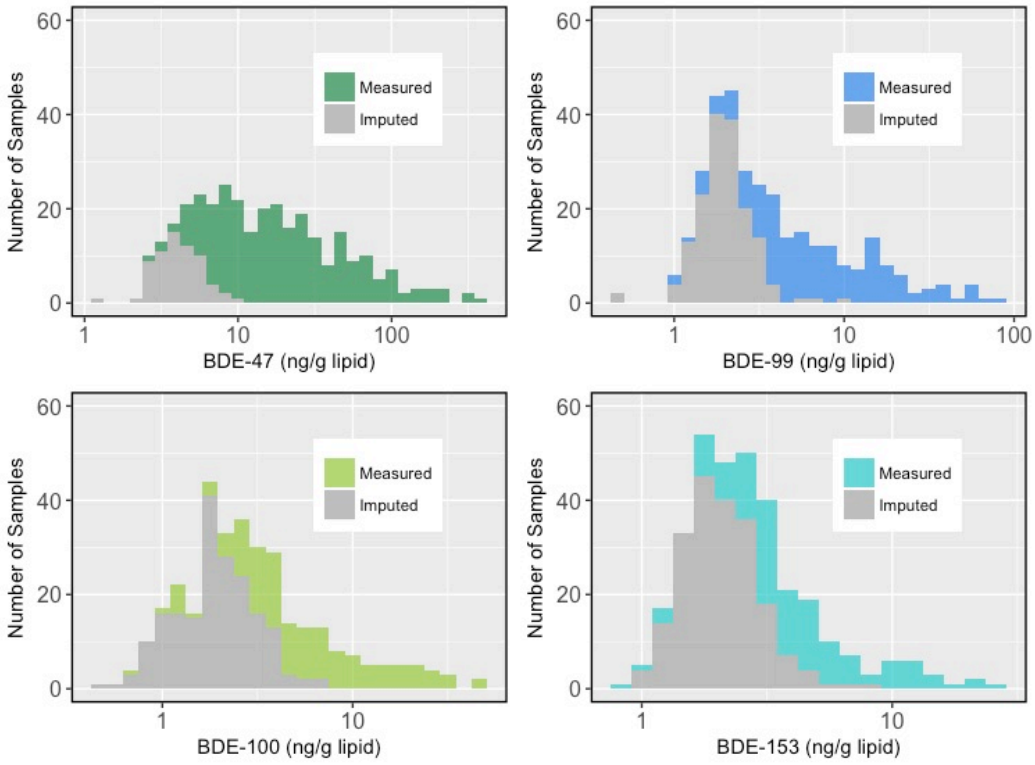
[a]Missing (n): pre-pregnancy BMI (10), weight gain (36), ever vacuum (11), ever damp mop (8), ever wet mop (9); [b]includes all previous pregnancies carried to the third trimester.

*African American and Dominican participants significantly different at the p=0.05 level.

Distribution of cord plasma PBDEs

We detected BDEs-47, -99, -100 and -153 in 80%, 50%, 42% and 38% of cord plasma samples, respectively. Supplemental Table 3 presents detection frequencies for all 11 congeners measured. As illustrated by Figure 1, BDEs-47, -99, -100, and -153 concentrations were log-normally distributed with geometric means of 14.1 ng/g lipid, 3.7 ng/g lipid, 2.9 ng/g lipid, and 2.6 ng/g lipid, respectively. As expected given their shared use in the PentaBDE mixture, these four congeners were moderately to highly correlated (mean $r_{spearman}$ between congeners across 10 imputed datasets: 0.42-0.80, $p<0.01$; see Supplemental Table 4, which provides correlation coefficients for each congener comparison).

Figure 1. Measured and imputed (non-detectable) PBDE concentrations (ng/g lipid) in cord plasma collected from infants born in New York City between 1998 and 2006.

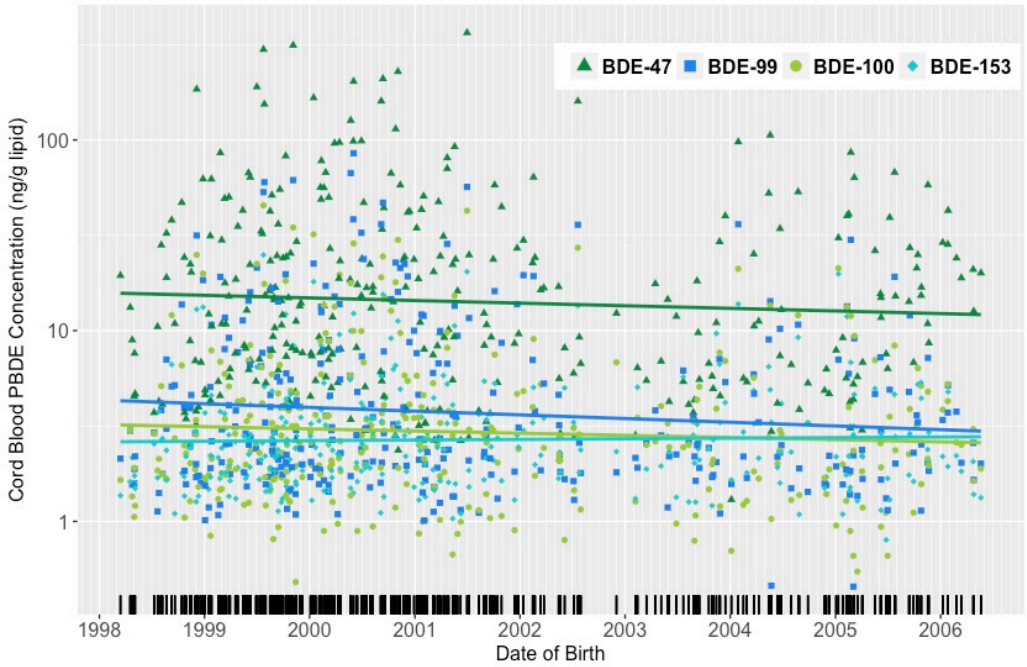


Note: Plots generated using data from 1 of the 10 imputed datasets.

Change in concentration of cord plasma PBDEs over time

As illustrated by Figure 2, we observed small reductions in BDEs-47 (3%), 99 (5%), and 100 (3%) per year of birth when examined over the entire enrollment period (1998 to 2006), however, this decrease was only statistically significant for BDE-99 (p=0.04). When we adjusted for ethnicity, decreases in concentrations were attenuated and the association with BDE-99 was no longer significant. When stratified by ethnicity, temporal decreases in concentration were larger among African American (2% decrease in BDE-47 per 8 years) compared to Dominican (0.5% decrease in BDE-47 per 8 years) infants (see Supplemental Material Figure 1). We detected no change in BDE-153 concentration over the enrollment period. We did not find significantly different PBDE concentrations among infants born before compared to after the PentaBDE phase-out in 2004 (p=0.13-0.60).

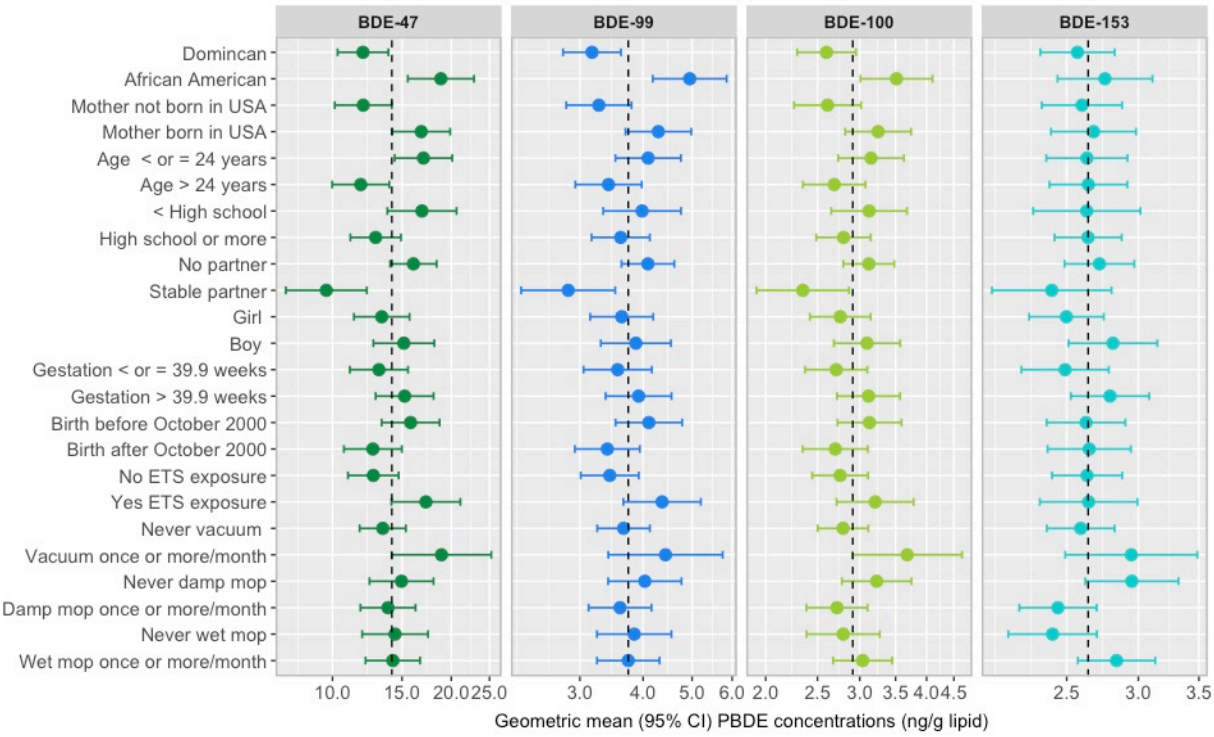
Figure 2. Temporal changes in PBDE concentrations measured in umbilical cord plasma collected from infants born in New York City between 1998 and 2006 (n=327)



Predictors of cord plasma PBDE concentrations.

Final multivariable models included 12 predictors, which are detailed below and collectively explained a low amount of variability in cord plasma BDE-47 (12%), BDE-99 (11%), BDE-100 (10%), and BDE-153 (8%) concentrations. Figure 3 presents geometric mean PBDE concentrations from univariate models between predictors and each congener. Higher concentrations were associated with: African American [vs. Dominican] ethnicity, maternal birth in [vs. outside] the United States, younger maternal age at delivery [continuous in days], lower maternal educational attainment [less than high school vs. high school degree or equivalent], single relationship status [vs. married or in a stable relationship for 7 or more years], male sex of the study child [vs. female], longer gestation [continuous in weeks], earlier date of birth of study child [continuous in days], and prenatal environmental tobacco smoke exposure [any vs. none]. With regard to maternal cleaning habits, vacuuming [ever vs. never] was generally associated with higher PBDE concentrations and damp mopping [ever vs. never] was associated with lower concentrations. Wet mopping [ever vs. never] was associated with higher BDE-153 concentrations, but not with the other three congeners (see Supplemental Table 5, which provides effect sizes and p-values for each association).

Figure 3. PBDE concentrations in umbilical cord plasma collected from children born in New York City between 1998 and 2006 stratified by lifestyle and demographic characteristics (n=327). Results are from bivariate models.



Ethnicity was the major determinant of PBDE exposure; African American infants had 58%, 56%, and 35% higher geometric mean cord plasma concentrations of BDE-47 ($p<0.01$), BDE-99 ($p<0.01$) and BDE-100 ($p<0.01$) compared to Dominican infants, respectively. Notably, African American mothers were more likely to be born in the United States, which itself was associated with 40% ($p<0.01$), 31% ($p=0.01$) and 24% ($p=0.04$) higher concentrations of BDE-47, BDE-99, and BDE-100. We did not find associations between BDE-153 and ethnicity or maternal birthplace. Among a subset of 167 Dominican mothers (81%) with information about lifetime residential history, we found that for every 10% of life lived in the United States, her infant's cord plasma BDE-47 concentration increased by 4% ($p=0.03$). We detected similar, but

statistically insignificant changes for BDE-99 (p=0.11) and BDE-100 (p=0.10). We did not detect an association between residential history and BDE-153.

Of the other 10 variables included in our final multivariable model, six significantly varied by ethnicity, with Dominican mothers typically having characteristics associated with lower PBDE concentrations (older, more likely to be in a stable relationship, less likely to be exposed to environmental tobacco smoke, less likely to vacuum). The exceptions were that Dominican mothers had longer pregnancies and were more likely to use a wet mop. When stratified by ethnicity, depending on the congener, our multivariable models explained 11-19% of variability in PBDE concentrations among Dominican mothers and 15-18% of variability among African American mothers. We did not detect significant associations between any variable included in our final models and cord plasma total lipid levels.

Discussion

We measured cord plasma PBDE concentrations in 327 minority infants born in New York City between 1998 and 2006; this is the largest United States-based study of umbilical cord PBDE concentrations. We detected BDE-47, which is typically the dominant congener found in humans, in 80% of samples and BDEs-99, -100, and -153 in at least 35% of samples. These four congeners are components of the PentaBDE mixture, which was voluntarily phased out of United States commerce in 2004 (9).

PBDE concentrations in our samples were similar to levels detected in umbilical cord (15, 19, 20) or maternal blood (17, 18, 21, 22) by seven other United States-based studies (see

Supplemental Table 6, which compares mean concentrations across studies). While enrollment in previous studies spanned only one to three years, we were able to examine time trends in exposure over an eight-year period that overlapped with the phase-out of PentaBDE. We found modest reductions in PBDE concentrations by year of birth (3-5% depending on the congener) and no significant difference in concentrations among infants born before versus after the PentaBDE phase-out in 2004. In a recent analysis of PBDE stocks and flows, Abbasi et al. estimated that approximately 46,000 tons of PentaBDE were used in consumer products in the United States and Canada between 1970 and 2004, with a peak application of approximately 17,000 tons in 2004 (1). Given these trends and the use of PBDEs in products that are infrequently replaced, it is not surprising that we detected only modest decreases over time and no significant difference between children born before versus after the 2004 phase-out. We expect exposure will continue to decrease and dominant sources of exposure to potentially shift as PBDE-containing products enter end-of-life stages and are removed from the indoor environment. For example, as PBDE-containing furniture enters waste streams, contamination of air and water may occur via volatilization and leaching from landfills. Given these potential exposure sources, monitoring landfills for PBDE emissions may be an important step in understanding the environmental burden of PBDEs in coming decades. Additionally, as time since the PBDE phase-out progresses, socioeconomic disparities in PBDE exposure may widen, as lower income families are likely to replace furniture less frequently or may purchase older, second-hand items manufactured before the PentaBDE phase-out.

Among the sociodemographic and lifestyle factors considered, ethnicity was the most important determinant of cord plasma BDE-47, -99 and -100 concentrations, with Dominican infants

typically having lower exposure. Notably, the majority of Dominican mothers were born in the Dominican Republic, where PBDEs may not have been routinely used as flame retardants in consumer products. The importance of the mother's birthplace is further supported by our finding of a positive association between cord plasma BDE-47 and proportion of lifetime lived in the United States. However, the modest size of this association (4% increase in concentration for every 10% increase in proportion of life lived in the United States) suggests that exposure conditions present during the mother's early life may be important determinants of body burden during adulthood. Our findings are consistent with results from a California-based study that found PBDE concentrations were 1) higher among pregnant women born in the United States compared to Mexico or Latin America and 2) increased with the duration of time lived in the United States (17). Likewise, an independent New York City-based study of pregnant women found PBDE concentrations in maternal blood were higher among African American women compared to Hispanic women (18).

In addition to birthplace, the PBDE exposure disparity we observed between African Americans and Dominicans may relate to differences in lifestyle and daily habits. For example, African American mothers were more likely to vacuum, which was associated with higher cord plasma PBDE concentrations. This is consistent with a recent study that reported household vacuuming frequency was positively associated with PBDE concentrations measured in silicon wristbands worn by children (23). It is plausible that the physical agitation of dust particles that occurs during the vacuuming process contributes to increased ingestion and inhalation of dust. Using a damp mop was associated with lower concentrations, especially for BDEs-100 and -153; in contrast to the direction observed with other predictors, this behavior was significantly more

common among African American mothers. Conversely, Dominican mothers were more likely to use a wet mop, which was associated with higher BDE-153 concentrations. While poorly understood, this finding is consistent with the results from a recent study based in Spain, which found housekeeping frequency was positively associated with cord blood BDE-153, but not BDEs-47, -99 or -100 (24).

Dominican mothers were significantly less likely to be single or exposed to environmental tobacco smoke, both of which were associated with higher PBDE concentrations. Our observation of lower concentrations among children born to older mothers is consistent with previous research (15). While it is plausible that older women would have lower body burdens due to increased child bearing, we did not find significant associations between PBDE concentrations and parity or time since previous delivery. Alternatively, our maternal age finding may reflect the older age of Dominican compared to African American mothers in our sample. In contrast to these patterns, African American infants had shorter gestations, which was associated with lower concentrations, possibly related to the reduced time opportunity for *in utero* transfer.

Other determinants of PBDE exposure in our final multivariable model that did not vary by ethnicity included maternal education and infant gender. We found mothers with less than a high school degree delivered infants with higher PBDE concentrations. This is consistent with the results of a Baltimore-based study (15), but in contrast to findings from a cohort in California (17). Notably, the California study found maternal education was positively associated with years of residency in the United States, which itself was associated with higher PBDE exposure. Conversely, they found that higher income, which was not related to residency, was associated

with lower exposure. Other studies measuring PBDEs in child or adult blood have reported similar trends-- with higher neighborhood poverty, lower household income and lower educational attainment typically associated with higher exposure (25, 26).

Finally, boys had higher cord plasma concentrations of BDE-153 compared to girls. While putative mechanisms underlying this finding are poorly understood, placental transfer of PBDEs is known to vary by bromination status, with lower brominated congeners characterized by faster and more extensive passage (4). Furthermore, while little information exists on placental gene expression in relation to PBDEs, expression of genes involved in nutrient transport has been shown to be sexually dimorphic (28), thus it is plausible that placental transfer of BDE-153, which is the highest molecular weight congener we studied, is impeded to a greater extent among girls compared to boys due to differential expression of transport genes. Importantly, however, this putative mechanism is inconsistent with findings from a recent study that reported higher concentrations of BDE-209, which is fully brominated, in placentas (suggesting it did not reach the fetus) from male pregnancies (29). More research is needed to understand how sex may contribute to differences in fetal exposure to PBDEs and other environmental chemicals.

In contrast to previous studies, we found no association between PBDE concentrations and pre-pregnancy body mass index (15, 17). Likewise, we found no relation between concentrations and weight gain during pregnancy or birthweight.

We identified several important predictors of PBDE exposure, including ethnicity, however, the majority of variation in cord plasma concentrations remained unexplained by our final models.

Additional factors that may be associated with PBDE exposure that we did not measure include breastfeeding of previous children, bedroom and living room floor type, the number of electronics and furniture pieces containing polyurethane foam in the home, and personal behaviors, such as handwashing and nail biting. While we did have information on kitchen floor type for 84% of participants, we found little variability between homes, with the majority (77%) having a smooth surface. We collected limited information on maternal diet, including meat and fish consumption, during pregnancy; however, these data focused on cooking styles rather than consumption quantity and did not include information on dairy products. In addition to these exposure sources and pathways, individual concentrations may vary due to inter-individual differences in genes involved in metabolism and excretion of PBDEs.

Over 95% of PentaBDE produced globally has been used in North America, where the general population has the highest body burden in the world (7). In 2004, PentaBDE was phased-out of United States commerce owing to evidence of environmental persistence and human toxicity. In 2009, it was added to the Stockholm Convention's list of persistent organic pollutants, prompting member countries to eliminate production and new use (3). Despite these measures, exposure to PBDEs is expected to continue for decades due to their ongoing release from furniture and other consumer products. Indeed, in the present study, we detected only modest reductions in cord plasma concentrations over an eight-year period. Further, we found that maternal birthplace was an important predictor of exposure, with foreign-born mothers delivering babies in the United States with significantly lower cord plasma PBDE concentrations compared to U.S. born mothers.

Future research investigating modifiable behaviors that may reduce exposure to PBDEs in the home environment is needed. For example, while vacuuming might reasonably be assumed to reduce exposure, we found vacuuming frequency during pregnancy was associated with higher cord plasma PBDE concentrations, likely due to the emission of dust into the air. We did not have information about personal vacuum characteristics, however, it is plausible that high efficiency particulate air (HEPA) filters may help reduce exposure during the vacuuming process. Importantly, HEPA vacuums are generally more expensive than standard filtration vacuums and may be less affordable for lower income households. Moreover, we found lower maternal education, an indicator of socioeconomic status and resource availability, was associated with higher exposure, echoing concerns by other researchers that disparities in PBDE exposure may be an environmental equity issue (26).

Finally, despite amendments to California's flammability standard (30) and the availability of chemical-free fire safety approaches, including the use of smoke detectors, sprinkler systems, reduced ignition propensity cigarettes and fire safe candles, evidence indicates companies have been replacing PBDEs with alternative flame retardant chemicals that have unknown toxicity (31). Development of federal and state requirements for testing replacement chemicals, especially those with structural or other properties similar to PBDEs and other POPs, before they are added to consumer products is critical for preventing future global contamination with toxic and environmentally persistent chemicals.

References

1. Abbasi G, Buser AM, Soehl A, Murray MW, Diamond ML. Stocks and flows of PBDEs in products from use to waste in the U.S. and Canada from 1970 to 2020. Environ Sci Technol 2015;49(3):1521-8.

2. Stapleton HM, Klosterhaus S, Keller A, Ferguson PL, van Bergen S, Cooper E, et al. Identification of flame retardants in polyurethane foam collected from baby products. Environ Sci Technol 2011;45(12):5323-31.

3. UNEP. Listing of POPs under the Stockholm convention. In. Chatelaine, Switzerland: The Stockholm Convention, United Nations Environmental Programme; 2012.

4. Frederiksen M, Vorkamp K, Thomsen M, Knudsen LE. Human internal and external exposure to PBDEs--a review of levels and sources. Int J Hyg Environ Health 2009;212(2):109-34.

5. Lorber M. Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol 2008;18(1):2-19.

6. Law RJ, Covaci A, Harrad S, Herzke D, Abdallah MA, Fernie K, et al. Levels and trends of PBDEs and HBCDs in the global environment: status at the end of 2012. Environ Int 2014;65:147-58.

7. Fromme H, Becher G, Hilger B, Volkel W. Brominated flame retardants - Exposure and risk assessment for the general population. Int J Hyg Environ Health 2016;219(1):1-23.

8. Linares V, Belles M, Domingo JL. Human exposure to PBDE and critical evaluation of health hazards. Arch Toxicol 2015;89(3):335-56.

9. Corportation GLC. Great Lakes Chemical Corporation completes phase-out of two flame retardants. In. Indianapolic, IN: Great Lakes Chemical Corportation; 2005.

10. USEPA. DecaBDE Phase-out Initiative. In; 2010.

11. Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, et al. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environ Health Perspect 2006;114(8):1287-92.

12. Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A, et al. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. Neurotoxicol Teratol 2004;26(3):373-85.

13. Jones R, Edenfield E, Anderson S, Zhang Y, Sjodin A. Semi-automated extraction and cleanup method for measuring persistent organic pollutants in human serum. Organohalogen Comp 2012;74:97-98.

14. Sjodin A, Jones RS, Lapeza CR, Focant JF, McGahee EE, 3rd, Patterson DG, Jr. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. Anal Chem 2004;76(7):1921-7.

15. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Patterson DG, Halden RU, et al. Determinants of prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in an urban population. Environ Health Perspect 2007;115(12):1794-800.

16. Kazemi SA, Sadeghzadeh M. Lipid Profile of Cord Blood in Term Newborns. J Compr Ped 2014;5(4):e23759.

17. Castorina R, Bradman A, Sjodin A, Fenster L, Jones RS, Harley KG, et al. Determinants of serum polybrominated diphenyl ether (PBDE) levels among pregnant women in the CHAMACOS cohort. Environ Sci Technol 2011;45(15):6553-60.

18. Horton MK, Bousleiman S, Jones R, Sjodin A, Liu X, Whyatt R, et al. Predictors of serum concentrations of polybrominated flame retardants among healthy pregnant women in an urban environment: a cross-sectional study. Environ Health 2013;12:23.

19. Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect 2010;118(5):712-9.

20. Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect 2003;111(9):1249-52.

21. Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. Environ Health Perspect 2011;119(10):1454-9.

22. Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, et al. Maternal Polybrominated Diphenyl Ether (PBDE) Exposure and Thyroid Hormones in Maternal and Cord Sera: The HOME Study, Cincinnati, USA. Environ Health Perspect 2015;123(10):1079-85.

23. Kile ML, Scott RP, O'Connell SG, Lipscomb S, MacDonald M, McClelland M, et al. Using silicone wristbands to evaluate preschool children's exposure to flame retardants. Environ Res 2016;147:365-72.

24. Costa O, Lopez-Espinosa MJ, Vizcaino E, Murcia M, Iniguez C, Navarrete-Munoz EM, et al. Dietary and Household Sources of Prenatal Exposure to Polybrominated Diphenyl Ethers (PBDEs) in the INMA Birth Cohort (Spain). Environ Sci Technol 2016;50(11):5935-5944.

25. Darrow LA, Jacobson MH, Preston EV, Lee GE, Panuwet P, Hunter RE, Jr., et al. Predictors of Serum Polybrominated Diphenyl Ether (PBDE) Concentrations among Children Aged 1-5 Years. Environ Sci Technol 2017;51(1):645-654.

26. Zota AR, Adamkiewicz G, Morello-Frosch RA. Are PBDEs an environmental equity concern? Exposure disparities by socioeconomic status. Environ Sci Technol 2010;44(15):5691-2.

27. Sjodin A, Wong LY, Jones RS, Park A, Zhang Y, Hodge C, et al. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. Environ Sci Technol 2008;42(4):1377-84.

28. Rosenfeld CS. Sex-Specific Placental Responses in Fetal Development. Endocrinology 2015;156(10):3422-34.

29. Leonetti C, Butt CM, Hoffman K, Hammel SC, Miranda ML, Stapleton HM. Brominated flame retardants in placental tissues: associations with infant sex and thyroid hormone endpoints. Environ Health 2016;15(1):113.

30. Technical Bulletin 117-2013. In. Sacramento, CA: State of California, Department of Consumer Affairs; 2013. p. 1-13.

31. Stapleton HM, Sharma S, Getzinger G, Ferguson PL, Gabriel M, Webster TF, et al. Novel and high volume use flame retardants in US couches reflective of the 2005 PentaBDE phase out. Environ Sci Technol 2012;46(24):13432-9.

Supplemental Information

Table of Contents

Supplemental Table 1 –Steps and R function for performing distribution-based multiple imputation

For each sample with a concentration less than the limit of detection (LOD), count the number of congener-specific detected samples with a concentration less than the LOD of the non-detected sample.

- If the number of available observations is ≥ 2
  - Calculate the mean and variance of ln(PBDE) from the available observations
  - Draw a random value from the normal distribution with the same mean and variance
    - BDE-47: 98%, BDE-99: 98%, BDE-100: 99.5%, BDE-153: 100%

- If the number of available observations = 1
  - Use the available ln(PBDE) to fill in the non-detected value
    - BDE-47: 0%, BDE-99: 0%, BDE-100: 0.5%, BDE-153: 0%

- If the number of available observations = 0
  - Replace non-detected concentration with ln(LOD/2)
    - BDE-47: 2%, BDE-99: 2%, BDE-100: 0%, BDE-153: 0%

R Function

PBDE: PBDE concentration
LOD: limit of detection
Ln_PBDE: natural log transformed PBDE concentration

```
impute.func=function(pbde, lod, ln_pbde){
  missing.index=which(is.na(pbde))
  for(i in missing.index){
    n.available=sum(pbde<lod[i], na.rm=T)
          if(n.available>1){
      mu=mean(ln_pbde[which(pbde<lod[i])], na.rm=T)
      sd=sd(ln_pbde[which(pbde<lod[i])], na.rm=T)
      ln_pbde[i]=rnorm(n=1, mean=mu, sd=sd)
    }else if(n.available==1){
      ln_pbde[i]=ln_pbde[which(pbde<lod[i])]
    }else if(n.available==0 & !is.na(lod[i])){
      ln_pbde[i]=log(lod[i]/2)
    }else if(is.na(lod[i])){
      ln_pbde[i]=mean(log(pbde), na.rm=T)
    }
  }
  return(ln_pbde)
```

Supplemental Table 2 – Limit of detection for detected and not detected concentrations of PBDE congeners measured in umbilical cord plasma collected between 1998 and 2006 (n=327)

	Detected range, ng/g lipid (n)	Not-detected range, ng/g lipid (n)	% Non-detected samples with LODs in the range of LODs from detected samples
BDE-47	0.69-9.14 (261)	2.60-11.59 (66)	98
BDE-99	0.41-5.46 (165)	0.91-5.40 (161)	98
BDE-100	0.29-5.46 (136)	0.94-5.40 (191)	100
BDE-153	0.29-5.10 (123)	1.14-5.46 (204)	100

Supplemental Table 3 - Detection frequencies for 11 PBDE congeners measured in cord plasma collected between 1998 and 2006 (n=327)

	Detected, n (%)	Not Detected, n (%)	Not Reportable, n (%)
BDE-17	0 (0)	327 (100)	0 (0)
BDE-28	48 (15)	279 (85)	0 (0)
BDE-47	261 (80)	66 (20)	0 (0)
BDE-66	16 (5)	310 (95)	1 (<1)
BDE-85	31 (9)	296 (91)	0 (0)
BDE-99	165 (50)	161 (49)	1 (<1)
BDE-100	136 (42)	191 (58)	0 (0)
BDE-153	123 (38)	204 (62)	0 (0)
BDE-154	11 (3)	316 (97)	0 (0)
BDE-183	10 (3)	317 (97)	0 (0)
BDE-209	6 (2)	321 (98)	0 (0)

Supplemental Table 4 – Correlations[a] ($R_{Spearman,}$ p-values) between PBDE congeners (ng/g lipid) measured in cord plasma collected between 1998 and 2006 in New York City (n=327)

	BDE-47	BDE-99	BDE-100	BDE-153
BDE-47		0.80 (<0.01)	0.70 (<0.01)	0.44 (<0.01)
BDE-99	0.80 (<0.01)		0.68 (<0.01)	0.42 (<0.01)
BDE-100	0.70 (<0.01)	0.68 (<0.01)		0.52 (<0.01)
BDE-153	0.44 (<0.01)	0.42 (<0.01)	0.52 (<0.01)	

[a]Values are the mean correlation coefficient across 10 imputed datasets

Supplemental Table 5. Associations between umbilical cord plasma PBDE concentrations (ng/g lipid) and several demographic and lifestyle factors measured between 1998 and 2006 (n=327)

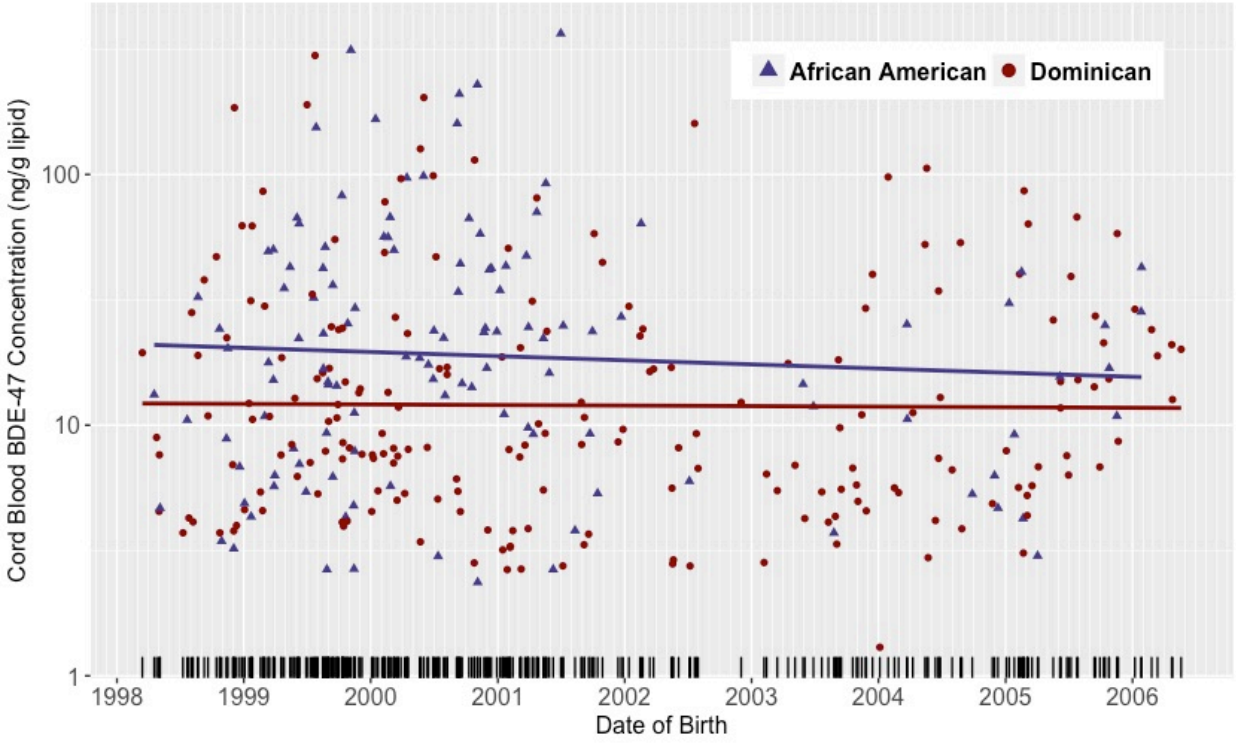
☐ Significantly higher levels at p ≤ 0.10	Ln BDE-47		Ln BDE-99		Ln BDE-100		Ln BDE-153	
☐ Significantly lower levels at p ≤ 0.10	β±SE	p	β±SE	p	β±SE	p	β±SE	p
African American vs. Dominican ethnicity	0.45±0.08	<0.01	0.44±0.11	<0.01	0.30±0.10	<0.01	0.07±0.08	0.36
Born in the United States vs. foreign born	0.34±0.12	<0.01	0.27±0.11	0.01	0.22±0.11	0.05	0.01±0.07	0.91
Age (per year)	-0.04±0.01	<0.01	-0.02±0.01	0.09	-0.01±0.01	0.28	0.00±0.01	0.72
High school or more vs. less	-0.27±0.13	0.03	-0.10±0.11	0.38	-0.11±0.10	0.28	0.00±0.08	0.97
Stable relationship	-0.51±0.14	<0.01	-0.36±0.12	<0.01	-0.28±0.12	0.02	-0.12±0.09	0.18
Office work during pregnancy	-0.26±0.19	0.18	-0.23±0.17	0.19	-0.11±0.16	0.51	-0.19±0.13	0.14
Pre-pregnancy body mass index (kg/m^2)	0.01±0.01	0.22	0.01±0.01	0.51	0.00±0.01	0.67	-0.01±0.01	0.17
Weight gain during pregnancy (kg)	0.00±0.01	0.92	0.01±0.01	0.55	0.00±0.01	0.92	0.00±0.01	0.58
Multiparous vs. nulliparous	-0.08±0.12	0.50	0.03±0.11	0.77	-0.03±0.10	0.74	0.02±0.07	0.78
Previous pregnancy within 4 years	0.09±0.12	0.47	0.10±0.11	0.34	0.04±0.10	0.70	-0.02±0.07	0.75
Male	0.13±0.12	0.29	0.06±0.11	0.55	0.12±0.10	0.24	0.12±0.07	0.10
Gestational age (weeks)	0.08±0.05	0.09	0.04±0.04	0.35	0.06±0.04	0.13	0.04±0.03	0.12
Birth weight (kg)	0.01±0.13	0.93	-0.06±0.12	0.61	0.00±0.11	0.97	-0.02±0.08	0.81
Born after October 18, 2000 (median)	-0.22±0.12	0.07	-0.19±0.11	0.08	-0.15±0.10	0.15	0.01±0.07	0.91
At least one material hardship vs. none	-0.14±0.13	0.27	-0.15±0.11	0.17	-0.10±0.10	0.33	-0.04±0.08	0.56
Yes environmental tobacco smoke vs. none	0.31±0.13	0.02	0.24±0.11	0.03	0.15±0.11	0.15	0.00±0.07	0.96
Ever vacuum during pregnancy vs. never	0.34±0.16	0.04	0.19±0.14	0.19	0.28±0.13	0.04	0.13±0.09	0.17
Ever damp mop during pregnancy vs. never	-0.08±0.13	0.53	-0.11±0.11	0.31	-0.17±0.10	0.10	-0.19±0.08	0.01
Ever wet mop during pregnancy vs. never	-0.01±0.13	0.92	-0.03±0.11	0.81	0.08±0.10	0.41	0.16±0.07	0.03

Supplemental Table 6 – PBDE concentrations (median, ng/g lipid) and detection frequencies (%) measured in maternal or cord blood collected by 8 U.S.-based studies conducted between 1998 and 2010							
Location (Ref)	Enrollment Period	Blood type	Study size	BDE-47	BDE-99	BDE-100	BDE-153
New York, NY[a]	1998-2006	UCB	327	14.1 (80)	3.7 (50)	2.9 (42)	2.6 (38)
Monterey, CA (1)[a]	1999-2000	MB	416	15.8 (100)	4.4 (99)	2.8 (98)	2.4 (98)
Indianapolis, IN (2)	2001	UCB	12	25 (100)	7.1 (100)	4.1 (100)	4.4 (100)
New York, NY (3)	2001-2002	UCB	201	11.2 (81)	3.2 (60)	1.4 (64)	0.7 (50)
Cincinnati, OH (4)[a]	2003-2006	MB	274[b]	20.5 (100)	4.9 (99)	4.1 (98)	5.5 (99)
Baltimore, MD (5)	2004-2005	UCB	297	13.6 (90)	4.3 (47)	2.3 (65)	2.6 (60)
Durham, NC (6)[a]	2008-2010	MB	137	16.5 (95)	4.7 (64)	4.2 (89)	5.9 (96)
New York, NY (7)	2009-2010	MB	316	7.9 (99)	1.6 (84)	1.7 (91)	3.0 (98)
MB: Maternal blood; UCB: Umbilical cord blood [a]Concentration is geometric mean, not median [b]Sample size: BDE-47=305, BDE-99=204, BDE-100=275							

Supplemental Table 6 References

1. Castorina R, Bradman A, Sjodin A, Fenster L, Jones RS, Harley KG, et al. Determinants of serum polybrominated diphenyl ether (PBDE) levels among pregnant women in the CHAMACOS cohort. Environ Sci Technol 2011;45(15):6553-60.
2. Mazdai A, Dodder NG, Abernathy MP, Hites RA, Bigsby RM. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect 2003;111(9):1249-52.
3. Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect 2010;118(5):712-9.
4. Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, et al. Maternal Polybrominated Diphenyl Ether (PBDE) Exposure and Thyroid Hormones in Maternal and Cord Sera: The HOME Study, Cincinnati, USA. Environ Health Perspect 2015;123(10):1079-85.
5. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Patterson DG, Halden RU, et al. Determinants of prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in an urban population. Environ Health Perspect 2007;115(12):1794-800.
6. Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. Environ Health Perspect 2011;119(10):1454-9.
7. Horton MK, Bousleiman S, Jones R, Sjodin A, Liu X, Whyatt R, et al. Predictors of serum concentrations of polybrominated flame retardants among healthy pregnant women in an urban environment: a cross-sectional study. Environ Health 2013;12:23.

Supplemental Figure 1. Ethnicity-stratified temporal changes in BDE-47 concentration measured in cord blood collected between 1998 and 2006 (n=327).



CHAPTER 3: Time trends and developmental patterns of plasma PBDE concentrations over a 15-year period between 1998 and 2013

Whitney J. Cowell, MPH[1,2], Andreas Sjödin, PhD[3], Richard Jones, MS[3], Ya Wang, BS[1,4] Shuang Wang, PhD[1,4] Julie B. Herbstman, PhD[1,2]

[1]Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, NY 10032 USA
[2]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032 USA
[3]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341 USA
[4]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY 10032 USA

Address correspondence to: Julie B. Herbstman, Columbia Center for Children's Environmental Health, 12th Fl, Mailman School of Public Health, 722 West 168th St, New York, NY 10032 USA. Telephone: (212) 304-7273. Fax: (212) 544-1943. E-mail: jh2678@cumc.columbia.edu

Acknowledgements: This research was supported by NIH R01 ES021806. During preparation of this manuscript, WJC was supported by NIH T32 ES023772, NIH T32 ES007322 and EPA FP-91779001. We gratefully acknowledge the contribution of Miss Shenika Christopher who helped to identify potential determinants of interest for investigation in this study.

Competing financial interests: The authors declare they have no actual or potential competing financial interests.

Publication status: Submitted to Journal of Exposure Science and Environmental Epidemiology

Abstract:

Polybrominated diphenyl ethers (PBDEs) were used extensively as flame retardants in furniture containing polyurethane foam. In this study, we examined temporal changes in PBDE concentrations from 1998 to 2013 and characterized patterns of exposure over the early lifecourse. We measured PBDEs in 903 plasma samples collected between birth and age 9 years among 334 children. We examined temporal trends in exposure by regressing PBDE concentrations on year of sample collection in age-adjusted models. We additionally characterized trajectories of exposure using latent class growth analysis (LCGA) and investigated potential determinants of trajectory assignment, including date of birth. We detected PBDEs in approximately 80% of cord plasma samples and 100% of child samples; BDE-47 was the predominant congener detected. Controlling for age, BDE-47 concentrations decreased by 5% per year between 1998 and 2013. When considering only postnatal samples, this reduction increased to 13%. The results of LCGA indicate that for the majority of children, PBDE concentrations peak during toddler years. Consistent with changes in concentration over time, year of birth was the most important determinant of PBDE trajectory assignment.

Keywords: PBDE, flame retardant, exposure, prenatal, childhood

Introduction

Polybrominated diphenyl ethers (PBDEs) are brominated chemicals that vary in the number and location of bromine atoms around a diphenyl ether backbone[1]. Due to the natural free-radical trapping properties of halogens[2], PBDEs were widely used as flame retardants to comply with California Technical Bulletin-117 (Cal-117), which was ratified in 1975 and required household consumer products to pass an open flame test before entering the marketplace[3]. Commercially, PBDEs were used as components of three technical mixtures known as pentaBDE, octaBDE and decaBDE[4]. The present study focuses on BDEs-47, -99, -100, and -153, which are the predominant congeners in the pentaBDE formulation and are estimated to make up 90% of the human body burden[5].

The United Nations Environmental Program (UNEP) estimates that 100,000 tons of pentaBDE were manufactured globally between 1975 and 2010[6], with approximately 85% used in North America[7], where exposure is ubiquitous and body burdens are the highest in the world[8]. PentaBDE was primarily used in couches, mattresses, carpet padding and other upholstered products[4] and typically comprised approximately 3% (by weight) of the polyurethane foam used in these products[9].

During production, PBDEs are not chemically bonded to base polymers, thus they have a propensity to migrate away from consumer products and accumulate in the indoor environment[10]. In the United States, human exposure occurs primarily through incidental ingestion of dust, with consumption of meat, fish and dairy products considered secondary sources[11, 12]. Owing to their lipophilic properties, PBDEs accumulate in adipose tissue[13], readily

cross the placenta[14], and partition into breast milk[15]. Adult half-lives are estimated to range from 1.6 (BDE-99) to 6.5 (BDE-153) years[16], however, little is known about how the unique exposure pathways (i.e. increased mouthing), metabolic differences, and other characteristics specific to children influence PBDE body burden.

Several studies have documented higher exposure to pentaBDE congeners among children compared to adults[17], likely owing to the greater amount of time infants and toddlers spend in close proximity to the floor and the frequency with which young children mouth fingers, toys and other objects[18]. In the present analysis, we investigated both time and age-specific changes in PBDE concentration over the early lifecourse.

Materials and Methods

Study participants

The study sample includes 334 of the 727 children enrolled in the Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns birth cohort. As previously described[19], healthy, non-smoking women living in Northern Manhattan or the South Bronx were enrolled during pregnancy between 1998 and 2006 and followed prospectively. Data analyzed in the present paper were collected between 1998 and 2013 at birth and at age 2, 3, 5, 7 and 9-year follow-up visits, resulting in a total of 903 data points. At each visit, a bilingual (English/Spanish) research worker conducted a structured interview with the mother to ascertain information related sociodemographic and lifestyle factors. Details related to housekeeping behaviors were collected by asking the mother about the frequency with which the home was cleaned with a vacuum, dust mop, damp mop or wet mop. Household material hardship was

assessed based on self-reported ability to afford adequate food, clothing, or housing[20]. Before each visit, mothers were informed about all study procedures and provided written informed consent to participate; after age 7 years, children additionally provided informed assent. Study protocols were approved by the Institutional Review Board of Columbia University; it was determined at the Centers for Disease Control and Prevention (CDC) that the agency was not engaged in human subjects' research.

Sample collection and laboratory analysis

At the child's birth, umbilical cord blood was collected by study staff, and at age 2, 3, 5, 7 and 9-year study visits child venous blood was collected by a pediatric phlebotomist. Following collection, blood was separated and stored at -70°C at the CCCEH laboratory. Aliquots of all available stored samples from each age period (N_{cord}=327, $N_{2\text{-}years}$=56, $N_{3\text{-}years}$=115, $N_{5\text{-}years}$=42, $N_{7\text{-}years}$=203, and $N_{9\text{-}years}$=160) were shipped to the CDC for measurement of 11 PBDE congeners (BDEs: 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, and 209). The present study examines BDEs 47, 99, 100 and 153, which were the most frequently detected congeners across study visits. Details of the analytic method have been previously published [21, 22]. Briefly, samples were processed using automatic fortification with internal standards and extracted by automated liquid liquid extraction (Gilson Inc.; Middleton, WI). Analytic determinations were made by gas chromatography isotope dilution high resolution mass spectrometry. Final data were corrected for the median concentration detected in blank samples included in each analytic run (3 blanks per 30 samples). Lipids were co-extracted and removed on a silica: silica/sulfuric acid column using the Rapid Trace equipment (Biotage; Uppsala, Sweden). Total cholesterol and triglyceride levels were determined by standard enzymatic methods using commercially available test kids

(Roche Diagnostics; Indianapolis, IN). Child total plasma lipids were estimated from these measured components using the short formula described by Phillips et al.[23] and cord plasma lipids were estimated using a recently-developed cord blood specific formula [total cord blood lipids = 2.66 × total cord blood cholesterol + cord blood triglycerides + 0.268, in g lipids/L plasma] (Sjödin A, personal communication).

Statistical analysis

We examined descriptive statistics and visualized age-specific distributions of BDEs-47, -99, -100, and -153 using histograms and boxplots. We calculated within congener correlations over time, as well as correlations between congeners at each time point. Concentrations below the limit of detection (LOD) were imputed using a distribution-based multiple imputation method that accounts for sample-specific LOD values[24]. Distribution-based methods for imputing non-detected concentrations have been shown to produce unbiased results, even in the presence of a large number of samples (50-70%) with non-detectable concentrations of certain analytes[25].

We examined temporal trends in exposure by regressing lipid-standardized, $\log_{10}$-transformed PBDE concentrations on year of sample collection. We built separate models for each congener and used the generalized estimating equations approach with an exchangeable working correlation to account for repeated measures within a child over time. We isolated effects driven by time, rather than age, by adjusting these models for exact age at blood collection, which we included as a time-varying covariate.

To examine trajectories of PBDE exposure over early life, we used latent class growth analysis (LCGA) to empirically estimate discrete groups of children with shared patterns of measured PBDE concentrations (ng/g lipid) from birth through age 9 years[26]. This approach models PBDE concentration as a continuous function of age at the time of blood collection and estimates the probability of trajectory membership for each child. It is well-suited for complicated data structures as it allows for inclusion of all children with PBDE concentrations measured at a minimum of one time point. We $\log_{10}$-transformed PBDE concentrations to better approximate a normal distribution and estimated models with varying numbers of groups (1–6) and shapes (linear, quadratic, cubic). We evaluated model fit using the Bayesian Information Criterion (BIC), as well as the magnitude of group membership posterior probabilities.

We examined predictors of trajectory assignment by re-estimating final models with potential predictors included as covariates. For each variable, the probability of belonging to each trajectory is estimated through a multinomial logistic regression in which trajectory membership is treated as the outcome variable. In addition to date of birth, which we evaluated as a continuous variable in 3-year increments (1998-2000, 2001-2003, 2004-2006), we explored the following variables in bivariate models: ethnicity (African American vs. Dominican), gender (male vs. female), parity (nulliparous vs. multiparous), maternal age at delivery (>24 years vs. ≤24 years), maternal level of education (high school vs. less than high school), household material hardship (inability to afford food, housing, or clothing vs. access to all), breastfeeding duration (≥12 weeks vs <12 weeks), presence of a smoker in the home (yes vs. no), and frequency of vacuuming (ever vs. never), dust mopping (ever vs. never), damp mopping (ever vs. never) and wet mopping (ever vs. never). We included variables in congener-specific

multivariable models if the p-value from bivariate associations was less than 0.10 for any one of the trajectories across the four congeners. We conducted regression analyses using SAS v9.4 (SAS Institute Inc., Cary, North Carolina) and performed LCGA using the Proc Traj procedure[27].

Results

We measured PBDE concentrations in 903 samples collected repeatedly from birth to age 9 years among 334 children born between 1998 and 2006. All children were African American or Dominican. Table 1 presents sociodemographic and lifestyle characteristics of maternal-child pairs included in the analysis. These 334 children did not significantly differ at the p=0.05 level from the fully enrolled cohort (n=727) on any sociodemographic or lifestyle factor examined in this analysis with the following exceptions: children with a measure of PBDEs were more likely to be born to a nulliparous mother (50% vs. 40%), were more likely to live in a household that used a dust mop at the prenatal period (12% vs. 7%), and were less likely to live in a household that used a dust mop at the 7-year period (20% vs. 27%) or a damp mop at the 3-year period (63% vs. 70%). To allow time to age in to the later study visits, children with PBDE measures were more likely to be born in 1998-2000 (n=183 vs. n=88 born 2001-2003 and n=63 born 2004-2006).

Table 1. Characteristics of maternal-child pairs (n=334).	
	n (%)
Child birth: 1998-2000	183 (55)
Child birth: 2001-2003	88 (26)
Child birth: 2004-2006	63 (19)
African American	124 (37)
Dominican	210 (63)
Maternal age[a]	129 (46)
Maternal <H.S. education[a]	117 (35)
Nulliparous[a]	168 (50)
Child sex (female)	182 (54)
Breastfed < 12 weeks	217 (66)
Smoker in home	
Prenatal	114 (34)
3 years	71 (21)
7 years	49 (15)
Material hardship (yes)	
Prenatal	129 (39)
3 years	96 (31)
7 years	104 (36)
Ever vacuum home	
Prenatal	57 (17)
3 years	51 (17)
7 years	70 (22)
Ever dust mop home	
Prenatal	41 (12)
3 years	52 (16)
7 years	67 (20)
Ever damp mop home	
Prenatal	190 (57)
3 years	209 (63)
7 years	202 (60)
Ever wet mop home	
Prenatal	174 (58)
3 years	194 (61)
7 years	194 (64)
[a]At delivery Abbreviations: high school (H.S.)	

PBDE concentrations

Across samples and congeners (BDE-47, -99, -100, -153), LODs for cord and child plasma PBDE concentrations ranged from 0.29 to 11.59 ng/g and 0.45 to 20.20 ng/g lipid, respectively. PBDE concentrations were more frequently detected in child compared to cord plasma samples and at all ages BDE-47 was the most frequently detected congener (Table 2).

Table 2. Summary of PBDE concentrations measured in umbilical cord and child plasma between birth and age 9 years (n=903 samples from 334 children).

	Cord (n=327)	Age 2 (n=56)	Age 3 (n=115)	Age 5 (n=42)	Age 7 (n=203)	Age 9 (n=160)
BDE-47						
GM±GSD (pg/g serum)	30.8±1.9	139.4±21.0	133.1±13.4	98.6±15.0	90.2±6.8	77.8±6.4
GM±GSD (ng/g lipid)	14.1±0.9	37.8±5.8	32.1±3.1	25.6±3.8	23.2±1.7	18.1±1.4
<LOD (n, %)	66 (20)	0 (0)	1 (1)	1 (2)	5 (2)	1 (1)
Non-reportable (n, %)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BDE-99						
GM±GSD (pg/g serum)	8.7±0.4	42.8±7.8	34.6±3.6	23.9±3.9	23.1±1.8	19.5±1.6
GM±GSD (ng/g lipid)	3.7±0.2	18.1±11.7	8.2±0.9	6.1±1.0	5.8±0.5	4.4±0.4
<LOD (n, %)	161 (49)	1 (2)	9 (8)	7 (17)	40 (20)	30 (19)
Non-reportable (n, %)	1 (0.3)	13 (23)	9 (8)	0 (0)	0 (0)	0 (0)
BDE-100						
GM±GSD (pg/g serum)	6.7±0.3	26.6±3.9	27.5±2.5	23.3±3.5	20.7±1.4	17.6±1.4
GM±GSD (ng/g lipid)	2.9±0.2	7.2±1.1	6.6±0.6	6.0±0.9	5.3±0.4	4.0±0.3
<LOD (n, %)	191 (58)	0 (0)	5 (4)	4 (10)	17 (8)	12 (8)
Non-reportable (n, %)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.6)
BDE-153						
GM±GSD (pg/g serum)	5.7±0.2	18.0±2.5	20.4±1.8	23.4±3.7	25.1±1.6	23.7±1.8
GM±GSD (ng/g lipid)	2.6±0.1	4.8±0.7	4.9±0.4	6.1±1.0	6.4±0.4	5.5±0.4
<LOD (n, %)	204 (62)	1 (2)	7 (6)	4 (10)	12 (6)	9 (6)
Non-reportable (n, %)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)
Total lipids (mg/dL)	236±11[a]	385±70[b]	433±77[b]	398±62[b]	406±79[b]	450±87[b]

Abbreviations: GM: geometric mean, GSD: geometric standard deviation, LOD: limit of detection; PBDE: polybrominated diphenyl ether.

[a]Estimated using: total cord blood lipids = 2.66 × cord blood total cholesterol + cord blood triglycerides + 0.268, in g lipid/L plasma

[b]Estimated using: total blood lipids = 2.27 × total cholesterol + triglycerides + 0.623, in g lipid/L plasma

Geometric mean BDE-47 concentration were highest in samples collected at age 2 years (38±6 ng/g lipid) and lowest in cord plasma samples (14±1 ng/g lipid); we observed a similar pattern for BDEs-99 and -100, however, while BDE-153 concentration were also lowest in cord plasma, it peaked at age 7 years (25.1±1.6 ng/g lipid vs. 5.7±0.2 in cord blood). Within congeners, PBDE concentration measured in cord plasma was poorly correlated with concentration measured in child plasma, however, concentrations measured between ages 2 and 9 years were moderately to highly correlated (see Supplemental Material, Table S1). Within age periods, BDEs-47, -99 and -100 were moderately to highly correlated (minimum $R_{Spearman}$: 0.76 in cord plasma to maximum $R_{Spearman}$ 0.96 in 3-year plasma) (see Supplemental Material, Table S2).

Changes over time

Controlling for child age at blood draw, BDEs-47, -99, -100 and -153 decreased by approximately 5%, 7%, 5%, and 2% per year between 1998 and 2013, respectively (Table 3 and Figure 1). When considering only samples collected during the postnatal period, which likely reflects direct exposure to PBDEs from the environment rather than from maternal transfer, concentrations decreased by 13%, 13%, 11%, and 11% per year between 2000 and 2013 for BDEs-47, -99, -100 and -153, respectively.

Figure 1. Changes in age-adjusted plasma PBDE concentration (ng/g lipid) between 1998 and 2013 (n=903 samples from 334 children). Prenatal concentration was measured in cord plasma.

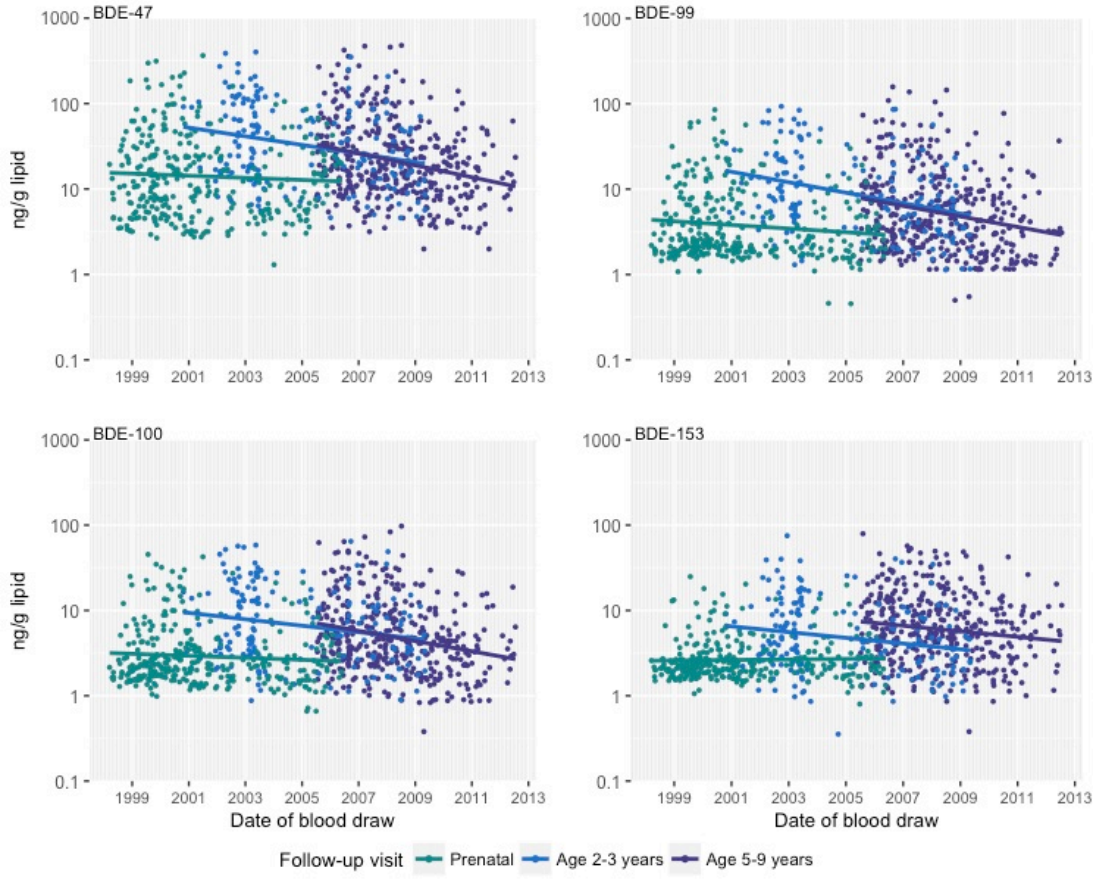


Table 3. Change in cord or child plasma PBDE concentrations (ng/g lipid) over time in GEE models adjusting for age at sample collection.			
	Percent change/year (95% CI)[a]	N children[b]	N observations[b]
Cord & child samples (1998-2013)			
BDE-47	-4.5 (-8.8, -2.3)	334	903
BDE-99	-6.7 (-8.8, -2.3)	334	880
BDE-100	-4.5 (-6.7, -0.9)	334	902
BDE-153	-2.3 (-6.7, 0.0)	334	901
Child samples only (2000-2013)			
BDE-47	-12.9 (-18.7, -8.8)	288	576
BDE-99	-12.9 (-18.7, -8.8)	285	554
BDE-100	-10.9 (-14.9, -6.7)	288	575
BDE-153	-10.9 (-14.9, -6.7)	281	574
[a]Percent change calculated using: $1-(10^{\beta})\times100$, where β coefficient was estimated by regressing $\log_{10}$-transformed PBDE concentration on year of sample collection. [b] Sample size varies due to non-reportable PBDE results (see Table 2)			

Early life trajectories

As illustrated by **Figure 2**, the best fitting LCGA model revealed four trajectories of BDEs-47, 99, and 100. One trajectory was characterized by low PBDE concentrations at all ages ('persistent low'). Two trajectories were defined by high concentrations during childhood, one of which showed a decrease after toddler years ('early postnatal peak') and a second that remained elevated throughout childhood ('sustained postnatal high'). The fourth trajectory was characterized by high prenatal concentrations that decreased after birth ('prenatal high'). Across these three congeners, the majority of children were assigned to the 'persistent low' (34-51%) or 'early postnatal peak' (24-38%) trajectories. We identified three relatively age-invariant trajectories of BDE-153, which we refer to as 'persistent low', 'sustained postnatal moderate', and 'sustained postnatal high'. Congener-specific sample sizes and frequencies for each trajectory are presented in Table 4. Owing to its small size (<10% of the sample), we do not plot the BDE-100 'prenatal high' trajectory, nor do we examine it in regression models, however, we retained the trajectory as it improved LCGA model fit. Across congeners, the mean posterior probability of trajectory membership (0.7-0.9) met or exceeded the widely-accepted threshold for satisfactory group assignment (mean of 0.7), indicating a high likelihood that a child's exposure pattern fit well within his or her assigned trajectory[28] (see Supplemental Material, Table S3).

Figure 2. Trajectories of plasma PBDE concentration (ng/g lipid) from birth through 9 years (n=334) estimated using latent class growth analysis; bands represent 95% confidence intervals.

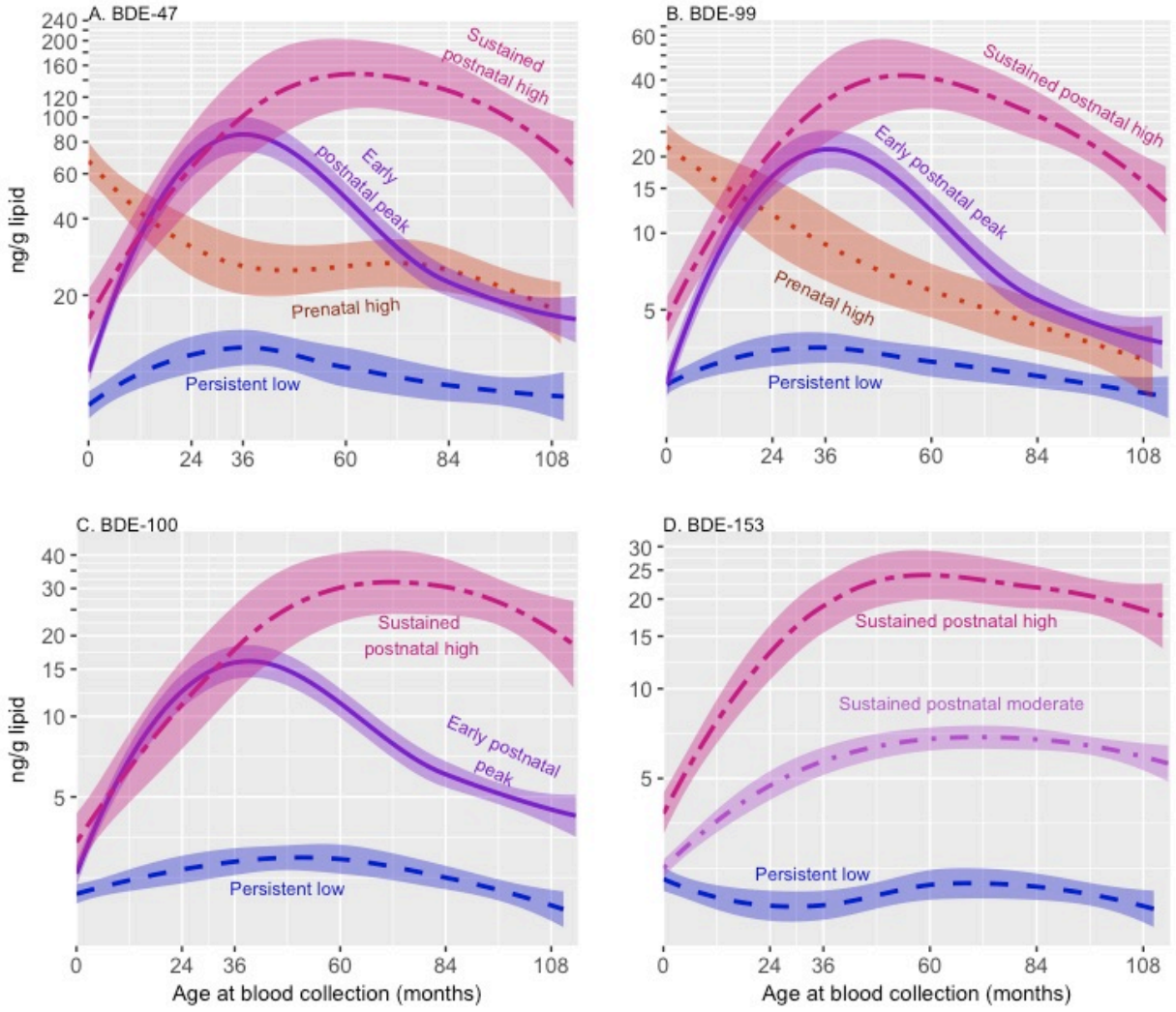


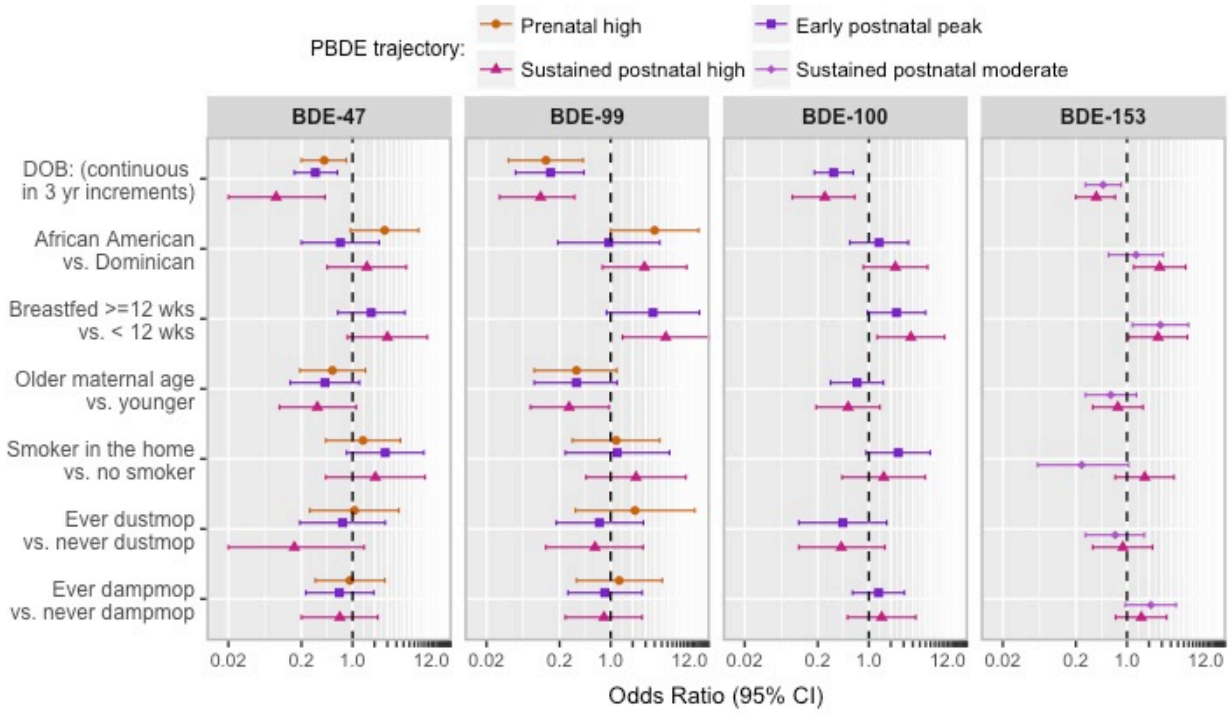
Table 4. Sample size of each PBDE exposure trajectory, N (%)

	Persistent low	Prenatal high	Early postnatal peak	Sustained postnatal moderate	Sustained postnatal high
BDE-47	113 (34)	68 (20)	116 (35)	NA	37 (11)
BDE-99	148 (44)	49 (15)	81 (24)	NA	56 (17)
BDE-100	155 (46)	NA	117 (35)	NA	32 (10)
BDE-153	88 (26)	NA	NA	185 (55)	61 (18)

Predictors of trajectory assignment

Figure 3 presents odds ratio (OR) estimates from multivariable multinomial models examining determinants of PBDE trajectory membership, which were fit within the LCGA modeling framework. In all models, the 'persistent low' trajectory serves as the reference category. Four children are excluded from these models due to missing information on breastfeeding history.

Figure 3. Odds ratios (ORs) from multivariable multinomial models examining determinants of PBDE trajectories over early life.



Legend: ORs from models examining breastfeeding as a predictor of the prenatal high trajectory not plotted due to the small number of breastfed children that were assigned to this trajectory and resulting wide confidence intervals.

Consistent with changes in concentration over time, year of birth was the most important determinant of trajectory assignment; across congeners, children born later in the cohort were significantly less likely to be assigned to the 'prenatal high' (OR_{BDE-47}=0.41, 95% CI: 0.20, 0.82; OR_{BDE-99}=0.16, 95% CI: 0.07, 0.37), 'early postnatal peak' (OR_{BDE-47}=0.31, 95% CI: 0.16, 0.62; OR_{BDE-99}=0.28, 95% CI: 0.13, 0.59, $OR_{BDE-100}$=0.33, 95% CI: 0.18, 0.61), or 'sustained postnatal high' (OR_{BDE-47}=0.09, 95% CI: 0.02, 0.42; OR_{BDE-99}=0.27, 95% CI: 0.13, 0.58, $OR_{BDE-100}$=0.25, 95% CI: 0.09, 0.64, $OR_{BDE-153}$=0.38, 95% CI: 0.20, 0.69) versus the 'persistent low' trajectory. In addition to year of birth, the following variables met our criteria (bivariate p-value <0.10) for inclusion in multivariable models: ethnicity, maternal age at delivery, breastfeeding duration, presence of a cigarette smoker residing in the home, dust mopping the home, and damp mopping the home. For time-varying covariates (smoker in the home and household cleaning behaviors), we modeled predictors collected at the prenatal, 3-year and 7-year study visits for the 'prenatal high', 'early postnatal peak' and 'sustained postnatal high' trajectories, respectively.

In general, across trajectories and congeners, African American (versus Dominican) ethnicity, younger maternal age at delivery, longer breastfeeding duration, and living in a household with an active smoker were associated with higher odds of assignment to the 'prenatal high', 'early postnatal peak' or 'sustained postnatal high' trajectories versus the 'persistent low' trajectory (see Figure 4). With regard to cleaning behaviors, dust mopping was associated with lower odds of assignment to the 'sustained postnatal high' BDE-47 trajectory, however, this association was imprecisely estimated given the relatively low prevalence of dust mopping in the cohort (20% at the 7-year visit). In contrast, while dust mopping was not associated with the sustained postnatal

moderate or high trajectories of BDE-153, children in households that used a damp mop were more likely to be assigned to these groups.

Discussion

In the present analysis, we measured plasma PBDE concentrations in children over a 15-year period. Given our relatively large sample size and the frequency of repeated measures, this study provides one of the most comprehensive PBDE exposure assessments that has been conducted among children. Plasma PBDE concentrations were generally similar to those detected by other U.S.-based studies, except that we detected slightly lower concentrations of BDEs-47, -99 and -100 at older ages and lower concentrations of BDE-153 at all ages (see Supplemental Material, Table S4). Controlling for age, we found that plasma concentrations of congeners in the pentaBDE technical mixture, which was phased out of U.S. commerce in 2004, significantly decreased between 1998 and 2013.

In addition to changes over time, we used the LCGA framework to identify children with similar developmental patterns of PBDE exposure over early life. While this method is used extensively in psychology and the social sciences (i.e. criminology, econometrics, sociology), it has rarely been used in the field of epidemiology. When it has been applied, it has typically been used to model exposure to social risk factors (i.e. violence[29], socioeconomic status[30]) or health outcomes (i.e. obesity[31], wheeze[32]) over time. Despite its applicability to the field of exposure science, we know of no studies that have used LCGA to model changes in biomarker concentrations over time. Our finding of peak PBDE concentration during toddler years is consistent with results from cross-sectional studies indicating exposure peaks at approximately 2-3 years for the

majority of children[33]. Other studies have found that PBDE concentration peaks between 4-6 years[34], which is consistent with our 'sustained postnatal high' trajectory. Overall, the presence of different developmental trajectories suggests that a single measure may not accurately reflect exposure to PBDEs throughout the early lifecourse. Further, while trajectories were generally similar for BDEs-47, -99 and -100, plasma concentrations of BDE-153 followed a unique pattern, indicating that summed measures of these congeners may reflect different proportional contributions from BDEs-47, -99, and -100 versus BDE-153 depending on the age at sample collection.

We found that maternal age was associated with lower odds of assignment to the BDE-47 and BDE-99 'prenatal high' trajectories, which is consistent with previous research[35] and suggests that, unlike other legacy persistent organic pollutants[36], PBDE body burdens may not increase with age among adults. Notably, lipophilic chemicals with long half-lives are not expected to differentiate from more rapidly eliminated chemicals until at least 20 years following peak exposure, thus it is plausible that the lack of an association between cord plasma PBDE concentrations and maternal age reflects the relatively limited temporal range of PBDE data that have been collected across studies, most of which were collected during the transition period following peak PBDE use[37].

Our finding that children born to African American (versus Dominican) mothers had higher odds of assignment to the 'prenatal high' trajectory likely reflect differences in maternal body burden related to lifetime residential history. Specifically, while all study children were born in New York City, the majority (67%) of Dominican mothers were born in the Dominican Republic,

where PBDEs may not have been used as extensively in consumer products. We observed a similar effect of ethnicity on assignment to the 'sustained postnatal high' trajectory, which may reflect differences in cleaning behaviors or other cultural differences between African American and Dominican households.

Consistent with previous research demonstrating breastfeeding as a pathway of PBDE exposure[11], children who were breastfed 12 weeks or longer were more likely to be assigned to the sustained postnatal high trajectory. Unexpectedly, breastfed children were also more likely to have high prenatal BDE plasma concentrations. In this cohort, breastfeeding (<12 weeks vs. ≥ 12 weeks) was associated with indicators of low socioeconomic status, such as material hardship (OR=1.66, 95% CI: 1.20, 2.82). It is possible that breastfeeding is serving as an indicator of unmeasured cultural or socioeconomic factors associated with PBDEs, such as the use of second-hand or deteriorating household furniture, which may be more likely to contain (due to older age) and leach (due to greater wear and tear) PBDEs. Children born into households with an active smoker were also more likely to be assigned to the 'prenatal high' trajectory. The direction of this finding is consistent with the results of a U.S.-based study that detected higher hand wipe PBDE concentrations among young children living in homes with an active smoker[38]. It is unlikely that cigarettes are a direct source of PBDEs, however, similar to our breastfeeding findings, it is possible that smoking may serve as an indicator of unmeasured socioeconomic factors related to PBDE exposure.

With regard to cleaning behaviors, children in households that used a dust mop were less likely to have high concentrations of BDEs-47, 99 and 100 throughout childhood. In contrast, children

in households that reported using a damp mop were more likely to have moderate or high BDE-153 concentrations throughout childhood. While unexpected, this later finding is consistent with results from the Spain-based INMA cohort, which found more frequent housekeeping (>1 times /week, including sweeping, vacuuming, dusting, and mopping) was associated with significantly higher serum concentrations of BDE-153, but not the other congeners, among pregnant women[39]. The U.S. Environmental Protection Agency recommends that parents dust, wet mop and use a vacuum with a high efficiency particulate air (HEPA) filter to reduce children's exposure to flame retardants in dust[40]; however, given our inconsistent findings related to cleaning behaviors, further research, including household intervention studies, is need to better understand what behavioral modifications are most effective for reducing exposure.

Strengths of our study include the large sample size, variation in both the chronological date and child age of blood collection, and the rich set of prospectively collected covariate data, including information on cleaning behaviors. Specific strengths of LCGA include the ability to retain all children with data at a minimum of one follow-up period, as well as the ability to include a wide range of variation in the age at blood draw within follow-up periods. However, although our data met the generally accepted posterior probability threshold for trajectory assignment, it is possible that some children were misclassified, which may have biased our findings towards the null.

Concurrent with the voluntary 2004 industry phase-out of pentaBDE, New York State passed an environmental law that codified the prohibition of pentaBDE production and use[41]. Despite these regulatory changes, PBDEs continue to leach from existing consumer products and migrate into house dust. Indeed, in the present study, we detected PBDE concentrations in approximately

80% of cord plasma samples collected between 1998 and 2006, and 100% of child (ages 2-9 years) samples collected between 2000 and 2013. Our findings of several unique PBDE trajectories may inform future research studies as well as interventions designed to target specific windows of peak exposure. Importantly, in the United States, the majority of furniture and other household items containing polyurethane foam are disposed of in landfills. For example, approximately 1.3 million tons of carpet/padding, furniture, and other bulky items were disposed of in California landfills in the year 2004 alone[42]. As more PBDE-containing items enter end-of-life waste streams in the coming decades, shifts in environmental contamination patterns due to leaching from outdoor reservoirs may trigger a transition in human exposure pathways from dust to dietary sources (fatty fish, seafood, meat, dairy)[43]. As time since the pentaBDE phase-out progresses, monitoring landfills, surrounding environmental media, and wildlife will be critical for understanding shifts in exposure pathways and reducing human exposure.

References

1. Bergman A, Ryden A, Law RJ, de Boer J, Covaci A, Alaee M *et al* A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environment international 2012; 49: 57-82.

2. D'Silva K, Fernandes A, Rose M Brominated organic micropollutants-igniting the flame retardant issue. Critcal reviews in environmental science and technology 2004; 34: 141-207.

3. Requirements, Test Procedure and Apparatus for Testing the Flame Retardance of Resilient Filling Materials used in Upholstered Furniture (Technical Bulletin 117). In: Furnishings CBoTIaH, (ed), 1975 (modified in 1990).

4. EPA. An Exposure Assessment of Polybrominated Diphenyl Ethers. In: Assessment NCfE, (ed). Washington, DC: Environmental Protection Agency, 2010.

5. Talsness CE Overview of toxicological aspects of polybrominated diphenyl ethers: a flame-retardant additive in several consumer products. Environmental research 2008; 108: 158-167.

6. UNEP. *United Nations Environment Programme. Technical review of the implications of reclycling commercial Penta and Octabromodiphenyl ethers.*, 2010.

7. Alcock RE, Sweetman AJ, Prevedouros K, Jones KC Understanding levels and trends of BDE-47 in the UK and North America: an assessment of principal reservoirs and source inputs. Environment international 2003; 29: 691-698.

8. Hites RA Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. Environmental science & technology 2004; 38: 945-956.

9. Cobb D Analysis of FR chemicals added to foams, fabric, batting, loose fill and barriers. . Memorandum to Dale R Ray, Project Manager, Upholstered Furniture, Consumer Products Safety Commission 2005.

10. Zhang X, Diamond ML, Robson M, Harrad S Sources, emissions, and fate of polybrominated diphenyl ethers and polychlorinated biphenyls indoors in Toronto, Canada. Environmental science & technology 2011; 45: 3268-3274.

11. Frederiksen M, Vorkamp K, Thomsen M, Knudsen LE Human internal and external exposure to PBDEs--a review of levels and sources. Int J Hyg Environ Health 2009; 212: 109-134.

12. Lorber M Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol 2008; 18: 2-19.

13. ATSDR. Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers (PBDEs). In: Registry AfTSD, (ed). Atlanta, GA, 2017.

14. Dassanayake RM, Wei H, Chen RC, Li A Optimization of the matrix solid phase dispersion extraction procedure for the analysis of polybrominated diphenyl ethers in human placenta. Analytical chemistry 2009; 81: 9795-9801.

15. Fang J, Nyberg E, Winnberg U, Bignert A, Bergman A Spatial and temporal trends of the Stockholm Convention POPs in mothers' milk -- a global review. Environ Sci Pollut Res Int 2015; 22: 8989-9041.

16. Geyer H, Schramm K, Darnerud P, Aune M, Feicht E, Fried K Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. Organohalogen Comp 2004; 66: 5.

17. Lunder S, Hovander L, Athanassiadis I, Bergman A Significantly higher polybrominated diphenyl ether levels in young U.S. children than in their mothers. Environmental science & technology 2010; 44: 5256-5262.

18. Hoffman K, Webster TF, Sjodin A, Stapleton HM Toddler's behavior and its impacts on exposure to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol 2017; 27: 193-197.

19. Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D *et al* Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environmental health perspectives 2006; 114: 1287-1292.

20. Mayer S, Jencks C Poverty and the distribition of material hardship. J Hum Resour 1988. 88-112.

21. Jones R, Edenfield E, Anderson S, Zhang Y, Sjodin A Semi-automated extraction and cleanup method for measuring persistent organic pollutants in human serum. Organohalogen Comp 2012; 74: 97-98.

22. Sjodin A, Jones RS, Lapeza CR, Focant JF, McGahee EE, 3rd, Patterson DG, Jr. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. Analytical chemistry 2004; 76: 1921-1927.

23. Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol 1989; 18: 495-500.

24. Cowell WJ, Sjodin A, Jones R, Wang Y, Wang S, Herbstman JB Determinants of prenatal exposure to polybrominated diphenyl ethers (PBDEs) among urban, minority infants born between 1998-2006. Environmental Pollution Under review.

25. Baccarelli A, Pfeiffer R, Consonni D, Pesatori AC, Bonzini M, Patterson DG, Jr. *et al* Handling of dioxin measurement data in the presence of non-detectable values: overview of available methods and their application in the Seveso chloracne study. Chemosphere 2005; 60: 898-906.

26. Nagin DS Group-based trajectory modeling: an overview. Ann Nutr Metab 2014; 65: 205-210.

27. Jones B, Nagin D, KA R SAS procedure based on mixture models for estimating developmental trajectories. Sociological Methodology Research 2001; 29: 374-393.

28. Nagin D. Group-based modeling of development. Harvard University Press: Cambridge, Massachusetts,, 2005.

29. Baskin D, Sommers I Trajectories of exposure to community violence and mental health symptoms among serious adolescent offenders. Criminal Justice and Behavior 2015; 42: 587-609.

30. Azad MB, Lissitsyn Y, Miller GE, Becker AB, HayGlass KT, Kozyrskyj AL Influence of socioeconomic status trajectories on innate immune responsiveness in children. PloS one 2012; 7: e38669.

31. Ziyab AH, Karmaus W, Kurukulaaratchy RJ, Zhang H, Arshad SH Developmental trajectories of Body Mass Index from infancy to 18 years of age: prenatal determinants and health consequences. Journal of epidemiology and community health 2014; 68: 934-941.

32. Chen Q, Just AC, Miller RL, Perzanowski MS, Goldstein IF, Perera FP *et al* Using latent class growth analysis to identify childhood wheeze phenotypes in an urban birth cohort. Ann Allergy Asthma Immunol 2012; 108: 311-315 e311.

33. Toms LM, Sjodin A, Harden F, Hobson P, Jones R, Edenfield E *et al* Serum polybrominated diphenyl ether (PBDE) levels are higher in children (2-5 years of age) than in infants and adults. Environmental health perspectives 2009; 117: 1461-1465.

34. Sjodin A, Schecter A, Jones R, Wong LY, Colacino JA, Malik-Bass N *et al* Polybrominated diphenyl ethers, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153), and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) concentrations in sera collected in 2009 from Texas children. Environmental science & technology 2014; 48: 8196-8202.

35. Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V *et al* Prenatal exposure to PBDEs and neurodevelopment. Environmental health perspectives 2010; 118: 712-719.

36. Gyalpo T, Toms LM, Mueller JF, Harden FA, Scheringer M, Hungerbuhler K Insights into PBDE Uptake, Body Burden, and Elimination Gained from Australian Age-Concentration

Trends Observed Shortly after Peak Exposure. Environmental health perspectives 2015; 123: 978-984.

37. Quinn CL, Wania F Understanding differences in the body burden-age relationships of bioaccumulating contaminants based on population cross sections versus individuals. Environmental health perspectives 2012; 120: 554-559.

38. Darrow LA, Jacobson MH, Preston EV, Lee GE, Panuwet P, Hunter RE, Jr. *et al* Predictors of Serum Polybrominated Diphenyl Ether (PBDE) Concentrations among Children Aged 1-5 Years. Environmental science & technology 2017; 51: 645-654.

39. Costa O, Lopez-Espinosa MJ, Vizcaino E, Murcia M, Iniguez C, Navarrete-Munoz EM *et al* Dietary and Household Sources of Prenatal Exposure to Polybrominated Diphenyl Ethers (PBDEs) in the INMA Birth Cohort (Spain). Environmental science & technology 2016; 50: 5935-5944.

40. EPA. Reducing your child's exposure to flame retardant chemicals. In, 2016.

41. S07621/A10050-A. NY State Senate Bill. In, 2004.

42. Petreas M, Oros D Polybrominated diphenyl ethers in California wastestreams. Chemosphere 2009; 74: 996-1001.

43. Shaw SD, Blum A, Weber R, Kannan K, Rich D, Lucas D *et al* Halogenated flame retardants: do the fire safety benefits justify the risks? Rev Environ Health 2010; 25: 261-305.

Supplemental Material

Table of Contents

Table S1. Within congener Spearman correlation coefficients (p-values) between plasma PBDE concentrations measured between birth and age 9 years.

	Birth	Age 2	Age 3	Age 5	Age 7	Age 9
BDE-47						
Birth	1.00					
Age 2	-0.03 (0.82)	1.00				
Age 3	0.09 (0.36)	0.79 (<0.01)	1.00			
Age 5	0.35 (0.02)	0.80 (<0.01)	0.77 (0.07)	1.00		
Age 7	0.17 (0.02)	0.33 (0.04)	0.33 (<0.01)	0.61 (<0.01)	1.00	
Age 9	0.12 (0.13)	0.39 (0.01)	0.15 (0.38)	0.31 (0.07)	0.80 (<0.01)	1.00
BDE-99						
Birth	1.00					
Age 2	0.04 (0.78)	1.00				
Age 3	0.09 (0.36)	0.83 (<0.01)	1.00			
Age 5	0.32 (0.04)	0.63 (0.02)	0.49 (0.33)	1.00		
Age 7	0.13 (0.06)	0.36 (0.04)	0.41 (<0.01)	0.62 (<0.01)	1.00	
Age 9	0.10 (0.20)	0.36 (0.06)	0.35 (0.06)	0.24 (0.16)	0.77 (<0.01)	1.00
BDE-100						
Birth	1.00					
Age 2	0.21 (0.13)	1.00				
Age 3	0.22 (0.02)	0.89 (<0.01)	1.00			
Age 5	0.18 (0.26)	0.82 (<0.01)	0.83 (0.04)	1.00		
Age 7	0.13 (0.07)	0.42 (<0.01)	0.36 (<0.01)	0.72 (<0.01)	1.00	
Age 9	0.10 (0.20)	0.45 (<0.01)	0.31 (0.07)	0.36 (0.03)	0.86 (<0.01)	1.00
BDE-153						
Birth	1.00					
Age 2	0.26 (0.05)	1.00				
Age 3	0.12 (0.22)	0.89 (<0.01)	1.00			
Age 5	0.40 (<0.01)	0.84 (<0.01)	0.71 (0.11)	1.00		
Age 7	0.10 (0.14)	0.70 (<0.01)	0.63 (<0.01)	0.87 (<0.01)	1.00	
Age 9	0.07 (0.39)	0.82 (<0.01)	0.67 (<0.01)	0.89 (<0.01)	0.93 (<0.01)	1.00

Table S2. Between congener Spearman correlation coefficients (p-values) for plasma PBDE concentrations measured repeatedly between birth and age 9 years.				
Birth (n=327)	BDE-47	BDE-99	BDE-100	BDE-153
BDE-47	1.00			
BDE-99	0.83 (<0.01)	1.00		
BDE-100	0.76 (<0.01)	0.79 (<0.01)	1.00	
BDE-153	0.47 (<0.01)	0.50 (<0.01)	0.66 (<0.01)	1.00
Age 2 (n=56)				
BDE-47	1.00			
BDE-99	0.94 (<0.01)	1.00		
BDE-100	0.93 (<0.01)	0.92 (<0.01)	1.00	
BDE-153	0.75 (<0.01)	0.71 (<0.01)	0.89 (<0.01)	1.00
Age 3 (n=115)				
BDE-47	1.00			
BDE-99	0.96 (<0.01)	1.00		
BDE-100	0.94 (<0.01)	0.91 (<0.01)	1.00	
BDE-153	0.76 (<0.01)	0.73 (<0.01)	0.90 (<0.01)	1.00
Age 5 (n=42)				
BDE-47	1.00			
BDE-99	0.92 (<0.01)	1.00		
BDE-100	0.88 (<0.01)	0.81 (<0.01)	1.00	
BDE-153	0.58 (<0.01)	0.55 (<0.01)	0.82 (<0.01)	1.00
Age 7 (n=203)				
BDE-47	1.00			
BDE-99	0.93 (<0.01)	1.00		
BDE-100	0.92 (<0.01)	0.90 (<0.01)	1.00	
BDE-153	0.49 (<0.01)	0.51 (<0.01)	0.67 (<0.01)	1.00
Age 9 (n=160)				
BDE-47	1.00			
BDE-99	0.94 (<0.01)	1.00		
BDE-100	0.90 (<0.01)	0.89 (<0.01)	1.00	
BDE-153	0.46 (<0.01)	0.43 (<0.01)	0.65 (<0.01)	1.00

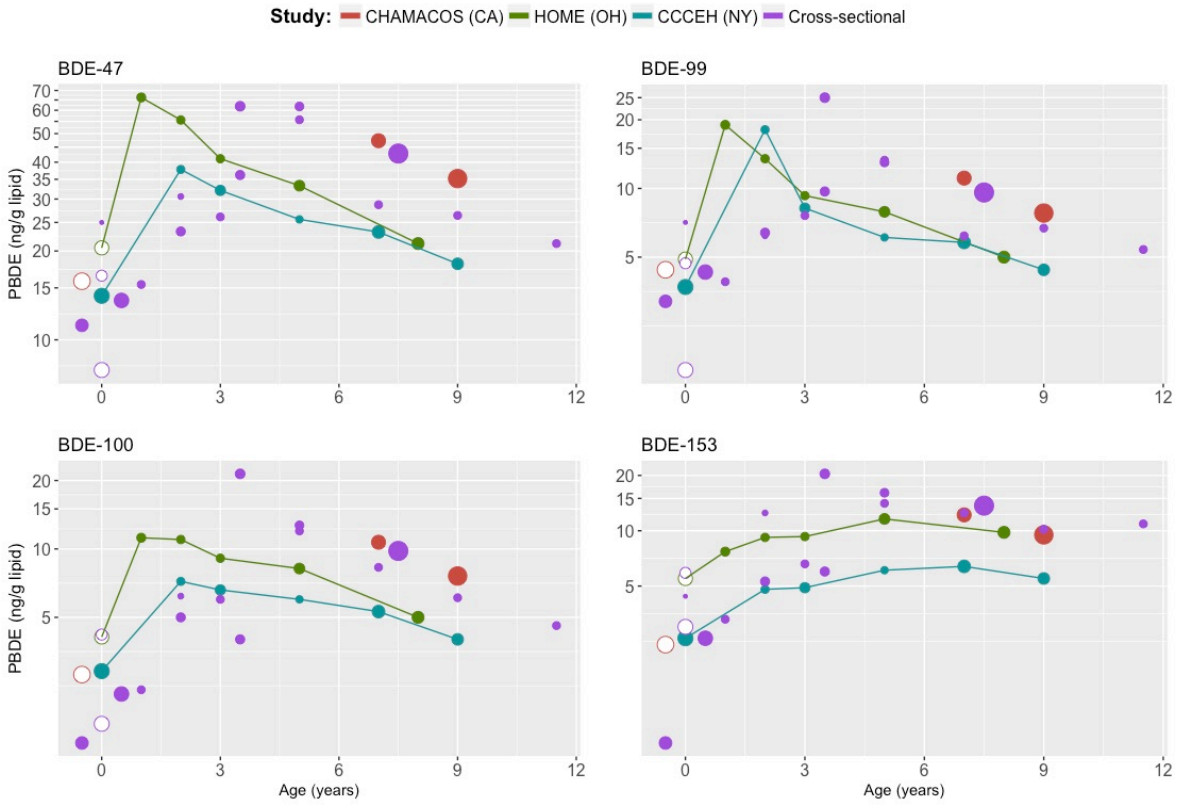
Table S3. BIC and $2\log_e(B_{10})$ for evaluating LCGA model fit		
Null vs. Alternative model	**BIC**	$\mathbf{2\log_e(B_{10}) \approx 2(\Delta BIC)}$
BDE-47		
2 vs 3	-579.59 vs -571.88	15.42
3 vs **4**	-571.88 vs -558.10	**27.56**
4 vs 5	-558.10 vs -552.37	11.46
5 vs 6	-552.37 vs -554.36	-3.98
BDE-99		
2 vs 3	-505.75 vs -496.44	18.62
3 vs **4**	-496.44 vs -483.93	**25.02**
4 vs 5	-483.93 vs -480.39	7.08
5 vs 6	-480.49 vs -475.42	10.14
BDE-100		
2 vs 3	-412.02 vs -399.98	24.08
3 vs **4**	-399.98 vs -363.40	**73.16**
4 vs 5	-363.40 vs -366.19	-5.58
5 vs 6	-366.19 vs -362.75	6.88
BDE-153		
2 vs **3**	-223.13 vs -192.72	**60.82**
3 vs 4	-192.72 vs -186.56	12.32
4 vs 5	N/A[a]	
5 vs 6	N/A[a]	
$2\log_e(B_{10})$: interpreted as the degree of evidence favoring the alternative model [a]5 and 6 group solutions were not possible to estimate for BDE-153		

Table S4. Comparison of geometric mean PBDE concentrations (ng/g lipid) measured in child plasma or serum samples by several recent U.S.-based studies.

Author, year	State	Study design	N	Age (yrs)	BDE-47	BDE-99	BDE-100	BDE-153
Vuong 2017	OH	Longitudinal	76	1	66.3	19.0	11.2	7.7
Sjodin 2014[a]	TX	Cross sectional	50	0-2	15.4	3.9	2.4	3.3
Vuong 2017	OH	Longitudinal	61	2	55.6	13.5	11.0	9.2
Cowell (present)	NY	Longitudinal	56	2	37.8	18.1	7.2	4.8
Lunder 2010[a]	U.S.	Cross sectional	20	1-3	30.6	6.2	6.2	12.5
Stapleton 2012	NC	Cross sectional	77	1-3	23.3	6.4	5.0	5.3
Vuong 2017	OH	Longitudinal	61	3	41.1	9.3	9.1	9.3
Cowell (present)	NY	Longitudinal	115	3	32.1	8.2	6.6	4.9
Sjodin 2014[a]	TX	Cross sectional	50	2-4	26.1	7.6	6.0	6.6
Jacobson 2016	GA	Cross sectional	80	1-5	36.2	9.7	4.0	6.0
Rose 2010	CA	Cross sectional	94	2-5	61.9	25.0	21.4	20.4
Sjodin 2014[a]	TX	Cross sectional	50	4-6	55.7	13.3	12.0	14.1
Wu 2015	CA	Cross sectional	67	2-8	61.8	13.0	12.7	16.1
Vuong 2017	OH	Longitudinal	127	5	33.3	7.9	8.2	11.6
Cowell (present)	NY	Longitudinal	42	5	25.6	6.1	6.0	6.1
Eskenazi 2013	CA	Longitudinal	270	7	47.3	11.1	10.7	12.2
Cowell (present)	NY	Longitudinal	203	7	23.2	5.8	5.3	6.4
Sjodin 2014[a]	TX	Cross sectional	50	6-8	28.7	6.2	8.3	12.5
Vuong 2017	OH	Longitudinal	173	8	21.2	5.0	5.0	9.8
Windham 2010	CA/OH	Cross sectional	599	6-9	42.8	9.6	9.8	13.7
Sagiv 2015	CA	Longitudinal*	546	9	35.2	7.8	7.6	9.5
Cowell (present)	NY	Longitudinal	160	9	18.1	4.4	4.0	5.5
Sjodin 2014[a]	TX	Cross sectional	50	8-10	26.4	6.7	6.1	10.2
Gump 2014[b]	NY	Cross sectional	43	10	8.5	2.3	0.9	NA
Sjodin 2014	TX	Cross sectional	50	10-13	21.2	5.4	4.6	10.9

[a]Median, [b]Arithmetic mean

Figure S1. Age-specific comparisons of infant and child PBDE concentrations across several U.S. based studies.



White circles outlined in color indicate concentration measured in maternal (versus cord) blood at the prenatal period. The size of the circle indicates relative sample size. References: CHAMACOS: Eskenazi 2013, Sagiv 2015; HOME: Vuong 2017.

Supplemental Material References

Gump, B.B., S. Yun, and K. Kannan. 2014. Polybrominated diphenyl ether (pbde) exposure in children: possible associations with cardiovascular and psychological functions. Environmental Research 132: 244-50.

Eskenazi B, Chevrier J, Rauch SA, Kogut K, Harley KG, Johnson C, et al. 2013. In utero and childhood polybrominated diphenyl ether (pbde) exposures and neurodevelopment in the CHAMACOS study. Environmental health perspectives 121:257-262.

Jacobson, M.H., Barr, D.B., Marcus, M., Muir, A.B., Lyles, R.H., Howards, P. P., et al. 2016. Serum polybrominated diphenyl ether concentrations and thyroid function in young children. Environmental Research 149: 222-30.

Lunder, S., Hovander, L., Athanassiadis, I., and Bergman, A. 2010. Significantly higher polybrominated diphenyl ether levels in young U.S. children than in their mothers. Environmental Science Technology 44(13): 5256-62.

Rose, M., Bennett, D.H., Bergman, A., Fangstrom, B., Pessah, I.N., and Hertz-Picciotto, I. 2010. PBDEs in 2-5 year-old children from California and associations with diet and indoor environment. Environmental Science Technology 44(7): 2648-53.

Sagiv SK, Kogut K, Gaspar FW, Gunier RB, Harley KG, Parra K, et al. 2015. Prenatal and childhood polybrominated diphenyl ether (pbde) exposure and attention and executive function at 9-12 years of age. Neurotoxicology and teratology 52:151-161

Sjodin, A., Schecter, A., Jones, R., Wong, L., Colacino, J.A., Malik-Bass, N., et al. 2014. Polybrominated diphenyl ethers, 2,2',4,4',5,5'-hexachlorobiphenyl (pcb-153), and p,p'-dichlorodiphenyldichloroethylene (p,p'-dde) concentrations in sera collected in 2009 from Texas children. Environmental Science Technology 48(14): 8196-202.

Stapleton, H.M., Eagle, S., Sjodin, A., and Webster, T.F. 2012. Serum PBDEs in a North Carolina toddler cohort: associations with handwipes, house dust, and socioeconomic variables. Environmental Health Perspectives 120(7): 1049-54.

Vuong AM, Braun JM, Yolton K, Xie C, Webster GM, Sjodin A, et al. 2017. Prenatal and postnatal polybrominated diphenyl ether exposure and visual spatial abilities in children. Environmental research 153:83-92

Windham, G.C., Pinney, S.M., Sjodin A, Lum R, Jones, R.S., Needham, L.L., Biro, F.M., et al. 2010. Body burdens of brominated flame retardants and other persistent organohalogenated compounds and their descriptors in U.S. girls. Environmental Resarch 110(3): 251-257.

CHAPTER 4: Associations between prenatal and childhood PBDE exposure and early adolescent visual, verbal and working memory

Whitney J. Cowell[1,2], Amy Margolis[1,3], Virginia A. Rauh[1,4], Andreas Sjödin[5], Richard Jones[5], Ya Wang[1,6], Wanda Garcia,[1,4] Frederica Perera[1,2], Shuang Wang[1,6], Julie B. Herbstman[1,2]

[1]Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, NY 10032 USA
[2]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032 USA
[3]Department of Child and Adolescent Psychiatry, Columbia University, New York, NY 10032 USA
[4]Heilbrunn Department of Population and Family Health, Mailman School of Public Health, Columbia University, New York, NY 10032 USA
[4]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341 USA
[6]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY 10032 USA

Address correspondence to: Julie B. Herbstman, Columbia Center for Children's Environmental Health, 12th Fl, Mailman School of Public Health, 722 West 168th St, New York, NY 10032 USA. Telephone: (212) 304-7273. Fax: (212) 544-1943. E-mail: jh2678@cumc.columbia.edu

Running title: Early life exposure to PBDEs and children's memory

Acknowledgements: This research was supported by: NIH R01 ES021806 and NIH R01DA027100. During preparation of this manuscript, WJC was supported by NIH T32 ES023772, NIH T32 ES007322 and EPA FP-91779001.

Disclaimer: The findings and conclusions in this publication are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services. This publication was developed under STAR Fellowship Assistance Agreement no. FP-91779001 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by the EPA. The views expressed in this publication are solely those of the authors.

Keywords: PBDE, flame retardant, prenatal, trajectory, childhood, memory

Publication status: Submitted to Environment International special issue on children's health and exposure to toxicants in the indoor environment

Abstract

Background: Prenatal and childhood exposure to polybrominated diphenyl ether (PBDE) flame retardants has been inversely associated with cognitive performance, however, few studies have measured PBDE concentrations in samples collected during both prenatal and postnatal periods.

Methods: The study population included a subset (n=212) of children enrolled in a New York City-based birth cohort. We examined prenatal (cord) and childhood (ages 2, 3, 5, 7 and 9 years) plasma PBDE concentrations in relation to memory outcomes assessed between the ages of 9 and 14 years. We used multivariable linear regression to examine associations between continuous $\log_{10}$-transformed PBDE concentrations and performance on tests of visual, verbal and working memory in age-stratified models. We additionally used latent class growth analysis to examine trajectories of PBDE exposure across early life in relation to memory outcomes. We examined interactions between PBDE exposure and sex using cross-product terms.

Results. Children with sustained high PBDE concentrations across childhood scored approximately 5-8 standard score points lower on tests of visual memory. Associations between prenatal exposure and working memory significantly varied by sex (*p-int*= 0.02), with a significant inverse relationship observed only among girls (i.e. $\beta_{BDE\text{-}47}$ = -7.55, 95% CI: -13.84, -1.24). Children with PBDE concentrations that peaked during toddler years performed better on verbal domains, however, this association was significant only among children breastfed > 12 weeks.

Conclusion. Exposure to PBDEs during both prenatal and postnatal periods may disrupt memory domains in early adolescence. These findings contribute to a substantial body of evidence supporting the developmental neurotoxicity of PBDEs.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of organohalogenated flame retardant chemicals that were used extensively in furniture and furnishings to meet United States fire safety standards until their phase-out between 2004 and 2013 (Abbasi et al. 2015). Exposure to PBDEs occurs primarily through incidental ingestion of dust (EPA 2010) and owing to their lipophilicity, PBDEs readily penetrate the placenta (Leonetti et al. 2016) and partition into breastmilk (Carrizo et al. 2007).

Mounting evidence supports an association between prenatal exposure to PBDEs and disrupted cognitive outcomes in children (Lam et al. 2017; Roth and Wilks 2014). Importantly, the brain continues to develop postnatally, remaining vulnerable to insult by environmental toxicants throughout childhood (Grandjean and Landrigan 2014). Additionally, research indicates PBDE exposure may peak during toddler years due to breastfeeding, as well as increased ingestion of dust from close proximity to the floor and frequent hand to mouth behavior (Fromme et al. 2016). Despite these factors, limited research has examined neurodevelopmental effects associated with PBDE concentrations measured during both prenatal and postnatal periods (Eskenazi et al. 2013; Gascon et al. 2011; Vuong et al. 2017).

In the present study, we examined associations between plasma PBDE concentrations measured repeatedly throughout the early lifecourse (birth through age 9 years) in relation to several subdomains of memory measured during early adolescence. We selected memory outcomes based on the results of animal research demonstrating inverse associations between PBDEs and performance on tests of memory and learning (Driscoll et al. 2009; Dufault et al. 2005; Viberg et

al. 2003; Viberg et al. 2006), as well as findings from human studies demonstrating inverse associations between PBDEs and cognitive performance (Eskenazi et al. 2013; Herbstman et al. 2010).

Methods

Study design and participants

The Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns study is a longitudinal birth cohort of African American and Dominican mother-child pairs in Northern Manhattan and the South Bronx. Additional details describing the study design and participant recruitment have been previously published (Perera et al. 2006). Eligible (healthy, 18-35-year old women free of tobacco and illicit drug use who initiated prenatal care by the 20th week of gestation) participants were recruited between 1998 and 2006 from two prenatal clinics. At the time of delivery, 727 mother-child pairs remained eligible and were fully enrolled into the cohort.

Bilingual research staff conducted structured participant interviews during the prenatal period, after delivery, and repeatedly during childhood (3 months, 6 months, 1, 2, 3, 5, 7, 9, 11, and 14 years) to collect information on demographic and lifestyle factors. As previously described (Rauh et al. 2004), prenatal exposure to environmental tobacco smoke was assessed using a combination of questions about smokers in the home and validated by blood cotinine concentrations. Maternal distress was evaluated using the Psychiatric Epidemiology Research Instrument- Demoralization (PERI-D) scale (Dohrenwend et al. 1981) and material hardship was indexed using a series of questions about access to basic needs (food, housing and clothing)

(Mayer and Jencks 1988). At the 3-year follow-up visit, maternal non-verbal intelligence was assessed using the Test of Nonverbal Intelligence, 2rd Edition (TONI-II) (Brown et al. 1997) and the child's living environment was evaluated using the Home Observation for Measurement of the Environment (HOME) Early Childhood Inventory, which was completed during a visit to the family's home (Caldwell and Bradley 2003). The HOME inventory is designed to evaluate stimulation and support available to children within their family surroundings and includes 8 areas of emphasis, including academic stimulation. Information on birth weight and gestational age was abstracted from medical records. Breast feeding history (initiation and duration) was assessed by questionnaire at multiple visits during early life (6 months – 36 months).

Before each visit, mothers signed a letter of informed consent and children ≥7 years additionally signed a letter of informed assent. Study procedures were approved by the Institutional Review Boards of Columbia University Medical Center and the New York State Psychiatric Institute. The involvement of the Centers for Disease Control and Prevention (CDC) was determined not to constitute engagement in human subjects' research.

Memory Assessment

Trained research staff administered the Children's Memory Scale (CMS) to children between the ages of 9 and 14 years (mean±SD: 11.1±1.1). The CMS is designed to measure memory and learning across three domains (auditory/verbal, visual/non-verbal and attention-concentration) in children and adolescents (Cohen 1997). We examined scores from the Attention-Concentration, Verbal Memory and Visual Memory indices, which are age-scaled and standardized against a normative population to reflect a mean±SD of 100±15 and range from 50 to 150. For verbal and

visual domains, we assessed scores on immediate indices, which reflect the ability to process, organize and hold material in short-term memory. Similarly, the Attention-Concentration Index assesses the ability to hold and simultaneously manipulate information and thus places a heavy demand on auditory working memory (Cohen 1997). Sixteen children were excluded from models examining the Attention-Concentration Index scale because they were not administered one of the two core subtests due to factors unrelated to the child. At the time of CMS testing, child self-report of anxiety was ascertained using the Revised Children's Manifest Anxiety Scale (RCMAS), which is a brief self-report inventory designed to measure the degree and nature of anxiety (Reynolds and Richond 1985).

PBDE exposure assessment

We measured PBDE concentrations in 903 stored plasma samples collected from 334 children between birth and age 9 years (N_{cord}=327, $N_{2\text{-}years}$=56, $N_{3\text{-}years}$=115, $N_{5\text{-}years}$=42, $N_{7\text{-}years}$=203, and $N_{9\text{-}years}$=160). Details pertaining to sample collection and analysis of PBDE concentrations in this cohort have been previously published (Cowell et al. under review). Briefly, hospital staff collected umbilical cord blood at the child's delivery and a pediatric phlebotomist collected child venous blood at 2, 3, 5, 7 and 9-year follow-up visits. All samples were immediately transported to the CCCEH laboratory, processed and stored in multiple aliquots at -70C.

Plasma PBDE concentrations were measured by the CDC using gas chromatography isotope dilution high-resolution mass spectrometry on a DFS instrument (ThermoFisher, Bremen, Germany) (Jones et al. 2012; Sjodin et al. 2004). Before final analytic determinations were made, samples were fortified with internal standards and extracted using a Gilson 215 liquid handler

(Gilson Inc., Middleton, WI). Blanks (N=3) were processed with every 30 samples and the median blank value was subtracted from the final result. Lipids were co-extracted using a Rapid Trace modular SPE work station (Biotage, Uppsala, Sweden) and total cholesterol and triglycerides were measured using commercially available test kits (Roche Diagnostics, Indianapolis, IN). We estimated total cord blood lipids using a recently developed cord blood-specific formula [total cord blood lipids = 2.66 × total cord blood cholesterol + cord blood triglycerides + 0.268, in g lipids/L plasma] (Sjodin A, unpublished data) and child blood lipids using the short formula described by Philips et al. 1989 (Phillips et al. 1989).

The limits of detections (LODs) for BDE-47 ranged from 0.69 to 11.59 ng/g lipid for cord plasma samples and 1.10 to 20.20 for child plasma samples. For the other three congeners investigated (BDEs-99, 100 and 153), LODs ranged from 0.29 to 5.46 ng/g lipid for cord plasma and 0.45 to 6.40 ng/g lipid for child plasma. As previously described (Cowell et al. in press), we imputed concentrations less than the sample-specific LOD from a normal probability distribution of natural-log transformed detected concentrations with an equal or lower LOD. To incorporate uncertainty introduced by imputation, we repeated this procedure 10 times. Given variation in detection frequencies for cord plasma (BDE-47: 80%, BDE-99: 51%, BDE-100: 42%, BDE-153: 38%) versus child plasma (across ages 2-9 years: BDE-47: 100%, BDE-99: 80-98%, BDE-100: 90-100%, BDE-153: 90-98%), we used congener-specific detected plasma concentrations from cord or child samples to impute concentrations for non-detectable concentrations in cord or child samples, respectively.

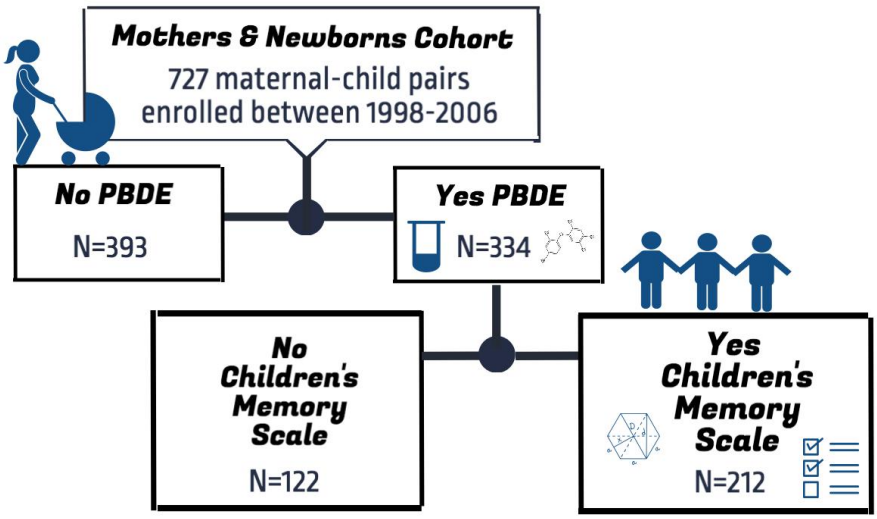
We additionally measured biomarkers of other developmental neurotoxicants with dietary

(polychlorinated biphenyls (PCBs)) or indoor (lead, chlorpyrifos) exposure sources. Cord plasma polychlorinated biphenyl (PCB) concentrations were measured simultaneous to PBDE measurement, chlorpyrifos concentrations were measured by gas chromatography isotope dilution high-resolution mass spectrometry as previously described (Rauh et al. 2006), and cord blood lead was measured by Zeeman graphite furnace atomic absorption spectrometry with a phosphate/Triton X-100/nitric acid matrix modifier.

Statistical analysis

As illustrated by Figure 1, of the 334 children with at least one measure of PBDEs, 212 also completed neurodevelopmental testing and were included in this analysis.

Figure 1. Diagram of study enrollment and follow-up.



We examined $\log_{10}$-transformed, lipid-standardized PBDE concentrations (ng PBDE/g lipid) measured in plasma collected at the prenatal (n=208), 3-year (n=70), 7-year (n=158), and 9-year (n=128) follow-up visits in relation to scores on the CMS Attention-Concentration, Verbal Memory and Visual Memory indices in separate models (n=12 models). We did not examine

continuous PBDE concentrations at the 2=year (n=41) or 5-year (n=35) follow-up visits given the small sample sizes at these ages. We performed regression analyses on each of the 10 datasets with imputed concentrations for non-detectable samples and pooled resulting parameter estimates following Rubin's rules (Rubin 1987).

To better understand the neurodevelopmental impacts of cumulative exposure across childhood, we additionally examined CMS scores in relation to trajectories of PBDE concentrations, which we estimated using latent glass growth analysis (LCGA) as previously described (Cowell et al. under review). Briefly, LCGA is a discrete mixture method for clustering individuals with similar patterns of a characteristic of interest (i.e. PBDE concentrations) over time (Nagin 2005; Nagin 2014). We fit trajectories using $\log_{10}$-transformed PBDE concentrations (ng/g lipid) and selected the best fitting model for each congener using the Bayesian Information Criterion, as well as the probability of correct trajectory membership. Before fitting trajectories, we replaced non-detectable PBDE concentrations with the child's age- and congener-specific mean concentration from the 10 multiply imputed datasets. We found that across the four congeners, one group of children was characterized by low exposure at all ages ("persistent low") and a second group was characterized by low prenatal exposure that increased during the toddler years and remained high throughout childhood ("sustained childhood high"). For BDEs 47, 99 and 100, concentrations among a third group of children increased between birth and toddler years, but subsequently decreased ("early postnatal peak") and concentrations among a fourth group were highest during the prenatal period ("prenatal high"). Finally, for BDE-153, we did not detect an "early postnatal peak" trajectory, but rather a group of children with "sustained moderate" concentrations throughout childhood (see Supplemental Material, Figure S1, which

plots PBDE trajectories). In the present analysis, we used multivariable regression to examine associations between trajectory membership, treated as a categorical variable, and continuous index scores from the CMS. In all models, we treated the 'persistent low' trajectory as the reference group.

We explored the following potential confounders: ethnicity (African American/Dominican), parity (nulliparous/multiparous), prenatal environmental tobacco smoke exposure (ever/never), maternal age at delivery (years), maternal education at the time of pregnancy (< high school/ ≥ high school degree or equivalent), maternal employment during pregnancy (employed/unemployed), maternal relationship status during pregnancy (married or with the same partner for more than 7 years/not in a stable relationship), gestational age (weeks), birth weight (grams), breastfeeding history of the study child (continuous, in weeks), maternal non-verbal intelligence, maternal demoralization during pregnancy, total and academic HOME Inventory scores at age 3 years, material hardship at delivery and at the time of CMS testing (inadequate access to food, housing or clothing/adequate access to all), primary language spoken in the home at the 3-year visit (English or English and Spanish/Spanish), child exact age at CMS testing (days), and child anxiety at the time of testing measured by the Total Anxiety score derived from the RCMAS.

We evaluated bivariate associations between covariates and each independent (continuous or trajectories of PBDE concentrations) and dependent (CMS index scores) variable and constructed congener-specific directed acyclic graphs (DAGs) to identify the set of observed covariates sufficient to close biasing paths across all models (Textor et al. 2011). We adjusted

final models for ethnicity, exact age at CMS testing, maternal nonverbal intelligence, maternal education, maternal employment, parity, birth weight, and breastfeeding history of the study child. We additionally *a priori* adjusted models for date of birth (continuous, in days) to account for differences in exposure among children born earlier versus later during the enrollment period, which spanned the U.S. pentaBDE phase-out. Given sexually dimorphic patterns of brain development, we examined interactions between sex and PBDE concentrations using cross product terms and stratified models.

We visually inspected residual plots to confirm normality of residuals and examined the impact high and low CMS scores by excluding children with an outlying score, defined as a value more than 1.5 times the interquartile range below or above the first or third quartile, respectively. In models examining PBDE trajectories, we evaluated the impact of potential misclassification by modeling associations subset on children with a high posterior probability of correct trajectory assignment (<60%). In sensitivity analyses, we evaluated correlations between cord plasma PBDE concentrations (ng/g lipid) and cord blood concentrations of PCBs, lead and chlorpyrifos. If significant correlations were observed, we examined models adjusting for the relevant co-exposure.

We constructed DAGs using DAGitty v2.3 and conducted regression analyses using SAS v9.4 (SAS Institute Inc., Cary, North Carolina). We performed LCGA model estimation using the SAS Proc Traj macro (Jones et al. 2001).

Results

Study population

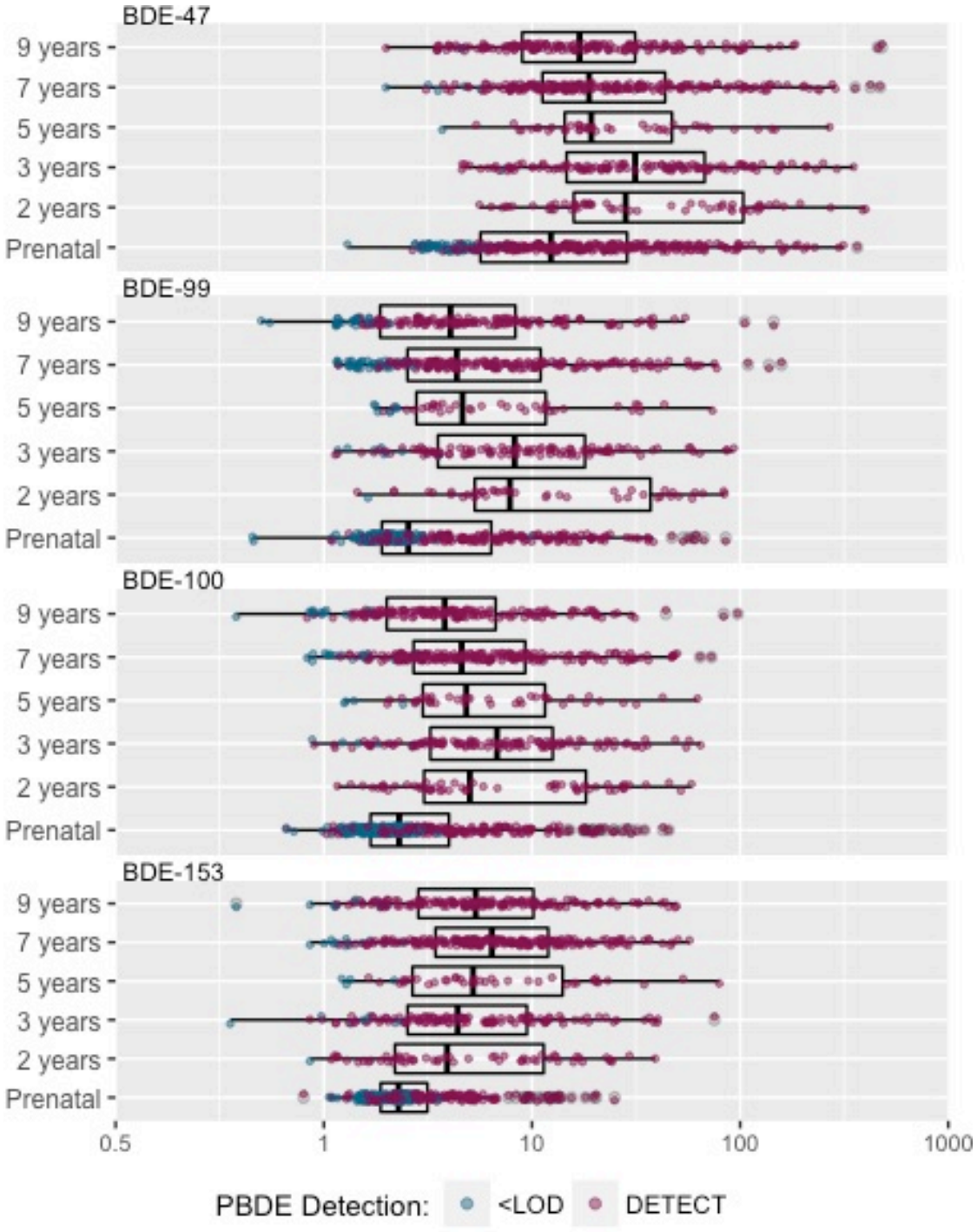
Children included in final models were African American (43%) or Dominican (57%). At delivery, 38% of mothers had less than a high school education, 51% were first time mothers, and 19% breastfed the study child for more than 12 weeks. Detailed characteristics of the study population are presented in Table 1. CMS scores were normally distributed with means±standard deviations (SD) of 94±16, 99±13 and 95±16 on the Attention-Concentration, Visual Memory and Verbal Memory indices, respectively. Scores were not significantly different between boys and girls for any of these indices. Compared to children without a measure of PBDEs or CMS testing (n=515), those included in final models weighed more at birth (mean: 145 grams) and were more frequently a firstborn child (51% versus 42%). We detected no other differences between the 334 maternal-child pairs with a measure of PBDEs and the 727 fully enrolled pairs, or between those with CMS scores and PBDEs (n=212) compared to those fully enrolled or compared to those with CMS scores but not PBDEs (n=122) (see Supplemental Material, Table S1).

Table 1. Characteristics of participants with plasma PBDE concentrations and CMS scores (n=212)	
Maternal characteristics[a]	Mean±SD or n (%)
Age	25.2±5.1
<High school education	80 (38)
Employed	120 (56)
Stable relationship	49 (23)
Nulliparous	108 (51)
Nonverbal intelligence	84.1±13.1
Demoralization	1.2±0.6
Child characteristics	
Birth weight (kg)	3.5±0.5
Gestational age (weeks)	39.4±1.3
African American	92 (43)
Dominican	120 (57)
Girl	119 (56)
Breastfed ≥ 12 weeks	67 (32)
Anxiety score at CMS testing[b]	9.5±6.2
Age at CMS testing (years)	11.1±1.1
Household characteristics	
Material hardship at delivery[b]	85 (41)
Material hardship at CMS testing[b]	81 (39)
Primarily Spanish-speaking home (age 3)	88 (42)
HOME academic score[b] (age 3)	4.3 (1.0)
HOME total score[b] (age 3)	39.3 (6.5)
Environmental tobacco smoke	77 (36)
HOME: Home Observation for Measurement of the Environment; RCMAS: Revised Children's Manifest Anxiety Scale. [a]Measured prenatally unless otherwise noted. [b]Missing (n): material hardship at delivery (5) and at CMS testing (2), HOME score (6), RCMAS Total Anxiety score (4).	

PBDE exposure

BDEs-47, 99, 100 and 153 were the most frequently detected congeners. At all ages, BDE-47 concentrations predominated samples; concentrations were lowest in cord blood (geometric mean±geometric standard deviation: 14.1±0.9) and highest during toddler years (age 2: 37.8±5.8, age 3: 32.1±3.1). As illustrated by Figure 2, across congeners, PBDE concentrations and detection frequencies were consistently higher in child (range across congeners and postnatal visits: 80-100%) compared to cord blood (range across congeners: 38-80%). Additional details describing PBDE detection frequencies and concentrations at each age, as well as correlations between congeners within age periods and within congeners over age periods among children in this cohort have been previously described (Cowell et al. under review).

Figure 2. Distribution of plasma PBDE concentrations (ng/g lipid) by study visit (n=903 data points from 334 children).



Legend: Value of PBDE concentrations less than the limit of detection (<LOD) is the mean of imputed concentration across 10 datasets

Attention-Concentration Index (ACI)

We detected a significant interaction between sex and log_{10}-transformed, continuous cord plasma BDE-47 (*p-interaction:* 0.02), BDE-99 (*p-interaction:* 0.02) and BDE-100 (*p-interaction:* 0.03) concentrations (ng/g lipid). In stratified models, higher exposure was associated with lower ACI scores among girls ($\beta_{BDE\text{-}47}$: -7.55, 95% CI: -13.87, -1.24, $\beta_{BDE\text{-}99}$: -8.14, 95% CI: -15.34, -0.93), but not boys (Figure 3). We did not find significant sex × PBDE interactions at later ages, nor did we detect inverse associations between continuous PBDE concentrations measured during childhood (ages 3, 7 or 9 years) and ACI scores. In contrast, continuous BDE-153 concentrations measured at the 9-year visit were marginally ($p<0.10$) associated with better performance on the ACI ($\beta_{BDE\text{-}153}$: 6.22, 95% CI: -1.10, 13.53). We did not investigate sex × 'prenatal high' trajectory interactions given the relatively small proportion of children assigned to this trajectory (BDE-47: 19%, BDE-99: 13%) in combination with the reduced power of models examining categorical variables. In unstratified models, we did not detect significant associations between any PBDE trajectory and ACI scores (Figure 4).

Visual Memory Index (ViMI)

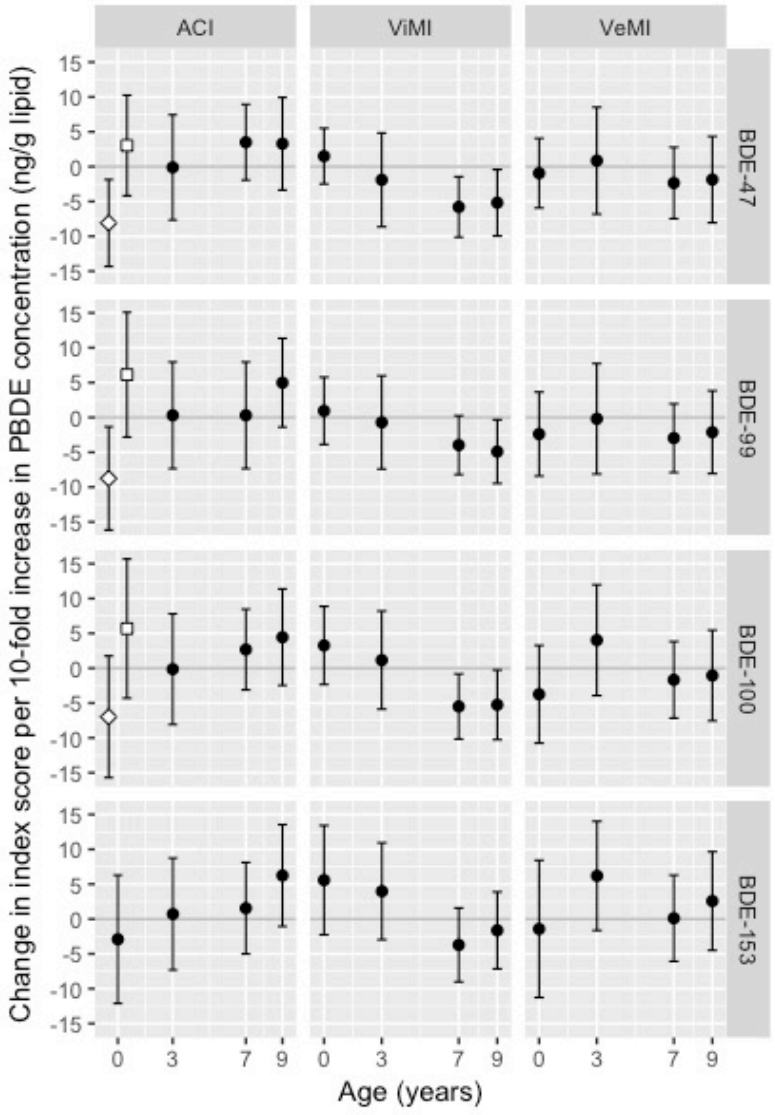
Continuous BDE-47, -99 and -100 concentrations measured at ages 7 and 9 years were inversely associated with ViMI scores (i.e. age 9: $\beta_{BDE\text{-}47}$: -5.18, 95% CI: -9.95, -0.42; $\beta_{BDE\text{-}99}$: -4.90, 95% CI: -9.47, -0.33; $\beta_{BDE\text{-}100}$: -5.24, 95% CI: -10.23, -0.25) (Figure 3). Likewise, children with sustained high concentrations of BDEs-47, -99 and -100 throughout the postnatal period, as indicated by trajectory membership, scored 5-8 standard score points lower on the ViMI compared to children with persistently low concentrations ($\beta_{BDE\text{-}47}$ = -8.44, 95% CI: -13.95, -

2.93; $\beta_{BDE\text{-}99}$ = -5.51, 95% CI: -10.41, -0.61; $\beta_{BDE\text{-}100}$ = -8.57, 95% CI: -14.19, -2.96) (Figure 4). We did not find a corresponding association for BDE-153.

Verbal Memory Index (VeMI)

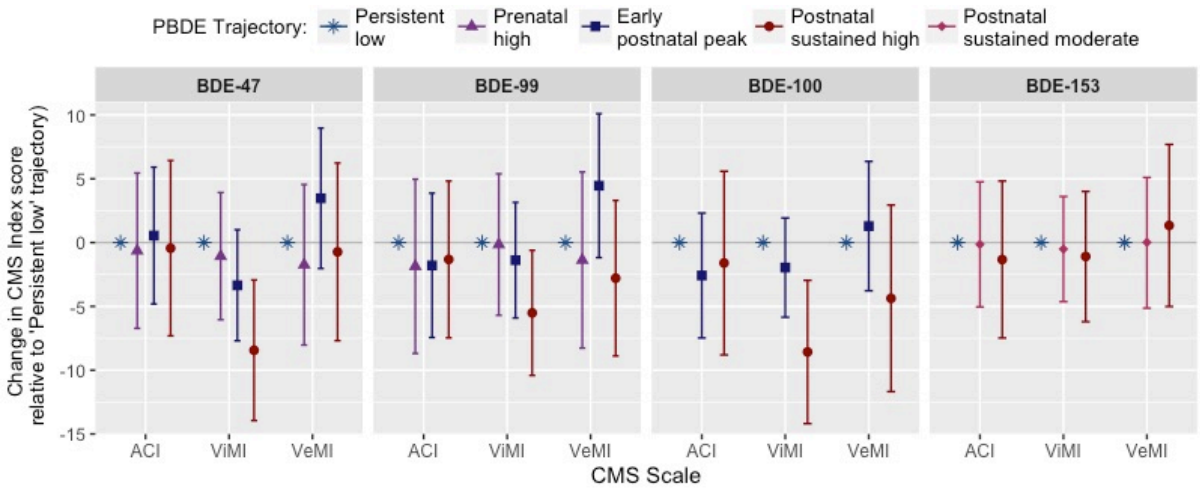
In contrast to attention concentration and visual memory domains, we did not detect significant inverse associations between continuous concentrations or trajectories of any PBDE congener and VeMI scores. However, we observed a trend of generally positive associations among children whose exposure peaked during toddler years (Figure 4, blue squares). Given the positive impact of breastfeeding on cognitive development (Fergusson et al. 1982), and the known transfer of PBDEs into breastmilk, we post-hoc explored interactions between breastfeeding and PBDE concentration using cross product terms and stratified models. We did not detect statistically significant interactions for any congener, however, in stratified models, positive associations were generally strengthened among children breastfed more than 12 weeks and attenuated among children breastfed 12 weeks or less (see Supplemental Material, Figure S2).

Figure 3. Point estimates (ß) and 95% CIs from adjusted models examining continuous, log_{10}-transformed plasma PBDE concentrations (ng/g lipid) in relation to Attention Concentration Index (ACI), Visual Memory Index (ViMI), and Verbal Memory Index (VeMI) scores.



Legend: Hollow diamonds indicate girls, hollow squares indicate boys, solid circles indicate boys and girls. PBDEs measured at ages 2 and 5 years not statistically analyzed due to small sample sizes. Sample size for ViMI and VeMI models: prenatal (n=208), age 3-years (n=70), age 7-years (n=158), age 9-years (n=128). Sample size for ACI models: prenatal (n=194), age 3-years (n=63), age 7-years (n=144), age 9-years (n=116).

Figure 4. Point estimates (ß) and 95% CIs from adjusted models examining trajectories of plasma PBDE concentrations in relation to Attention Concentration Index (ACI), Visual Memory Index (ViMI), and Verbal Memory Index (VeMI) scores (n=212).



Legend: See Supplemental Material, Figure S1 for visualizations of each PBDE trajectory

Supplemental and sensitivity analyses

Results from models 1) excluding influential observations, or 2) subset on children with a high probability of correct trajectory assignment were marginally strengthened or unchanged compared to our main findings (see Supplemental Material, Table S3a-c), except that the positive association between continuous BDE-153 concentrations measured at age 9-years with ACI scores was attenuated when outlying values (n=3) were excluded ($\beta_{BDE\text{-}153}$: 4.25, 95% CI: -2.96, 11.47).

BDE-47 (ng/g lipid) was weakly correlated (all Spearman) with lead (r=0.02, p=0.78, n=192), chlorpyrifos (r=0.17, p=0.01, n=261), and $\Sigma PCB_{118,\ 153,\ 138\text{-}158,\ 180}$ (r=0.06, p=0.33, n=288). Correlations between each of these chemicals and BDEs-99, -100 and -153 were similar or smaller. Given the small, but significant correlation with chlorpyrifos, a pesticide that we previously demonstrated to be associated with working memory deficits among children enrolled in this cohort (Rauh et al. 2006), we further investigated whether its inclusion as a covariate altered our findings. Despite the reduced sample size (n=90), inclusion of chlorpyrifos (>4.15 pg/g vs. ≤4.15 pg/g) did not substantially change the magnitude or direction of associations between continuous cord plasma BDEs-47 or BDE-99 concentrations with Attention-Concentration Index scores among girls ($\beta_{BDE\text{-}47}$=-8.81, 95% CI: -15.28, -2.35; $\beta_{BDE\text{-}99}$: -9.20, 95% CI: -17.02, -1.38), suggesting that the observed association was not driven by concurrent exposure to chlorpyrifos.

Discussion

We detected significant inverse associations between cord plasma PBDE concentrations (BDE-47 and BDE-99) and performance on the Attention-Concentration Index, a measure of auditory working memory, among girls but not boys. We did not detect corresponding associations between exposure during childhood, suggesting that working memory may be most sensitive to disruption by PBDEs during gestation, when rapid anatomical and functional development (Antonelli et al. 2017), including normal differentiation of sexually dimorphic regions (Andreano and Cahill 2009) occurs. Animal research has demonstrated associations between gestational exposure to PBDEs and changes in sexually dimorphic brain regions (Faass et al. 2013), with effects on sexual maturation that persist into adolescence (Lilienthal et al. 2006). Our findings are further supported by research demonstrating stronger inverse associations between prenatal exposure to the persistent organohalogenated insecticide DDT and working memory among girls compared to boys (Ribas-Fito et al. 2006). In contrast, while Eskenazi et al. found prenatal exposure to PBDEs was associated with decrements on the WISC-IV Working Memory Index among a California-based cohort of Mexican-American children at age 7-years (β per 10-fold increase in ΣBDEs-47, -99, -100, -153 = -2.4, 95% CI: -7.2, 2.3, n= 231), they did not detect a significant sex interaction at the level of $p<0.10$ (Eskenazi et al. 2013). This inconsistency may relate to differences in the study populations (geography, age at assessment), exposure metrics (ΣPBDE versus congener-specific models), tests administered (WISC-IV versus CMS) or other factors that differ between cohorts.

We found visual memory, which develops rapidly between toddler years and adolescence (Bauer and Fivush 2014), may be affected by sustained exposure to PBDEs during childhood. Unlike

verbal memory, few human studies have investigated PBDEs in relation to visual domains; however, our findings are consistent with animal research demonstrating that adult male mice exposed to PBDEs during the neonatal period perform worse on tests of spatial learning and memory (i.e. swim maze) compared to vehicle-exposed controls (Eriksson et al. 2001; He et al. 2011). The only other epidemiologic study to examine visual domains found that PBDE concentrations measured prenatally and at several ages postnatally (1-8 years) were generally associated with improved visual spatial abilities (memory retention and visual learning) at age 8-years as assessed by performance on the Virtual Morris Water Maze (Vuong et al. 2017). Given the virtual nature of this test, the authors suggest that their findings may reflect uncontrolled confounding by video game proficiency, which may be associated with higher PBDE exposure due to increased time spent indoors. Notably, Vuong et al did not adjust for breastfeeding, which may have masked inverse associations between PBDEs and scores on visual outcomes.

Several previous epidemiological studies have detected inverse or null associations between PBDE concentrations measured during the prenatal period or childhood and performance on tests of verbal comprehension (Eskenazi et al. 2013; Gascon et al. 2011; Herbstman et al. 2010; Sagiv et al. 2015). In contrast, we found exposure during toddler years was generally associated with better performance on the Verbal Memory Index. Importantly, positive associations were attenuated among children who were breastfed for less than 12 weeks and strengthened among children breastfed for 12 weeks or more, suggesting that the positive effects we observed may relate to neurodevelopmental benefits conferred by breast milk, breastfeeding behavior, or its socioeconomic correlates. Given the lack of a statistically significant interaction between breastfeeding and PBDE exposure, it is likely that the appearance of effect-measure modification

is attributable to residual confounding due to the limited resolution of our breastfeeding variable (Rothman et al. 2008). For example, while we had information on the duration of breastfeeding, we were not able to control for frequency of breastfeeding or quantity of breastmilk consumed. The potential positive influence of breastfeeding is further supported by Adgent et al, who found that verbal domains (expressive and receptive language scores) assessed by the Mullen Scales of Early Learning were generally positively associated with PBDE concentrations measured in breast milk (Adgent et al. 2014). Notably, a recent systematic review concluded that the positive effects of breastfeeding may be largely attributable to the typically higher intelligence and favorable socioeconomic circumstances of mothers who choose to breastfeed (Walfisch et al. 2013). In our sample, breastfeeding was associated with lower maternal non-verbal intelligence ($p=0.06$), and indicators of lower socioeconomic status, such as material hardship ($p=0.02$), suggesting that the positive trend we observed may relate to the nutritional content, immuno-protective or other biological benefits of breastmilk (APA 1997). Alternatively, the discrepancy between our finding and those of previous studies may relate to differences in the specific verbal domains assessed (i.e. comprehension versus memory) or variation in the age at assessment (approximately 4-7 years in other studies versus approximately 11 years here).

The present paper has several strengths. We measured PBDEs in plasma samples collected repeatedly from birth through age 9 years; thus, despite the limited size of our sample ($n=212$), we had high resolution to examine memory outcomes in relation to changes in PBDE plasma concentrations over time. We additionally collected extensive information on sociodemographic and lifestyle factors, enabling us to explore the impact of many potential confounders and effect measure modifiers. Unfortunately, while we were able to examine correlations between PBDEs

and several other developmental neurotoxicants, given the reduced sample size of children with overlapping exposures, we were insufficiently powered to investigate interactions between PBDEs and co-exposures. Similarly, while we previously measured thyroid hormone concentrations, the limited number of children with plasma PBDE concentrations, thyroid hormone concentrations and CMS Index scores precluded our ability to explore mediation by pathways related to thyroid hormone disruption.

Conclusion

We found that children with low prenatal, but high postnatal plasma PBDE concentrations throughout childhood performed significantly worse on tests of visual memory assessed during early adolescence compared to children with persistent low plasma PBDE concentrations. In addition, girls with high cord plasma PBDE concentrations performed significantly worse on tests of auditory working memory assessed during early adolescence. Taken together, these results suggest that the developing brain remains vulnerable to insult by PBDEs from gestation through childhood and that the effects of PBDEs on memory may vary by the duration and developmental period(s) during which exposure occurs. Our findings contribute to a substantial body of evidence (Lam et al. 2017) demonstrating the developmental neurotoxicity of PBDEs and underscore the need to reduce exposure among pregnant women and children.

References

Abbasi G, Buser AM, Soehl A, Murray MW, Diamond ML. 2015. Stocks and flows of pbdes in products from use to waste in the u.S. And canada from 1970 to 2020. Environmental science & technology 49:1521-1528.

Adgent MA, Hoffman K, Goldman BD, Sjodin A, Daniels JL. 2014. Brominated flame retardants in breast milk and behavioural and cognitive development at 36 months. Paediatric and perinatal epidemiology 28:48-57.

Andreano JM, Cahill L. 2009. Sex influences on the neurobiology of learning and memory. Learn Mem 16:248-266.

Antonelli MC, Pallares ME, Ceccatelli S, Spulber S. 2017. Long-term consequences of prenatal stress and neurotoxicants exposure on neurodevelopment. Prog Neurobiol 155:21-35.

APA. 1997. American academy of pediatrics policy statement. Breastfeeding and the use of human milk. Pediatrics 100:5.

Bauer PJ, Fivush R. 2014. The wiley handbook on the development of children's memory:John Wiley & Sons, Ltd.

Brown L, Sherbenou R, Johnsen S. 1997. Test of nonverbal intelligence, third edition examiner's manual. Austin, TX:Pro-Ed.

Caldwell BM, Bradley RH. 2003. Home observation for measurement of the environment: Administration manual. Tempe, AZ:Family & Human Dynamics Resarch Institute, Arizona University.

Carrizo D, Grimalt JO, Ribas-Fito N, Sunyer J, Torrent M. 2007. Influence of breastfeeding in the accumulation of polybromodiphenyl ethers during the first years of child growth. Environmental science & technology 41:4907-4912.

Cohen M. 1997. Children's memory scale (cms) manual. San Antonio, Texas:Pearson.

Cowell WJ, Sjodin A, Jones R, Wang Y, Wang S, Herbstman JB. under review-a. Determinants of prenatal exposure to polybrominated diphenyl ethers (pbdes) among urban, minority infants born between 1998-2006. Environmental Pollution.

Cowell WJ, Sjodin A, Jones R, Wang Y, Wang S, Herbstman JB. under review-b. Time trends and developmental patterns of polybrominated diphenyl ether concentrations over a 15-year period between 1998 and 2013 JESEE.

Dohrenwend BP, Dohrenwend BS, Warheit GJ, Bartlett GS, Goldsteen RL, Goldsteen K, et al. 1981. Stress in the community: A report to the president's commission on the accident at three mile island. Annals of the New York Academy of Sciences 365:159-174.

Driscoll LL, Gibson AM, Hieb A. 2009. Chronic postnatal de-71 exposure: Effects on learning, attention and thyroxine levels. Neurotoxicology and teratology 31:76-84.

Dufault C, Poles G, Driscoll LL. 2005. Brief postnatal pbde exposure alters learning and the cholinergic modulation of attention in rats. Toxicological sciences: an official journal of the Society of Toxicology 88:172-180.

EPA. 2010. An exposure assessment of polybrominated diphenyl ethers. EPA/600/R-08/086F. Washington, DC:U.S. Environmental Protection Agency.

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.

Eskenazi B, Chevrier J, Rauch SA, Kogut K, Harley KG, Johnson C, et al. 2013. In utero and childhood polybrominated diphenyl ether (pbde) exposures and neurodevelopment in the chamacos study. Environmental health perspectives 121:257-262.

Faass O, Ceccatelli R, Schlumpf M, Lichtensteiger W. 2013. Developmental effects of perinatal exposure to pbde and pcb on gene expression in sexually dimorphic rat brain regions and female sexual behavior. Gen Comp Endocrinol 188:232-241.

Fergusson DM, Beautrais AL, Silva PA. 1982. Breast-feeding and cognitive development in the first seven years of life. Soc Sci Med 16:1705-1708.

Fromme H, Becher G, Hilger B, Volkel W. 2016. Brominated flame retardants - exposure and risk assessment for the general population. Int J Hyg Environ Health 219:1-23.

Gascon M, Vrijheid M, Martinez D, Forns J, Grimalt JO, Torrent M, et al. 2011. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. Environment international 37:605-611.

Grandjean P, Landrigan PJ. 2014. Neurobehavioural effects of developmental toxicity. Lancet Neurol 13:330-338.

He P, Wang A, Niu Q, Guo L, Xia T, Chen X. 2011. Toxic effect of pbde-47 on thyroid development, learning, and memory, and the interaction between pbde-47 and pcb153 that enhances toxicity in rats. Toxicol Ind Health 27:279-288.

Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. 2010. Prenatal exposure to pbdes and neurodevelopment. Environmental health perspectives 118:712-719.

Jones B, Nagin D, KA R. 2001. Sas procedure based on mixture models for estimating developmental trajectories. Sociological Methodology Research 29:374-393.

Jones R, Edenfield E, Anderson S, Zhang Y, Sjodin A. 2012. Semi-automated extraction and cleanup method for measuring persistent organic pollutants in human serum. Organohalogen Comp 74:97-98.

Lam J, Lanphear BP, Bellinger DC, Axelrad DA, McPartland J, Sutton P, et al. 2017. Developmental pbde exposure and iq/adhd in childhood: A systematic review and meta-analysis. Environmental health perspectives 125.

Leonetti C, Butt CM, Hoffman K, Miranda ML, Stapleton HM. 2016. Concentrations of polybrominated diphenyl ethers (pbdes) and 2,4,6-tribromophenol in human placental tissues. Environment international 88:23-29.

Lilienthal H, Hack A, Roth-Harer A, Grande SW, Talsness CE. 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.

Mayer S, Jencks C. 1988. Poverty and the distribition of material hardship. J Hum Resour:88-112.

Nagin D. 2005. Group-based modeling of development. Cambridge, Massachusetts,:Harvard University Press.

Nagin DS. 2014. Group-based trajectory modeling: An overview. Ann Nutr Metab 65:205-210.

Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, et al. 2006. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environmental health perspectives 114:1287-1292.

Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL. 1989. Chlorinated hydrocarbon levels in human serum: Effects of fasting and feeding. Arch Environ Contam Toxicol 18:495-500.

Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A, et al. 2004. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. Neurotoxicology and teratology 26:373-385.

Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, et al. 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118:e1845-1859.

Reynolds C, Richond B. 1985. Revised children's manifest anxiety scale manual. Los Angeles:Western Psychological Services.

Ribas-Fito N, Torrent M, Carrizo D, Munoz-Ortiz L, Julvez J, Grimalt JO, et al. 2006. In utero exposure to background concentrations of ddt and cognitive functioning among preschoolers. American journal of epidemiology 164:955-962.

Roth N, Wilks MF. 2014. Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: A systematic review of the epidemiological literature using a quality assessment scheme. Toxicology letters 230:271-281.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. Part 3rd. Philadelphia:Wolters Kluwer Health/Lippincott Williams & Wilkins,.

Rubin DB. 1987. Multiple imputation for nonresponse in surveys. New York:John Wiley and Sons.

Sagiv SK, Kogut K, Gaspar FW, Gunier RB, Harley KG, Parra K, et al. 2015. Prenatal and childhood polybrominated diphenyl ether (pbde) exposure and attention and executive function at 9-12 years of age. Neurotoxicology and teratology 52:151-161.

Sjodin A, Jones RS, Lapeza CR, Focant JF, McGahee EE, 3rd, Patterson DG, Jr. 2004. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. Analytical chemistry 76:1921-1927.

Textor J, Hardt J, Knuppel S. 2011. Dagitty: A graphical tool for analyzing causal diagrams. Epidemiology 22:745.

Viberg H, Fredriksson A, Eriksson P. 2003. Neonatal exposure to polybrominated diphenyl ether (pbde 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. Toxicol Appl Pharmacol 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: an official journal of the Society of Toxicology 92:211-218.

Vuong AM, Braun JM, Yolton K, Xie C, Webster GM, Sjodin A, et al. 2017. Prenatal and postnatal polybrominated diphenyl ether exposure and visual spatial abilities in children. Environmental research 153:83-92.

Walfisch A, Sermer C, Cressman A, Koren G. 2013. Breast milk and cognitive development--the role of confounders: A systematic review. BMJ Open 3:e003259.

Supplemental Material

Table of contents

Abbreviations used in supplemental material:
CMS: Children's Memory Scale
HOME: Home Observation for Measurement of the Environment
LCGA: Latent class growth analysis
PBDE: Polybrominated diphenyl ether
RCMAS: Revised Children's Manifest Anxiety Scale
TONI-3: Test of Nonverbal Intelligence, 3rd Edition

Table S1. Characteristics (mean±SD or n, %) of the study population among all maternal-child pairs enrolled, those with plasma PBDEs, and those administered the CMS.

	All enrolled		PBDE subset		CMS & PBDE subset	
Maternal characteristics[a]	N	Mean±SD or N, %	N	Mean±SD or N, %	N	Mean±SD or N, %
Age	727	25.2±4.9	334	25.1±4.9	212	25.2±5.1
<High school education	726	262 (36)	334	117 (35)	212	80 (38)
Employed	724	387 (53)	334	184 (55)	212	120 (57)
Stable relationship	723	194 (27)	334	83 (25)	212	49 (23)
Nulliparous	723	324 (45)	334	168 (50)[*]	212	108 (51)[#]
Nonverbal intelligence	581	85.5±13.3	312	84.9±13.0	212	84.1±13.1
Demoralization	693	1.2±0.6	327	1.2±0.6	212	1.2±0.6
Child characteristics						
Birth weight (kg)	717	3.4±0.5	333	3.5±0.5[*]	212	3.5±0.5[#]
Gestational age (weeks)	726	39.3±1.4	334	39.4±1.3	212	39.4±1.3
Breastfed ≥ 12 weeks	687	522 (76)	330	258 (78)	212	67 (32)
African American	727	254 (35)	334	124 (37)	212	92 (43)
Dominican	727	473 (65)	334	210 (63)	212	120 (57)
Girl	727	376 (52)	334	182 (54)	212	119 (56)
Anxiety at testing (RCMAS)	384	9.7±6.1	209	9.5±6.2	208	9.5±6.2
Household characteristics						
Material hardship at delivery	712	285 (40)	327	127 (39)	207	85 (41)
Material hardship at testing	384	141 (37)	213	81 (38)	210	81 (39)
Spanish speaking home at 3 yrs	543	251 (46)	301	130 (43)	212	88 (42)
HOME academic score at 3 yrs	543	4.3±1.0	297	4.3±1.0	206	4.3 (1.0)
HOME total score at 3 yrs	543	39.4±6.3	297	39.3±6.3	206	39.3 (6.5)
Environmental tobacco smoke	725	254 (35)	334	118 (35)	212	77 (36)

[a]Measured at the prenatal period unless otherwise noted.
[*]Significantly different from children without PBDEs (n=393) at p=0.05
[#]Significantly different from children without PBDEs or CMS scores (n=515) at p=0.05

Figure S1. Trajectories of PBDE exposure from birth to age 9 years estimated using LCGA.

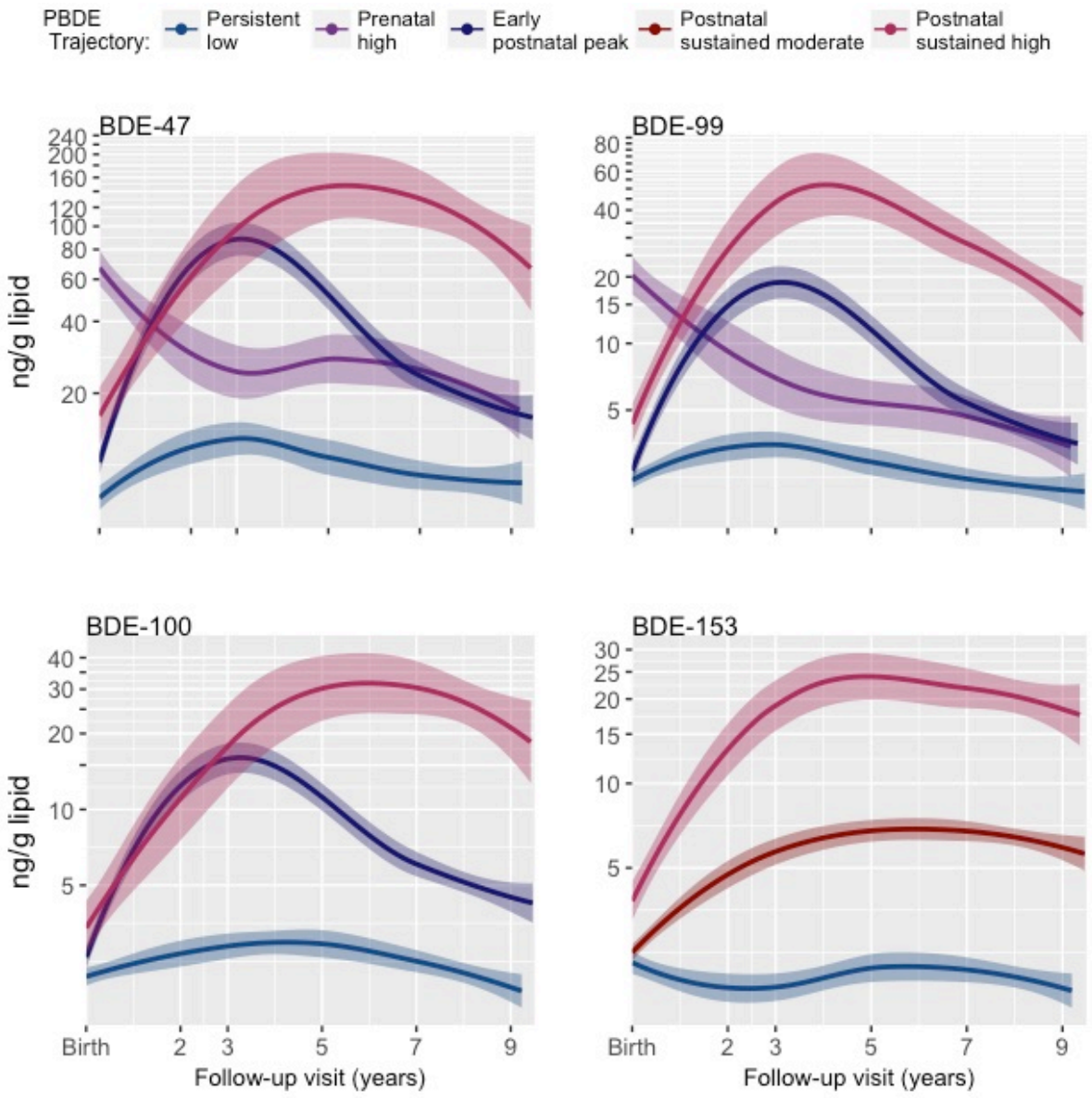


Table S2. Sample size of each PBDE exposure trajectory subset on children with CMS scores, N (%)

	Persistent low	Prenatal high	Early postnatal peak	Sustained postnatal moderate	Sustained postnatal high
BDE-47	74 (35)	40 (19)	65 (31)	NA	33 (16)
BDE-99	87 (41)	28 (13)	52 (25)	NA	45 (21)
BDE-100	91 (46)	13 (6)[a]	80 (40)	NA	28 (14)
BDE-153	62 (29)	NA	NA	107 (50)	43 (20)

[a]Not plotted or analyzed in regression models due to small sample size.

Information relevant to Tables S3a-c. Associations (β, 95% confidence interval) between plasma PBDE concentrations (ng/g lipid) and CMS index scores.

All regression models are adjusted for: date of birth, ethnicity, age at CMS testing, maternal non-verbal intelligence, maternal employment (prenatal), maternal education (prenatal), maternal demoralization (prenatal), parity, birthweight, and breastfeeding duration.

Trajectories were fit using LCGA; continuous PBDE concentrations were $\log_{10}$-transformed before LCGA modeling. In all models examining trajectories, the 'persistent low' group serves as the reference category. Posterior probabilities indicate the probability of correct trajectory assignment for a given child. In sensitivity analyses examining potential misclassification of trajectory membership, we excluded children with a posterior probability <0.6.

We defined outliers as a value more than 1.5 times the interquartile range below or above the first or third quartile, respectively

Colors corresponding to significant findings in tables:

- Green shading highlights significant ($p<0.05$) inverse associations
- Light green shading highlights marginally significant ($p<0.10$) inverse associations
- Blue shading highlights significant ($p<0.05$) positive associations
- Light blue shading highlights marginally significant ($p<0.10$) positive associations

Table S3a. Attention Concentration Index

	Final adjusted model	Exclude outliers (n=3)	Exclude posterior probability < 0.6
Log_{10}BDE-47 continuous			
Prenatal – Girls	-7.55 (-13.84, -1.24)	-7.30 (-13.34, -1.27)	N/A
Prenatal – Boys	3.10 (-4.00, 10.19)	1.84 (-5.02, 8.70)	N/A
	p-int=0.02	*p-int=0.04*	
Age 3 years	-0.11 (-7.67, 7.44)	-2.20 (-9.10, 4.70)	N/A
Age 7 years	3.49 (-1.94, 8.93)	2.68 (-2.64, 8.01)	N/A
Age 9 years	3.28 (-3.73, 10.30)	2.00 (-4.82, 8.83)	N/A
BDE-47 trajectory			
Prenatal high			
Early postnatal peak	0.55 (-4.81, 5.91)	0.19 (-4.95, 5.34)	2.10 (-3.86, 8.05)
Sustained postnatal high	-0.44(-7.31, 6.43)	-1.71 (-8.29, 4.86)	0.86 (-6.88, 8.60)
Log_{10}BDE-99 continuous			
Prenatal – Girls	-8.14 (-15.34, -0.93)	-8.02 (-14.90, -1.15)	N/A
Prenatal – Boys	6.47 (-2.18, 15.12)	4.99 (-3.44, 13.43)	N/A
	p-int=0.02	*p-int=0.03*	
Age 3 years	0.29 (-7.35, 7.93)	-2.17 (-9.23, 4.89)	N/A
Age 7 years	2.13 (-3.16, 7.43)	1.52 (-3.65, 6.69)	N/A
Age 9 years	4.96 (-1.40, 11.33)	3.96 (-2.22, 10.14)	N/A
BDE-99 trajectory			
Prenatal high			
Early postnatal peak	-1.79 (-7.44, 3.87)	-2.05 (-7.48, 3.38)	-3.73 (-10.72, 3.25)
Sustained postnatal high	-1.32 (-7.47, 4.82)	-2.56 (-8.44, 3.32)	0.48 (-6.45, 7.41)
Log_{10}BDE-100 continuous			
Prenatal – Girls	-6.15 (-14.26, 1.97)	-8.14 (-15.90, -0.38)	N/A
Prenatal – Boys	6.12 (-3.44, 15.69)	4.82 (-4.38, 14.07)	N/A
	p-int=0.03	*p-int=0.03*	
Age 3 years	-0.14 (-8.08, 7.79)	-3.66 (-10.99, 3.66)	N/A
Age 7 years	2.67 (-3.13, 8.47)	1.79 (-3.89, 7.48)	N/A
Age 9 years	4.44 (-2.47, 11.35)	2.80 (-3.97, 9.58)	N/A
BDE-100 trajectory			
Early postnatal peak	-2.58 (-7.48, 2.31)	-3.03 (-7.70, 1.65)	-1.70 (-6.82, 3.41)
Sustained postnatal high	-1.60 (-8.80, 5.59)	-2.44 (-9.29, 4.41)	-0.01 (-7.21, 7.18)
Log_{10}-BDE-153 continuous			
Prenatal	-2.38 (-12.11, 7.36)	-2.82 (-11.89, 6.26)	N/A
Age 3 years	0.70 (-7.32, 8.72)	-3.08 (-10.50, 4.33)	N/A
Age 7 years	1.55 (-5.01, 8.10)	0.41 (-6.03, 6.86)	N/A
Age 9 years	6.22 (-1.10, 13.53)	4.25 (-2.96, 11.47)	N/A
BDE-153 trajectory			
Sustained postnatal mod	-0.14 (-5.04, 4.76)	-0.10 (-4.81, 4.62)	-0.92 (-6.00, 4.17)
Sustained postnatal high	-1.33 (-7.48, 4.82)	-1.90 (-7.79, 3.99)	-2.16 (-8.46, 4.14)

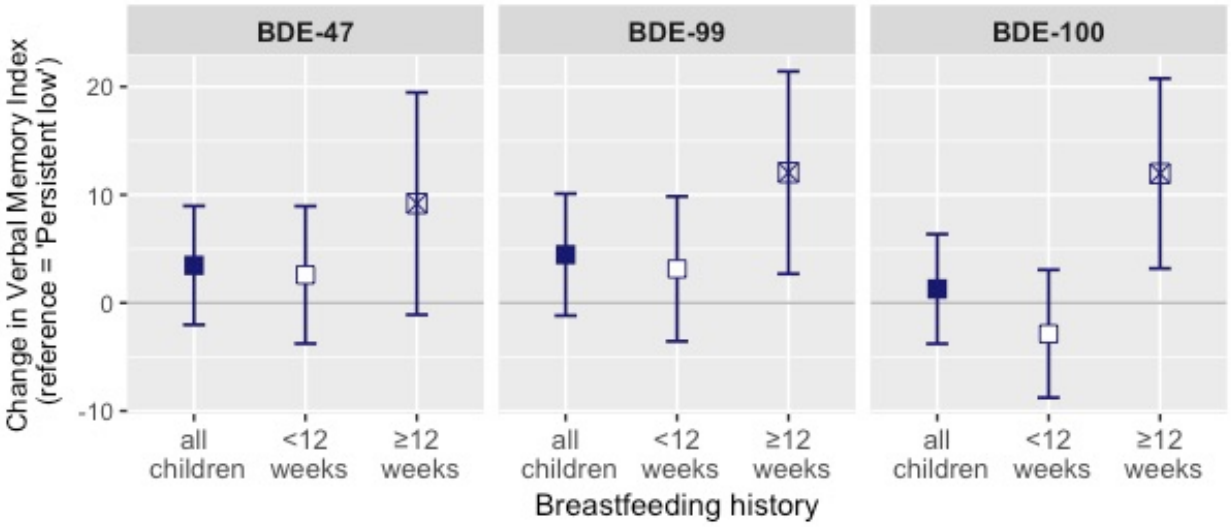
Table S3b. Visual Memory Index

	Final adjusted model	Exclude outliers (n=2)	Exclude posterior probability < 0.6
Log_{10}BDE-47 continuous			
Prenatal	1.49 (-2.50, 5.49)	1.25 (-2.59, 5.08)	N/A
Age 3 years	-1.90 (-8.64, 4.83)	-1.90 (-8.64, 4.83)	N/A
Age 7 years	-5.28 (-10.13, -1.44)	-5.31 (-9.45, -1.16)	N/A
Age 9 years	-5.18 (-9.95, -0.42)	-4.32 (-8.99, 0.36)	N/A
BDE-47 trajectory			
Prenatal high	-1.07 (-6.05, 3.92)	-1.32 (-6.14, 3.51)	-1.96 (-7.64, 3.72)
Early postnatal peak	-3.35 (-7.70, 1.02)	-3.37 (-7.48, 0.84)	-2.36 (-7.14, 2.43)
Sustained postnatal high	-8.44 (-13.95, -2.93)	-6.59 (-12.00, -1.18)	-10.45 (-16.48, -4.42)
Log_{10}BDE-99 continuous			
Prenatal	0.94 (-3.95, 5.65)	0.69 (-3.86, 5.24)	N/A
Age 3 years	-0.71 (-7.42, 6.00)	-0.71 (-7.42, 6.00)	N/A
Age 7 years	-3.96 (-8.18, 0.27)	-3.45 (-7.49, 0.59)	N/A
Age 9 years	-4.90 (-9.47, -0.33)	-4.12 (-8.60, 0.36)	N/A
BDE-99 trajectory			
Prenatal high	-0.15 (-5.70, 5.39)	-0.36 (-5.72, 5.00)	1.24 (-4.97, 7.46)
Early postnatal peak	-1.38 (-5.91, 3.15)	-1.36 (-5.74, 3.01)	-2.76 (-8.29, 2.76)
Sustained postnatal high	-5.51 (-10.41, -0.61)	-4.11 (-8.59, 0.37)	-4.93 (-10.39, 0.53)
Log_{10}BDE-100 continuous			
Prenatal	2.83 (-3.31, 8.96)	2.12 (-3.93, 8.19)	N/A
Age 3 years	1.16 (-5.91, 8.22)	1.16 (-5.91, 8.22)	N/A
Age 7 years	-5.47 (-10.16, -0.77)	-4.77 (-9.26, -0.28)	N/A
Age 9 years	-5.24 (-10.25, -0.25)	-4.68 (-9.54, 0.18)	N/A
BDE-100 trajectory			
Early postnatal peak	-1.96 (-5.85, 1.93)	-1.65 (-5.41, 2.11)	-1.93 (-6.28, 2.43)
Sustained postnatal high	-8.57 (-14.19, -2.96)	-7.57 (-13.05, -2.10)	-7.86 (-13.83, -1.88)
Log_{10}BDE-153 continuous			
Prenatal	4.61 (-3.16, 12.38)	4.34 (-3.36, 12.05)	N/A
Age 3 years	3.96 (-2.98, 10.90)	3.96 (-2.98, 10.90)	N/A
Age 7 years	-3.71 (-9.03, 1.61)	-3.17 (-8.24, 1.90)	N/A
Age 9 years	-1.63 (-7.15, 3.89)	-1.27 (-6.63, 4.08)	N/A
BDE-153 trajectory			
Sustained postnatal moderate	-0.51 (-4.63, 3.61)	0.33 (-3.64, 4.30)	-1.17 (-5.51, 3.16)
Sustained postnatal high	-1.09 (-6.20, 4.01)	-1.15 (-6.05, 3.75)	-0.29 (-5.60, 5.02)

Table S3c. Verbal Memory Index

	Final adjusted model	Exclude outliers (n=2)	Exclude posterior probability < 0.6
Log_{10}BDE-47 continuous			
Prenatal	-0.93 (-5.94, 4.08)	-1.63 (-6.55, 3.30)	N/A
Age 3 years	0.85 (-6.88, 8.52)	0.85 (-6.82, 8.52)	N/A
Age 7 years	-2.35 (-7.47, 2.77)	-2.38 (-7.28, 2.52)	N/A
Age 9 years	-1.86 (-8.05, 4.34)	-2.16 (-8.03, 3.71)	N/A
BDE-47 trajectory			
Prenatal high	-1.74 (-8.03, 4.55)	-1.43 (-7.62, 4.77)	0.54 (-6.26, 7.34)
Early postnatal peak	3.47 (-2.03, 8.97)	3.57 (-1.84, 8.98)	7.02 (1.29, 12.75)
Sustained postnatal high	-0.73 (-7.69, 6.23)	-0.65 (-7.47, 6.16)	1.69 (-5.53, 8.91)
Log_{10}BDE-99 continuous			
Prenatal	-2.27 (-8.24 3.70)	-2.52 (-8.36, 3.32)	N/A
Age 3 years	-0.20 (-8.12, 7.72)	-0.20 (-8.12, 7.72)	N/A
Age 7 years	-2.96 (-7.88, 1.96)	-2.79 (-7.50, 1.92)	N/A
Age 9 years	-2.11 (-8.05, 3.82)	-2.35 (-7.98, 3.27)	N/A
BDE-99 trajectory			
Prenatal high	-1.37 (-8.27, 5.53)	-1.23 (-7.98, 5.53)	-2.46 (-9.78, 4.87)
Early postnatal peak	4.46 (-1.18, 10.10)	4.44 (-1.08, 9.96)	4.47 (-2.04, 10.98)
Sustained postnatal high	-2.79 (-8.89, 3.30)	-2.80 (-8.77, 3.16)	-3.44 (-9.88, 3.00)
Log_{10}BDE-100 continuous			
Prenatal	-3.20 (-10.27, 3.88)	-3.17 (-10.12, 3.78)	N/A
Age 3 years	4.01 (-3.93, 11.96)	4.01 (-3.93, 11.96)	N/A
Age 7 years	-1.66 (-7.16, 3.84)	-1.86 (-7.13, 3.40)	N/A
Age 9 years	-1.05 (-7.54, 5.44)	-1.20 (-7.36, 4.95)	N/A
BDE-100 trajectory			
Early postnatal peak	1.29 (-3.78, 6.36)	1.43 (-3.53, 6.39)	4.47 (-0.87, 9.80)
Sustained postnatal high	-4.37 (-11.68, 2.94)	-4.35 (-11.48, 2.77)	-3.94 (-11.26, 3.39)
Log_{10}BDE-153 continuous			
Prenatal	-1.14 (-11.36, 9.07)	-1.72 (-11.68, 7.44)	N/A
Age 3 years	6.15 (-1.66, 13.97)	6.15 (-1.66, 13.97)	N/A
Age 7 years	0.11 (-6.06, 6.29)	-1.22 (-7.17, 4.73)	N/A
Age 9 years	2.57 (-4.51, 9.65)	1.40 (-5.36, 8.16)	N/A
BDE-153 trajectory			
Sustained postnatal moderate	2.58 (-4.50, 9.66)	-0.83 (-5.82, 4.16)	0.04 (-5.31, 5.40)
Sustained postnatal high	1.34 (-5.01, 7.69)	0.69 (-5.51, 6.88)	1.59 (-4.97, 8.15)

Figure S2. Associations (β, 95% confidence interval) from adjusted models examining the 'early postnatal peak' trajectory of plasma PBDE concentrations in relation to Verbal Memory Index scores stratified by breastfeeding history of the study child (n=212)



Sample sizes of the 'early postnatal peak' + 'persistent low' trajectories:
BDE-47: all children (n=139), breastfed <12 weeks (n=102), breastfed ≥12 weeks (n=37)
BDE-99: all children (n=139), breastfed <12 weeks (n=101), breastfed ≥12 weeks (n=38)
BDE-100: all children (n=171), breastfed <12 weeks (n=122), breastfed ≥12 weeks (n=49)

CHAPTER 5: Prenatal Exposure to PBDEs and Serum Thyroid Parameters Measured during Childhood

Whitney J. Cowell[a,b], Andreas Sjödin[c], Richard Jones[c], Ya Wang[d], Shuang Wang[d], Robin M. Whyatt[a,b], Pam Factor-Litvak[e], Gary Bradwin[f], Abeer Hassoun[g], Sharon Oberfield[g], Julie B. Herbstman[a,b]*

[a]Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, NY
[b]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY
[c]Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA
[d]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY
[e]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY
[f]Department of Laboratory Medicine, Harvard Medical School and Boston Children's Hospital, Boston, MA
[g]Division of Pediatric Endocrinology, Diabetes and Metabolism, Department of Pediatrics, New York-Presbyterian Morgan Stanley Children's Hospital, New York, NY

Address correspondence to: Julie Herbstman, Columbia Center for Children's Environmental Health, 12th Fl, Mailman School of Public Health, 722 West 168th St, New York, NY 10032 USA. Telephone: (212) 304-7273. E-mail: jh2678@cumc.columbia.edu

Funding: This work was supported by National Institutes of Health [grant numbers R03ES0218, R01ES013543, P50ES009600, T32ES023772 and T32ES007322]; and Environmental Protection Agency FP-91779001.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services. This publication was developed under STAR Fellowship Assistance Agreement no. FP-91779001 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by the EPA. The views expressed in this publication are solely those of the study authors.

Manuscript status: Submitted to *Environmental Health Perspectives*

Acknowledgements: We thank Dr. Lori Hoepner for assistance with data management and Dr.

Antonia Calafat and Dr. Ben Blount for analysis of urinary endocrine disrupting chemicals.

Abstract

Background: Findings from observational studies examining prenatal exposure to polybrominated diphenyl ethers (PBDEs), a class of flame retardants, in relation to thyroid hormone parameters measured during and after pregnancy have been inconsistent.

Methods: This analysis included 205 children enrolled in the Columbia Center for Children's Environmental Health Mothers and Newborns birth cohort, which recruited pregnant African American and Dominican women between 1998 and 2006. We measured PBDEs in umbilical cord plasma and thyroid parameters (thyroid stimulating hormone (TSH), free thyroxine (free T_4), and total thyroxine (total T_4)) in child serum collected at ages 3, 5, 7 and 9 years. We used multivariable linear regression to examine associations between prenatal PBDE plasma concentrations (BDE-47, BDE-99, BDE-100, BDE-153) and each thyroid parameter.

Results: BDE-47 was the predominant congener detected. Compared to children with BDE-47 concentrations in the first quartile of the exposure distribution, children in quartiles 2, 3 and 4 had 17% (95% confidence interval (CI) -31, -2), 22% (CI -35, -7) and 17% (CI -29, -2) lower geometric mean TSH levels. Likewise, children in the third and fourth quartiles had 1.13 pmol/L (CI -1.96, -0.29) and 0.77 pmol/L (CI -1.51, -0.03) lower mean free T_4 levels, respectively. We did not detect associations between BDE-47 and total T_4 levels or between the 3 other congeners and any thyroid parameter.

Conclusions: This is the first study to prospectively examine associations between cord plasma PBDE concentrations and childhood thyroid parameters. We found that prenatal BDE-47 exposure may be associated with lower TSH and free T_4 levels during childhood.

Introduction

Thyroid hormones are essential for fetal and postnatal brain development and for regulating neuropsychological functioning in children (Williams 2008). Mounting evidence from animal research suggests that polybrominated diphenyl ethers (PBDEs), which structurally resemble triiodothyronine (T_3) and thyroxine (T_4), may disrupt thyroid hormone homeostasis (Costa et al. 2014). PBDEs are a class of flame retardant chemicals that were widely used in household consumer products until their phase-out between 2004 and 2013 (USEPA 2010). Exposure occurs primarily via incidental ingestion of dust and due to their lipophilicity, PBDEs readily cross the placenta and enter fetal circulation (Herbstman et al. 2008; Leonetti et al. 2016).

Despite findings from animal research demonstrating inverse associations between prenatal PBDE exposure and circulating thyroid hormone levels in newborn offspring (Costa et al. 2014), findings from human studies have been inconsistent (Abdelouahab et al. 2013b; Chevrier et al. 2010; Chevrier et al. 2011; Herbstman et al. 2008; Stapleton et al. 2011; Vuong et al. 2015). Notably, previous epidemiologic studies have measured thyroid parameters in maternal, umbilical cord, or infant blood collected during pregnancy, delivery or within hours to days of birth; these are periods when transient, yet substantial endocrine system changes occur, including profound alterations to the thyroid regulatory system (Glinoer 1997). In the present analysis, we prospectively examined associations between umbilical cord plasma PBDE concentrations and serum thyroid parameters (TSH, total T_4, and free T_4) measured during childhood.

Methods

We conducted this analysis among a subset of participants enrolled in the Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns birth cohort, which recruited African American and Dominican women from New York City between 1998 and 2006. The cohort was designed to examine sub-clinical health effects in relation to several environmental exposures among healthy children; therefore, women were excluded if they were outside the ages of 18-35 years, initiated prenatal care after the 20th week of pregnancy, had a multiple pregnancy, used tobacco products or illicit drugs, had diabetes, had hypertension, or were HIV positive. Women were considered fully enrolled if a maternal or umbilical cord blood sample was collected at the child's delivery.

During pregnancy and at each postnatal study visit a bilingual research worker conducted a structured interview to collect information about sociodemographic and lifestyle factors. At delivery, study staff collected umbilical cord blood and at the 2, 3, 5, 7 and 9 year visits a pediatric phlebotomist collected child venous blood. All blood samples were transported to the CCCEH laboratory immediately following collection, where the buffy coat, packed red blood cells, and plasma were separated and frozen at -70C. The study protocol was approved by the Institutional Review Board of Columbia University Medical Center. It was determined at the Centers for Disease Control and Prevention (CDC) that the agency was not engaged in human subjects' research. Before each study visit, mothers gave written informed consent for herself and her child until the age of 7 years, after which the child also gave informed assent.

Laboratory analyses

The CDC's Persistent Organic Pollutants Biomonitoring Laboratory measured concentrations of 11 PBDE congeners in cord plasma samples collected from 327 neonates. Detailed analytic methods are published elsewhere (Jones et al. 2012; Sjodin et al. 2004). Briefly, samples were fortified with internal standards followed by automated liquid-liquid extraction using a Gilson 215 liquid handler (Gilson Inc., Middleton, WI). Final analytical determinations were made by gas chromatography isotope dilution high-resolution mass spectrometry using a DFS instrument (Thermo Fisher Scientific, Bremen, Germany). Each analytical batch was comprised of method blanks (N=3), quality control samples (N=3) and study samples (N=26). All reported data were subtracted from the median concentration detected in method blank samples. Co-extracted lipids were removed using a silica: silica/sulfuric acid column with automation on a Rapid Trace SPE work station (Biotage, Uppsala, Sweden) and total cholesterol and triglycerides were determined on a Roche Hitachi 912 Chemistry Analyzer (GMI Inc, Ramsey, MN). Total blood lipid levels, including unmeasured free cholesterol and phospholipids, were estimated by summation of individual lipid components using an umbilical cord blood-specific formula (A Sjödin, unpublished data, 2016).

The Clinical and Epidemiologic Research Laboratory at Boston Children's Hospital analyzed TSH, free T_4, and total T_4 in umbilical cord plasma (n=195) and repeatedly collected child serum (n_{total}=361: $n_{3\ years}$=150, $n_{5\ years}$=73, $n_{7\ years}$=67, $n_{9\ years}$=9) samples. All analyses were performed by automated immunoassay using a competitive electrochemiluminescence detection system (Roche Diagnostics, Indianapolis, IN). The lowest detection limits were 0.005 μlU/mL, 0.26 pmol/L, and 5.4 nmol/L for TSH, free T_4, and total T_4, respectively. Day-to-day imprecision

values ranged from 1.8%-5.4% for 0.09-3.96 μIU/mL of TSH, 3.5%-6.6% for 8.75-50.70 pmol/L of free T_4, and 3.0%-6.9% for 33.4-237 nmol/L of total T_4.

We measured several other environmental chemicals that have been shown or suspected to disrupt the thyroid regulatory system, including: bisphenol A (BPA), mono-2-ethylhexyl phthalate (MEHP), triclosan, perchlorate, thiocyanate, nitrate, polychlorinated biphenyls (PCBs), and p'p'-dichlorodiphenyldichloroethylene (p'p'-DDE) (Johns et al. 2016; Pearce and Braverman 2009; Zoeller 2007). BPA, MEHP, triclosan, perchlorate, thiocyanate, and nitrate (all ng/mL) were measured at the CDC in maternal spot urine samples collected during the third trimester. PCBs and p'p'-DDE (ng/lipid) were measured in umbilical cord plasma simultaneous to PBDE measurement.

Statistical analysis

We focused on BDEs-47, -99, -100 and -153, which were the most frequently detected congeners. We adapted a previously described (Baccarelli et al. 2005) distribution-based multiple imputation method to estimate values for samples with PBDE concentrations less than the limit of detection (Cowell et al. in press). Briefly, for each sample with a non-detected concentration, we first calculated the mean and variance of the set of log-transformed detected results with an equal or lower limit of detection. We then randomly and repeatedly (n=10) drew a value from the normal distribution with the same mean and variance. We pooled parameter estimates from each of the 10 resulting datasets for all subsequent analyses.

We used linear regression to examine associations between cord plasma PBDE concentrations and serum thyroid parameters measured during childhood and employed the generalized estimating equations (GEE) approach with an exchangeable working correlation to account for repeated thyroid measures within a child over time. We expressed TSH, free T_4, and total T_4 as continuous variables and $\log_{10}$-transformed TSH to better approximate a normal distribution. We expressed PBDE congeners as lipid-standardized, continuous variables and applied a $\log_{10}$-transformation to stabilize the variance. We additionally examined PBDEs as a quartile variable and assessed linear dose-response trends by treating the quartiles as a continuous measure.

Thyroid hormone concentrations decrease with age (Elmlinger et al. 2001); therefore, we *a priori* included exact age at blood draw as a time-varying covariate. We considered sex, ethnicity (African American/Dominican), date of birth, gestational age (in weeks), birth weight (in grams), prenatal environmental tobacco smoke exposure (yes/no as previously described (Rauh et al. 2004)), breastfeeding history (ever/never), parity (nulliparous/multiparous), relationship status (unmarried/married or with the same partner for 7 or more years), maternal age (in years), material hardship (none/unable to afford food, clothing or housing) and maternal education (less than high school/high school or equivalent) as potential confounders. All maternal variables were collected during the prenatal period. We examined associations between these covariates and each PBDE congener and thyroid parameter in separate bivariate models and constructed a Directed Acyclic Graph (DAG) to identify the minimally sufficient set of covariates for adjustment, which included only ethnicity (see Figure S1). We further evaluated the influence of covariate selection by examining *a priori* (age at blood draw-only) and fully adjusted models.

We performed several supplemental analyses focused on quartiles of BDE-47. First, we assessed the impact of outlying data points by excluding observations with internally studentized residuals greater than the absolute value of 3 (TSH n=1, free T_4 n=2, total T_4 n=3). We also evaluated our decision to lipid standardize PBDE concentrations by examining models with BDE-47 expressed on a plasma volume basis with and without total blood lipids included as a covariate. To potentially reduce thyroid parameter variability and increase the precision of our estimates, we explored the impact of adjusting for several co-exposures, including: perchlorate, thiocyanate, nitrate, BPA, DEHP (using the metabolite MEHP), triclosan, ΣPCB_4, and p'p-DDE. With the exception of perchlorate, thiocyanate, and nitrate, we examined each co-exposure in a separate model. Perchlorate, thiocyanate and nitrate have been shown to disrupt thyroid function through a shared mechanism of action, therefore we created a single variable to reflect exposure to these compounds, which we coded as "high" if the mother's urinary concentration was in the top quartile of the exposure distribution for any 1 of the 3. We summed PCBs 118, 153, 180 and co-eluting 138-158 (ΣPCB_4), which were the 4 most frequently detected congeners in our sample. We expressed BPA, MEHP, triclosan, ΣPCB_4, and p'p-DDE as $\log_{10}$-transformed continuous measures and replaced non-detected concentrations with the LOD over the square root of 2.

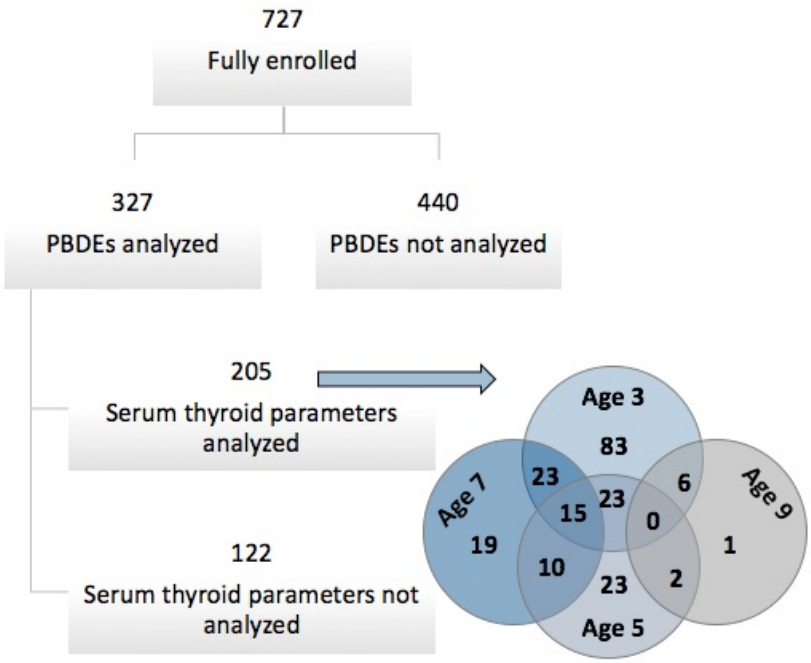
To better understand the role of exposure timing, we adjusted models for BDE-47 concentration measured in child blood collected at ages 2 to 3 years or 5 to 9 years. Within these 2 models, BDE-47 and thyroid parameters were measured cross sectionally and no measures were repeated over time. Finally, to facilitate comparison of our results to other published studies, we examined cross sectional associations between cord blood PBDEs and thyroid parameters. In addition to ethnicity, we adjusted these models for gestational age and mode of delivery (cesarean section

versus vaginal birth). We performed all statistical analyses using SAS v9.4 (SAS Institute) or RStudio v0.99.891 and constructed the DAG using DAGitty v2.3.

Results

At delivery, 727 mothers remained eligible for study enrollment and provided an umbilical cord or maternal blood sample. The current analysis was restricted to 205 children (28% of those fully enrolled) with prenatal PBDE and postnatal thyroid parameter data. As illustrated by Figure 1, 126 children had a TSH, free T_4 and total T_4 sample at either age 3-years (n=83), 5-years (n=23), 7-years (n=19) or 9-years (n=1). An additional 64 children had measures at 2 of the 4 age periods and 15 children had measures at 3 of the 4 age periods.

Figure 1. Diagram of participant selection from the CCCEH Mothers and Newborns birth cohort



Characteristics of the study population are presented in **Table 1**. Maternal-child pairs were African American (44%) and Dominican (56%). At delivery, 36% of mothers had less than a

high school education, 24% were married or in a stable relationship, and 37% reported experiencing material hardship. Sociodemographic and lifestyle characteristics were similarly distributed between children included in the analysis and those excluded due to missing PBDE or thyroid parameter data, with the following exceptions: the excluded sample had a higher proportion of Dominican participants (69% versus 56%), fewer nulliparous women (42% versus 51%), and on average infants had lower birthweights (123 grams).

Table 1. Demographic and lifestyle characteristics, umbilical cord plasma PBDE concentrations, and child thyroid parameter concentrations of maternal-child pairs included in the analysis (n=205).

	N (%) or mean±SD
African American	90 (44)
Dominican	115 (56)
Nulliparous	105 (51)
Maternal age (years)	25.0±4.9
< High school education	73 (36)
Stable relationship	49 (24)
Material hardship	75 (37)
Male	92 (45)
Prenatal ETS exposure	73 (36)
Gestational age (weeks)	39.3±1.3
Birth weight (grams)	3461±473
Ever breastfed	115 (56)
PBDEs (ng/g lipid)	
BDE-47[a]	13.9±3.1
BDE-99[a]	3.9±2.6
BDE-100[a]	2.9±2.3
BDE-153[a]	2.6±1.8
Child thyroid parameters	
TSH (μIU/mL)[a]	2.2±1.1
Free T_4 (pmol/L)	18.0±2.2
Total T_4 (nmol/L)	142.5±28.2

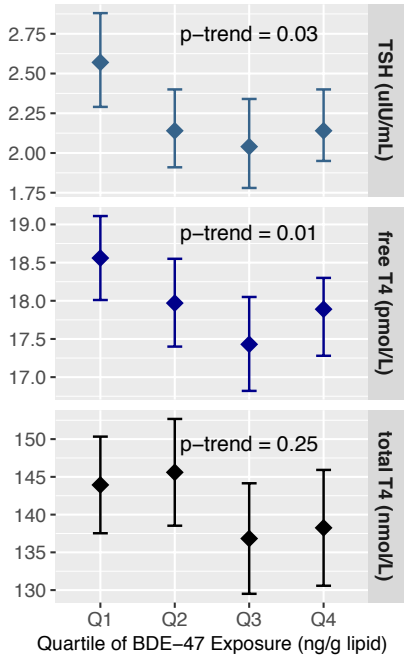
ETS: environmental tobacco smoke exposure, PBDE: polybrominated diphenyl ether, TSH: thyroid stimulating hormone, T_4: thyroxine. [a]Geometric mean and geometric standard deviation.

Among children with thyroid data, we detected BDEs-47, -99, -100, and -153 in 78%, 47%, 36%, and 28% of samples. The geometric mean (±GSD) concentration of BDE-47 (13.9±3.1) was more than 3 times that of BDE-99 (3.9±2.6), BDE-100 (2.9±2.3), and BDE-153 (2.6±1.8). These 4 congeners were moderately to highly correlated (average Spearman rho across 10 imputed datasets: 0.42 – 0.80, $p<0.01$). The distributions of PBDE concentrations were similar between the 123 children excluded due to missing thyroid parameter data and the 205 children included in the analysis. Likewise, thyroid parameter concentrations among the 156 children excluded due to not having a measure of PBDEs were similar to the 205 children included (see Table S1). Across ages, the geometric mean (±GSD) TSH concentration was 2.2±1.1 μIU/mL and the arithmetic mean free T_4 and total T_4 concentrations were 18.0±2.2 pmol/L and 142.5±28.2 nmol/L, respectively. Age-specific thyroid parameter concentrations are presented in Table S2.

Table 2 presents associations from GEE models examining cord plasma PBDEs (ng/g lipid) and child serum thyroid parameters and Figure 2 plots adjusted mean thyroid parameter concentrations stratified by quartile of BDE-47 concentration. Compared to children with BDE-47 concentrations in the first quartile of the exposure distribution, children in quartiles 2, 3 and 4 had 17% (95% CI: -31, -2), 22% (-35, -7) and 17% (-29, -2) lower geometric mean TSH levels; each quartile increase in BDE-47 concentration was associated with an average 7% decrease in TSH concentration (95% CI: -11, -0.7). When expressed as a continuous variable, each 10% increase in BDE-47 concentration was associated with a 0.3% decrease in TSH concentration (95% CI: -0.9, 0.02). Likewise, compared to children in the first BDE-47 quartile, children in the third and fourth quartiles had 1.13 pmol/L (95% CI: -1.96, -0.29) and 0.77 pmol/L (95% CI: -

1.51, -0.03) lower mean free T_4 levels, respectively. On average, each quartile increase in concentration was associated with a 0.28 pmol/L decrease in free T_4 concentration (95% CI: -0.52, -0.05). When expressed as a continuous variable, every 10-fold increase in BDE-47 was associated with a 0.56 pmol/L (95% CI: -1.07, -0.06) decrease in free T_4 concentration. We did not detect associations between BDE-47 and total T_4 levels. Likewise, we did not detect associations between the 3 other congeners and any childhood thyroid parameter. Results from *a priori*-only models, fully adjusted models, and models excluding observations with an absolute internally studentized residual >3 were essentially unchanged from the results presented in Table 2. Similarly, expression of BDE-47 on a volume basis with or without total lipid levels included as a covariate did not impact the magnitude or direction of associations (see Figure S2).

Figure 2. Age and ethnicity-adjusted mean thyroid parameter concentrations by quartile of plasma BDE-47 (ng/g lipid).



Legend: *P*-trend estimates are from models treating BDE-47 quartiles as a continuous variable.

Table 2. Age- and ethnicity-adjusted associations[a] between cord plasma PBDEs (ng/g lipid) and serum thyroid parameters; N=205 children and 299 thyroid measurements.

	$Log_{10}TSH$[b] (μIU/mL)	Free T_4 (pmol/L)	Total T_4 (nmol/L)
BDE-47			
Continuous	-0.03 (-0.09, 0.02)	-0.56 (-1.07, -0.06)	-4.36 (-11.72, 3.01)
Quartile 2	-0.08 (-0.16, -0.01)	-0.59 (-1.44, 0.26)	1.67 (-7.71, 11.05)
Quartile 3	-0.11 (-0.19, -0.03)	-1.13 (-1.96, -0.29)	-7.10 (-16.99, 2.78)
Quartile 4	-0.08 (-0.15, -0.01)	-0.77 (-1.51, -0.03)	-5.68 (-15.61, 4.25)
P-trend[c]	0.03	0.02	0.13
BDE-99[d]			
Continuous	-0.02 (-0.08, 0.05)	-0.18 (-0.82, 0.45)	-3.35 (-12.45, 5.74)
Quartile 2	0.00 (-0.08, 0.07)	0.08 (-0.74, 0.91)	-1.72 (-12.81, 9.37)
Quartile 3	0.01 (-0.08, 0.09)	0.23 (-0.59, 1.05)	3.27 (-8.09, 14.63)
Quartile 4	0.04 (-0.06, 0.13)	0.23 (-0.66, 1.13)	2.58 (-8.83, 13.99)
P-trend[c]	0.39	0.53	0.45
BDE-100			
Continuous	0.02 (-0.05, 0.08)	-0.28 (-1.09, 0.53)	-4.39 (-15.12, 6.35)
Quartile 2	0.02 (-0.06, 0.09)	-0.33 (-1.27, 0.60)	0.86 (-12.80, 14.52)
Quartile 3	0.01 (-0.08, 0.10)	-0.02 (-0.94, 0.91)	1.87 (-9.58, 13.33)
Quartile 4	0.02 (-0.06, 0.10)	0.31 (-0.56, 1.18)	5.90 (-5.12, 16.92)
P-trend[c]	0.71	0.41	0.33
BDE-153			
Continuous	-0.03 (-0.13, 0.07)	-0.52 (-1.50, 0.45)	-8.24 (-24.07, 7.59)
Quartile 2	0.03 (-0.06, 0.12)	-0.05 (-1.00, 0.87)	-1.57 (-12.16, 9.01)
Quartile 3	0.05 (-0.04, 0.15)	0.11 (-1.03, 1.24)	2.48 (-11.44, 16.39)
Quartile 4	0.05 (-0.04, 0.15)	0.34 (-0.50, 1.18)	5.60 (-5.90, 17.10)
P-trend[c]	0.20	0.40	0.28

[a]Continuous PBDE concentrations are log_{10} transformed and quartile 1 is the reference group for all quartile analyses; beta coefficients and 95% confidence intervals are presented. [b]Percent change calculated using $[1-10^{\beta}*100]$; [c]Tests for trend treat quartiles as a continuous variable. [d]N=204 due to 1 sample with non-reportable concentrations.

We measured several other suspected endocrine disrupting chemicals among 80 of the 205 children included in this analysis (n=116 repeated thyroid observations). In models adjusted for perchlorate, nitrate and thiocyanate, the association between BDE-47 and free T_4 was marginally reduced across all quartiles. Adjustment for other co-exposures did not change the direction or substantially alter the magnitude of effect estimates, nor did their inclusion improve precision, which we evaluated by the width of the 95% confidence interval (see Figure S3).

In cross sectional analyses, we found BDE-47 measured in toddlers (age 2-3 years, n=87) was positively associated with total T_4 (β (95% CI): Q_2 vs $Q_{1:}$ 20.9 (5.7, 36.2), Q_3 vs $Q_{1:}$ 6.3 (-8.6, 21.3), Q_4 vs $Q_{1:}$ 14.5 (-0.82, 29.88)). Conversely, we found no association between BDE-47 and total T_4 measured during middle childhood (age 5-9 years, n=88), or with TSH and free T_4 measured in toddlers or during middle childhood, however, adjusting prenatal BDE-47 models for postnatal BDE-47 concentrations did not substantially change the direction or magnitude of observed associations (see Figure S4). We did not detect associations between cord plasma BDE-47 and any cord serum thyroid hormone parameters (n=65) (Fig S5).

Discussion

This is the first study to examine associations between prenatal exposure to PBDEs and serum thyroid parameters measured during childhood and the largest study of cord plasma PBDE concentrations in the United States. We observed inverse associations between cord plasma BDE-47 and serum TSH and free T_4 levels measured between the ages of 3 and 9 years.

Five birth cohort studies (n>100) with comparable maternal or cord blood PBDE concentrations in North America have investigated associations with thyroid parameters measured during pregnancy or infancy (see Figure S5). Results across these studies include a mix of positive, negative and null associations (Abdelouahab et al. 2013b; Chevrier et al. 2010; Herbstman et al. 2008; Stapleton et al. 2011). As has been previously discussed (Abdelouahab et al. 2013a; Chevrier 2013), these inconsistent findings may relate to several factors, including differences in source populations, covariate selection, timing of sample collection, assay design, and blood source (i.e. cord versus venous). Notably, previous studies have measured thyroid parameters in maternal blood (Abdelouahab et al. 2013b; Chevrier et al. 2010; Vuong et al. 2015) collected during pregnancy or delivery, cord blood (Abdelouahab et al. 2013b; Herbstman et al. 2008; Vuong et al. 2015), or infant blood (Chevrier et al. 2011; Herbstman et al. 2008) collected within hours to weeks of birth. To meet the demands of the developing fetus, thyroid system homeostasis changes drastically during pregnancy and parturition. For example, in response to an estrogen-induced elevation of thyroid binding globulin and placental production of chorionic gonadotrophin, maternal T_4 levels increase sharply and TSH levels fall during the first trimester. During delivery, a stress and cold-evoked surge in TSH occurs in the newborn, followed by a reflexive increase in T_4 over the next 24-48 hours (Braverman et al. 2013; Fisher and Klein 1981; Glinoer 1997). It is possible that the inconsistent findings observed across previous studies may be partially attributable to these time-dependent fluctuations in thyroid system functioning.

Although thyroid hormones have short half-lives (7-10 days), circulating levels within an individual are stable over time (Andersen et al. 2002; Braverman et al. 2013). This consistency is maintained by the hypothalamic-pituitary-thyroid (HPT) axis, which uses a negative feedback

mechanism to regulate circulating levels around an intra-individual set point. Briefly, low circulating T_3 and T_4 levels signal the hypothalamus to release thyroid regulating hormone (TRH), which triggers pituitary production of TSH. This hormone subsequently stimulates increased production and secretion of T_3 and T_4 (Braverman et al. 2013). While the molecular events involved in maturation of the HPT axis are incompletely understood, evidence from animal models and human clinical studies suggests that the setpoint around which this axis responds is programmed during gestation (Azizi et al. 1974; Bagattini et al. 2014; Cavaliere et al. 1985; Walker and Courtin 1985). It is plausible that prenatal disruption of this setpoint underlies our findings of an inverse association between BDE-47 with both TSH and free T_4. Specifically, our findings suggest that BDE-47 may play a role in lowering the HPT setpoint, thereby reducing hypothalamic and pituitary responsiveness to low T_4 levels.

The impact of prenatal PBDE exposure on TSH and free T_4 is further supported by our finding of similar effect sizes in models adjusting for childhood PBDE exposure and no direct association between childhood exposure and TSH or free T_4 levels. Conversely, we detected a positive, cross sectional association between BDE-47 and total T_4 measured in toddlers, the age period at which PBDE exposure peaked for the majority of children. Total T_4 reflects hormone bound to transport proteins and both animal and *in-vitro* studies indicate PBDEs may bind and displace T_4 from protein transporters, providing a potential mechanism underlying these observed results (Costa et al. 2014). We identified 5 cross-sectional studies that have examined childhood PBDE exposure in relation to thyroid parameters (Gascon et al. 2011; Jacobson et al. 2016; P Xu et al. 2014; X Xu et al. 2014). Similar to studies focused on pregnancy and infancy, results from studies examining childhood PBDE exposure have been inconsistent and difficult to compare

due to differences in study design, a wide range of concentrations, and variation in the distribution of PBDE congeners detected.

In addition to the longitudinal design, the present study has several strengths. First, PBDE concentrations were comparable to other geographically and temporally similar birth cohorts and reflect general population exposure (Cowell et al. 2015; Foster et al. 2011; Herbstman et al. 2007; Vuong et al. 2015). Additionally, our results were robust to several modeling choices, including our decision to express PBDEs on a lipid-standardized basis. Finally, we were able to examine many potential confounders, precision variables and effect measure modifiers, including demographics, lifestyle factors, and biomarkers of prenatal exposure to several endocrine-disrupting chemicals, none of which substantially altered our findings. Unfortunately, we were not able to evaluate selenium, which is known to be an important determinant of thyroid status (Arthur et al. 1999). Additional limitations include our lack of T_3 levels, thyroid binding protein levels, and PBDE metabolite data, which may more closely resemble endogenous thyroid hormones compared to parent congeners (Costa et al. 2014). Finally, we analyzed thyroid parameters by immunoassay, which may be affected by variation in serum thyroid binding protein levels (Braverman et al. 2013).

Conclusions

We detected an inverse association between cord plasma BDE-47 concentrations and childhood thyroid parameters (TSH and free T_4) among a cohort of African American and Dominican children. This is the first study to prospectively examine these relations and the largest North American study of cord blood PBDE concentrations. Our findings suggest that prenatal PBDE

may disrupt programming of the thyroid regulatory system resulting in disrupted thyroid hormone homeostasis that persists into childhood.

References

Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. 2013a. Abdelouahab et al. Respond to "maternal pbdes and thyroid hormones". American journal of epidemiology 178:720-721.

Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. 2013b. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. American journal of epidemiology 178:701-713.

Andersen S, Pedersen KM, Bruun NH, Laurberg P. 2002. Narrow individual variations in serum t(4) and t(3) in normal subjects: A clue to the understanding of subclinical thyroid disease. The Journal of clinical endocrinology and metabolism 87:1068-1072.

Arthur JR, Beckett GJ, Mitchell JH. 1999. The interactions between selenium and iodine deficiencies in man and animals. Nutr Res Rev 12:55-73.

Azizi F, Vagenakis AG, Bollinger J, Reichlin S, Braverman LE, Ingbar SH. 1974. Persistent abnormalities in pituitary function following neonatal thyrotoxicosis in the rat. Endocrinology 94:1681-1688.

Baccarelli A, Pfeiffer R, Consonni D, Pesatori AC, Bonzini M, Patterson DG, Jr., et al. 2005. Handling of dioxin measurement data in the presence of non-detectable values: Overview of available methods and their application in the seveso chloracne study. Chemosphere 60:898-906.

Bagattini B, Cosmo CD, Montanelli L, Piaggi P, Ciampi M, Agretti P, et al. 2014. The different requirement of l-t4 therapy in congenital athyreosis compared with adult-acquired hypothyroidism suggests a persisting thyroid hormone resistance at the hypothalamic-pituitary level. European journal of endocrinology / European Federation of Endocrine Societies 171:615-621.

Braverman LE, Cooper DS, Werner SC, Ingbar SH. 2013. Werner & ingbar's the thyroid : A fundamental and clinical text. 10th ed. Philadelphia:Wolters Kluwer/Lippincott Williams & Wilkins Health.

Cavaliere H, Medeiros-Neto GA, Rosner W, Kourides IA. 1985. Persistent pituitary resistance to thyroid hormone in congenital versus later-onset hypothyroidism. J Endocrinol Invest 8:527-532.

Chevrier J, Harley KG, Bradman A, Gharbi M, Sjodin A, Eskenazi B. 2010. Polybrominated diphenyl ether (pbde) flame retardants and thyroid hormone during pregnancy. Environmental health perspectives 118:1444-1449.

Chevrier J, Harley KG, Bradman A, Sjodin A, Eskenazi B. 2011. Prenatal exposure to polybrominated diphenyl ether flame retardants and neonatal thyroid-stimulating hormone levels in the chamacos study. American journal of epidemiology 174:1166-1174.

Chevrier J. 2013. Invited commentary: Maternal plasma polybrominated diphenyl ethers and thyroid hormones--challenges and opportunities. American journal of epidemiology 178:714-719.

Costa LG, de Laat R, Tagliaferri S, Pellacani C. 2014. A mechanistic view of polybrominated diphenyl ether (pbde) developmental neurotoxicity. Toxicology letters 230:282-294.

Cowell WJ, Lederman SA, Sjodin A, Jones R, Wang S, Perera FP, et al. 2015. Prenatal exposure to polybrominated diphenyl ethers and child attention problems at 3-7 years. Neurotoxicology and teratology 52:143-150.

Elmlinger MW, Kuhnel W, Lambrecht HG, Ranke MB. 2001. Reference intervals from birth to adulthood for serum thyroxine (t4), triiodothyronine (t3), free t3, free t4, thyroxine binding globulin (tbg) and thyrotropin (tsh). Clinical chemistry and laboratory medicine : CCLM / FESCC 39:973-979.

Fisher DA, Klein AH. 1981. Thyroid development and disorders of thyroid function in the newborn. The New England journal of medicine 304:702-712.

Foster WG, Gregorovich S, Morrison KM, Atkinson SA, Kubwabo C, Stewart B, et al. 2011. Human maternal and umbilical cord blood concentrations of polybrominated diphenyl ethers. Chemosphere 84:1301-1309.

Gascon M, Vrijheid M, Martinez D, Forns J, Grimalt JO, Torrent M, et al. 2011. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. Environment international 37:605-611.

Glinoer D. 1997. The regulation of thyroid function in pregnancy: Pathways of endocrine adaptation from physiology to pathology. Endocr Rev 18:404-433.

Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Patterson DG, Halden RU, et al. 2007. Determinants of prenatal exposure to polychlorinated biphenyls (pcbs) and polybrominated diphenyl ethers (pbdes) in an urban population. Environmental health perspectives 115:1794-1800.

Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, et al. 2008. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (pcb) and polybrominated diphenyl ether (pbde) and neonatal thyroid hormone levels. Environmental health perspectives 116:1376-1382.

Jacobson MH, Barr DB, Marcus M, Muir AB, Lyles RH, Howards PP, et al. 2016. Serum polybrominated diphenyl ether concentrations and thyroid function in young children. Environmental research 149:222-230.

Johns LE, Ferguson KK, McElrath TF, Mukherjee B, Meeker JD. 2016. Associations between repeated measures of maternal urinary phthalate metabolites and thyroid hormone parameters during pregnancy. Environmental health perspectives.

Jones R, Edenfield E, Anderson S, Zhang Y, Sjodin A. 2012. Semi-automated extraction and cleanup method for measuring persistent organic pollutants in human serum. Organohalogen Comp 74:97-98.

Leonetti C, Butt CM, Hoffman K, Miranda ML, Stapleton HM. 2016. Concentrations of polybrominated diphenyl ethers (pbdes) and 2,4,6-tribromophenol in human placental tissues. Environment international 88:23-29.

Pearce EN, Braverman LE. 2009. Environmental pollutants and the thyroid. Best Pract Res Clin Endocrinol Metab 23:801-813.

Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A, et al. 2004. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. Neurotoxicology and teratology 26:373-385.

Sjodin A, Jones RS, Lapeza CR, Focant JF, McGahee EE, 3rd, Patterson DG, Jr. 2004. Semiautomated high-throughput extraction and cleanup method for the measurement of polybrominated diphenyl ethers, polybrominated biphenyls, and polychlorinated biphenyls in human serum. Analytical chemistry 76:1921-1927.

Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. 2011. Associations between polybrominated diphenyl ether (pbde) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. Environmental health perspectives 119:1454-1459.

USEPA. 2010. An exposure assessment of polybrominated diphenyl ethers. EPA/600/R-08/086F. Washington, DC:U.S. Environmental Protection Agency.

Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, et al. 2015. Maternal polybrominated diphenyl ether (pbde) exposure and thyroid hormones in maternal and cord sera: The home study, cincinnati, USA. Environmental health perspectives 123:1079-1085.

Walker P, Courtin F. 1985. Transient neonatal hyperthyroidism results in hypothyroidism in the adult rat. Endocrinology 116:2246-2250.

Williams GR. 2008. Neurodevelopmental and neurophysiological actions of thyroid hormone. Journal of neuroendocrinology 20:784-794.

Xu P, Lou X, Ding G, Shen H, Wu L, Chen Z, et al. 2014. Association of pcb, pbde and pcdd/f body burdens with hormone levels for children in an e-waste dismantling area of zhejiang province, china. The Science of the total environment 499:55-61.

Xu X, Liu J, Zeng X, Lu F, Chen A, Huo X. 2014. Elevated serum polybrominated diphenyl ethers and alteration of thyroid hormones in children from guiyu, china. PloS one 9:e113699.

Zoeller RT. 2007. Environmental chemicals impacting the thyroid: Targets and consequences. Thyroid : official journal of the American Thyroid Association 17:811-817.

Supplemental Material

Prenatal Exposure to PBDEs and Serum Thyroid Parameters Measured during Childhood

Whitney J. Cowell, Andreas Sjödin, Richard Jones, Ya Wang, Shuang Wang, Robin M. Whyatt, Pam Factor-Litvak, Gary Bradwin, Abeer Hassoun, Sharon Oberfield, Julie B. Herbstman

Figure S1. Figure of the Directed Acyclic Graph illustrating relations between cord plasma BDE-47, serum thyroid parameters and measured covariates**.**

Table S1. Table of mean±SD PBDE and thyroid parameter concentrations for children included and excluded from the analysis.

Table S2. Table of mean±SD childhood thyroid hormone levels stratified by age at blood draw, n=205 children and 299 measurements.

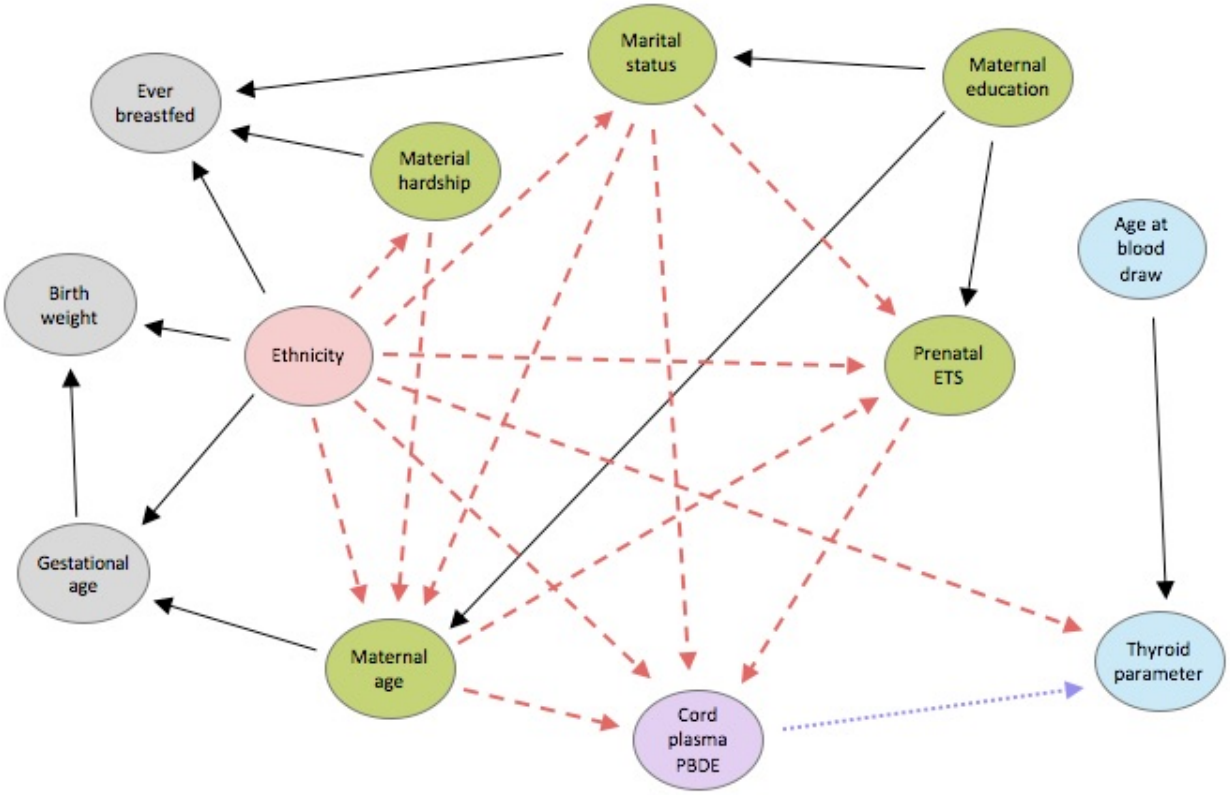
Figure S2. Results from models examining quartiles of cord plasma BDE-47 expressed on a wet weight basis, with and without adjustment for total blood lipids.

Figure S3. Associations between quartiles of prenatal BDE-47 exposure and childhood thyroid parameters adjusting for prenatal exposure to several suspected endocrine disrupting chemicals.

Figure S4. Results examining quartiles of cord plasma BDE-47 exposure and thyroid hormone parameters, adjusted for childhood BDE-47 exposure.

Figure S5. Figure of results from multiple linear regression models examining the relation between $\log_{10}$BDE-47 (ng/g lipid) and thyroid hormone parameters ($\log_{10}$TSH: μIU/mL; free T_4: ng/dL, total T_4: μg/dL) measured as continuous variables reported by six North American birth cohort studies.

Figure S1. Directed acyclic graph illustrating associations between cord plasma PBDEs, serum thyroid hormones and measured covariates.



The dotted purple line represents the causal path between exposure (PBDEs) and outcome (thyroid parameter concentration). Dashed red lines represent biasing paths. Green circles represent ancestors of the exposure, blue circles represent ancestors of the outcome, grey circles represent variables not on a biasing path but related to an ancestor of exposure, red circles represent ancestors of the exposure and the outcome. As illustrated, adjusting for ethnicity is sufficient to block biasing paths between the exposure and the outcome. We additionally examined sex and parity, however, these variables are excluded from the diagram as they were not associated with the exposure, the outcome, or any ancestor of these variables. Diagram generated in DAGitty v2.3 available at: http://www.dagitty.net/

Table S1. Mean±SD PBDE and thyroid parameter concentrations for children included and excluded from the analysis.

	Included	Excluded
Thyroid Parameters	(n=205 children, 299 observation)	(n=156 children, 214 observation)
TSH (μIU/mL)[a]	2.2±1.1	2.3±1.4
fT4 (pmol/L)	18.0±2.2	18.1±2.4
Total T4 (nmol/L)	142.5±28.2	144.4±29.0
PBDEs[a]	(n=205 children)	(n=122 children)
BDE-47	13.9±3.1	14.6±2.9
BDE-99	3.9±2.6	3.5±2.6
BDE-100	2.9±2.3	2.9±2.4
BDE-153	2.6±1.8	2.7±1.9

[a]Geometric mean and geometric standard deviation

Table S2. Mean±SD childhood thyroid parameter levels stratified by age at blood draw, n=205 children and 299 measurements.

Age[a] (years)	N	TSH (μIU/mL)	free T_4 (pmol/L)	total T_4 (nmol/L)
		GM±GSD[a]	Mean±SD	Mean±SD
3.1±0.2	150	2.4±1.2	18.3±2.2	140.6±25.3
5.0±0.1	73	2.1±1.1	18.1±2.3	145.4±31.8
7.1±0.2	67	2.1±0.9	17.6±2.1	144.5±30.8
9.1±0.2	9	1.7±1.2	16.5±2.0	129.9±19.0
Total	299	2.2±1.1	18.0±2.2	142.5±28.2

[a]GM: geometric mean; GSD: geometric standard deviation

Figure S2. Results from models examining quartiles of cord plasma BDE-47 expressed on a wet weight basis, with and without adjustment for total blood lipids. Markers represent beta coefficients and error bars represent 95% confidence intervals. Models are adjusted for age at blood draw and ethnicity. TSH is expressed as a $\log_{10}$ transformed variable.

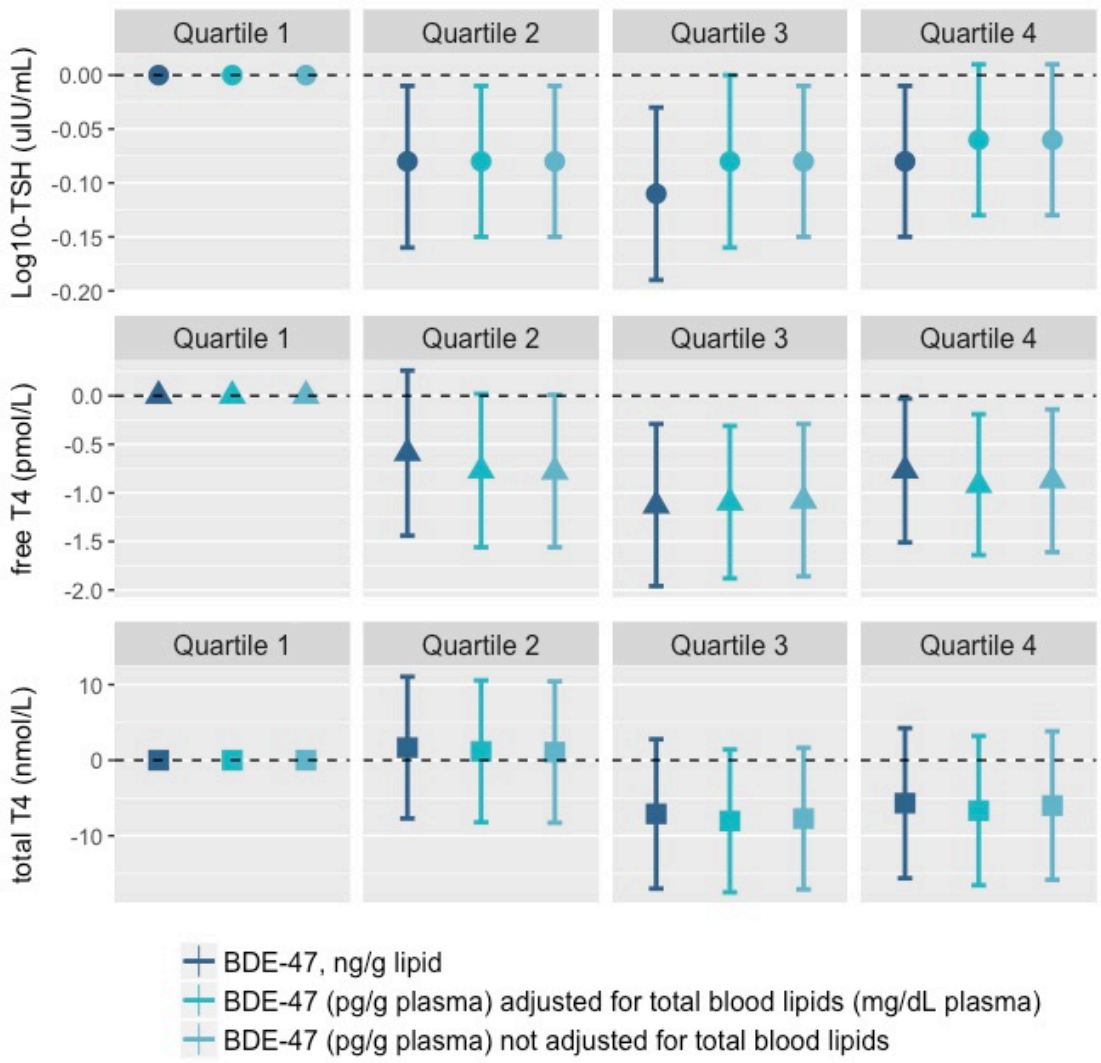
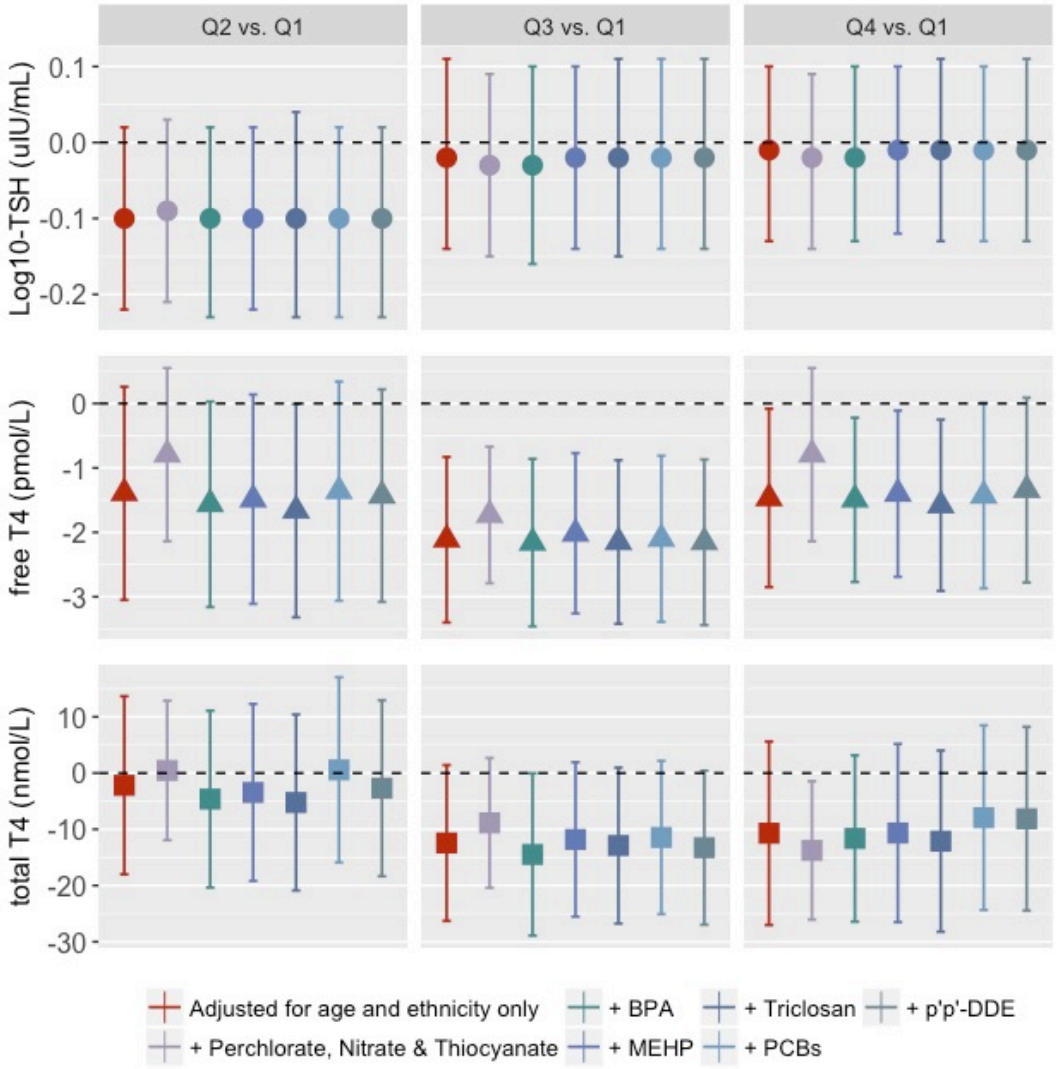
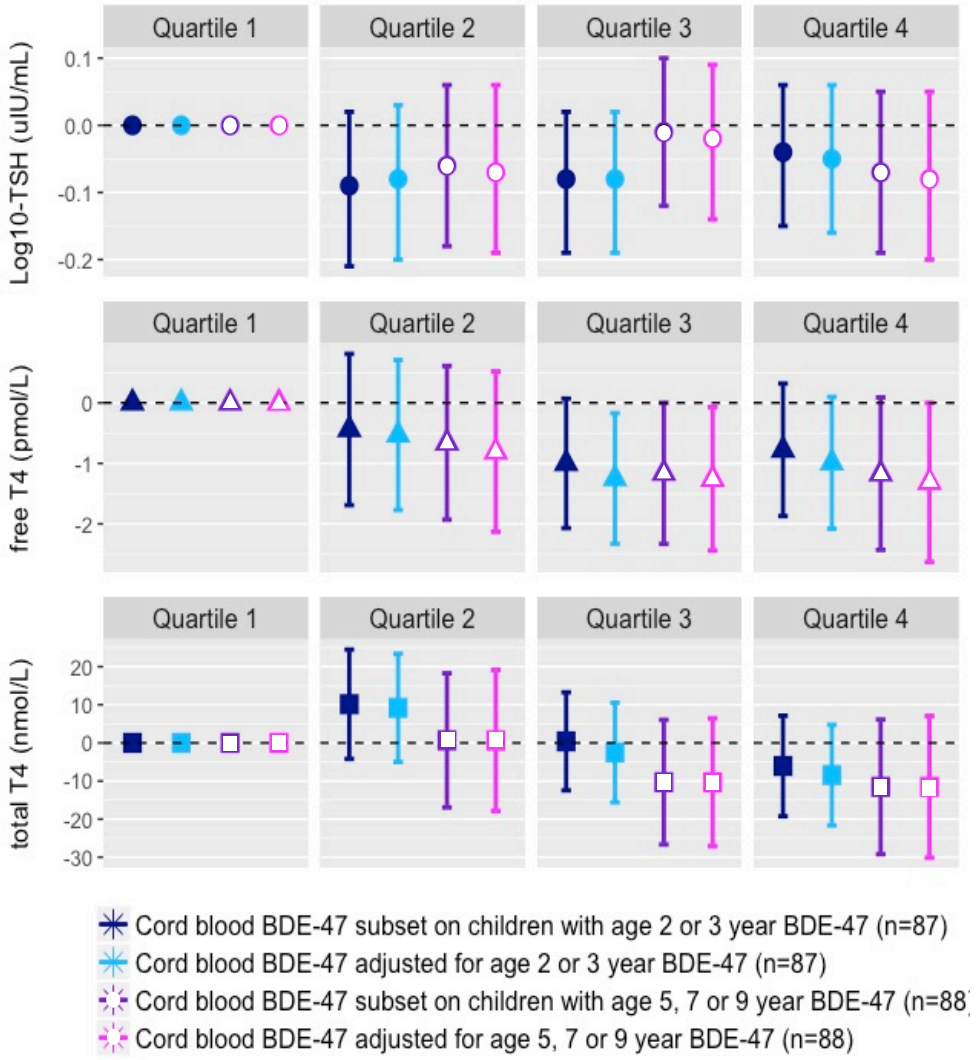


Figure S3. Associations between quartiles (Q) of prenatal BDE-47 (ng/g lipid) and childhood thyroid parameters adjusting for prenatal exposure to several suspected endocrine disrupting chemicals.



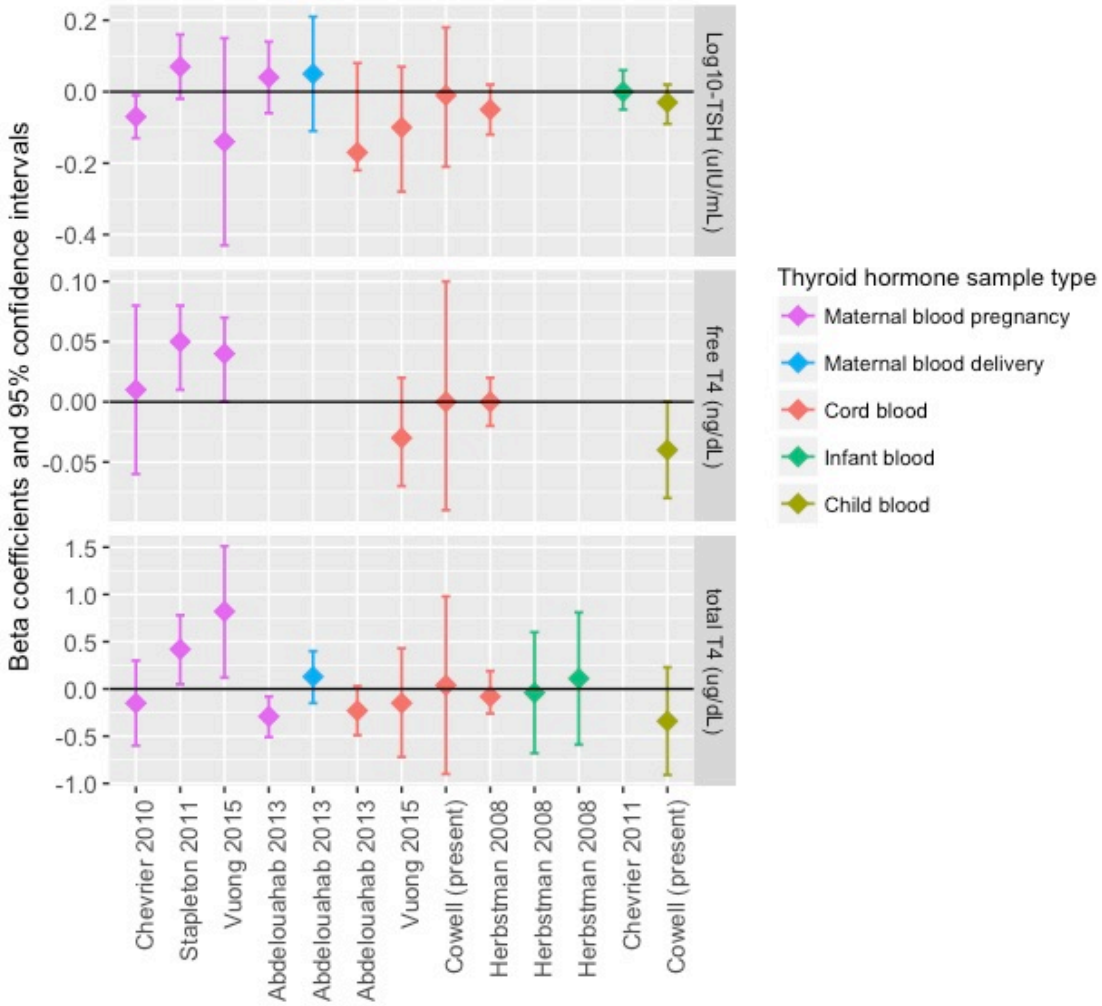
Sample includes n= 80 children (116 repeated thyroid measures) with data on all co-exposures. Markers represent beta coefficients and error bars represent 95% confidence intervals. All models are adjusted for age at blood draw and ethnicity; models examining BPA, MEHP, triclosan, and perchlorate, thiocyanate and nitrate are additionally adjusted for urine specific gravity. TSH is expressed as a $\log_{10}$ transformed variable.

Figure S4. Results examining quartiles of cord plasma BDE-47 (ng/g lipid) and thyroid hormone parameters, adjusted for childhood BDE-47 (ng/g lipid) exposure.



Markers represent beta coefficients and error bars represent 95% confidence intervals. Models are adjusted for age at blood draw and ethnicity. TSH is expressed as a $\log_{10}$ transformed variable.

Figure S5. Results from multiple linear regression models examining the relation between continuous log10BDE-47 (ng/g lipid) and thyroid parameters reported by six North American birth cohort studies.



BDE-47 was measured in maternal blood collected during pregnancy by all studies except Herbstman 2008 and the present study (measured in cord blood). Herbstman 2008 and Stapleton 2011 applied a natural-log transformation to BDE-47 and TSH rather than a $\log_{10}$-transformation. Stapleton 2011 additionally natural-log transformed free T_4. To facilitate comparison of our results to others, we re-ran our final models expressing free T_4 and total T_4 in units of ng/dL and µg/dL, respectively. Abdelouahab 2013 modeled cord blood free T_4 on a pmol/L basis, therefore, these results are excluded to accommodate the y-axis scale.

Supplemental material references

1. Chevrier J, Harley KG, Bradman A, Gharbi M, Sjodin A, Eskenazi B. Polybrominated diphenyl ether (PBDE) flame retardants and thyroid hormone during pregnancy. *Environ Health Perspect* 2010;**118**(10):1444-9.

2. Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. *Environ Health Perspect* 2011;**119**(10):1454-9.

3. Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, Sjodin A, Yolton K, Lanphear BP, Chen A. Maternal Polybrominated Diphenyl Ether (PBDE) Exposure and Thyroid Hormones in Maternal and Cord Sera: The HOME Study, Cincinnati, USA. *Environ Health Perspect* 2015;**123**(10):1079-85.

4. Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. *Am J Epidemiol* 2013;**178**(5):701-13.

5. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, Panny SR, Needham LL, Goldman LR. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. *Environ Health Perspect* 2008;**116**(10):1376-82.

6. Chevrier J, Harley KG, Bradman A, Sjodin A, Eskenazi B. Prenatal exposure to polybrominated diphenyl ether flame retardants and neonatal thyroid-stimulating hormone levels in the CHAMACOS study. *Am J Epidemiol* 2011;**174**(10):1166

CHAPTER 6: Associations between relative leukocyte telomere length in paired maternal-newborn samples and material hardship, perceived stress and demoralization

Whitney J. Cowell[a,b], Deliang Tang[a,b], Jie Yue[a,b], Lirong Qu[a,b], Julie B. Herbstman[a,b]

[a]Columbia Center for Children's Environmental Health, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032 USA

[b]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York, 10032, United States

Address correspondence to: Julie Herbstman, Columbia Center for Children's Environmental Health, 12th Fl, Mailman School of Public Health, 722 West 168th St, New York, NY 10032 USA. Telephone: (212) 304-7273. E-mail: jh2678@cumc.columbia.edu

Funding: This research was supported by: NIH R03ES026416 and NIH P50ES009600. During preparation of this manuscript, WJC was supported by NIH T32 ES023772, NIH T32 ES007322 and EPA FP-91779001.

Disclaimer: This publication was developed under STAR Fellowship Assistance Agreement no. FP-91779001 awarded by the U.S. Environmental Protection Agency (EPA). It has not been formally reviewed by EPA. The views expressed in this publication are solely those of the authors.

Keywords: telomere, cord blood, maternal-newborn pair, stress

Manuscript status: In preparation for submission to Pediatric and Perinatal Epidemiology

Abstract

Introduction: Telomeres are repetitive nucleotide sequences located at terminal chromosome ends. On average, telomere length shortens with age as a result of base pair loss with each mitotic cell division that occur across the lifespan. Substantial evidence supports the importance of early life in setting lifelong telomere dynamics, and points to a relationship whereby higher psychosocial stress is associated with shorter telomere length.

Methods: We investigated associations between maternal and newborn telomere length collected at delivery, as well as associations between telomere length and measures of financial strain, perceived stress and psychological distress experienced by the mother during her pregnancy or within 6-months of the child's birth. We measured relative leukocyte telomere length (rLTL) in paired maternal (venous blood) and newborn (cord blood) samples using monochrome multiplex quantitative polymerase chain reaction. This study was performed within a large, well-characterized and prospectively followed birth cohort.

Results: Maternal blood and cord blood rLTL were moderately correlated (partial Spearman ρ= 0.30, $p<0.001$) and maternal ln-rLTL explained 8% of the variability (R^2) in cord blood ln-rLTL. We did not detect an association between maternal or newborn rLTL and 1) material hardship, an indicator of financial strain, or 2) demoralization, an indicator of non-specific psychological distress. In contrast, we detected an inverse, albeit not statistically significant, association between maternal perceived stress and newborn rLTL, but not with maternal rLTL.

Conclusions: Our results examining perceived stress are consistent with previous research and suggest that telomere dynamics during gestation may be sensitive to maternal perceptions of stress.

Introduction

Telomeres are repetitive, non-coding hexameric nucleotide sequences (T_2AG_3 in vertebrates) located at terminal chromosome ends (Blackburn 1990). During cell division, chromosomes erode owing to limitations of DNA replication machinery, thus telomeres serve a self-sacrificing role against damage and degradation of protein coding regions (Bonetti et al. 2013). On average, leukocyte telomere length shortens with age as a result of the continuous mitotic cell divisions that occur across the lifespan. Several large epidemiological studies indicate that shorter telomere length is associated with age-adjusted increased mortality, as well as earlier onset of several diseases of aging (reviewed by: D'Mello et al. 2015). Likewise, research conducted in birds, a species that is commonly used in telomere research owing to its moderate lifespan—a relevant and useful feature for studying the biological impact of aging--has demonstrated that telomere length measured in early life predicts lifespan (Heidinger et al. 2012). In humans, as newborn telomere length decreases, susceptibility to developing chronic disease in later life significantly increases (Biron-Shental et al. 2016). These findings have sparked widespread scientific interest in understanding whether telomere length at a given moment during the lifespan reflects the cumulative biologic toll, or cellular 'wear and tear', that progressively and inevitably occurs with age.

Telomere length is highly heritable (~70%), however, genetic variants identified through candidate gene and genome-wide association studies indicate only a small proportion of inter-individual difference in telomere length is attributable to genetic variability (Broer et al. 2013). For example, healthy children with normal telomerase born to parents with defective telomerase, an enzyme capable of extending telomere length that is expressed by stem and progenitor cells,

have shorter telomeres at birth compared to children with parents that have normal functioning telomerase (Aubert and Lansdorp 2008; Collopy et al. 2015).

In adults, mounting evidence links shorter telomere length with experiences of socioeconomic deprivation, stress and psychiatric disorders (primarily depression) (Darrow et al. 2016; Lindqvist et al. 2015). Many of these studies, which are largely cross-sectional by design, have concluded that shorter telomere length in adulthood is attributable to adversities experienced during early life. For example, young adults born to mothers who experienced a severe stressor during pregnancy (n=45), assessed by retrospective maternal-report, were found to have significantly shorter telomeres compared to young adults born to mothers who had a healthy, uneventful pregnancy (n=49) (Entringer et al. 2011).

Despite mounting evidence supporting the importance of early life in setting telomere length across the lifespan, few studies have investigated maternal and newborn telomeres in relation to maternal stress and adversity experienced during pregnancy. In the present study, we investigated associations between telomere length in paired maternal-newborn samples collected at delivery. We additionally investigated associations between telomere length and measures of self-reported financial strain, perceived stress and psychological distress.

Materials and Methods

Study population

This study was conducted among a subset of the 727 maternal-child pairs enrolled in the Columbia Center for Children's Environmental Health (CCCEH) Mothers and Newborns birth

cohort, which recruited pregnant women from two prenatal clinics in Northern Manhattan between 1998 and 2006 (Perera et al. 2006; Whyatt et al. 2002). Women were excluded if they were carrying multiples (e.g., twins, triplets), were outside the ages of 18-35, initiated prenatal care after the 20th week of pregnancy, used tobacco products or illicit drugs, or had diabetes, hypertension or HIV. All newborns were healthy at birth.

Study interview

During the third trimester of pregnancy, trained research workers conducted a structured interview with the mother to ascertain information on demographic, socioeconomic and lifestyle factors. Research workers additionally extracted information related to the pregnancy and delivery (birthweight, gestational age, delivery mode) from maternal and newborn medical records. We calculated maternal pre-pregnancy body mass index (BMI) in kg/m^2 using maternal report of pre-pregnancy weight and height. As previously described, we validated self-reported height against measures extracted from maternal medical records and measures taken at postnatal study visits (Widen et al. 2016). Information on exposure to environmental tobacco smoke during pregnancy was assessed by maternal report of smokers living in the home, as well as by maternal and cord blood concentrations of cotinine, a short half-life biomarker that reflects recent exposure to tobacco smoke. In cases where either cord (n=17) or maternal (n=21) cotinine was missing, we imputed one from the other using a regression model as previously described (Rauh et al. 2004).

Stress is a multidimensional construct in which objective stressors are appraised and subsequently yield emotional responses (Lobel and Dunkel-Schetter 1990). Structural equation modeling suggests a single latent construct does not underlie these objective, perceptive and

emotional dimensions of stress, supporting evaluation of each as a distinct exposure (Lobel et al. 1992). In the present study, we operationalized stress using scales that span these dimensions. We examined objective stress using an index of material hardship adapted from a survey designed to compare poverty and hardship distributions in urban environments; specific questions related to unmet basic needs in the areas of food, housing and clothing (Mayer and Jencks 1988). We evaluated maternal stress appraisal using the 4-item Cohen's Perceived Stress Scale (PSS-4), which was administered within 6-months of the child's birth. The PSS-4 is a validated self-report instrument that provides a subjective measure of how stressful one finds her life over the preceding month across the dimensions of unpredictability, uncontrollability and overload; it is completed by answering how often (never to very often) each of four items applies using a 5-point scale (Cohen et al. 1983). While the PSS-4 was not administered until the 6-month follow-up visit, repeated measures collected during the child's early life (12, 24, 36 months) indicate that scores are generally stable across time ($R_{Pearson}$: 6-months vs. 12-months = 0.51, 6-months vs. 24-months = 0.48, 6-months vs. 36-months = 0.32, all $p<0.0001$) in this cohort, suggesting that stress perceptions during pregnancy may be reasonably captured by data collected at the 6-month follow-up visit. Additionally, while we administered only the 4-item version during early life, the full 14-item version (PSS-14) was administered to mothers at 5- and 7-year follow-up visits. PSS-4 scores at the 6-month visit were moderately correlated with the PSS-14 scores administered at these later ages ($R_{Pearson}$: 5 years: 0.45, 7 years: 0.46, $p<0.0001$). We examined maternal demoralization using the Psychiatric Epidemiology Research Instrument Demoralization scale (PERI-D), which was completed during the prenatal study visit. The PERI-D is a validated self-report instrument designed to measure nonspecific psychological distress across eight domains, including anxiety, sadness, psychophysiological symptoms, perceived

physical symptoms, poor self-esteem, hopelessness-helplessness, confused thinking and dread (Dohrenwend et al. 1981). The scale includes 27 items that are answered based on the degree to which they apply (never to very often) and scored by averaging the score across all items resulting in a possible range from 0 to 4, with higher scores indicating greater demoralization.

Blood collection, processing and storage

Immediately following delivery, blood was collected from the umbilical cord (30-60 ml) by a member of the research staff; within two days of the child's birth, maternal blood was collected by venipuncture. Buffy coat was separated from whole blood by centrifugation, processed, and stored at -70^{C} in the CCCEH laboratory space. Genomic DNA (100-500 ng) was isolated from leukocytes using the standard phenol-chloroform extraction protocol.

Telomere measurement

We measured relative leukocyte telomere length (rLTL) using multiplex monochrome quantitative polymerase chain reaction (MMqPCR) on a BioRad CFX96 Lightcycler (Life Science Group, Hercules, CA, USA). All paired maternal-cord samples were assayed on the same plate. In addition to experimental samples, each plate contained a 6-point standard curve derived from serially diluted reference DNA (0.625-150 ng), a calibrator sample comprised of pooled DNA, and a no template control (water). All experimental and control samples were assayed in triplicate. The primers for the telomere PCR were *telc*: 5′-TGTTAGGTA$(TCCCTA)_5$ACA-3′ and *telg*: 5′-ACACTAAGG$(TTT\ GGG)_4$TTAGTG T-3′, each used at a final concentration of 600 nM (Life Technologies). The primers for the single-copy gene (human albumin) were *albd*: 5′-

GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAGCATGGTCGCCTGTT-3′ and *albu*: 5′-CGGCGGCGGGCGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG-3′, each used at a final concentration of 250 nM (Life Technologies). The final reaction mixture was comprised of 12.5 µl of 1x iQ SYBR Green Supermix (BioRad), 6.4 µl water, and 5 µl of genomic DNA at a concentration of 2 ng/µl. The thermal cycling profile consisted of: stage 1) 1 cycle at 95°C for 3 min, stage 2) 2 cycles of 94°C for 15 sec and 49°C for 15 sec, and stage 3) 32 cycles of 94°C for 15 sec, 62°C for 10 sec, 74°C for 10 sec (with telomere signal acquisition), 84°C for 10 sec, 88°C for 10 sec (with albumin signal acquisition).

We divided the telomere (T) copy number by albumin (S) copy number to calculate the well-specific T/S ratio before taking the mean across triplicates. The T/S ratio is a unitless measure proportional to the average telomere length across all chromosomes for all cells sampled. If one triplicate did not produce results or if the triplicate failed quality assurance/quality control (QA/QC) criteria, defined as a triplicate coefficient of variation (CV) greater than 1.5 times the interquartile range of triplicates across all samples, we averaged the results from the remaining two wells (n=13 cord blood samples, n=20 maternal samples). If two of the three triplicates did not produce results or failed QA/QC, we excluded the sample from statistical analyses.

The inter-plate cycle threshold (C_t) and copy number (C_n) CVs for the pooled 'calibrator' sample included on each plate were 1.2% and 10.6%, respectively. Across plates, the rLTL for the calibrator sample ranged from 0.54 to 0.89 with a mean±standard deviation of 0.74±0.08. To reduce batch variability, we calculated a plate-specific normalizing factor by dividing the

'calibrator' sample on a given plate by the mean of calibrator samples across all plates. We then standardized experimental rLTLs by this plate-specific 'normalizing' factor.

Total WBCs are a heterogeneous group of nucleated cells and telomere length has been shown to vary in these different cell types (Robertson et al. 2000, Rufer et al. 1999). We used an algorithm for estimating cell proportions in cord blood based on genome-wide DNA methylation data (Koestler et al. 2013, Bakulski 2016), which we previously measured in a subset of our samples (n=98) using the Illumina Infinium 450K HumanMethylation array. We did not find that cell distribution was a significant predictor of rLTL using a storage time-adjusted multivariable linear regression model excluding granulocytes (p-values for each cell type in model: B cells (0.56), CD4T cells (0.44), CD8T cells (0.95), monocytes (0.24), natural killer cells (0.79), nucleated red blood cells (0.39)). Methylation data have not been measured in maternal samples, therefore, we were unable to examine associations between cell type and maternal rLTL.

As indicated by NanoDrop 260/280 absorbance ratios, 98% of samples exceeded the generally accepted threshold (1.8) for DNA purity; the remaining 3 samples had a ratio of 1.7. Previous research has demonstrated that storage conditions and other pre-analytical conditions can impact telomere length as measured by qPCR (Dagnall et al. 2017; Reichert et al. 2017; Tolios et al. 2015). No changes to our study protocol changed over the 8-year cohort enrollment period (1998-2006), however, we found that rLTL was non-linearly associated with date of sample collection, such that rLTL increased steeply during the first two years of the study (5% increase in rLTL per month between 1998 and 1999, $p<0.001$), after which the slope flattened and became relatively constant in maternal samples and showed a small, linear increase in cord blood

samples (see Supplemental Material, Figure S2). Given this finding, we excluded samples collected during the first two years of the study (n=46) from all statistical analyses and *a priori* adjusted regression models by storage time as a continuous variable.

Statistical analysis

We examined descriptive statistics for maternal and cord blood rLTL and visualized distributions using boxplots and histograms. We calculated Spearman correlation coefficients between maternal and newborn rLTL and visualized this relationship using a scatter plot. We examined the percent of variability in cord blood rLTL attributable to maternal rLTL (R^2) by regressing ln-transformed cord blood rLTL on ln-transformed maternal rLTL.

We examined associations between rLTL and several demographic and lifestyle characteristics that have been previously related to adult and/or cord blood rLTL, including maternal age (continuous, in days), ethnicity (African American vs. Dominican), pre-pregnancy BMI (>25 kg/m^2 vs. ≤ 25 kg/m^2), presence of a smoker in the home (yes vs. no), maternal or cord blood cotinine (continuous, in ng/ml), child sex, gestational age (weeks), birthweight (grams), mode of delivery (vaginal vs. cesarean section), and parity (multiparous vs. nulliparous).

We used linear regression to examine associations between ln-transformed maternal or newborn rLTL with material hardship (2 or 3 vs. 0 or 1 hardships), maternal psychological distress (fourth quartile of PERI-D scores vs. lower three quartiles), and maternal perceived stress (fourth quartile of PSS-4 scores vs. lower three quartiles), in separate models adjusted for storage time. We additionally examined maternal rLTL models adjusting for age at delivery, ethnicity and pre-

pregnancy BMI, each of which was marginally statistically significantly related to rLTL in bivariate models (Table 1). To investigate whether maternal experiences may indirectly affect fetal telomere length without affecting maternal telomere length, we examined newborn rLTL in models adjusting for maternal rLTL. We additionally adjusted this model for birthweight, which was marginally statistically significantly associated with cord blood rLTL. We investigated the influence of outlying points by examining models excluding observations with a rLTL greater than 1.5 times the interquartile range above the 75th percentile of ln-transformed values (maternal blood n=2, cord blood n=2).

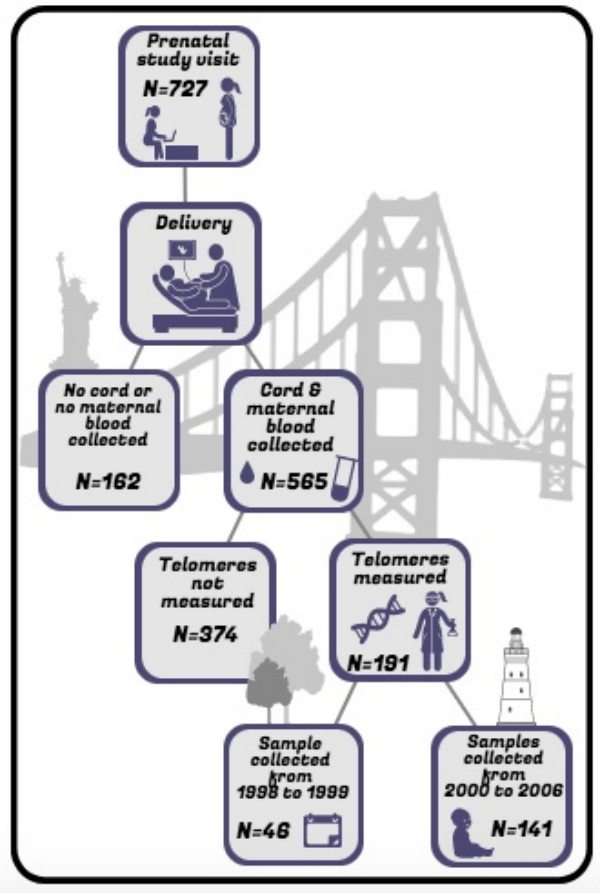
Results

Participant characteristics

This analysis includes rLTL measured in 145 paired maternal (venous) and newborn (umbilical cord) blood samples collected between 2000 and 2006 (Figure 1). We excluded four of these 145 pairs from final models due to missing information on covariates. All maternal-newborn pairs were African American (33%) or Dominican (67%). At delivery, mothers were, on average, 25 years of age, 40% had less than a high school education, 33% reported living with a cigarette smoker, and 21% reported experiencing high material hardship, defined as two or more unmet basic needs in the areas of food, clothing, and housing. Before becoming pregnant, 49% of mothers were overweight or obese (BMI > 25 kg/m^2). Three children were born prematurely (at 36 weeks gestation), however, none of these newborns were classified as low birthweight based on a threshold of 2,500 grams. Maternal demoralization scores ranged from 0 to 3.1 (maximum possible: 4) with a mean±standard deviation score of 1.2±0.7 and perceived stress scores ranged from 0 to 14 (maximum possible: 16) with a mean±standard deviation score of 5.1±3.6. On

average, children included in the analysis were born two days later (p=0.05) and weighted 144 grams more at birth (p<0.001) compared to children enrolled in the cohort but not included in the present analysis. Included children did not significantly differ from excluded children by any other sociodemographic, lifestyle or stress variables investigated.

Figure 1. Overview of study design and sample selection.

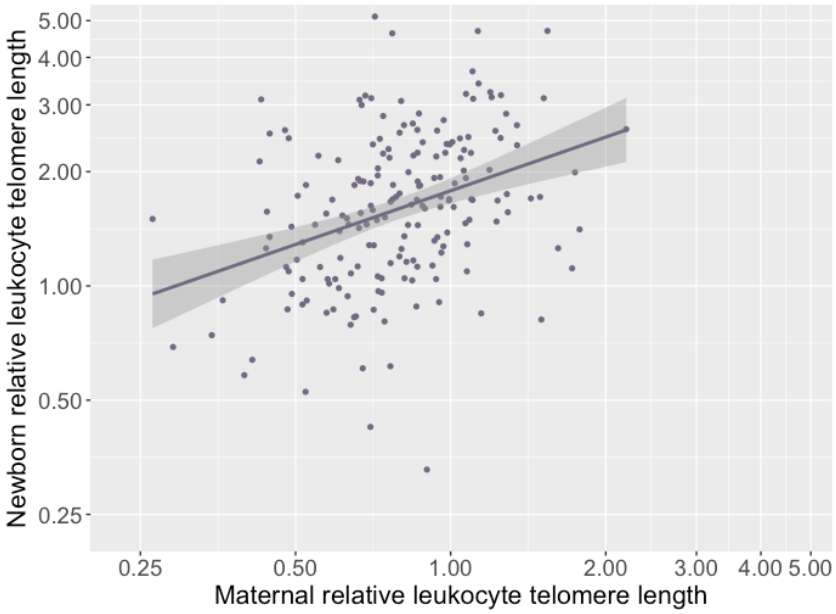


Maternal and cord blood rLTL at delivery

Relative LTL measured in maternal and cord blood samples was approximately log-normally distributed (see Supplemental Material, Figure S1). As expected, rLTL was generally longer and more variable in cord blood (storage-time adjusted geometric mean and geometric mean standard

deviation: aGM±GSE: 1.83±1.04, range 0.32-5.12) compared to maternal blood (0.81±1.03, range: 0.26-2.19). Maternal blood and cord blood rLTL were moderately correlated (partial Spearman ρ= 0.30, $p<0.001$); maternal ln-rLTL at delivery explained 8% of the variability (R^2) in cord blood ln-rLTL (**Figure 2**).

Figure 2. Relationship between maternal (venous) versus newborn (cord blood) rLTL collected at the time of delivery.



Relative LTL in relation to physical and sociodemographic variables

Table 1 presents storage time-adjusted geometric mean rLTLs for mothers and newborns stratified by several sociodemographic and other participant characteristics. Maternal age was inversely associated with maternal rLTL (0.3% decrease per year, 95% CI: -1.4, 0.7); the oldest 25% of mothers in the cohort (30-36 years at delivery) had rLTLs that were approximately 7% shorter (95% CI: -20.3, 9.1) compared to the youngest 25% of mothers (18-20 years); however,

these associations were not statistically significant at the p=0.05 level. On average, rLTL was approximately 8% shorter (95% CI: -17.6, 3.4) among African American compared to Dominican mothers. We observed no difference by ethnicity (p=0.35) among newborns. Mothers who were overweight or obese (BMI ≥ 25, n=69) before pregnancy had rLTLs that were approximately 6% (95% CI: -15.3, 4.8) shorter compared to healthy (n=68) and underweight (n=7) mothers. For each unit increase in maternal pre-pregnancy BMI, maternal rLTL decreased by 0.7% (95% CI: -1.6, 0.1) and newborn rLTL decreased by 0.9% (95% CI: -1.9, 0.2).

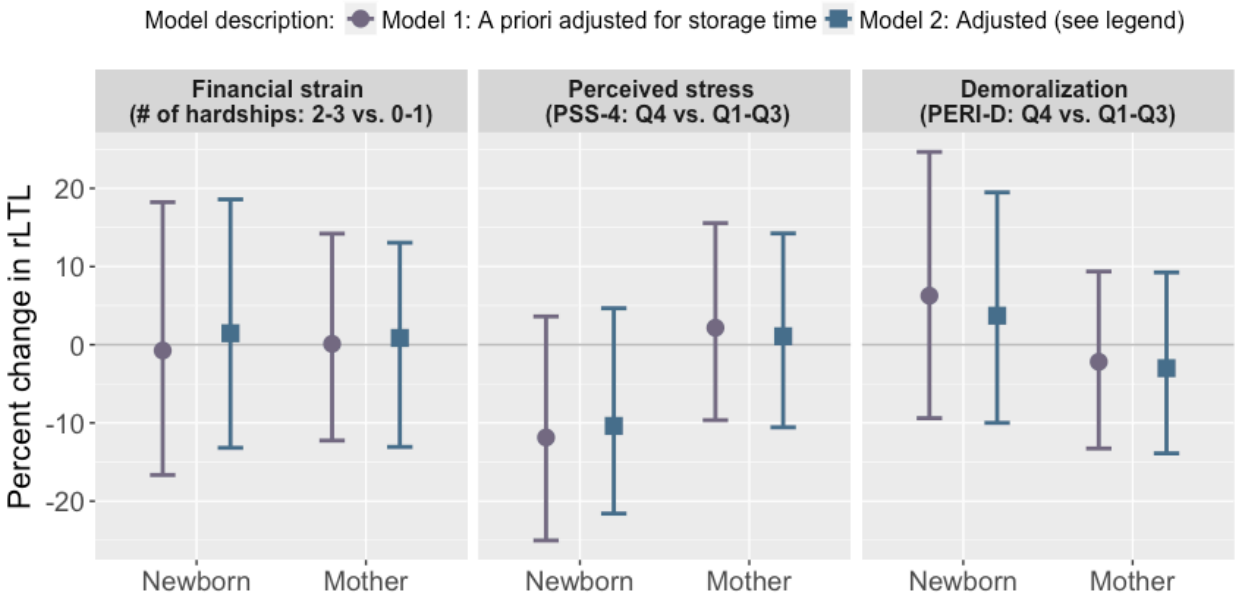
Maternal rLTL did not vary by maternal level of education (less than high school vs. high school degree or equivalent) (p=0.54), however, newborns born to mothers with less than a high school degree had 16% (95% CI: -27.1, -3.0) shorter rLTL compared to newborns born to mothers with a high school degree. We observed no differences in rLTL by smoker living in the home (mother: p=0.46, newborn: p=0.89) or cotinine level (mother: p=0.86, newborn: p=0.26) among mothers or newborns. Among newborns, each additional kilogram of birthweight was associated with 13.5% shorter rLTL (95% CI: -26.6, 2.0). Cord blood rLTL was approximately 16% shorter (95% CI: -29.5, 1.3) among newborns born to multiparous versus nulliparous mothers, however, this finding was attenuated (8% shorter, 95% CI: -21.1, 6.1) when adjusted for birth weight, which was on average 127 grams greater among newborns born to multiparous mothers (p=0.08). Adjusting for age did not change the results between parity and rLTL in models with or without birthweight. Newborn rLTL did not significantly vary by mode of delivery (vaginal vs. cesarean section) (p=0.89), gestational age (weeks) (p=0.42) or sex (p=0.63).

Associations between rLTL and financial strain, perceived stress and psychological distress

We did not detect statistically significant associations between material hardship, maternal perceived stress or maternal demoralization in relation to maternal or newborn rLTL (see Figure 3 and Supplemental Material, Table S1). In adjusted (maternal rLTL, education, birthweight) models, newborns born to mothers with high perceived stress scores (quartile 4) had rLTLs that were approximately 13% shorter (95% CI: -25.7, 1.3), compared to newborns born to mothers with low scores (quartiles 1-3). This association was attenuated, yet still large (percent change: -9.8, 95% CI: -21.9, 4.4) in models excluding 2 newborns with outlying rLTLs. Excluding these newborns did not substantially change the direction or magnitude of effects across other models (Supplemental Material, Table S1).

Table 1. Geometric mean±geometric standard error[a] rLTL stratified by sociodemographic and other characteristics of maternal-child pairs in the CCCEH cohort (n=141)			
	N	Mother	Newborn
All	141	0.81±1.03	1.83±1.04
Ethnicity			
African American	47	0.77±1.05	1.74±1.07
Dominican	94	0.83±1.04	1.88±1.05
Age (years)			
18-20	34	0.81±1.06	1.84±1.08
21-24.5	38	0.82±1.05	1.86±1.07
24.5-29	37	0.84±1.06	1.75±1.08
30-36	32	0.76±1.06	1.87±1.08
Body mass index			
< 25 mg/kg^2	72	0.83±1.04	1.91±1.05
≥ 25 mg/kg^2	69	0.78±1.04	1.75±1.06
Parity			
Nulliparous	61	0.83±1.06	1.93±1.06
Multiparous	79	0.79±1.04	1.73±1.06
Maternal education			
< high school	57	0.79±1.05	1.65±1.06
≥ high school	84	0.82±1.04	1.96±1.05
Sex			
Girls	78	—	1.86±1.05
Boys	63	—	1.79±1.06
Gestational age (weeks)			
< 39	26	—	1.79±1.09
≥ 39	115	—	1.84±1.04
Birthweight (grams)			
≤ 3190	35	—	1.96±1.08
3191 -3480	38	—	1.82±1.08
3481-3818	33	—	1.78±1.11
> 3818	35	—	1.77±1.08
Material hardship			
Yes (2-3 hardships)	30	0.81±1.06	1.82±1.09
No (0-1 hardships)	111	0.81±1.03	1.83±1.04
Perceived stress			
Low/moderate (Q_1-Q_3)	104	0.80±1.04	1.90±1.09
High (Q_4)	37	0.82±1.06	1.67±1.09
Demoralization			
Low/moderate (Q_1-Q_3)	102	0.81±1.04	1.80±1.05
High (Q_4)	39	0.80±1.05	1.91±1.07
[a]All geometric means adjusted for storage time (years)			

Figure 3. Percent change in maternal and newborn rLTL by high vs. low material hardship, maternal perceived stress and maternal demoralization.



Model 2: maternal model adjusted for storage time, maternal age at delivery, ethnicity and pre-pregnancy BMI; newborn model adjusted for storage time, birthweight, maternal level of education, and maternal rLTL.

Discussion

In the present study, we investigated rLTL in samples collected from maternal-newborn pairs in relation to objective, perceptive and emotional measures of stress. We detected no association between maternal or newborn rLTL and material hardship or demoralization-- an indicator of non-specific psychological distress. Interestingly, while we did not detect an association with material hardship, we found that rLTL was significantly lower (-16%, 95% CI: -27.1, -3.0) among newborns born to mothers with less than a high school education, an indicator of low socioeconomic status, compared to mothers with a high school degree or more. This is consistent with previous research examining parental education in relation to telomere length in children. For example, Needham et al. found that children (n=70) ages 7-13 years old of parents who never attended college had significantly shorter (1,178 base pairs, p=0.01) telomeres compared to children with at least one college educated parent (Needham et al. 2012).

We detected an inverse, albeit not statistically significant, association between maternal perceived stress and newborn rLTL. This finding is consistent with results reported by Send et al. who found that maternal perceived stress, measured using the PSS-14 administered during the third trimester, was associated with significantly shorter rLTL in cord blood collected from 318 newborns, but not in saliva collected from mothers at the time of delivery (Send et al. 2017). Likewise, a meta-analysis of 8,724 individuals from 22 studies detected only a small inverse association between perceived stress and age-adjusted telomere length in adults (Mathur et al. 2016). Three smaller studies have reported similar findings between maternal pregnancy-specific stress (n=27) (Entringer et al. 2013), perceived stress (n=71) (Salihu et al. 2016), or experiences of negative life events (n=24) (Marchetto et al. 2016) during or in the year prior to pregnancy

and shorter newborn telomere length.

Our finding of an association between maternal perceived stress and newborn rLTL, independent of maternal rLTL, as well no association between stress and maternal rLTL suggests that maternal stress may indirectly affect the developing fetus (versus direct transmission of maternal stress-induced shortened germline telomeres). Indirect transmission is supported by the results of experimental research conducted in birds. For example, Haussmann et al. showed that chicks exposed to elevated glucocorticoids (cortisol in humans, corticosterone in birds) during embryogenesis via direct injection of corticosterone into egg yolks had shorter telomeres and higher baseline plasma reactive oxygen metabolite levels after hatching compared to control chicks (Haussmann et al. 2015). A similar study showed that mother zebra finches exposed to glucocorticoids during egg production had higher levels of reactive oxygen metabolites, accelerated telomere attrition and hatched chicks with shorter telomeres compared to control birds (Tissier et al. 2014). While this later finding suggests that maternally-transferred hormone levels can impact telomere dynamics during embryogenesis, it also leaves open the possibility of direct maternal transmission. Research using a cross-fostering design in which telomere length could be measured directly in maternal gametes would be required to more fully understand the pathways underlying the intergenerational transmission of stress-induced shortened telomeres. Notably, both studies found glucocorticoid exposure was associated with greater reactive oxygen metabolites, suggesting that the observed telomere attrition may be in part driven through glucocorticoid-mediated oxidative stress pathways. Telomeres (TTAGGG) are known to be particularly susceptible to damage by reactive oxygen species, which preferentially bind to guanines nucleotides (von Zglinicki 2002).

Our finding of a marginally statistically significant association between higher maternal pre-pregnancy BMI and lower maternal rLTL (0.7% decrease per BMI unit increase, 95% CI: -1.6, 0.1) is consistent with results of previous research showing BMI, percent body fat and waist circumference were significantly inversely associated with rLTL in a sample of obese women (n=54) (Shin and Lee 2016). Likewise, the association we observed with newborn rLTL (0.9% decrease per BMI unit increase, 95% CI: -2.0, 0.2) is consistent with results from a large cohort study (n=743) that found an inverse association (0.7% decrease per unit BMI increase, 95% CI: -2.0, 0.2) between maternal pre-pregnancy BMI and cord blood rLTL (Martens et al. 2016). Obesity is associated with higher chronic oxidative stress, for example, as indicated by increased lipid peroxidation products among obese individuals (Szokalska et al. 2016; Zelzer et al. 2011). This suggests that oxidative stress pathways may underlie associations between psychosocial stress and decreased telomere length. Newborn birthweight was inversely associated with rLTL and this association remained marginally significant after adjusting for parity, pre-pregnancy BMI and maternal rLTL in separate models.

With regard to demographic variables, we did not observe significant differences in rLTL by age, sex or ethnicity, however, the inverse direction of the maternal age-maternal rLTL association was consistent with the general trend of average telomere shortening across the lifespan. While longer telomere lengths have been consistently observed among adult women compared to men (Gardner 2014), findings in newborns have been less consistent, suggesting that the telomere length by sex divergence in adults may reflect differential telomere erosion. For example, in a cohort (n=165) of primarily black and Hispanic (68%) newborns, Okuda et al. detected no

association between sex and LTL measured by southern blotting (Okuda et al. 2002). Conversely, in a cohort (n=318) of primarily white infants (86%), Send et al found that telomeres were significantly longer in female compared to male newborns (Send et al. 2017). Similarly, patterns between telomere length and race/ethnicity have varied across studies. In our cohort, rLTL was generally shorter among African American mothers and newborns compared to Dominican mothers and newborns; however, these associations were not statistically significant. This is consistent with results from a large multiethnic cohort of adults, which found blacks and Hispanics had shorter rLTLs compared to whites, but no difference in rLTL when compared to each other (Diez Roux et al. 2009). Likewise, Okuda et al. did not detect differences in telomere length between black, white and Hispanic newborns (Okuda et al. 2002). In contrast, among 5,360 adults enrolled in the National Health and Nutrition Examination Survey, African American participants had significantly longer rLTLs compared to Mexican-Americans and whites (Needham et al. 2015).

Strengths of this study include the relatively large sample size compared to the majority of previous research examining gestational stress in relation to newborn telomere length (Entringer et al. 2013; Marchetto et al. 2016; Salihu et al. 2016). Additionally, the only other large study of paired maternal-newborn rLTLs was conducted in a largely white population (Send et al. 2017); the present study extends this research to a minority population. While we were able to investigate the influence of many sociodemographic and other lifestyle characteristics, we did not have information on paternal age, which is a well-established determinant of newborn telomere length, likely due to the upregulation of telomerase in sperm that occurs with age (Aviv and Susser 2013).

We assessed objective and emotional dimensions of stress collected during the prenatal period, however, our measure of perceived stress was not ascertained until 6 months following the child's birth. While the correlation between PSS-4 scores from repeatedly administered exams between 6 months and 36 months were moderate to high in this cohort, it is possible that some mothers were misclassified as having high stress during the prenatal visit or vice versa. Given this important limitation, the inverse association we observed between PSS-4 scores and newborn rLTL should be interpreted with caution.

We measured rLTL in samples that had been stored between 10 and 18 years. As described, rLTL measured in samples collected during the earliest two years of the study (1998 and 1999) was significantly associated with date of collection. To our knowledge, no major study protocols or laboratory conditions changed during the first two study years compared to the later six years, giving rise to the possibility that the non-random variation by date of collection that we observed may relate to the longer storage duration of the earliest samples (i.e. a storage time threshold effect). Multiple studies have been conducted with the goal of investigating the influence of pre-analytic variables on telomere length (Cunningham et al. 2013; Raschenberger et al. 2016; Tolios et al. 2015), however, few have examined the influence of long-term (>5 years) storage (Dagnall et al. 2017; Reichert et al. 2017). If long-term storage does impact telomere length, this would have important implications for future longitudinal research aiming to investigate telomere length in archived samples. Due to the potential for batch effects, it is common practice in longitudinal studies to run all samples collected within an individual on the same plate, which requires the oldest samples to be stored, leading to collinearity of within-subject storage duration

and chronological aging over time. While it is possible to statistically adjust for storage time, when age-related change (i.e. telomere erosion) is the variable of interest, a statistical conundrum arises if variability introduced by storage duration co-varies with the chronological age-related changes of interest. While little evidence is available that concretely indicates storage time is a concern when analyzing telomere length by qPCR, our results suggest researchers planning to use archived samples should consider the possibility of non-random variation by long-term storage.

In conclusion, our study supports previous research showing that maternal perceived stress during pregnancy may be inversely associated with telomere length among newborns, however, our findings should be interpreted with caution as our measure of perceived stress was not collected until 6 months following the child's birth. Telomere length at birth and attrition during early childhood are thought to be the most important determinants of telomere length in adulthood. Given our findings, this pattern suggests programs and interventions designed to support mothers and minimize stress and stress perception during pregnancy may have lasting impacts for her developing child.

References

Aubert G, Lansdorp PM. 2008. Telomeres and aging. Physiol Rev 88:557-579.

Aviv A, Susser E. 2013. Leukocyte telomere length and the father's age enigma: Implications for population health and for life course. Int J Epidemiol 42:457-462.

Bakulski KM, Feinberg JI, Andrews SV, Yang J, Brown S, S LM, et al. 2016. DNA methylation of cord blood cell types: Applications for mixed cell birth studies. Epigenetics 11:354-362.

Benetos A, Kark JD, Susser E, Kimura M, Sinnreich R, Chen W, et al. 2013. Tracking and fixed ranking of leukocyte telomere length across the adult life course. Aging Cell 12:615-621.

Biron-Shental T, Liberman M, Elbaz M, Laish I, Sharony R, Amiel A. 2016. Telomere homeostasis in placentas from pregnancies with uncontrolled diabetes. Placenta 44:13-18.

Blackburn EH. 1990. Telomeres: Structure and synthesis. J Biol Chem 265:5919-5921.

Bonetti D, Martina M, Falcettoni M, Longhese MP. 2013. Telomere-end processing: Mechanisms and regulation. Chromosoma.

Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, et al. 2013. Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. Eur J Hum Genet 21:1163-1168.

Cohen S, Kamarck T, Mermelstein R. 1983. A global measure of perceived stress. Journal of health and social behavior 24:385-396.

Collopy LC, Walne AJ, Cardoso S, de la Fuente J, Mohamed M, Toriello H, et al. 2015. Triallelic and epigenetic-like inheritance in human disorders of telomerase. Blood 126:176-184.

Cunningham JM, Johnson RA, Litzelman K, Skinner HG, Seo S, Engelman CD, et al. 2013. Telomere length varies by DNA extraction method: Implications for epidemiologic research. Cancer Epidemiol Biomarkers Prev 22:2047-2054.

D'Mello MJ, Ross SA, Briel M, Anand SS, Gerstein H, Pare G. 2015. Association between shortened leukocyte telomere length and cardiometabolic outcomes: Systematic review and meta-analysis. Circ Cardiovasc Genet 8:82-90.

Dagnall CL, Hicks B, Teshome K, Hutchinson AA, Gadalla SM, Khincha PP, et al. 2017. Effect of pre-analytic variables on the reproducibility of qpcr relative telomere length measurement. PloS one 12:e0184098.

Darrow SM, Verhoeven JE, Revesz D, Lindqvist D, Penninx BW, Delucchi KL, et al. 2016. The association between psychiatric disorders and telomere length: A meta-analysis involving 14,827 persons. Psychosomatic medicine 78:776-787.

Diez Roux AV, Ranjit N, Jenny NS, Shea S, Cushman M, Fitzpatrick A, et al. 2009. Race/ethnicity and telomere length in the multi-ethnic study of atherosclerosis. Aging Cell 8:251-257.

Dohrenwend BP, Dohrenwend BS, Warheit GJ, Bartlett GS, Goldsteen RL, Goldsteen K, et al. 1981. Stress in the community: A report to the president's commission on the accident at three mile island. Annals of the New York Academy of Sciences 365:159-174.

Entringer S, Epel ES, Kumsta R, Lin J, Hellhammer DH, Blackburn EH, et al. 2011. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proceedings of the National Academy of Sciences of the United States of America 108:E513-518.

Entringer S, Epel ES, Lin J, Buss C, Shahbaba B, Blackburn EH, et al. 2013. Maternal psychosocial stress during pregnancy is associated with newborn leukocyte telomere length. Am J Obstet Gynecol 208:134 e131-137.

Haussmann MF, Longenecker AS, Marchetto NM, Juliano SA, Bowden RM. 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. Proc Biol Sci 279:1447-1456.

Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB, Monaghan P. 2012. Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences of the United States of America 109:1743-1748.

Koestler DC, Christensen B, Karagas MR, Marsit CJ, Langevin SM, Kelsey KT, et al. 2013. Blood-based profiles of DNA methylation predict the underlying distribution of cell types: A validation analysis. Epigenetics 8:816-826.

Lindqvist D, Epel ES, Mellon SH, Penninx BW, Revesz D, Verhoeven JE, et al. 2015. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neurosci Biobehav Rev 55:333-364.

Lobel M, Dunkel-Schetter C. 1990. Conceptualizing stress to study effects on health: Environmental, perceptual, and emotional components. Anxiety Research 3:213-230.

Lobel M, Dunkel-Schetter C, Scrimshaw SC. 1992. Prenatal maternal stress and prematurity: A prospective study of socioeconomically disadvantaged women. Health Psychol 11:32-40.

Marchetto NM, Glynn RA, Ferry ML, Ostojic M, Wolff SM, Yao R, et al. 2016. Prenatal stress and newborn telomere length. Am J Obstet Gynecol 215:94 e91-98.

Martens DS, Plusquin M, Gyselaers W, De Vivo I, Nawrot TS. 2016. Maternal pre-pregnancy body mass index and newborn telomere length. BMC Med 14:148.

Mathur MB, Epel E, Kind S, Desai M, Parks CG, Sandler DP, et al. 2016. Perceived stress and telomere length: A systematic review, meta-analysis, and methodologic considerations for advancing the field. Brain Behav Immun 54:158-169.

Mayer S, Jencks C. 1988. Poverty and the distribition of material hardship. J Hum Resour:88-112.

Needham BL, Fernandez J, Lin J, Epel ES, Blackburn EH. 2012. Socioeconomic status and cell aging in children. Social Science and Medicine. 74:1948-1951.

Needham BL, Mezuk B, Bareis N, Lin J, Blackburn EH, Epel ES. 2015. Depression, anxiety and telomere length in young adults: Evidence from the national health and nutrition examination survey. Molecular psychiatry 20:520-528.

Okuda K, Bardeguez A, Gardner JP, Rodriguez P, Ganesh V, Kimura M, et al. 2002. Telomere length in the newborn. Pediatric research 52:377-381.

Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, et al. 2006. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. Environmental health perspectives 114:1287-1292.

Raschenberger J, Lamina C, Haun M, Kollerits B, Coassin S, Boes E, et al. 2016. Influence of DNA extraction methods on relative telomere length measurements and its impact on epidemiological studies. Sci Rep 6:25398.

Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A, et al. 2004. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. Neurotoxicology and teratology 26:373-385.

Reichert S, Froy H, Boner W, Burg TM, Daunt F, Gillespie R, et al. 2017. Telomere length measurement by qpcr in birds is affected by storage method of blood samples. Oecologia 184:341-350.

Robertson JD, Gale RE, Wynn RF, Dougal M, Linch DC, Testa NG, et al. 2000. Dynamics of telomere shortening in neutrophils and t lymphocytes during ageing and the relationship to skewed x chromosome inactivation patterns. Br J Haematol 109:272-279.

Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, et al. 1999. Telomere fluorescence measurements in granulocytes and t lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory t cells in early childhood. J Exp Med 190:157-167.

Salihu HM, King LM, Nwoga C, Paothong A, Pradhan A, Marty PJ, et al. 2016. Association between maternal-perceived psychological stress and fetal telomere length. South Med J 109:767-772.

Send TS, Gilles M, Codd V, Wolf I, Bardtke S, Streit F, et al. 2017. Telomere length in newborns is related to maternal stress during pregnancy. Neuropsychopharmacology 42:2407-2413.

Shin YA, Lee KY. 2016. Low estrogen levels and obesity are associated with shorter telomere lengths in pre- and postmenopausal women. J Exerc Rehabil 12:238-246.

Szokalska K, Stepniak J, Karbownik-Lewinska M. 2016. Lipid peroxidation evaluated in epidermis exfoliated during microdermabrasion is a reliable marker of oxidative stress related to obesity. J Eur Acad Dermatol Venereol 30:1429-1431.

Tissier ML, Williams TD, Criscuolo F. 2014. Maternal effects underlie ageing costs of growth in the zebra finch (taeniopygia guttata). PloS one 9:e97705.

Tolios A, Teupser D, Holdt LM. 2015. Preanalytical conditions and DNA isolation methods affect telomere length quantification in whole blood. PloS one 10:e0143889.

von Zglinicki T. 2002. Oxidative stress shortens telomeres. Trends Biochem Sci 27:339-344.

Whyatt RM, Camann DE, Kinney PL, Reyes A, Ramirez J, Dietrich J, et al. 2002. Residential pesticide use during pregnancy among a cohort of urban minority women. Environmental health perspectives 110:507-514.

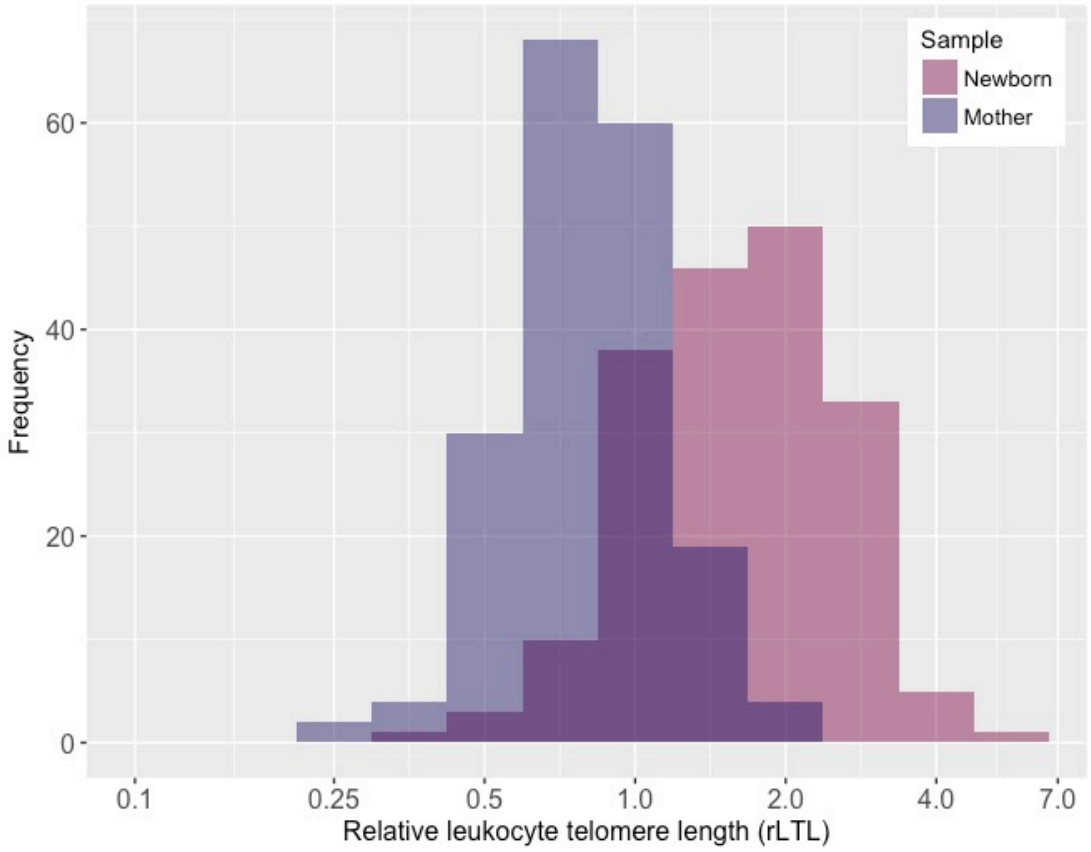
Widen EM, Whyatt RM, Hoepner LA, Mueller NT, Ramirez-Carvey J, Oberfield SE, et al. 2016. Gestational weight gain and obesity, adiposity and body size in african-american and dominican children in the bronx and northern manhattan. Matern Child Nutr 12:918-928.

Zelzer S, Fuchs N, Almer G, Raggam RB, Pruller F, Truschnig-Wilders M, et al. 2011. High density lipoprotein cholesterol level is a robust predictor of lipid peroxidation irrespective of gender, age, obesity, and inflammatory or metabolic biomarkers. Clin Chim Acta 412:1345-1349.

Supplemental Material

Table of Contents

Supplemental Figure S1. Distribution of newborn and maternal rLTL.



Supplemental Table S1. Percent change[a] (95% confidence interval) in paired maternal and newborn ln-rLTL by level of financial strain, perceived stress and demoralization (n=141)

	Material hardships (2-3 vs. 0-1)	Perceived stress (Q_4 vs Q_{1-3})	Demoralization (Q_4 vs Q_{1-3})
Maternal (venous blood)			
Model 1[b]	0.11 (-12.25, 14.21)	2.17 (-9.65, 15.55)	-2.18(-13.27, 9.37)
Model 2[c]	0.87 (-13.07, 13.05)	1.08 (-10.56, 14.24)	-3.00 (-13.89, 9.25)
Model 3[d]	-0.84 (-12.27, 12.09)	0.81 (-10.08, 13.03)	-3.18 (-13.36, 7.58)
Newborn (cord blood)			
Model 1[b]	-0.74 (-16.66, 18.23)	-11.86 (-25.03, 3.62)	6.27 (-9.39, 24.66)
Model 2[c]	-1.28 (-16.75, 17.06)	-12.9 3 (-25.40, 1.62)	5.46 (-9.57, 23.00)
Model 3[d]	1.47 (-13.18, 18.59)	-10.38 (-21.59, 4.67)	3.72 (-9.99, 19.49)

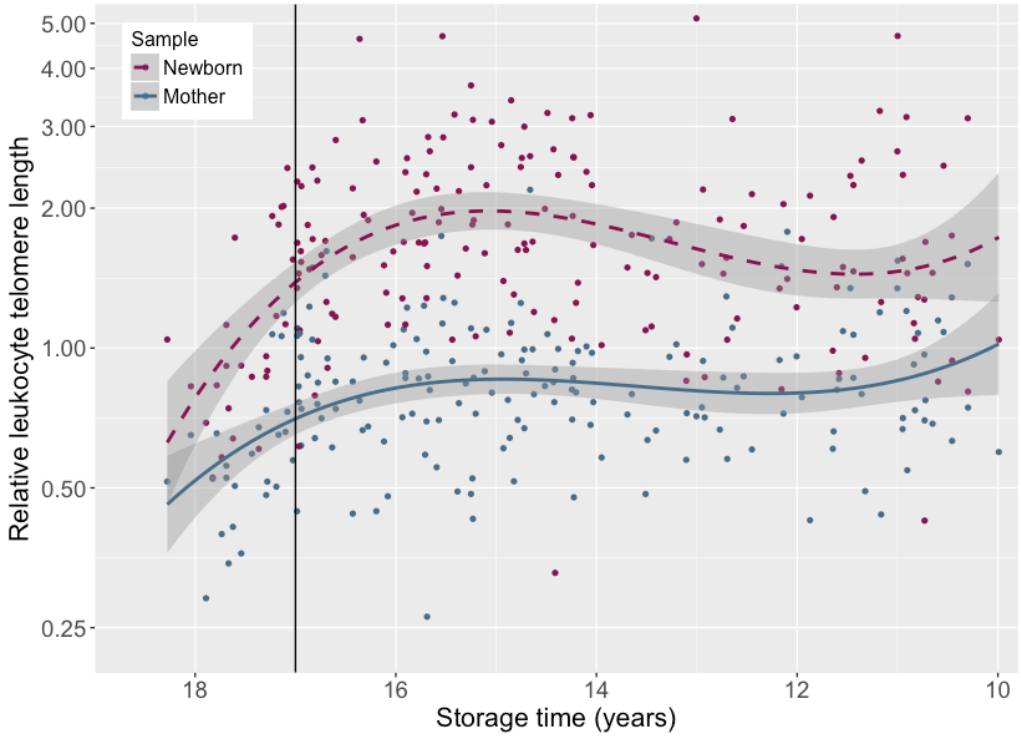
[a]Percent change calculated as: Positve: $\left(\left(e^{\beta}\right)-1\right)\times 100$; Negative: $\left(1-\left(e^{\beta}\right)\right)\times 100$

[b]Model 1: *A priori* adjusted for storage time

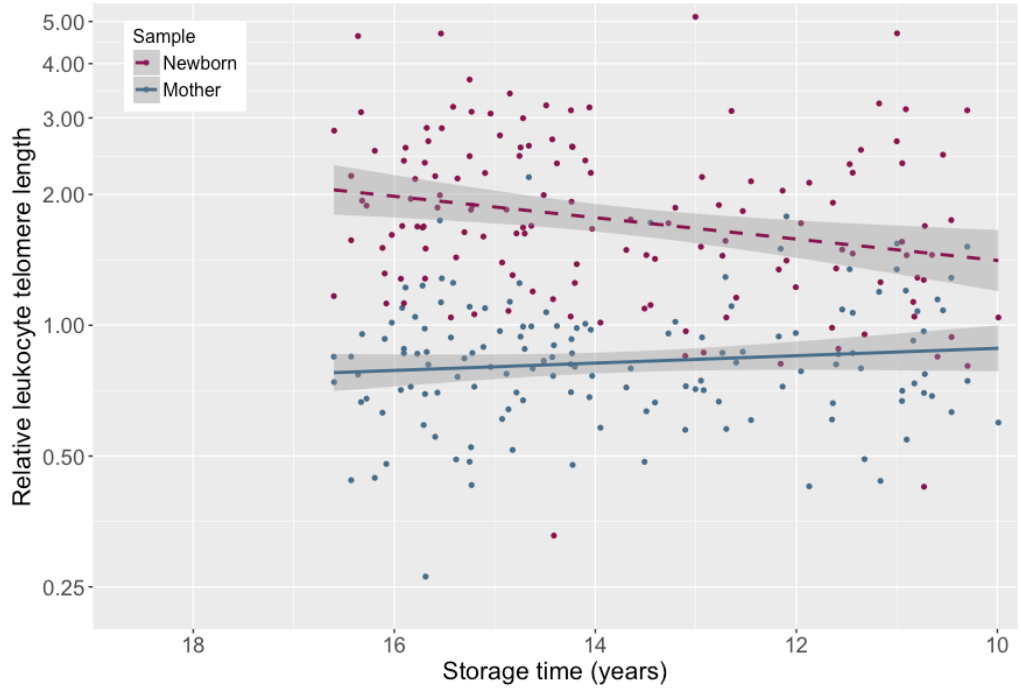
[c]Model 2: Maternal model adjusted for storage time, age, ethnicity and pre-pregnancy BMI; newborn model adjusted for storage time, birthweight, maternal education and maternal rLTL.

[d]Model 3: Adjusted models excluding outlying rLTL values: newborn (n=2), maternal (n=2)

Supplemental Figure S2a. Relative LTL by storage time modeled as a cubic polynomial.



Supplemental Figure S2b. Relative LTL by storage time modeled as a linear variable, excluding samples collected between 1998 and 1999.



CONCLUSION AND FUTURE DIRECTIONS

We conducted the research presented in Chapters 2 through 6 to address the unifying question: "Can telomeres be used as a biomarker of cumulative 'wear and tear' indicative of disease susceptibility in environmental epidemiology studies examining neurotoxicant exposure and associated developmental outcomes?" We operationalized this research project into three specific aims and seven corresponding hypotheses. The main results of these aims and hypotheses, as well as challenges associated with fully completing Aims 2 and 3, are summarized in the following pages.

KEY FINDINGS FROM PROJECT I: PBDE EXPOSURE OVER THE EARLY LIFECOURSE IN RELATION TO NEUROENDOCRINE OUTCOMES

Our research on prenatal and postnatal exposure to PBDEs contributes new information on important determinants of exposure, including modifiable factors such as cleaning behaviors. The work additionally contributes an improved understanding of how exposure changes during early life, as well as how body burdens in young children have changed since the phase-out of these relatively long half-life compounds. Lastly, this work adds to the growing number of studies linking PBDE exposure with neuroendocrine disruption. The aims of Project I were 1) to test the hypothesis that in samples collected between birth and age 9 years, PBDE detection frequencies would be nearly 100% and concentrations would peak during toddler years (Specific Aim 1.1); 2) to test the hypothesis that high exposure to PBDEs during prenatal and/or postnatal development would be associated with reduced memory performance among children (Specific Aim 1.2); and 3) to test the hypothesis that prenatal, but not postnatal, exposure to PBDEs would be associated with decreased T_4 and increased TSH levels during childhood.

Our analysis of determinants of cord blood PBDE concentrations (Chapter 2) showed that over 80% of newborns born in New York City between 1998 and 2006 were exposed to PBDEs during gestation. We found that African American newborns had significantly higher cord blood concentrations compared to Dominican infants, likely due the generally greater amount of time African American mothers had lived in the United States before giving birth, where PBDE concentrations are the highest in the world. Cord plasma PBDE concentrations decreased only modestly between 1998 and 2006, indicating that despite their phase-out in 2004, fetal exposure due to maternal body burdens is likely to continue for years owing to the lipophilic properties of

these chemicals.

Our longitudinal investigation of prenatal and postnatal plasma PBDE concentrations (Chapter 3) is the largest and highest resolution study of PBDE exposure during childhood. The study contributes to our understanding of how PBDEs are changing across both time and age. We found that over a 15-year period between 1998 and 2013, concentrations generally decreased independent of child age. Consistent with our hypothesis that children would be ubiquitously exposed, we detected pentaBDE concentrations in 100% of plasma samples collected between the ages of 2 and 9 years.

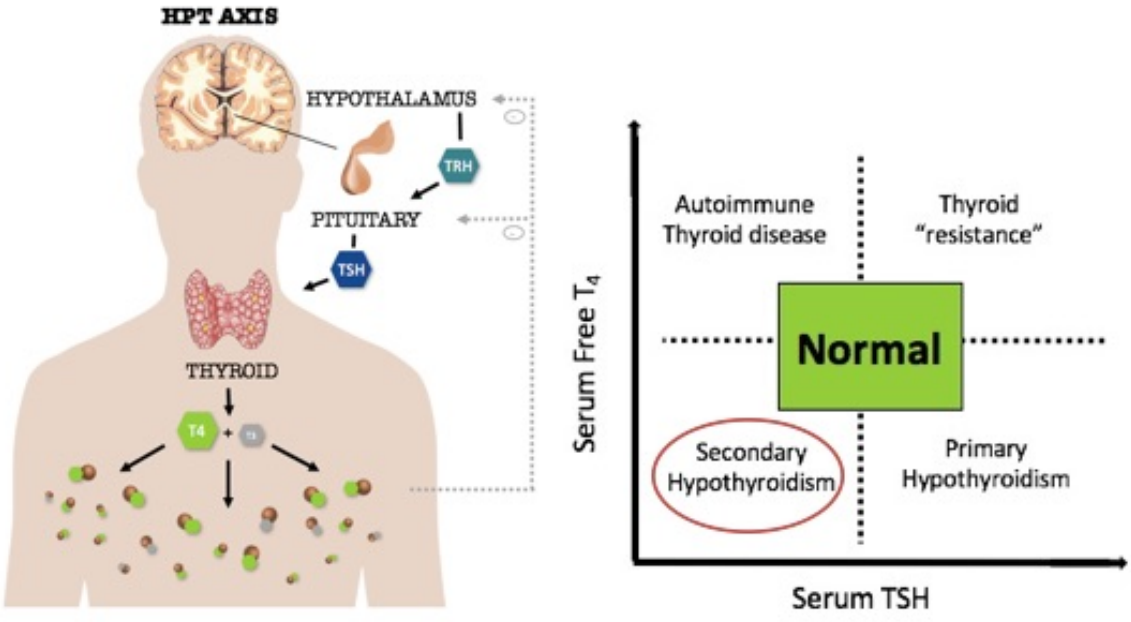
Additionally, we used LCGA, also known as group-based trajectory modeling (GBTM), to estimate trajectories of PBDE exposure across early life. LCGA is a statistical approach that was developed in the fields of criminology and econometrics (Nagin 2005; Nagin 2014). It has been used extensively by psychologists and child developmental scientists, however, despite its applicability to exposure science and environmental epidemiology, it has not been widely used in these fields. The approach allows researchers to model changes in exposure over time and is robust to variation in timing of exposure between subjects within a follow-up period, as well as to missing data across follow-up periods. Here, we demonstrate how this approach can be used in the environmental health sciences to study determinants of exposure patterns, as well as exposure patterns in relation to distal health outcomes. We estimated several different trajectories of PBDE exposure during childhood. Consistent with our hypothesis, the majority of children were characterized by either low exposure throughout early life or exposure that peaked during toddler years. We further found that several modifiable factors predicted patterns of exposure during early life, thereby providing information that may be used by researchers studying and/or implementing interventions to reduce exposure.

Our investigation of PBDE trajectories in relation to early adolescent memory domains (Chapter 4) aimed to tease apart the importance of PBDE exposure timing. Several previous studies have found associations between prenatal or postnatal exposure to PBDEs and disrupted neurodevelopmental outcomes (across both cognitive and behavioral domains), however, few studies have measured concentrations at both time periods. We aimed to better understand whether exposure during gestation, when the brain undergoes its most rapid development, is as or more important than exposure during early childhood- a period of continued brain development and also the timing of peak exposure for the majority of children. Our results suggest that different domains of the developing brain may be most sensitive to disruption by PBDE exposure during prenatal (working memory) and postnatal (visual memory) periods. Further, we detected a significant interaction between prenatal PBDE exposure and sex on working memory outcomes, highlighting the importance of investigating sex-specific effects when investigating exposure to developmental neurotoxicants.

Our analysis of prenatal exposure to PBDEs in relation to thyroid hormone levels measured repeatedly during childhood (Chapter 5) is the first study to prospectively investigate these associations. Previous research in humans has measured thyroid hormone parameters in cord blood or maternal blood collected during pregnancy or delivery, with conflicting results. Our prospective design is advantageous as thyroid hormone levels during pregnancy and parturition are known to fluctuate widely, which may introduce measurement error in studies measuring thyroid hormone levels during these periods. Based on findings in animal research, as well as the negative feedback mechanism through which circulating thyroid hormone are regulated (Figure

1), we hypothesized that PBDE exposure would be associated with decreased T_4 levels and increased TSH levels.

Figure 1. Overview of the HPT axis and feedback relationship between TSH and free T_4



However, we found that prenatal BDE-47 exposure was associated with lower levels of both T_4 and TSH. Although concentrations in our study were generally within the healthy range of thyroid hormone levels, this pattern is consistent with a 'secondary hypothyroidism' phenotype, in which dysfunction occurs at the level of the hypothalamus or pituitary (Zoeller et al 2007). As illustrated by Figure 1, circulating thyroid hormones are regulated under the control of the HPT axis, which responds to low levels of T_3 and T_4 by stimulating hypothalamic and pituitary production of TSH. The set point at which this negative feedback mechanism responds is thought to be programmed during gestation (Fisher and Klein 1981), thus it is possible that prenatal PBDE exposure interferes with prenatal programming of this setpoint, resulting in dysregulated thyroid hormone levels that persist after birth.

KEY FINDINGS FOR PROJECT II: CHARACTERIZATION OF TELOMERE DYNAMICS OVER THE EARLY LIFECOURSE AND ASSOCIATIONS WITH HARDSHIP, PERCEIVED STRESS AND PSYCHOLOGICAL DISTRESS

Our goals for the project investigating telomere dynamics across early life were: 1) to characterize baseline variation (cord blood) and patterns of leukocyte telomere length change between birth and age 9 years, as well as to understand how newborn telomere length relates to maternal telomere length at the time of delivery (Specific Aim 2.1); 2) to test the hypothesis that elevated maternal stress during pregnancy would be associated with shorter maternal and newborn telomere length in samples collected at delivery (Specific Aim 2.2); and 3) to test the hypothesis that elevated maternal stress during the child's early life would be associated with greater telomere erosion between birth and age 9 years (Specific Aim 2.3).

In Chapter 6, we present results examining telomere length measured in maternal and newborn samples collected at the time of birth in relation to measures of objective and perceptive stress and emotional distress. As hypothesized, we detected high variability in rLTL measured in cord blood samples collected from newborns (aGM±GSE:1.83±1.04, range: 0.3 to 5.1). Consistent with our hypotheses, we found that higher maternal perceived stress was associated (marginally statistically significant) with shorter cord blood rLTL and that this association was not affected by adjusting for maternal rLTL. However, contrary to our hypothesis, we did not detect associations between maternal perceived stress and maternal rLTL, or between material hardship or maternal demoralization and newborn or maternal rLTL. These findings suggest maternal stress perception may affect telomere dynamics in the developing fetus, and that this may occur via indirect transmission (i.e. relating to conditions of the *in-utero* environment) rather than through direct effects on maternal germline telomere length prior to conception.

In addition to the n=141 cord and maternal blood samples presented in Chapter 6, we analyzed rLTL in an additional n=113 cord blood samples and n=85 maternal sample. These participants were excluded from the analyses conducted in Chapter 6 due to missing measures of stress. The purpose of analyzing these samples was to increase the sample size for analyses examining change in rLTL over time. In addition to samples collected at delivery, we measured rLTL in samples from children collected at ages 2- or 3-years (n=254), 5-years (n=205), 7-years (n=189), and 9-years (n=202), resulting in a total of 1330 samples collected from 225 maternal-child pairs. All samples were analyzed by MMqPCR as described in Chapter 6. Repeatedly collected samples within a maternal-child pair were run on the same plate (n=58 plates total), however, maternal-child pairs were not plated in chronological order by date of study enrollment. We aimed to examine changes in rLTL within a child over time and differences in telomere attrition between children in relation to measures of stressful conditions (material hardship), maternal perceived stress, and maternal emotional distress (demoralization). In turn, these conditions may create a more stressful living environment for the child, for example, through reduced parental responsiveness (Bradley et al. 2001) (Specific Aim 2.3). Given the multi-year enrollment design of the CCCEH Mothers and Newborns cohort (children were born between 1998-2006), samples were stored between 1 (last included 9-year old sample collected in June 2015) and 18 (first included cord blood sample collected in April 1998) years. As described in the following paragraphs, we were unable to fully complete Specific Aim 2 (and also Specific Aim 3, discussed later), due to the observation of a non-linear change in rLTL by date of sample collection (Figure 1), age (Figure 2), and date of birth (Figure 3).

Figure 1. Relative LTL by years since study start (0 = 1998, 17 = 2014).

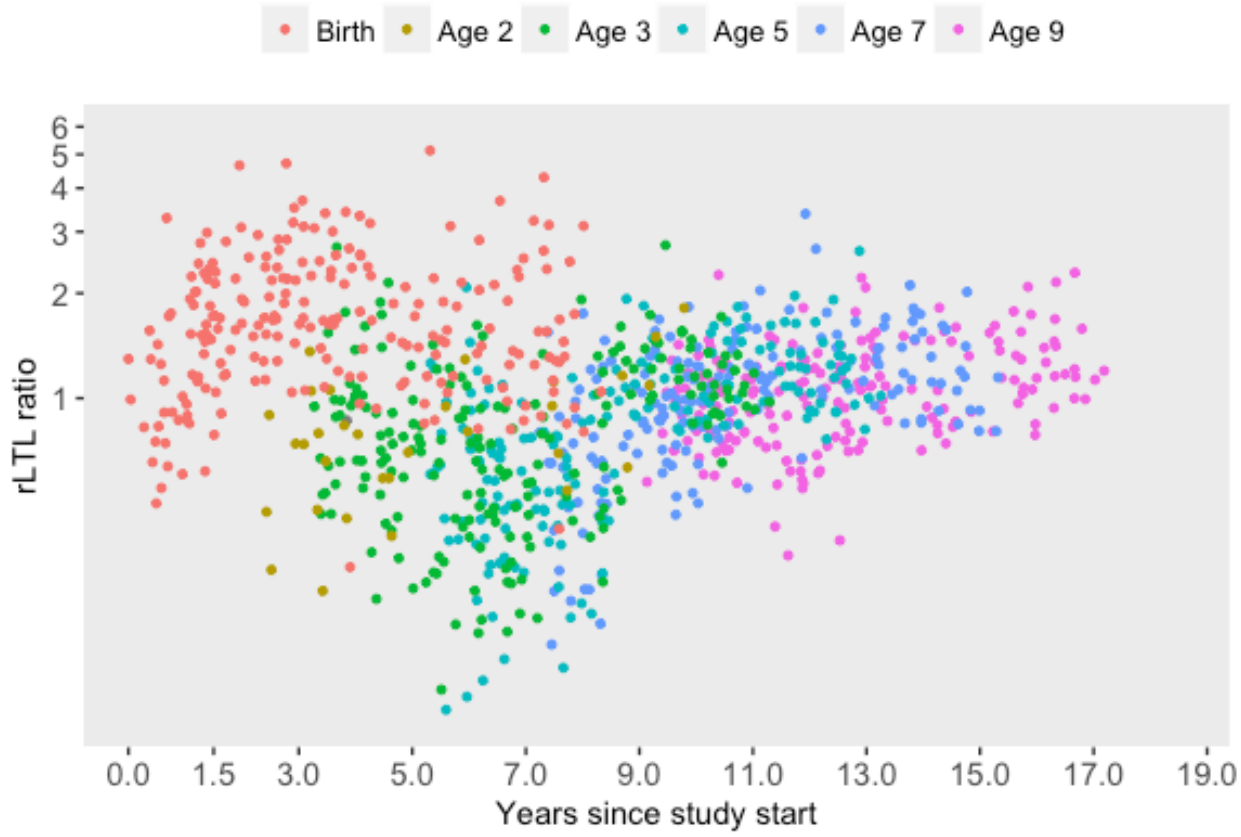


Figure 1 shows that in general, rLTL in postnatal samples (primarily between ages 3-5 years) collected in years 5-7 of the study (2003-2005) is lower compared to postnatal samples collected later during the study period.

Figure 2. Relative LTL by child age between birth and 9-years.

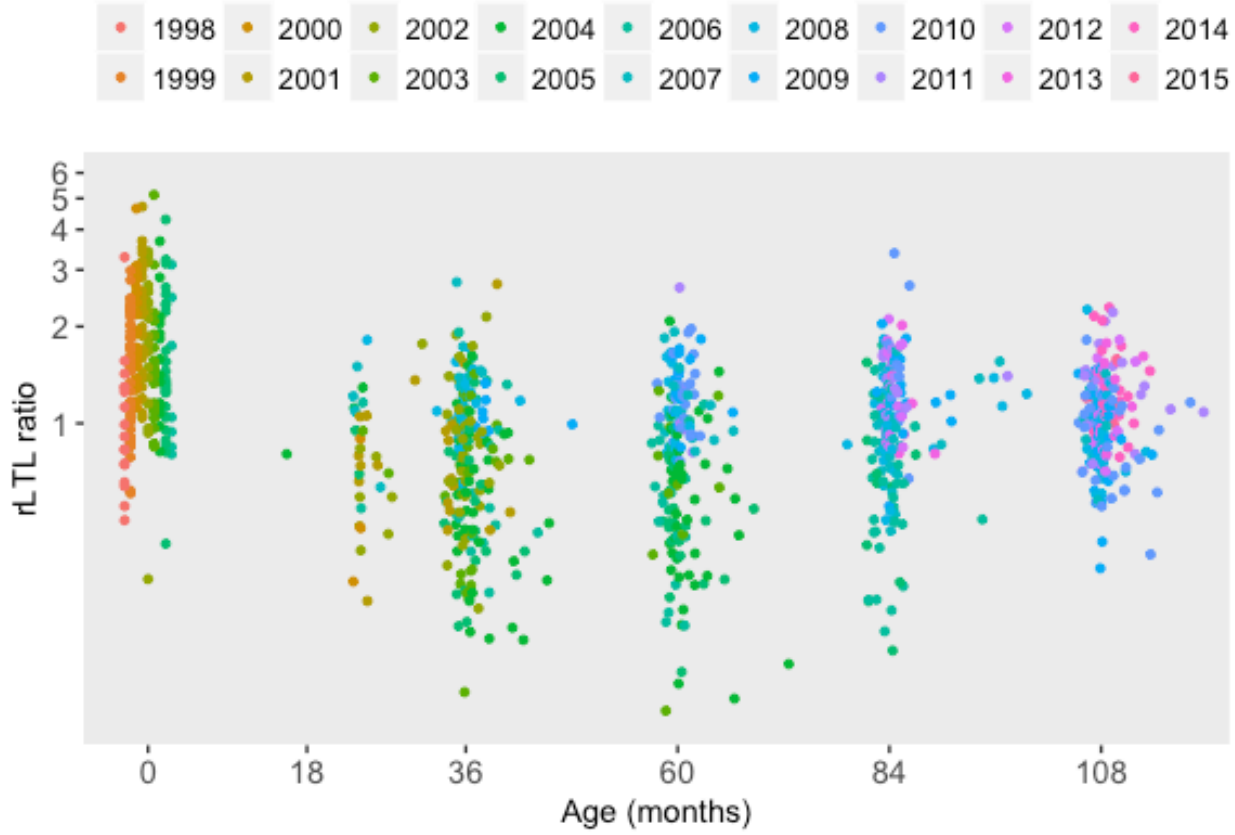


Figure 2 shows that regardless of sample collection date, we observed the steepest decline in rLTL between birth and ages 2- to 3-years (as expected), however, at approximately age 5-years (60 months), we observed an average increase in rLTL with age. Between the ages of 2-years (or 3-years if age 2 was missing) and 9-years (or age 7 if 9 years was missing), rLTL increased by more than 10% in 67% of children.

Figure 3. Relative LTL between birth and 9 years, stratified by year of birth.

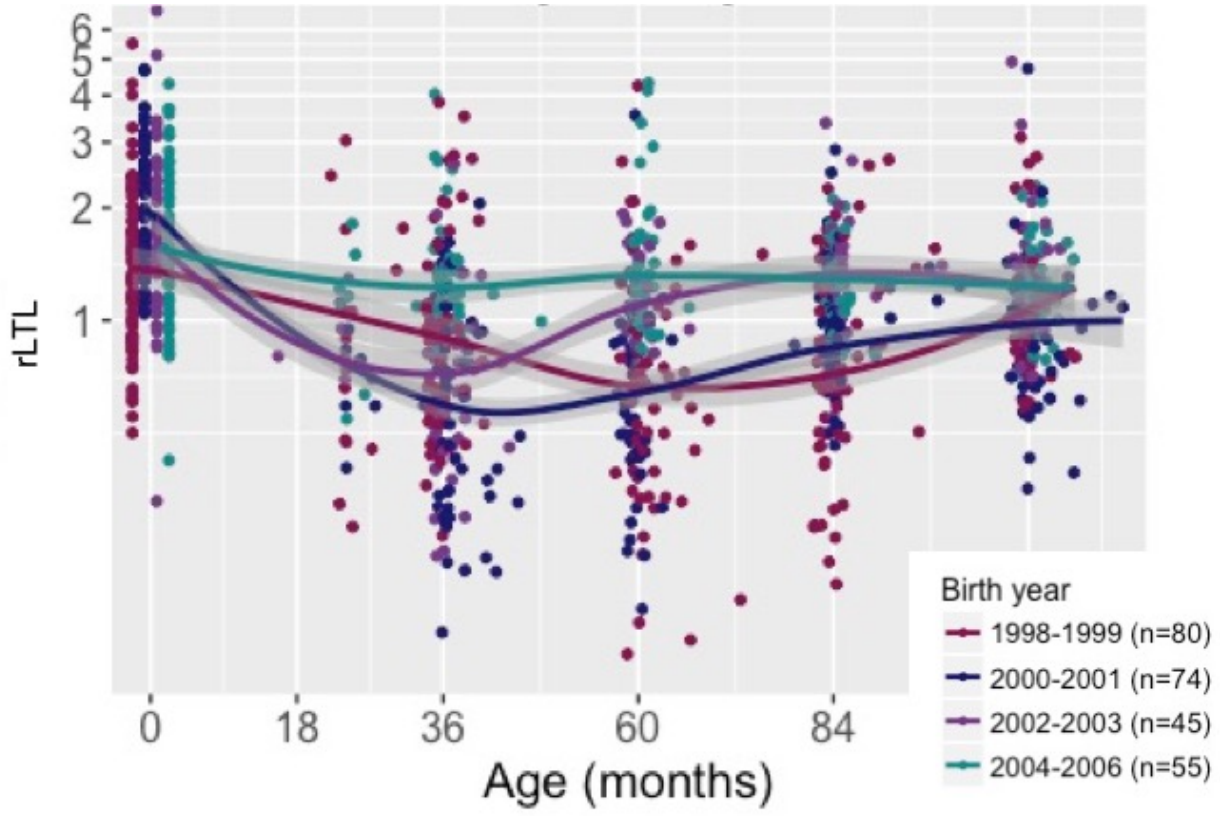
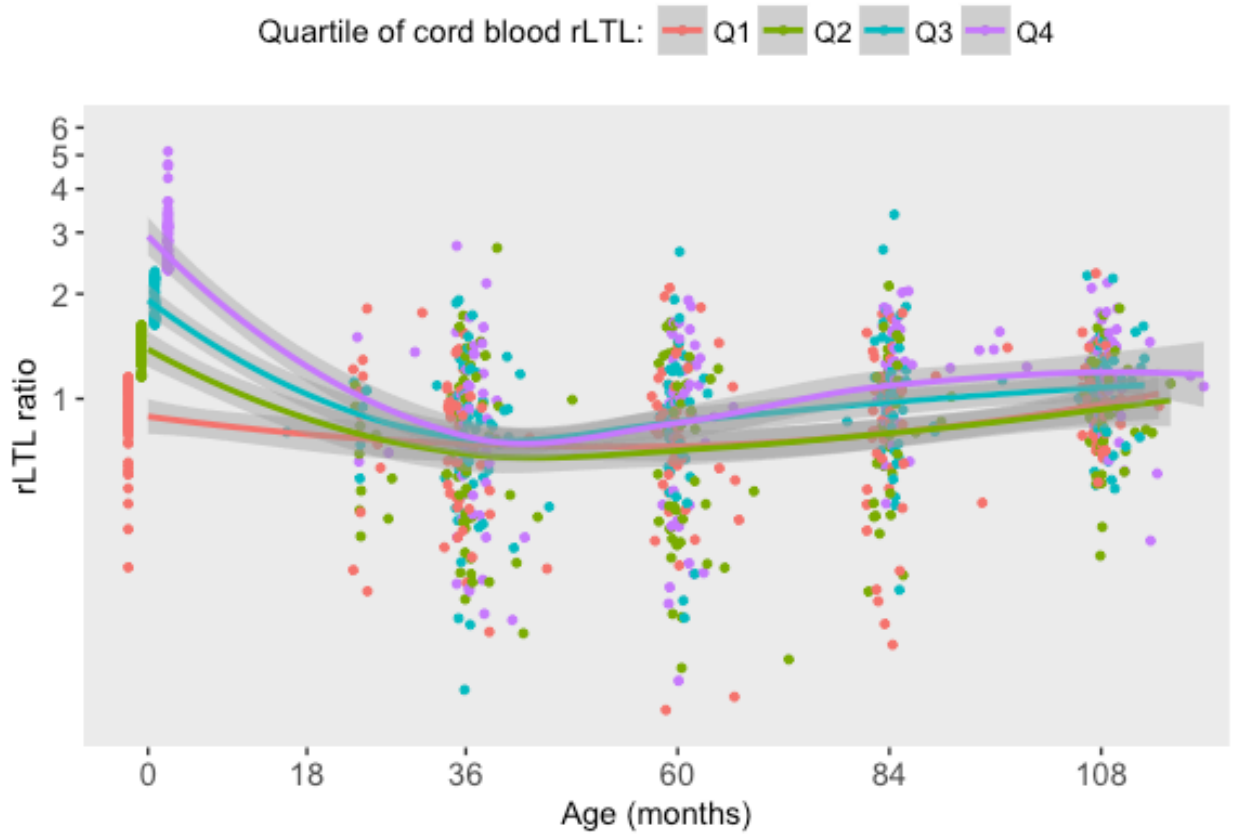


Figure 3. shows that the trajectory of rLTL change over time varied to a large degree by the child's year of birth. We do not have reason to believe any major changes in New York City occurred over the enrollment period that would result in this pattern of rLTL change, suggesting that the pattern may relate to non-random variation attributable to laboratory conditions, however, blood processing and DNA extraction protocols and procedures remained unchanged throughout the duration of the study.

The majority of cross-sectional research examining telomere length at various ages indicates telomeres shorten across the lifespan (Aubert and Lansdorp 2008). Likewise, the small number of longitudinal studies that have examined within-person change over time indicate that, on average, telomeres erode with age (Muezzinler et al. 2013). However, consistent with our results, the majority of longitudinal studies have observed telomere elongation among a subset of individuals. For example, a review of 10 studies with repeated measures of telomere length in adults, found lengthening occurred in 0-50% of subjects (Bateson and Nettle 2017). Likewise, one of three studies that has examined telomere change during childhood found that length increased by more than 15% between the ages of 5 and 10 years in 16% of children (Shalev et al. 2013). In the second study to examine change in children, Wojcicki et al. measured telomere length twice over a one year period (age 4 to 5 years) among 77 healthy Latino children (n=153 observations) recruited from two San Francisco hospitals. Using a cut-point of 10% to define a 'change' in telomere length between baseline and follow-up, the researchers found that 66% of children showed telomere maintenance (no change), while 31% demonstrated lengthening and only 3% showed shortening. Using a 5% cut point, the percent of children with shortening increased to 16% (Wojcicki et al. 2016). The third study to examine these relationships in children did not report information on lengthening (Humphreys et al. 2016).

In our sample, we observed that children with the longest baseline rLTLs had the greatest erosion between birth and age 2- or 3-years and the steepest increase through age 9 years (Figure 4).

Figure 4. Change in rLTL between birth and 9-years, stratified by quartile of rLTL at birth. Curves fit using local smoothing (LOESS) with an 80% data span.



This finding is consistent with the results of research conducted in children (Shalev et al. 2012) and adults (Epel et al. 2008; Farzaneh-Far et al. 2010; Puterman et al. 2015) indicating that shorter telomere length at baseline predicts greater lengthening at follow-up and vice versa. To investigate whether this pattern reflects regression to the mean versus a true biological relationship, Verhulst et al. performed simulation modeling to estimate plausible changes using repeatedly collected measures of telomere length in adults (Verhulst et al. 2013). After correcting

for regression to the mean using the approach outlined by Berry (Berry et al. 1984), the authors concluded that a modest, but statistically significant effect between longer baseline telomere length and greater attrition remains. This finding suggests that the dependency of attrition on baseline length reflects a true biological phenomenon, but that the magnitude of this effect is likely inflated. These findings highlight the importance of assessing and adjusting for potential 'regression to the mean' when examining repeated telomere measures.

It is currently debated whether telomere lengthening detected by observational epidemiology studies reflects true biological change or error introduced by imprecision of telomere measurement assays (Bateson and Nettle 2017; Nussey et al. 2014; Steenstrup et al. 2013; Verhulst et al. 2015). Recently, Bateson et al. investigated this question by fitting empirical data to several scenarios modeled assuming varying degrees of measurement error in combination with varying degrees of true lengthening. They found that the empirical data fit several scenarios that allowed for true lengthening equally well or better than scenarios that assumed only measurement error. The authors concluded that while measurement error likely contributes to apparent telomere lengthening, a biological basis for true lengthening should not be ruled out (Bateson and Nettle 2017).

Experimental and observational evidence supporting a biological basis of telomere lengthening stems from observations that average telomere length within an individual oscillates over relatively short periods (i.e. 6 months) of time in response to natural environmental (i.e. season) and modifiable lifestyle (i.e. dietary intervention) changes. These oscillations are likely due to a combination of telomerase upregulation and shifts in the distribution of leukocyte cell types. For example, *in vitro* research has shown that resveratrol, a plant-derived phenol with antioxidant

properties, upregulates telomerase activity in endothelial progenitor cells (Wang et al. 2011) and that aspirin, an anti-inflammatory drug, upregulate telomerase in mesenchymal stem cells (Chen et al. 2014). In humans, statins, which have anti-inflammatory properties, have also been shown to promote telomerase activity (Janic et al. 2016). Further, among a sample of overweight and obese adolescents, telomere length was found to significantly increase in 88% of individuals following participation in an intensive 'healthy lifestyle' intervention program (Garcia-Calzon et al. 2014); similar results have been observed in adults (Carulli et al. 2016).

Similarly, in a population-based observational study of 581 adults living in Costa Rica, Rehkopf et al. showed that telomere length fluctuated with season, such that shortening occurred in the wet season (when infections are most common) and lengthening occurred during the dry season (Rehkopf et al. 2014). During an immune response, circulating T cells, which have been shown to express low levels of telomerase (Yang et al. 2008), undergo clonal expansion, resulting in shortened telomeres among the new cells. Eventually, the majority of these cells undergo apoptosis and are cleared from the body (Akbar et al. 1993), however, because most telomere assays provide a measure of average leukocyte telomere length across all chromosomes from all cells, blood collected during an infection may reflect the increased number of T cells with reduced telomere length as a result of recent cell division. Unsurprisingly, this pattern is complicated by research demonstrating that memory T cell telomere shortening varies by antigen (Akbar et al. 2004) and that in some cell populations, infections may upregulate telomerase, resulting in an increase rather than a decrease in telomere length (Hathcock et al. 2003; Maini et al. 1999).

Collectively, these findings suggest that, depending on the magnitude and timing of an oscillation (i.e. increase or decrease in telomere length) relative to an individual's overall trajectory, telomere length within an individual may appear to increase over time. Importantly, it is unknown whether more permanent changes in telomerase expression can occur, or what circumstances would promote this type of change. Further, in addition to acute, infection-triggered shifts, the distribution of leukocyte cell types is known to change with age (see Table 1) and possibly in response to other conditions (i.e. diet (Babio et al. 2013) and medication (Li et al. 2017)). Therefore, it is possible that atypical (increasing or fluctuating) changes in telomere length over time could reflect a shift in the distribution of cell types.

Table 1. Pediatric differential white blood cell count reference ranges

	WBC ($x\ 10^3$/uL)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)
Birth	9-30	41-81	26-36	0.4-3.1
1-3 years	6-17	15-45	44-74	
3-5 years	6-16	25-57	35-65	0-0.8
6-10 years	5-13	38-68	25-54	

Source: Mayo Medical Laboratories (http://a1.mayomedicallaboratories.com/webjc/attachments/110/30a2131-complete-blood-count-normal-pediatric-values.pdf)

Few large studies have examined changes in leukocyte sub-populations over age and studies are especially limited in young children, potentially due to challenges associated with obtaining enough whole blood to allow for separation of cell types. In one cross-sectional study that examined neurotrophil and T lymphocyte telomeres among individuals between the ages of 10 and 31 years, telomeres were significantly longer in T cells compared to neutrophils at all ages (Robertson et al. 2000). In a similar study that examined cell-specific changes between birth and age 90 years (n=436), telomeres in both granulocytes and T cells shortened rapidly during early life; this decline continued with age in memory T cells, but not in naïve T cells (Rufer et al.

1999). The difference in attrition between memory and naïve T cells has recently been replicated in three large cohort studies (Chen et al. 2017). However, other studies have detected no association between telomere length and cell type (Mollica et al. 2009). A large study capable of investigating cell-specific changes in telomere length, ideally within individuals over time, is needed to more fully understand whether observed telomere lengthening is an artifact of measurement error or a true biological phenomenon related to cell distribution. However, as described below, researchers planning to conduct longitudinal research on telomeres should carefully consider the potential impacts of storage time and other conditions (i.e. temperature, form of sample).

In our samples, we found an unusual pattern whereby average telomere length declined rapidly during the first years of life, after which length gradually increased with age. Given that samples collected from children at older ages were also collected most recently, it is possible that the observed increase relates to the shorter storage time of samples collected at older ages; however, because of collinearity in age and storage time, we are not able to statistically adjust for this variable. While research indicates that short-term (i.e. 6 month) storage time does not impact telomere length measured by qPCR (Dagnall et al. 2017), we are not aware of studies that have investigated longer-term storage. Conversely, research examining the impact of other pre-analytic variables on telomere length assays indicates that the method of DNA isolation is an important factor (Cunningham et al. 2013, Raschenberger et al. 2016). In our study, DNA was isolated following the same standard phenol-chloroform protocol across all years, however, the timing of DNA extraction varied, with some samples stored primarily as buffy coat at -70C and other samples stored primarily as isolated DNA at -20C. For a subset of samples (46%, all postnatal), we isolated DNA contemporaneous with performing MMqPCR. Information on the

date of DNA isolation for the remaining 54% of samples is not available, however, it is likely that more recently collected samples were also more recently extracted. To investigate the potential influence of extraction timing and DNA storage conditions, we examined differences in rLTL among samples for which DNA was isolated at the time of telomere analysis compared to all other samples. As illustrated by Figure 5, we observed that samples extracted most proximate to the time of telomere analysis had telomere lengths at the top of each age-specific distribution between the ages of 3 and 9 years. This finding suggests that storage conditions (i.e. stored as buffy coat versus isolated DNA) may impact telomere length measurement by qPCR.

Figure 5. Change in rLTL between birth and 9 years, stratified by timing of DNA isolation

To further investigate this issue, we designed several additional experiments that we aim to complete using archived samples collected from the same cohort of mothers and children, as well as from a second cohort of their younger siblings. The research questions and corresponding approaches include:

1. Research question: Does storage as buffy coat versus isolated DNA impact *cord* telomere length? To investigate this question, we will analyze rLTL in split cord blood samples collected from the same child that have been stored as both buffy coat and isolated DNA for 16 to 17 years (n=98 samples from 48 children).
2. Research question: Does storage as buffy coat versus isolated DNA impact *child* telomere length? To investigate this question, we will analyze rLTL in samples collected from children repeatedly at ages 9 and 11 years and stored between 8 and 10 years as buffy coat or isolated DNA, respectively (n=72 samples from 36 children).
3. Research question: Does storage time as isolated DNA impact child telomere length? To investigate this question, we will analyze samples from 3-year old children who were enrolled at either end of the cohort, such that samples were stored for 10 years or 16 years. The exact date of DNA isolation is unknown, however, any child for whom 3-year DNA was isolated at the time of telomere analysis was excluded (n=72 samples from 72 children).
4. Research question: Is the trend of increasing telomere length that we previously observed also observed in more recently collected samples from similarly aged children? To investigate this question, we will analyze rLTL in cord blood, age 3-year, 5-year, 7-year and 9-year samples collected repeatedly from children enrolled in the Sibling-

Hermanos Birth Cohort. The oldest child enrolled in this cohort was born in 2008 (n=96 samples from 29 children).

Additionally, if we again observe a trend of increasing telomere length, despite shorter storage time, in samples collected by the Sibling-Hermanos birth cohort, a next step will be to consider options for statistically accounting for measurement error. For example, Bateson et al. recently developed a computational model of telomere dynamics available in the R statistical software, that enables researchers to estimate the percent of individuals in an empirical dataset predicted to have telomere lengthening under varying assumptions about 1) measurement error and 2) true change in telomere length (Bateson and Nettle 2017). These estimates can then potentially be used to correct for non-random measurement error related to assay or pre-analytical conditions. Ultimately, while we generally observe variation in the rate of telomere erosion between children, given these pending research questions, we are unable to draw concrete conclusions about change over time during early life.

CHALLENGES FACED IN COMPLETING AIM 3: TELOMERE DYNAMICS AS AN INDICATOR OF SUSCEPTIBILITY TO NEUROTOXICANT EXPOSURE

The overarching goal of this dissertation was to determine if early life telomere dynamics can serve as an indicator of susceptibility to the adverse effects of concurrent or subsequent neurotoxicant exposure. We hypothesized that the effect of PBDEs on memory outcomes would be greatest among children experiencing the greatest early life stress, indicated by the shortest baseline telomere lengths and greatest erosion during childhood (Specific Aim 3.1).

As summarized by the results of Aim 1.1, we found that one of the largest determinants of plasma PBDE concentration was date of birth, likely attributable to the phase-out of pentaBDE from U.S. commerce in 2004. As illustrated by Figure 3 (above), date of birth also predicted change in rLTL over time, likely attributable to a currently unknown source of measurement error relating to storage time, storage conditions, or another unidentified variable. Furthermore, we found that for the majority of children, PBDE concentrations peaked during toddler years (Chapter 3), which corresponds with the age we would expect the rate of rLTL erosion to begin slowing. Given this pattern, as well as the shared variability attributable to time (i.e. date), we were unable to statistically investigate interactions between PBDE concentrations and rLTL, and thus unable to test the hypothesis in Aim 3.1.

OVERALL IMPLICATIONS OF THIS WORK AND DIRECTIONS FOR FUTURE WORK

Over the past 40 years, we've seen U.S. state regulations transition from essentially mandating the use of flame retardant chemicals to prohibiting them. This shift was driven by decades of research indicating that organohalogenated chemicals, the most common class of flame retardant that has been used in the United States, are persistent in the environment, capable of long-range transport, and toxic to wildlife and humans (Law et al 2012). Indeed, in the present work we detected PBDE flame retardants in 100% of child samples and found significant associations between higher exposure and both dysregulated thyroid hormone homeostasis and impaired memory functioning in children. Ostensibly, flame retardants are used as a means of preventing fires and protecting human health. However, putting these goals in the context of risks to the environment and to children raises questions about the costs and benefits of using synthetic chemicals in everyday consumer products. Exposure to PBDEs is likely modifiable through washing hands, toys and other objects, limiting hand to mouth behaviors (nail biting, cigarette smoking, thumb sucking) and regularly cleaning the home using water-based methods. A large-scale intervention study may be useful in identifying what behavioral modifications for reducing exposure are most effective. Notably, research on human behavior suggests that providing people with accurate information does not consistently change perceptions about truth or lead to changed behaviors (Mercier H and Sperber D). Appealing to human emotions has been shown to be more effective in changing behavior, however, within the context of environmental health, this runs the risk of raising alarm and creating panic. If we as public health professionals truly hope to reduce exposure, perhaps a future direction will be a shift towards 'behavioral environmental health' approaches whereby knowledge of human behavior and decision making is investigated together with the sources and pathways of exposure.

We found that plasma PBDE concentrations have been decreasing among children over the past decade, likely as a result of their phase-out in 2004. However, despite legislative amendments that allow furniture manufacturers to pass fire safety tests without the use of added chemicals (Cal-117 2013), evidence suggests that companies continue to add an ever-expanding cadre of chemical flame retardants to products manufactured in the United States (Stapleton et al. 2012). Future research in this area will involve understanding the sources, exposure pathways and health effects associated with these replacement flame retardants.

The recent development of high throughput assays for measuring telomere length has led to an explosion of experimental and observational research across numerous disciplines (i.e. aging, psychology, epidemiology) investigating both the causes and consequences of telomere change across the lifecourse. The rapid publication of research has spawned debate and disagreement among scientists in the field over aspects of study design (i.e. should we measure telomere length in buccal cells or leukocytes?), approach (i.e. are results generated via southern blotting and qPCR equally good?), and interpretation (i.e. is apparent telomere elongation a true biological phenomenon or an artifact of measurement error?). A review of discussion sections from recently published manuscripts spanning the telomere field quickly conveys a common theme: the call for a large, prospective study examining telomere length analyzed by multiple techniques in samples collected repeatedly within individuals over a long period of time, with measurement ideally beginning at birth. Additionally, there is a general consensus that standardized references need to be developed so that results across studies and laboratories can be more readily compared.

The National Institutes of Health (NIH) recently convened a group of leading scientists to discuss whether telomeres can be used as "sentinels for environmental exposures, psychosocial stress and disease susceptibility." Reflecting on the early days of epigenetics, a senior scientist in the room (Colter Mitchell, PhD), offered that "developing a new biomarker always involves a lengthy scientific process, starting with initial excitement about the method, then disillusionment as challenges are realized, and then solid methods emerge." It seems the field of telomere science has entered the second stage of this process, with members of the scientific meeting generally concluding that many questions remain about how to precisely and reliably measure telomere length. It is our hope that the challenges we faced relating to analysis of archived samples, as well as the additional experiments we aim to complete, will contribute insight into some of the outstanding issues currently being grappled with by the field.

With regard to stress, the question of whether telomere dynamics can serve as a marker of cellular 'wear and tear' remains unanswered. As summarized by this dissertation, numerous studies have identified associations between various metrics of psychosocial stress (i.e. adversity, negative life events, trauma), stress correlates (i.e. socioeconomic hardship), and psychological state (primarily depression) with shorter age-adjusted telomere length. However, the vast majority of these studies are constrained by the same two methodological limitations: small sample sizes (typically $n<100$) and retrospective reporting of childhood experiences during adulthood. There is general consensus that telomere length during childhood is one of the most important determinants of telomere length in adults, thus emphasizing the importance of accurately measuring stressors as they occur during early life. We attempted to address these limitations in our study of telomere length in 141 paired maternal-newborn samples collected

from a prospectively followed cohort. Of the three predictors (material hardship, demoralization, perceived stress) we studied, only perceived stress, which was measured 6-months following the child's birth rather than during pregnancy, was marginally associated with newborn telomere length. While perceived stress was moderately to highly correlated within mothers across time, this problem of temporality suggests our findings should be interpreted with caution.

There is a trend in environmental health discourse towards a definition of environment that includes not only the chemical and physical aspects of our ambient surroundings, but also the stressors and social relationships experienced across life. This dissertation sought to contribute to this evolving discourse by testing whether telomeres can be used as a biological indicator of cellular 'wear and tear' in observational research investigating interactions between chemical and non-chemical stressors. While we were ultimately unable to answer this question, the research presented here expands our understanding of 1) exposure pathways, body burdens and neuroendocrine effects associated with PBDE flame retardants, 2) suggests telomeres may be sensitive to maternal stress during pregnancy and 3) provides insights on potential pitfalls of analyzing telomere length in archived DNA samples.

REFERENCES

Akbar AN, Salmon M, Savill J, Janossy G. 1993. A possible role for bcl-2 in regulating t-cell memory--a 'balancing act' between cell death and survival. Immunol Today 14:526-532.

Akbar AN, Beverley PC, Salmon M. 2004. Will telomere erosion lead to a loss of t-cell memory? Nat Rev Immunol 4:737-743.

Aubert G, Lansdorp PM. 2008. Telomeres and aging. Physiol Rev 88:557-579.

Babio N, Ibarrola-Jurado N, Bullo M, Martinez-Gonzalez MA, Warnberg J, Salaverria I, et al. 2013. White blood cell counts as risk markers of developing metabolic syndrome and its components in the predimed study. PloS one 8:e58354.

Bateson M, Nettle D. 2017. The telomere lengthening conundrum - it could be biology. Aging Cell 16:312-319.

Berry DA, Eaton ML, Ekholm BP, Fox TL. 1984. Assessing differential drug effect. Biometrics 40:1109-1115.

Bradley RH, Corwyn RF, McAdoo HP, Coll, CG. The home environments of children in the United States part I: variations by age, ethnicity, and poverty status. Child Dev. 2001; 72(6):1844-67.

Carulli L, Anzivino C, Baldelli E, Zenobii MF, Rocchi MB, Bertolotti M. 2016. Telomere length elongation after weight loss intervention in obese adults. Mol Genet Metab 118:138-142.

Chen BH, Carty CL, Kimura M, Kark JD, Chen W, Li S, et al. 2017. Leukocyte telomere length, t cell composition and DNA methylation age. Aging (Albany NY) 9:1983-1995.

Chen C, Akiyama K, Yamaza T, You YO, Xu X, Li B, et al. 2014. Telomerase governs immunomodulatory properties of mesenchymal stem cells by regulating fas ligand expression. EMBO Mol Med 6:322-334.

Cunningham JM, Johnson RA, Litzelman K, Skinner HG, Seo S, Engelman CD, et al. 2013. Telomere length varies by DNA extraction method: Implications for epidemiologic research. Cancer Epidemiol Biomarkers Prev 22:2047-2054.

Dagnall CL, Hicks B, Teshome K, Hutchinson AA, Gadalla SM, Khincha PP, et al. 2017. Effect of pre-analytic variables on the reproducibility of qpcr relative telomere length measurement. PloS one 12:e0184098.

Epel ES, Merkin SS, Cawthon R, Blackburn EH, Adler NE, Pletcher MJ, et al. 2008. The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. Aging (Albany NY) 1:81-88.

Farzaneh-Far R, Lin J, Epel E, Lapham K, Blackburn E, Whooley MA. 2010. Telomere length trajectory and its determinants in persons with coronary artery disease: Longitudinal findings from the heart and soul study. PloS one 5:e8612.

Fisher DA, Klein AH. 1981. Thyroid development and disorders of thyroid function in the newborn. The New England journal of medicine 304:702-712.

Garcia-Calzon S, Moleres A, Marcos A, Campoy C, Moreno LA, Azcona-Sanjulian MC, et al. 2014. Telomere length as a biomarker for adiposity changes after a multidisciplinary intervention in overweight/obese adolescents: The evasyon study. PloS one 9:e89828.

Hathcock KS, Kaech SM, Ahmed R, Hodes RJ. 2003. Induction of telomerase activity and maintenance of telomere length in virus-specific effector and memory cd8+ t cells. J Immunol 170:147-152.

Humphreys KL, Esteves K, Zeanah CH, Fox NA, Nelson CA, 3rd, Drury SS. 2016. Accelerated telomere shortening: Tracking the lasting impact of early institutional care at the cellular level. Psychiatry Res 246:95-100.

Janic M, Lunder M, Cerkovnik P, Prosenc Zmrzljak U, Novakovic S, Sabovic M. 2016. Low-dose fluvastatin and valsartan rejuvenate the arterial wall through telomerase activity increase in middle-aged men. Rejuvenation Res 19:115-119.

Law RJ, Covavi A, Harrad S, Herzke D, Abdallah MA, Fernie K, et al. Levels and trends of PBDEs and HBCDs in the global environment: status at the end of 2012. Environ Int 2014;65:147-58.

Li Q, Yu HY, Chen M, Jiang F, Zhou J, Bao YQ, et al. 2017. Waist circumference-dependent peripheral monocytes change after gliclazide treatment for chinese type 2 diabetic patients. J Huazhong Univ Sci Technolog Med Sci 37:204-209.

Maini MK, Soares MV, Zilch CF, Akbar AN, Beverley PC. 1999. Virus-induced cd8+ t cell clonal expansion is associated with telomerase up-regulation and telomere length preservation: A mechanism for rescue from replicative senescence. J Immunol 162:4521-4526.

Mercier H, Sperber D. 2017. The enigma of reason. Cambridge, Massachusetts:Harvard University Press.

Mollica L, Fleury I, Belisle C, Provost S, Roy DC, Busque L. 2009. No association between telomere length and blood cell counts in elderly individuals. J Gerontol A Biol Sci Med Sci 64:965-967.

Muezzinler A, Zaineddin AK, Brenner H. 2013. A systematic review of leukocyte telomere length and age in adults. Ageing Res Rev 12:509-519.

Nussey DH, Baird D, Barrett E, Boner W, Fairlie J, Gemmell N, et al. 2014. Measuring telomere length and telomere dynamics in evolutionary biology and ecology. Methods Ecol Evol 5:299-310.

Puterman E, Lin J, Krauss J, Blackburn EH, Epel ES. 2015. Determinants of telomere attrition over 1 year in healthy older women: Stress and health behaviors matter. Molecular psychiatry 20:529-535.

Raschenberger J, Lamina C, Haun M, Kollerits B, Coassin S, Boes E, et al. 2016. Influence of DNA extraction methods on relative telomere length measurements and its impact on epidemiological studies. Sci Rep 6:25398.

Rehkopf DH, Dow WH, Rosero-Bixby L, Lin J, Epel ES, Blackburn EH. 2014. Seasonal variation of peripheral blood leukocyte telomere length in costa rica: A population-based observational study. Am J Hum Biol 26:367-375.

Robertson JD, Gale RE, Wynn RF, Dougal M, Linch DC, Testa NG, et al. 2000. Dynamics of telomere shortening in neutrophils and t lymphocytes during ageing and the relationship to skewed x chromosome inactivation patterns. Br J Haematol 109:272-279.

Rufer N, Brummendorf TH, Kolvraa S, Bischoff C, Christensen K, Wadsworth L, et al. 1999. Telomere fluorescence measurements in granulocytes and t lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory t cells in early childhood. J Exp Med 190:157-167.

Shalev I. 2012. Early life stress and telomere length: Investigating the connection and possible mechanisms: A critical survey of the evidence base, research methodology and basic biology. BioEssays : news and reviews in molecular, cellular and developmental biology 34:943-952.

Shalev I, Moffitt TE, Sugden K, Williams B, Houts RM, Danese A, et al. 2013. Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: A longitudinal study. Molecular psychiatry 18:576-581.

Stapleton HM, Sharma S, Getzinger G, Ferguson PL, Gabriel M, Webster TF, et al. Novel and high volume use flame retardants in US couches relfective of the 2005 PentaBDE phase out. Environ Sci Technol 2012;46(24)13432-9.

Steenstrup T, Hjelmborg JV, Kark JD, Christensen K, Aviv A. 2013. The telomere lengthening conundrum--artifact or biology? Nucleic acids research 41:e131.

Verhulst S, Aviv A, Benetos A, Berenson GS, Kark JD. 2013. Do leukocyte telomere length dynamics depend on baseline telomere length? An analysis that corrects for 'regression to the mean'. Eur J Epidemiol 28:859-866.

Verhulst S, Susser E, Factor-Litvak PR, Simons MJ, Benetos A, Steenstrup T, et al. 2015. Commentary: The reliability of telomere length measurements. Int J Epidemiol 44:1683-1686.

Wang XB, Zhu L, Huang J, Yin YG, Kong XQ, Rong QF, et al. 2011. Resveratrol-induced augmentation of telomerase activity delays senescence of endothelial progenitor cells. Chin Med J (Engl) 124:4310-4315.

Wojcicki JM, Shiboski S, Heyman MB, Elwan D, Lin J, Blackburn E, et al. 2016. Telomere length change plateaus at 4 years of age in latino children: Associations with baseline length and maternal change. Mol Genet Genomics 291:1379-1389.

Yang Y, An J, Weng NP. 2008. Telomerase is involved in il-7-mediated differential survival of naive and memory cd4+ t cells. J Immunol 180:3775-3781.

Zoeller RT, Tan SW, Tyl RW. 2007. General background on the hypothalamic-pituitary-thyroid (hpt) axis. Critical reviews in toxicology 37:11-53.

APPENDIX: PROTOCOL FOR RELATIVE TELOMERE LENGTH MEASUREMENT

This MMqPCR protocol was optimized between January-October 2016. It was adapted from the method developed by Cawthon 2009 and modified by Pavanello 2011. The assay provides a measure of relative telomere length (rLTL) in genomic DNA by determining the ratio of telomere repeat copy number (T) to single copy gene (S) copy number (T/S ratio) in experimental samples relative to a reference sample. We use albumin as the single copy gene. In multiplexing, the telomere (telc/telg) and albumin (albd/albu) primers are combined in the same reaction mix.

Instrument: BioRad CFX96 Thermal Cycler

Primers
telc: 5′-TGT TAG GTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA ACA-3′
telg: 5′-ACA CTA AGG TTT GGG TTT GGG TTT GGG TTT GGG TTA GTG T-3′
albd: 5′-GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAGCATGGTCGCCTGTT-3′
albu: 5′-CGGCGGCGGGCGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG-3′

Multiplex PCR Mix	µl	Final conc	
iQ SYBR Green Supermix	12.5	1x	BioRad
40 µM telg	0.375	600 nM	Life Technologies, purity by desalt
40 µM telc	0.375	600 nM	Life Technologies, purity by desalt
40 µM albu	0.15625	250 nM	Life Technologies, purity by desalt
40 µM albd	0.15625	250 nM	Life Technologies, purity by desalt
H_20	6.4375		
DNA (2 ng/µl)	5		
Total	20		
Plates: BioRad white hard-shell thin-wall skirted plates for higher fluorescent signals			

Thermal cycling profile:

Stage 1	Stage 2: 2 cycles	Stage 3: 32 cycles
3 min at 95 °C	15 sec at 94 °C	15 sec at 94 °C
	15 sec at 49 °C	10 sec at 62 °C
		10 sec at 74 °C with telomere signal acquisition
		10 sec at 84 °C
		10 sec at 88 °C with albumin signal acquisition
Melting curve (optional) from 72 to 95 degrees, 0.5 degrees per step, 5 seconds per step		

Plate set-up

Each 96-well plate included (all in triplicate):

- 24 experimental samples
- No template control (NTC)
- Positive control (cc2)
- 6-point standard curve (S1-S6) generated from serially diluted DNA (S1: 150 ng/well, S2: 50 ng/well, S3: 16.7 ng/well, S4 5.55 ng/well, S5 1.85 ng/well, S6: 0.62 ng/well). Two standard curves are generated for each plate, one for the telomere signal and one for the albumin signal.

1	1	1	**NTC**	**NTC**	**NTC**	9	9	9	17	17	17
2	2	2	**S1**	**S1**	**S1**	10	10	10	18	18	18
3	3	3	**S2**	**S2**	**S2**	11	11	11	19	19	19
4	4	4	**S3**	**S3**	**S3**	12	12	12	20	20	20
5	5	5	**S4**	**S4**	**S4**	13	13	13	21	21	21
6	6	6	**S5**	**S5**	**S5**	14	14	14	22	22	22
7	7	7	**S6**	**S6**	**S6**	15	15	15	23	23	23
8	8	8	**cc2**	**cc2**	**cc2**	16	16	16	24	24	24