Phenols, Parabens and Triclocarban During Pregnancy: Associations with Maternal Hormones and Birth Outcomes and the Modifying Effect of Maternal Stress

by

Ameera M. Aker

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Environmental Health Sciences)
in The University of Michigan
2019

Doctoral Committee:

Professor John D. Meeker, Chair
Professor Rita Loch-Caruso
Professor Bhramar Mukherjee
Professor Sung Kyun Park

Ameera Mazen Aker

aaker@umich.edu

ORCID iD: 0000-0002-0063-7955

DEDICATION

I dedicate this defense to my husband, Faris.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. John Meeker, for his unwavering support, guidance and understanding throughout this program. His mentorship allowed me to get through this program even under trying times. I am forever grateful. I would also like to thank my committee members, Drs. Rita Loch-Caruso, Bhramar Mukherjee and Sung Kyun Park for their constant support and enthusiasm throughout the years. I would like to acknowledge my funding sources: National Institute of Environmental Health Sciences' (NIEHS) grants P42ES017198, P50ES026049, P30ES017885 and UG3OD023251. And a special heartfelt thank you to my family and friends for keeping me sane, especially my incredible husband, who believed in me more than I ever did, my beautiful children, and the best parents and siblings a girl could ask for. I couldn't have made it without you!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Gestational age at delivery and birth weight are important predictors of fetal and infant health. Preterm infants and infants born with a low birth weight are at risk to a host of adverse health effects including neurological disabilities, breathing problems and chronic diseases later in life. Puerto Rico has one of the highest rates of preterm birth in the U.S. and the reasons behind this elevated rate are unclear. Chemical exposure during pregnancy could impact the mother, leading to adverse birth outcomes, or could pass through the placenta and directly impact the fetus. These adverse effects could potentially occur with very low concentrations of chemicals due to the vulnerable period of development. Phenols, parabens and triclocarban are a group of ubiquitous chemicals commonly found in personal care products and household items. These chemicals have been detected in the majority of the U.S. population, and a cohort in Puerto Rico, PROTECT, has higher concentrations of these chemicals as compared to the U.S. mainland. Phenols, parabens and triclocarban have also been linked to a number of growth parameters during pregnancy and at birth, including birth weight and gestational length. Endocrine disruption is a suspected pathway leading to these health outcomes. This dissertation focuses on the associations between urinary biomarkers of phenols, parabens and triclocarban on birth outcomes. The association between the exposure biomarkers and maternal hormones is also explored as a potential pathway. Maternal stress could modify the association between the exposure biomarkers and birth outcomes given the similar pathways involved between stress and gestational length. Therefore, the interaction between stress and the exposure biomarkers was explored.

Aim 1 focused on the associations between the exposure biomarkers and maternal hormones in two study populations of pregnant women. The exposure biomarkers were associated with altered thyroid and reproductive hormones in the mother. Aim 2 and 3 were focused on the main

dissertation cohort in Puerto Rico, PROTECT. Aim 2 found changes in gestational length with higher concentrations of some of the biomarkers of exposure. Benzophenone-3, bisphenol-A, methylparaben and propylparaben were associated with an increase in gestational length, with stronger associations observed at the 16-20 weeks gestation time point in comparison to 24-28 weeks. There was also evidence of a change in birth size with increased concentrations of the exposure biomarkers. Triclosan was associated with a higher odds of small for gestational age (SGA), and benzophenone-3 was associated with a higher odds of being large for gestational age (LGA). The relationship between bisphenol-S and birth size differed by study visit. At 16-20 weeks gestation, bisphenol-S was associated with LGA; however, at 24-28 weeks gestation, bisphenol-S was associated with SGA. Aim 3 examined the modifying effect of maternal stress on the association between the exposure biomarkers and gestational length. Associations between exposure biomarkers and gestational length were stronger in the presence of an overall negative score of the life event survey, indicating maternal stress due to negative life events may make pregnancies more vulnerable to chemical exposure. Overall, these results add to the growing literature on the effects of phenols, parabens and triclocarban on pregnancy. Further studies are required to confirm these findings, particularly the temporal differences observed. This is the first study to look into the modifying effect of mater nal stress and these associations, and more studies are needed to confirm the findings observed.

CHAPTER I. Introduction

Background

Gestational age at delivery and birth weight are important predictors of fetal and infant health. Preterm birth, defined as gestational length less than 37 weeks, is a leading cause of neonatal death in the United States and most high and middle income countries, accounting for over a third of the 3.1 million global neonatal deaths every year [1,2]. Preterm infants and infants born with a low birth weight (less than 2500 grams at birth) remain at risk to a host of adverse health effects. For example, preterm birth is the second leading cause of pneumonia for children below the age of five, and the survivors of preterm birth are subject to an increased risk of cerebral palsy, neurological disabilities, vision problems, hearing impairment, breathing problems, and chronic diseases later in life [2–5]. As such, identifying risk factors of preterm birth to implement effective preventative measures is essential. The U.S. preterm birth rate has declined since 1997, yet remains at one of the highest rates in the developed world at 9.6% [6,7] .

On the other hand, pregnancies lasting over 40 weeks are associated with their own risks, to both the baby and the mother. The rate of stillbirth, birth injuries, and neonatal complications are increased as well as maternal complications including perineal lacerations, postpartum hemorrhage, and increase rate of caesarean deliveries [8,9]. In the United States, the post-term birth rate incidence was 7% in 2005 [10], but the induction of labor practices is thought to have decreased this rate of post-term births [9].

Puerto Rico has one of the highest rates of preterm birth in the U.S. at approximately 12% of all live births [11], and the reasons behind this elevated rate are unclear [12]. The island is a non-incorporated territory of the U.S.; this ambiguous status leads to political and social issues that partly explain Puerto Rico’s health disparities and crumbling health infrastructure [13]. While

the social and political aspects are likely to indirectly influence the preterm birth rate, other environmental exposures likely play an important role in describing this high rate.

Hefty tax incentives over the last few decades drove industry and manufacturers to Puerto Rico, leading to a subsequent growth in population and urban development in parts of the island. However, the rise in industry and their reliance on some hazardous materials also led to environmental contamination from accidental and/or deliberate spills in the groundwater [14]. Puerto Rico has an area of approximately 3,500 miles (the third smallest territory/state in the U.S.), yet currently has 16 active Superfund sites, and over 200 hazardous waste sites. In addition, many of these sites lie on unlined limestone aquifers, increasing the chance of exposure to contaminants via groundwater.

The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) Program, funded by the Environmental Health Sciences' Superfund Research Program, is a multi-disciplinary center set up in order to explore the role of environmental contamination with birth outcomes, provide insight on environmental routes and exposure pathways, and develop mitigation strategies to reduce the levels of contamination. To achieve this, a cohort of pregnant women is being followed to birth, and urinary and serum samples are being collected from the women at several time points during their pregnancy to account for the varying levels of exposures and biological markers throughout gestation. This large cohort provides an opportunity to study the effects of prenatal/in-utero exposure to various chemicals on birth outcomes in a vulnerable population. Details of the study population are described later in this Chapter.

The prenatal period is an important window of vulnerability due to the various stages involved in rapid development. Chemical exposure during pregnancy could impact the mother, leading to adverse birth outcomes, or could pass through the placenta and directly impact the fetus [15,16]. This is especially important because even low concentrations could lead to adverse health effects that manifest from the fetal stage to adulthood, and potentially to future generations [17,18]. Among chemical classes associated with adverse health effects with gestational exposure are phenols and parabens.

Phenols, parabens and triclocarban as exposures of interest. Phenols, parabens and triclocarban are a group of ubiquitous chemicals commonly found in personal care products and household items. Reports from the National Health and Nutrition Examination Survey (NHANES) show

that the majority of the U.S. population have detectable levels of these contaminants in their bodies [19], and the levels of phenols and triclocarban found in the PROTECT cohort are higher than the levels of women of reproductive age in NHANES [20]. Triclocarban, in particular, was found at concentrations 37 times greater in PROTECT compared to NHANES concentrations. The exposure biomarkers of interest include: 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), bisphenol-A (BPA), bisphenol-S (BPS), bisphenol-F (BPF), benzophenone-3 (BP-3), triclosan, triclocarban, and ethyl- (EPB), methyl- (MPB), butyl- (BPB) and propyl-paraben (PPB).

BPA is a high volume chemical used in the manufacture of polycarbonate plastics and epoxy resins in many consumer products such as food containers and cans. BPS and BPF are common substitutes for BPA. BP-3 is a UV-filter used in cosmetics and sunscreens and some plastics [21]. Triclosan and parabens have anti-microbial properties and are commonly added to personal care products to reduce bacterial contamination [21,22]. Triclocarban is not a phenol, but is also used in products similar to triclosan due to its antiseptic properties [23]. Conversely, 2,4-DCP is a metabolite of the widely used herbicide 2,4-dichlorophenoxyacetic acid, and 2,5-DCP is a metabolite of 1,4-dichlorobenzene, a compound used in mothballs and room deodorizers [21]. Results from a product use questionnaire administered to the PROTECT cohort also showed that the phenol, paraben and triclocarban biomarkers were associated with consumer product use [20,24]. The urinary paraben mean concentration was higher among PROTECT women who reported using cosmetics and lotions; the urinary triclosan and triclocarban levels were higher among those who used toothpaste, hairspray and soap; the urinary BPA level was higher among those who used mouthwash; and the urinary BP-3 level was higher among those who reported use of sunscreen and lotion.

Exposure to phenols, parabens and triclocarban could occur in a multitude of pathways given these chemicals' widespread use, most notably through dermal absorption via use of personal care products, and ingestion in the case of contamination of food products. Multiple exposure science studies have documented these chemicals' detection in human urine, nails, plasma, breast milk, placental tissue and umbilical cord blood [25–31].

Although the research is limited, phenols, parabens and triclocarban have also been linked to a number of growth parameters during pregnancy and at birth, including birth weight, birth length

and gestational length [32–36]. There are, however, many discrepancies across the studies. While BPA positively increased the odds of preterm birth among only males in one study [37], another reported an association with preterm birth among only females [38]. BPA was also associated with low birth weight or smaller size for gestational age [39–45]. 2,4-DCP and 2,5-DCP were associated with low birth weight in human and animal studies [36,46–48], whereas BP-3 was associated with both low and high birthweight in two separate studies [36,46]. Triclosan was not associated with birthweight in human neonates [33,34]; however, an animal study found evidence of reduced birthweight after prenatal triclosan exposure to rats [49]. Parabens were associated with high birthweight in a human study [34], but animal studies in rats and mice found reduced [50] or no effect [51–53] on fetal birthweight.

There were inherent differences across the studies that may have led to the differences in the results. Phenols and parabens are non-persistent and can be highly variable across gender, lifestyle and time of day [19,54,55]. The majority of human studies that looked at phenol and parabens' association with adverse birth outcomes were cross-sectional in nature, and included urinary samples from a single time point during pregnancy. However, evidence suggests biomarker concentrations of these chemicals, particularly BPA and parabens is highly variable throughout pregnancy, likely due to the intermittent exposure of these chemicals and their rapid metabolism [56]. Pregnancy is not a constant biological state; maternal and fetal hormonal and inflammation pathways vary throughout the developmental stages; therefore, exposure to environmental chemicals could impact birth outcomes differently depending on the time of exposure, which was not accounted for in most epidemiological studies [57–61]. In turn, exposure measurement deficiencies, the varying study populations, as well as unaccounted for residual confounding could explain the discrepancies observed. The lack of strong study methodologies, and limited number of studies in the literature warrant further studies to be conducted. The PROTECT cohort provides a unique and rigorous opportunity to do so.

Hormone disruption as a potential biological mechanism. A potential toxicity pathway for these chemicals is through endocrine disruption, and pregnancy is a vulnerable window for endocrine disruption due to the varying levels of hormones involved in the growing organism [62]. Reproductive hormones have an important role in maintaining pregnancy; in turn, disruption of the complex interplay between the hormones could lead to adverse effects during gestation.

Estriol is named the “estrogen of pregnancy” because 90% of estriol precursors in maternal circulation arise from fetal precursors [63]. Thus, estriol is a marker for fetal adrenal activity, wherein elevated levels of estriol in the maternal circulation could indicate fetal distress. At low levels, estriol is an inhibitor of estradiol, but the level of estriol increases drastically approximately 2-4 weeks before delivery. Once the estriol:estradiol ratio exceeds 10:1, estriol becomes an effective agonist of estradiol- which leads to a decrease in progesterone levels and a surge in CRH [64,65]. CRH (synthesized by the placenta) is not only associated with myometrial contractions, but stimulates the production of dehydroepiandrosterone sulfate (DHEAS) – an estriol precursor – in the fetal adrenal, causing the feedback loop to produce more estriol [65]. Consequently, an exponential rise in CRH correlates with timing of birth. Progesterone is normally implicated as the hormone responsible for maintaining pregnancy status, particularly in animals. However, evidence suggests that in humans, the ratio between progesterone and estriol is more important in maintaining pregnancy, deferring labor by promoting uterine relaxation and reducing prostaglandin synthesis, than the level of progesterone on its own [63,66].

Thyroid hormones also play an important role in pregnancy. Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are produced in the thyroid gland, and their levels are negatively controlled by thyroid stimulating hormone (TSH) from the pituitary gland via the hypothalamic-pituitary-thyroid (HPA) axis [67]. The fetal thyroid only begins to produce hormones at 10-12 weeks gestation, and is completely dependent on maternal thyroid hormones for neurodevelopment in its first weeks of life, particularly T4 [68–70]. Additionally, thyroid hormones (maternal and fetal) are essential to the development of fetal tissues, and fetal growth promotion [71]. With the rise of estrogens, T3 and T4 rise in early pregnancy, stabilizing by the first half of pregnancy [72]. During the second half of pregnancy, maternal thyroid hormones help maintain fetal thyroid homeostasis [73]. Subclinical maternal thyroid dysfunction has been associated with low birth weight, low Apgar scores, and neurological disabilities [74–76].

It is clear that because of this complexity in hormone levels, a slight disruption could potentially lead to adverse effects. Endocrine disrupting chemicals could act through several pathways, including hormone synthesis, regulation, transport and metabolism, and/or interference with receptors. Although research on most of the phenols, parabens and triclocarban mechanism of action is limited, a brief summary of the findings is described below.

2,4-DCP and 2,5-DCP are suspected endocrine disruptors. 2,4-DCP was associated with an increase in progesterone among non-menopausal women [77], and a decrease in serum FT4 among pregnant woman in a large cohort measured at two time points in pregnancy [78]. There was also evidence of altered steroidogenesis with 2,4-DCP exposure to zebrafish, causing lesser rates of hatching of eggs [79]. 2,5-DCP, on the other hand, was associated with earlier menarche [80] and earlier breast development among a cohort of U.S. girls, pointing to evidence of thyroid agonist tendencies and/or reproductive hormone disruption [59]. This was further supported by an NHANES study that reported an increase in TSH and hypothyroidism with an increase in 2,5-DCP among adolescents [81].

BPA has been associated with changes in thyroid and reproductive hormone levels [82,83]. More specifically, BPA has weakly estrogenic properties [19], and increased estradiol and SHBG in rat pups and male adults [84,85]. Additionally, BPA increased T4 after perinatal exposure in rats, however, pregnant ewes demonstrated lower levels of thyroid hormones with dietary exposure to BPA [86–88]. A number of human studies have also been conducted. A review of epidemiology studies found relatively consistent results in males, wherein BPA exposure tended to increase FSH and testosterone, but decrease estradiol [89]. However, the results pertaining to women were more variable. This is likely due to the differences in the ages of women across the studies (pre-pubertal versus pregnant women) and differences in the reproductive health status (polycystic ovary syndrome versus non-polycystic ovary syndrome).

BPS and BPF are common replacements for BPA, but have also been associated with similar effects to BPA [90,91]. Although less researched, BPF and BPS interfered with thyroid hormone signaling by binding to thyroid hormone receptors TRα and TRβ, but with lower binding potencies [83].

BP-3 is suggested to be weakly estrogenic and antiandrogenic [59]. In *in vitro* studies, BP-3 exerted an agonist effect on human ERα and ERβ, an antagonistic effect on the androgen receptor and progesterone receptors, and thyroid receptor agonistic effects [92–95]. *In vivo* studies mirrored some of these effects. BP-3 altered transcription of hormonal receptors in invertebrates [96], and decreased spermatozoa among male fish [97]. The potential endocrine disrupting effects of BP-3 is not as well documented in humans; however endocrine-mediated

endpoint changes were observed in connection to BP-3, including earlier menarche [80] and breast development [59] among girls.

One of triclosan's modes of action is disruption of steroidogenesis [22], and this was shown to occur in the placenta as well [98]. Triclosan's chemical structure is similar to that of anthropogenic estrogens, and evidence suggests trislocan disrupts hormone metabolism, displaces hormones from receptors and disrupts steroidogenic enzyme activity [58]. Moreover, triclosan is structurally similar to thyroid hormones T3 and T4, and has been shown to decrease T4 in rodent studies [49,99–101]. This effect was also observed, albeit not significantly, in a cohort of pregnant women in the U.S. [78].

Triclocarban has not been studied in detail, although studies have demonstrated its estrogenic and androgenic effects in rodents and aquatic animals, and these findings were supported by *in vitro* studies [23]. It was recently described as a "new type" of endocrine disruptor because it is thought to augment endogenous hormone action rather than activate hormone receptors [102].

With regards to parabens, most animal studies have focused on effects of BPB, likely due to the fact that BPB has the highest estrogenic potency [103]. It is important to note that while MPB has the lowest estrogenic potency, it also penetrates through the skin at the highest rate as compared to other parabens, and can accumulate in skin layers [103]. While parabens have low binding affinity to estrogen receptors, they have been documented to have full agonist properties, particularly with longer exposure durations [60]. Two rat studies found no difference in estradiol levels in dam ovaries after in utero exposure to BPB and iso-butylparaben [104,105]. However, estradiol levels decreased after exposing human adrenocortical carcinoma cells (H295R cells) to parabens to test their interference with steroid hormone biosynthesis, affirming the weak estrogenic properties of parabens [104]. Likewise, researchers found decreases in estradiol and progesterone levels after paraben exposure in prepubertal female rats, particularly with ethyl- and isopropyl paraben [106]. Other endocrine-mediated effects have been linked to MPB and BPB, including decreased odds of live births [107], increases in dam uterine weights [104], and changes in various reproductive hormone levels .

Given the growing evidence of the endocrine disrupting properties of these exposure biomarkers, in addition to the evidence linking the biomarkers to adverse birth outcomes, I was interested in exploring the associations between phenols, parabens and triclocarban with maternal hormones,

and to look for potential windows of susceptibility. As described below in, this analysis was carried out in two pregnancy cohorts to help validate observed associations.

*Interaction between chemical exposure and psychosocial stress***.** There is a growing interest in looking at the combined effect of stressors in the environment on human health, particularly with the introduction of the exposome concept [108,109]. In addition to looking at the effect of chemical mixtures on health, interactions between chemical and non-chemical stressors are important to consider in deepening our understanding on how the environment impacts humans. Although this area of research is relatively new and limited, evidence supports the notion of an increased risk in the presence of an exposure to high levels of environmental chemicals, as well as high psychosocial stress levels, indicating a modifying effect of stress on the relationship between chemicals and health. For example, a systematic review found the combined effect of chemical exposures such as smoking and traffic pollution in combination with high stress or socio-economic stressors were associated with worse fetal growth parameters than with either chemical or psychosocial stressors on their own [110].

With this in mind, I was interested in examining the potential effect of chemical exposure in combination with maternal stress as a potential effect modifier, given that maternal stress has been associated with adverse birth outcomes in multiple studies. A population-based case control study reported a 60% increased odds of a very low birth weight among women who reported always feeling stressed as compared to women reporting no stress [111]. Other studies have also reported a shortened gestational length with maternal stress or a larger number of negative life events [112–115].

The effects of maternal stress on the fetus could also extend beyond the pregnancy and birth experience, and potentially cause long-term adverse effects on the cognitive, behavioral and psychomotor development of the child [116,117]. Maternal stress influences the HPA axis [118], and this intra-uterine programming of the HPA axis is the suspected mechanism between low birth weight and adult HPA axis regulation abnormalities, and adult insulin resistance [119–121].

In conclusion, this dissertation focuses on the association between phenols and parabens on birth outcomes, the association between phenols and parabens and hormones as a potential mechanism of action, and the modifying effect of psychosocial factors in the association between phenols

and parabens and gestational length. Figure I.1 is a conceptual figure summarizing the study aims described below.

Aim 1

Associations between phenols and parabens on hormones during pregnancy is a largely understudied area of research. Endocrine disruption is an important pathway that could lead to a myriad of adverse health effects, including birth outcomes. Most human studies only looked at the associations between phenols and parabens at one time point during pregnancy, particularly at the end of pregnancy. However, changes at the start of pregnancy may also be important since some maternal hormones play an important role in fetal development in early pregnancy as well.

To this end, my first aim was to investigate the extent to which urinary concentrations of phenols, parabens and triclocarban are individually associated with serum reproductive and thyroid hormone levels, including estriol, progesterone, corticotropin-releasing hormone (CRH), sex hormone-binding globulin (SHBG), total thyroxine (T4), total triiodothyronine (T3), free thyroxine (FT4), and thyroid stimulating hormone.

A preliminary analysis on the first 106 women in the PROTECT cohort showed some associations between the chemical biomarkers and serum hormones. The direction of association between the chemical biomarkers and hormone levels was dependent on the specific hormone and biomarker. Based on this analysis and existing research, I hypothesized an increase in circulating thyroid hormone concentrations in association with BPA and parabens, a decrease in thyroid hormones in association with triclosan, a decrease in SHBG in association with phenols and parabens, and a change in the direction of associations between the two study visits in relationships with progesterone. I was unable to hypothesize directions of associations for the remaining associations due to the lack of human health data, so a goal of Aim 1 is to fill this gap in the literature.

For Aim 1, I repeated the analysis on two cohorts: a larger sample of women from the PROTECT cohort, and a large sample of women from a second cohort, LifeCodes, based in Boston, MA. I ran the analyses in two cohorts to help validate the associations observed. However, LifeCodes only had data on maternal thyroid hormones.

Aim 2

Aim 2 investigated the association between phenols, parabens and triclocarban in relation to the birth outcomes, gestational length and birth weight. Studies regarding the associations between the exposure biomarkers have varied in their conclusions; therefore I wanted to investigate this association in a larger cohort, and investigate how the timing of exposure would impact the strength of associations between the chemicals and birth outcomes.

I hypothesized that phenols were associated with a decrease in gestational length, whereas the parabens were associated with an increase in gestational length and birthweight.

Aim 3

In concert with the growing trend of looking at the impact of multiple exposures on birth outcomes, I wanted to investigate the interaction between chemicals and a non-chemical stressor. Therefore, my third aim examines the extent to which pyschosocial factors modify the associations between the chemical biomarkers and gestational length. Preliminary analysis showed that gestational length was the outcome most strongly associated with the exposure biomarkers, so Aim 3 focused on this birth outcome. I hypothesized that the associations between exposure biomarkers and gestational length will be stronger in the presence of high levels of psychosocial factors.

Study Populations

PROTECT [Aims 1-3]

As mentioned previously, the study included participants from the ongoing prospective cohort of pregnant women, PROTECT. The study participants (aged 18-40 years) have been recruited from 2010-present at 14 ± 2 weeks gestation from two hospitals (Manatí Medical Center and Arecibo's Cayetano Coll y Toste Hospital) and five affiliated prenatal clinics in Northern Puerto Rico. The recruitment target was 1,000 live births, and the recruitment rate was 78% from 2011-2018. Women received $30 for participating in the study. The exclusion criteria include: women who live outside the region, have multiple gestations, used oral contraceptives within three months prior to getting pregnant, got pregnant using in vitro fertilization, or have known medical health conditions (e.g., diabetes, hypertension, liver/kidney disease).

Demographic information was collected via questionnaires at the initial study visit. A nurse-administered questionnaire was conducted during an in-home visit to collect information on household exposures, water source and usage, job history, hobbies, housing characteristics, family situation, mental health, and life experiences. Spot urine samples were collected at three separate study visits (Visit 1: 16-20; Visit 2: 20-24; Visit 3: 24-28 gestation weeks), and blood samples are collected during the first and third visits. Figure I.2 shows the timeline for recruitment and data collection from the cohort. The timing of the visits were aimed to coincide with routine clinical visits, thus also coinciding with periods of rapid fetal growth and reducing costs. This study was approved by the research and ethics committees of the University of Michigan School of Public Health, University of Puerto Rico, Northeastern University, and participating hospitals and clinics. All study participants provided full informed consent prior to participation.

LifeCodes [Aim 1]

Participants were part of a nested case-control study within the LifeCodes cohort. LifeCodes is comprised of a prospective birth cohort of pregnant women planning on giving birth at the Brigham and Women's Hospital in Boston, MA. LifeCodes enrolled 1600 women from 2006-2008, and 1181 were followed to deliver live singletons. Women aged ≥ 18 years old were recruited prior to 15 weeks gestation. Relevant health information, urine and blood samples were collected at up to four time points, Visit 1: median 10 weeks gestation, Visit 2 median 18 weeks gestation, Visit 3 median 26 weeks gestation, and Visit 4 median 35 weeks gestation. The visits were meant to coincide with clinically relevant pregnancy characteristics. Demographic and lifestyle details were collected at the first study visit. From the LifeCodes cohort, 130 women who delivered preterm (< 37 weeks) and 352 randomly selected controls were included in the nested case-control study. An additional 41 women were excluded with self-reported pre-existing and/or gestational thyroid diseases (such as hyper- or hypothyroidism, Graves' disease, or thyroid cancer). The final study population included 116 cases of preterm birth and 323 controls. The study protocols were approved by the ethics and research committees from Brigham and Women's Hospital and the University of Michigan, and all study participants provided written informed consent.

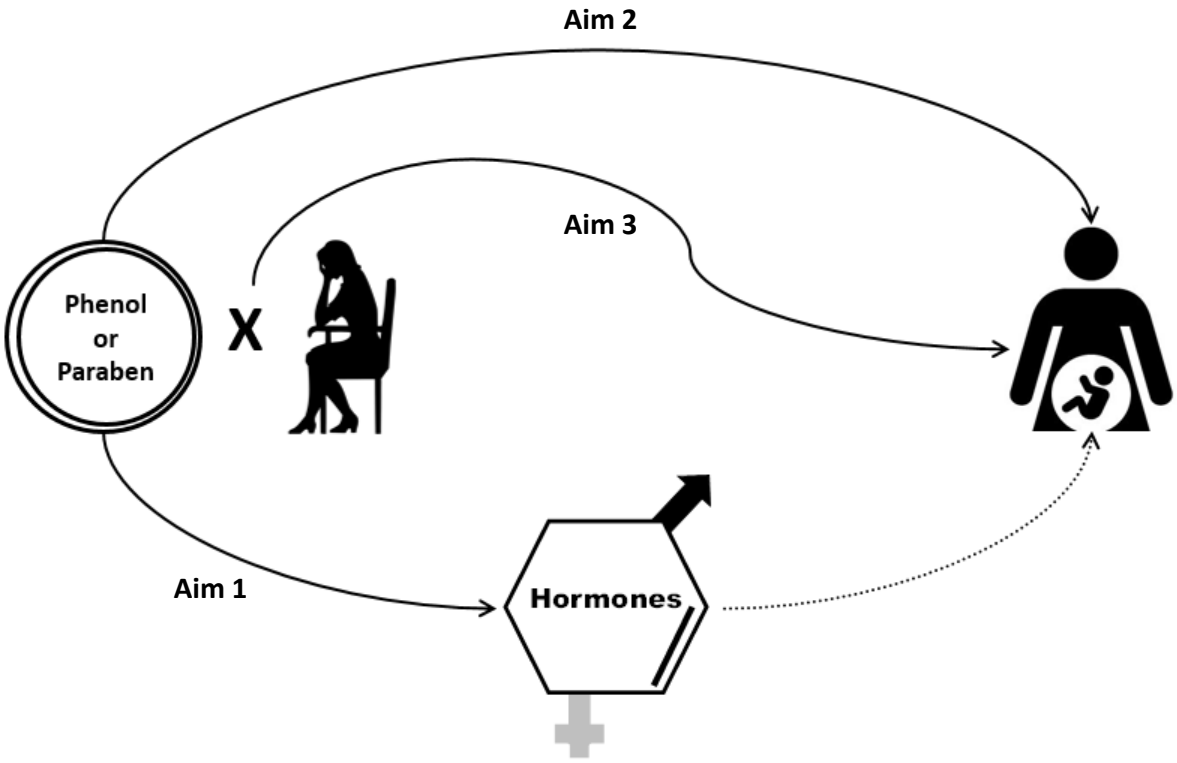


Figure I.1 Conceptual figure of Dissertation Study Aims

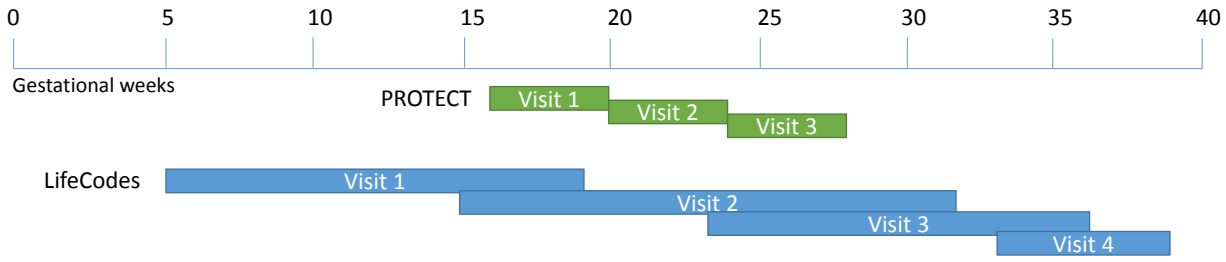


Figure I.2 Study visit timelines by gestational age (weeks) for the PROTECT and LifeCodes cohorts

References

[1] L. Liu, H.L. Johnson, S. Cousens, J. Perin, S. Scott, J.E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers, R.E. Black, Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000, The Lancet. 379 (2012) 2151–2161. doi:10.1016/S0140-6736(12)60560-1.

[2] H. Blencowe, S. Cousens, D. Chou, M. Oestergaard, L. Say, A.-B. Moller, M. Kinney, J. Lawn, Born Too Soon: The global epidemiology of 15 million preterm births, Reprod. Health. 10 (2013) S2. doi:10.1186/1742-4755-10-S1-S2.

[3] Centers for Disease Control and Prevention, Preterm Birth | Maternal and Infant Health | Reproductive Health | CDC, Preterm Birth. (2015). http://www.cdc.gov/reproductivehealth/maternalinfanthealth/pretermbirth.htm (accessed February 8, 2016).

[4] N. Marlow, D. Wolke, M.A. Bracewell, M. Samara, Neurologic and Developmental Disability at Six Years of Age after Extremely Preterm Birth, N. Engl. J. Med. 352 (2005) 9–19. doi:10.1056/NEJMoa041367.

[5] T.M. Luu, S.L. Katz, P. Leeson, B. Thébaud, A.-M. Nuyt, Preterm birth: risk factor for early-onset chronic diseases, CMAJ Can. Med. Assoc. J. J. Assoc. Medicale Can. (2015). doi:10.1503/cmaj.150450.

[6] C.N. Schoen, S. Tabbah, J.D. Iams, A.B. Caughey, V. Berghella, Why the United States preterm birth rate is declining, Am. J. Obstet. Gynecol. 213 (2015) 175–180. doi:10.1016/j.ajog.2014.12.011.

[7] B.E. Hamilton, J.A. Martin, M.J.K. Osterman, S.C. Curtin, T.J. Matthews, Births: Final Data for 2014, Natl. Vital Stat. Rep. Cent. Dis. Control Prev. Natl. Cent. Health Stat. Natl. Vital Stat. Syst. 64 (2015) 1–64.

[8] A.B. Caughey, N.E. Stotland, A.E. Washington, G.J. Escobar, Maternal and obstetric complications of pregnancy are associated with increasing gestational age at term, Am. J. Obstet. Gynecol. 196 (2007) 155.e1-6. doi:10.1016/j.ajog.2006.08.040.

[9] M. Galal, I. Symonds, H. Murray, F. Petraglia, R. Smith, Postterm pregnancy, Facts Views Vis. ObGyn. 4 (2012) 175–187.

[10] J.A. Martin, B.E. Hamilton, P.D. Sutton, S.J. Ventura, F. Menacker, S. Kirmeyer, M.L. Munson, Centers for Disease Control and Prevention National Center for Health Statistics National Vital Statistics System, Births: final data for 2005, Natl. Vital Stat. Rep. Cent. Dis. Control Prev. Natl. Cent. Health Stat. Natl. Vital Stat. Syst. 56 (2007) 1–103.

[11] Premature birt report card, March of Dimes, 2015. http://www.marchofdimes.org/materials/premature-birth-report-card-puerto-rico.pdf.

[12] H. Blencowe, S. Cousens, M.Z. Oestergaard, D. Chou, A.-B. Moller, R. Narwal, A. Adler, C. Vera Garcia, S. Rohde, L. Say, J.E. Lawn, National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications, Lancet Lond. Engl. 379 (2012) 2162–2172. doi:10.1016/S0140-6736(12)60820-4.

[13] J. Roman, The Puerto Rico Healthcare Crisis, Ann. Am. Thorac. Soc. 12 (2015) 1760–1763. doi:10.1513/AnnalsATS.201508-531PS.

[14] I. Padilla, C. Irizarry, K. Steele, Historical contamination of groundwater resrouces in the North Coast karst aquifers of Puerto Rico, Rev. Dimens. 3 (2011) 7.

[15] S.D. Mitro, T. Johnson, A.R. Zota, Cumulative Chemical Exposures During Pregnancy and Early Development, Curr. Environ. Health Rep. 2 (2015) 367–378. doi:10.1007/s40572-015-0064-x.
[16] L.L. Aylward, S.M. Hays, C.R. Kirman, S.A. Marchitti, J.F. Kenneke, C. English, D.R. Mattison, R.A. Becker, Relationships of chemical concentrations in maternal and cord blood: a review of available data, J. Toxicol. Environ. Health B Crit. Rev. 17 (2014) 175–203. doi:10.1080/10937404.2014.884956.
[17] E. Diamanti-Kandarakis, J.-P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, R.T. Zoeller, A.C. Gore, Endocrine-disrupting chemicals: an Endocrine Society scientific statement, Endocr. Rev. 30 (2009) 293–342. doi:10.1210/er.2009-0002.
[18] A. Wang, A. Padula, M. Sirota, T.J. Woodruff, Environmental Influences on Reproductive Health, the Importance of Chemical Exposures, Fertil. Steril. 106 (2016) 905–929. doi:10.1016/j.fertnstert.2016.07.1076.
[19] Centers for Disease Control and Prevention, Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2017, Centers for Disease Control and Prevention (CDC), Atlanta, GA, 2017. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf (accessed May 9, 2017).
[20] P. Ashrap, D.J. Watkins, A.M. Calafat, X. Ye, Z. Rosario, P. Brown, C.M. Vélez-Vega, A. Alshawabkeh, J.F. Cordero, J.D. Meeker, Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends, Environ. Int. (2018). doi:10.1016/j.envint.2018.08.020.
[21] Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, Centers for Disease Control and Prevention (CDC), 2015.
[22] A.B. Dann, A. Hontela, Triclosan: environmental exposure, toxicity and mechanisms of action, J. Appl. Toxicol. JAT. 31 (2011) 285–311. doi:10.1002/jat.1660.
[23] R.U. Halden, A.E. Lindeman, A.E. Aiello, D. Andrews, W.A. Arnold, P. Fair, R.E. Fuoco, L.A. Geer, P.I. Johnson, R. Lohmann, K. McNeill, V.P. Sacks, T. Schettler, R. Weber, R.T. Zoeller, A. Blum, The Florence Statement on Triclosan and Triclocarban, Environ. Health Perspect. 125 (2017) 064501. doi:10.1289/EHP1788.
[24] J.D. Meeker, D.E. Cantonwine, L.O. Rivera-González, K.K. Ferguson, B. Mukherjee, A.M. Calafat, X. Ye, L.V. Anzalota Del Toro, N. Crespo-Hernández, B. Jiménez-Vélez, A.N. Alshawabkeh, J.F. Cordero, Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico, Environ. Sci. Technol. 47 (2013) 3439–3447. doi:10.1021/es400510g.
[25] M. Fisher, S. MacPherson, J.M. Braun, R. Hauser, M. Walker, M. Feeley, R. Mallick, R. Bérubé, T.E. Arbuckle, Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product Use, Environ. Sci. Technol. 51 (2017) 4009–4017. doi:10.1021/acs.est.6b04302.
[26] L.A. Geer, B.F.G. Pycke, J. Waxenbaum, D.M. Sherer, O. Abulafia, R.U. Halden, Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York, J. Hazard. Mater. 323, Part A (2017) 177–183. doi:10.1016/j.jhazmat.2016.03.028.
[27] B.F.G. Pycke, L.A. Geer, M. Dalloul, O. Abulafia, R.U. Halden, Maternal and fetal exposure to parabens in a multiethnic urban U.S. population, Environ. Int. 84 (2015) 193–200. doi:10.1016/j.envint.2015.08.012.

[28] J.R. Rochester, A.L. Bolden, K.E. Pelch, C.F. Kwiatkowski, Potential Developmental and Reproductive Impacts of Triclocarban: A Scoping Review, J. Toxicol. 2017 (2017). doi:10.1155/2017/9679738.
[29] L.N. Vandenberg, I. Chahoud, J.J. Heindel, V. Padmanabhan, F.J.R. Paumgartten, G. Schoenfelder, Urinary, Circulating, and Tissue Biomonitoring Studies Indicate Widespread Exposure to Bisphenol A, Environ. Health Perspect. 118 (2010) 1055–1070. doi:10.1289/ehp.0901716.
[30] F. Vela-Soria, I. Jiménez-Díaz, R. Rodríguez-Gómez, A. Zafra-Gómez, O. Ballesteros, A. Navalón, J.L. Vílchez, M.F. Fernández, N. Olea, Determination of benzophenones in human placental tissue samples by liquid chromatography-tandem mass spectrometry, Talanta. 85 (2011) 1848–1855. doi:10.1016/j.talanta.2011.07.030.
[31] T.J. Woodruff, A.R. Zota, J.M. Schwartz, Environmental chemicals in pregnant women in the United States: NHANES 2003-2004, Environ. Health Perspect. 119 (2011) 878–885. doi:10.1289/ehp.1002727.
[32] K.K. Ferguson, J.D. Meeker, D.E. Cantonwine, B. Mukherjee, G.G. Pace, D. Weller, T.F. McElrath, Environmental phenol associations with ultrasound and delivery measures of fetal growth, Environ. Int. 112 (2018) 243–250. doi:10.1016/j.envint.2017.12.011.
[33] T.H. Lassen, H. Frederiksen, H.B. Kyhl, S.H. Swan, K.M. Main, A.-M. Andersson, D.V. Lind, S. Husby, C. Wohlfahrt-Veje, N.E. Skakkebæk, T.K. Jensen, Prenatal Triclosan Exposure and Anthropometric Measures including Anogenital Distance in Danish Infants, Environ. Health Perspect. (2016). doi:10.1289/ehp.1409637.
[34] C. Philippat, J. Botton, A.M. Calafat, X. Ye, M.-A. Charles, R. Slama, EDEN Study Group, Prenatal exposure to phenols and growth in boys, Epidemiol. Camb. Mass. 25 (2014) 625–635. doi:10.1097/EDE.0000000000000132.
[35] R. Tang, M.-J. Chen, G.-D. Ding, X.-J. Chen, X.-M. Han, K. Zhou, L.-M. Chen, Y.-K. Xia, Y. Tian, X.-R. Wang, Associations of prenatal exposure to phenols with birth outcomes, Environ. Pollut. Barking Essex 1987. 178 (2013) 115–120. doi:10.1016/j.envpol.2013.03.023.
[36] M.S. Wolff, S.M. Engel, G.S. Berkowitz, X. Ye, M.J. Silva, C. Zhu, J. Wetmur, A.M. Calafat, Prenatal phenol and phthalate exposures and birth outcomes, Environ. Health Perspect. 116 (2008) 1092–1097. doi:10.1289/ehp.11007.
[37] B. Weinberger, A.M. Vetrano, F.E. Archer, S.W. Marcella, B. Buckley, D. Wartenberg, M.G. Robson, J. Klim, S. Azhar, S. Cavin, L. Wang, D.Q. Rich, Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age, J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. 27 (2014) 323–327. doi:10.3109/14767058.2013.815718.
[38] D.E. Cantonwine, K.K. Ferguson, B. Mukherjee, T.F. McElrath, J.D. Meeker, Urinary Bisphenol A Levels during Pregnancy and Risk of Preterm Birth, Environ. Health Perspect. 123 (2015) 895–901. doi:10.1289/ehp.1408126.
[39] J. Troisi, C. Mikelson, S. Richards, S. Symes, D. Adair, F. Zullo, M. Guida, Placental concentrations of bisphenol A and birth weight from births in the Southeastern U.S, Placenta. 35 (2014) 947–952. doi:10.1016/j.placenta.2014.08.091.
[40] C.A. Snijder, D. Heederik, F.H. Pierik, A. Hofman, V.W. Jaddoe, H.M. Koch, M.P. Longnecker, A. Burdorf, Fetal Growth and Prenatal Exposure to Bisphenol A: The Generation R Study, Environ. Health Perspect. 121 (2013) 393–398. doi:10.1289/ehp.1205296.

[41] W.-C. Chou, J.-L. Chen, C.-F. Lin, Y.-C. Chen, F.-C. Shih, C.-Y. Chuang, Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan, Environ. Health Glob. Access Sci. Source. 10 (2011) 94. doi:10.1186/1476-069X-10-94.

[42] M. Miao, W. Yuan, G. Zhu, X. He, D.-K. Li, In utero exposure to bisphenol-A and its effect on birth weight of offspring, Reprod. Toxicol. Elmsford N. 32 (2011) 64–68. doi:10.1016/j.reprotox.2011.03.002.

[43] W. Huo, W. Xia, Y. Wan, B. Zhang, A. Zhou, Y. Zhang, K. Huang, Y. Zhu, C. Wu, Y. Peng, M. Jiang, J. Hu, H. Chang, B. Xu, Y. Li, S. Xu, Maternal urinary bisphenol A levels and infant low birth weight: A nested case-control study of the Health Baby Cohort in China, Environ. Int. 85 (2015) 96–103. doi:10.1016/j.envint.2015.09.005.

[44] D. Cantonwine, J.D. Meeker, H. Hu, B.N. Sánchez, H. Lamadrid-Figueroa, A. Mercado-García, G.Z. Fortenberry, A.M. Calafat, M.M. Téllez-Rojo, Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study, Environ. Health Glob. Access Sci. Source. 9 (2010) 62. doi:10.1186/1476-069X-9-62.

[45] A. Veiga-Lopez, K. Kannan, C. Liao, W. Ye, S.E. Domino, V. Padmanabhan, Gender-Specific Effects on Gestational Length and Birth Weight by Early Pregnancy BPA Exposure, J. Clin. Endocrinol. Metab. 100 (2015) E1394-1403. doi:10.1210/jc.2015-1724.

[46] C. Philippat, M. Mortamais, C. Chevrier, C. Petit, A.M. Calafat, X. Ye, M.J. Silva, C. Brambilla, I. Pin, M.-A. Charles, S. Cordier, R. Slama, Exposure to phthalates and phenols during pregnancy and offspring size at birth, Environ. Health Perspect. 120 (2012) 464–470. doi:10.1289/ehp.1103634.

[47] H. Aoyama, H. Hojo, K.L. Takahashi, N. Shimizu, M. Araki, M. Harigae, N. Tanaka, N. Shirasaka, M. Kuwahara, N. Nakashima, E. Yamamoto, M. Saka, S. Teramoto, A two-generation reproductive toxicity study of 2,4-dichlorophenol in rats, J. Toxicol. Sci. 30 Spec No. (2005) 59–78.

[48] D.E. Rodwell, R.D. Wilson, M.D. Nemec, M.D. Mercieca, Teratogenic assessment of 2,4-dichlorophenol in Fischer 344 rats, Fundam. Appl. Toxicol. Off. J. Soc. Toxicol. 13 (1989) 635–640.

[49] P.E.A. Rodríguez, M.S. Sanchez, Maternal exposure to triclosan impairs thyroid homeostasis and female pubertal development in Wistar rat offspring, J. Toxicol. Environ. Health A. 73 (2010) 1678–1688. doi:10.1080/15287394.2010.516241.

[50] L. Zhang, L. Dong, S. Ding, P. Qiao, C. Wang, M. Zhang, L. Zhang, Q. Du, Y. Li, N. Tang, B. Chang, Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed E_2 levels in male rat offspring, Environ. Toxicol. Pharmacol. 37 (2014) 705–717. doi:10.1016/j.etap.2014.01.016.

[51] F. Manservisi, K. Gopalakrishnan, E. Tibaldi, A. Hysi, M. Iezzi, L. Lambertini, S. Teitelbaum, J. Chen, F. Belpoggi, Effect of maternal exposure to endocrine disrupting chemicals on reproduction and mammary gland development in female Sprague-Dawley rats, Reprod. Toxicol. 54 (2015) 110–119. doi:10.1016/j.reprotox.2014.12.013.

[52] J. Shaw, D. deCatanzaro, Estrogenicity of parabens revisited: impact of parabens on early pregnancy and an uterotrophic assay in mice, Reprod. Toxicol. Elmsford N. 28 (2009) 26–31. doi:10.1016/j.reprotox.2009.03.003.

[53] J. Boberg, S. Metzdorff, R. Wortziger, M. Axelstad, L. Brokken, A.M. Vinggaard, M. Dalgaard, C. Nellemann, Impact of diisobutyl phthalate and other PPAR agonists on

steroidogenesis and plasma insulin and leptin levels in fetal rats, Toxicology. 250 (2008) 75–81. doi:10.1016/j.tox.2008.05.020.
[54] K.W. Smith, J.M. Braun, P.L. Williams, S. Ehrlich, K.F. Correia, A.M. Calafat, X. Ye, J. Ford, M. Keller, J.D. Meeker, R. Hauser, Predictors and Variability of Urinary Paraben Concentrations in Men and Women, Including before and during Pregnancy, Environ. Health Perspect. 120 (2012) 1538–1543. doi:10.1289/ehp.1104614.
[55] J.D. Meeker, T. Yang, X. Ye, A.M. Calafat, R. Hauser, Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage, Environ. Health Perspect. 119 (2011) 252–257. doi:10.1289/ehp.1002238.
[56] J.D. Meeker, D.E. Cantonwine, L.O. Rivera-González, K.K. Ferguson, B. Mukherjee, A.M. Calafat, X. Ye, L.V. Anzalota Del Toro, N. Crespo-Hernández, B. Jiménez-Vélez, A.N. Alshawabkeh, J.F. Cordero, Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico, Environ. Sci. Technol. 47 (2013) 3439–3447. doi:10.1021/es400510g.
[57] M. Krause, A. Klit, M. Blomberg Jensen, T. Søeborg, H. Frederiksen, M. Schlumpf, W. Lichtensteiger, N.E. Skakkebaek, K.T. Drzewiecki, Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters, Int. J. Androl. 35 (2012) 424–436. doi:10.1111/j.1365-2605.2012.01280.x.
[58] C.-F. Wang, Y. Tian, Reproductive endocrine-disrupting effects of triclosan: Population exposure, present evidence and potential mechanisms, Environ. Pollut. Barking Essex 1987. 206 (2015) 195–201. doi:10.1016/j.envpol.2015.07.001.
[59] M.S. Wolff, S.L. Teitelbaum, K. McGovern, S.M. Pinney, G.C. Windham, M. Galvez, A. Pajak, M. Rybak, A.M. Calafat, L.H. Kushi, F.M. Biro, Breast Cancer and Environment Research Program. Electronic address: http://www.bcerp.org/index.htm, Environmental phenols and pubertal development in girls, Environ. Int. 84 (2015) 174–180. doi:10.1016/j.envint.2015.08.008.
[60] P.D. Darbre, P.W. Harvey, Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks, J. Appl. Toxicol. JAT. 28 (2008) 561–578. doi:10.1002/jat.1358.
[61] E. Karpuzoglu, S.D. Holladay, R.M. Gogal, Parabens: potential impact of low-affinity estrogen receptor binding chemicals on human health, J. Toxicol. Environ. Health B Crit. Rev. 16 (2013) 321–335. doi:10.1080/10937404.2013.809252.
[62] T.T. Schug, A. Janesick, B. Blumberg, J.J. Heindel, Endocrine Disrupting Chemicals and Disease Susceptibility, J. Steroid Biochem. Mol. Biol. 127 (2011) 204–215. doi:10.1016/j.jsbmb.2011.08.007.
[63] V.D. Castracane, Endocrinology of preterm labor, Clin. Obstet. Gynecol. 43 (2000) 717–726.
[64] M. Melamed, E. Castaño, A.C. Notides, S. Sasson, Molecular and kinetic basis for the mixed agonist/antagonist activity of estriol, Mol. Endocrinol. Baltim. Md. 11 (1997) 1868–1878. doi:10.1210/mend.11.12.0025.
[65] R. Smith, J.I. Smith, X. Shen, P.J. Engel, M.E. Bowman, S.A. McGrath, A.M. Bisits, P. McElduff, W.B. Giles, D.W. Smith, Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor, J. Clin. Endocrinol. Metab. 94 (2009) 2066–2074. doi:10.1210/jc.2008-2257.
[66] G.C.L. Lachelin, H.H.G. McGarrigle, P.T. Seed, A. Briley, A.H. Shennan, L. Poston, Low saliva progesterone concentrations are associated with spontaneous early preterm

labour (before 34 weeks of gestation) in women at increased risk of preterm delivery, BJOG Int. J. Obstet. Gynaecol. 116 (2009) 1515–1519. doi:10.1111/j.1471-0528.2009.02293.x.
[67] E. Tingi, A.A. Syed, A. Kyriacou, G. Mastorakos, A. Kyriacou, Benign thyroid disease in pregnancy: A state of the art review, J. Clin. Transl. Endocrinol. 6 (2016) 37–49. doi:10.1016/j.jcte.2016.11.001.
[68] G. Mastorakos, E.I. Karoutsou, M. Mizamtsidi, G. Creatsas, The menace of endocrine disruptors on thyroid hormone physiology and their impact on intrauterine development, Endocrine. 31 (2007) 219–237.
[69] G.R. Williams, Neurodevelopmental and Neurophysiological Actions of Thyroid Hormone, J. Neuroendocrinol. 20 (2008) 784–794. doi:10.1111/j.1365-2826.2008.01733.x.
[70] T.R. Zoeller, A.L.S. Dowling, C.T.A. Herzig, E.A. Iannacone, K.J. Gauger, R. Bansal, Thyroid hormone, brain development, and the environment, Environ. Health Perspect. 110 Suppl 3 (2002) 355–361.
[71] A.J. Forhead, A.L. Fowden, Thyroid hormones in fetal growth and prepartum maturation, J. Endocrinol. 221 (2014) R87–R103. doi:10.1530/JOE-14-0025.
[72] D. Glinoer, The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology, Endocr. Rev. 18 (1997) 404–433. doi:10.1210/edrv.18.3.0300.
[73] M.-L. Hartoft-Nielsen, M. Boas, S. Bliddal, Å.K. Rasmussen, K. Main, U. Feldt-Rasmussen, M.-L. Hartoft-Nielsen, M. Boas, S. Bliddal, Å.K. Rasmussen, K. Main, U. Feldt-Rasmussen, Do Thyroid Disrupting Chemicals Influence Foetal Development during Pregnancy?, Do Thyroid Disrupting Chemicals Influence Foetal Development during Pregnancy?, J. Thyroid Res. J. Thyroid Res. 2011, 2011 (2011) e342189. doi:10.4061/2011/342189, 10.4061/2011/342189.
[74] J.M. Braun, Early-life exposure to EDCs: role in childhood obesity and neurodevelopment, Nat. Rev. Endocrinol. 13 (2017) 161–173. doi:10.1038/nrendo.2016.186.
[75] L.-M. Chen, W.-J. Du, J. Dai, Q. Zhang, G.-X. Si, H. Yang, E.-L. Ye, Q.-S. Chen, L.-C. Yu, C. Zhang, X.-M. Lu, Effects of subclinical hypothyroidism on maternal and perinatal outcomes during pregnancy: a single-center cohort study of a Chinese population, PloS One. 9 (2014) e109364. doi:10.1371/journal.pone.0109364.
[76] F. Saki, M.H. Dabbaghmanesh, S.Z. Ghaemi, S. Forouhari, G. Ranjbar Omrani, M. Bakhshayeshkaram, Thyroid function in pregnancy and its influences on maternal and fetal outcomes, Int. J. Endocrinol. Metab. 12 (2014) e19378. doi:10.5812/ijem.19378.
[77] A.Z. Pollack, S.L. Mumford, J.R. Krall, A.E. Carmichael, L.A. Sjaarda, N.J. Perkins, K. Kannan, E.F. Schisterman, Exposure to bisphenol A, chlorophenols, benzophenones, and parabens in relation to reproductive hormones in healthy women: A chemical mixture approach, Environ. Int. 120 (2018) 137–144. doi:10.1016/j.envint.2018.07.028.
[78] K. Berger, R.B. Gunier, J. Chevrier, A.M. Calafat, X. Ye, B. Eskenazi, K.G. Harley, Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels, Environ. Res. 165 (2018) 379–386. doi:10.1016/j.envres.2018.05.005.
[79] Y. Ma, J. Han, Y. Guo, P.K.S. Lam, R.S.S. Wu, J.P. Giesy, X. Zhang, B. Zhou, Disruption of endocrine function in in vitro H295R cell-based and in in vivo assay in

zebrafish by 2,4-dichlorophenol, Aquat. Toxicol. Amst. Neth. 106–107 (2012) 173–181. doi:10.1016/j.aquatox.2011.11.006.
[80] A.M. Binder, C. Corvalan, A.M. Calafat, X. Ye, V. Mericq, A. Pereira, K.B. Michels, Childhood and adolescent phenol and phthalate exposure and the age of menarche in Latina girls, Environ. Health Glob. Access Sci. Source. 17 (2018) 32. doi:10.1186/s12940-018-0376-z.
[81] Y. Wei, J. Zhu, Para-Dichlorobenzene Exposure Is Associated with Thyroid Dysfunction in US Adolescents, J. Pediatr. 177 (2016) 238–243. doi:10.1016/j.jpeds.2016.06.085.
[82] J. Peretz, L. Vrooman, W.A. Ricke, P.A. Hunt, S. Ehrlich, R. Hauser, V. Padmanabhan, H.S. Taylor, S.H. Swan, C.A. VandeVoort, J.A. Flaws, Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013, Environ. Health Perspect. 122 (2014) 775–786. doi:10.1289/ehp.1307728.
[83] Y.-F. Zhang, X.-M. Ren, Y.-Y. Li, X.-F. Yao, C.-H. Li, Z.-F. Qin, L.-H. Guo, Bisphenol A alternatives bisphenol S and bisphenol F interfere with thyroid hormone signaling pathway in vitro and in vivo, Environ. Pollut. Barking Essex 1987. 237 (2018) 1072–1079. doi:10.1016/j.envpol.2017.11.027.
[84] J.M. Gámez, R. Penalba, N. Cardoso, P.S. Bernasconi, S. Carbone, O. Ponzo, M. Pandolfi, P. Scacchi, R. Reynoso, Exposure to a low dose of bisphenol A impairs pituitary-ovarian axis in prepubertal rats: effects on early folliculogenesis, Environ. Toxicol. Pharmacol. 39 (2015) 9–15. doi:10.1016/j.etap.2014.10.015.
[85] X. Liu, M. Miao, Z. Zhou, E. Gao, J. Chen, J. Wang, F. Sun, W. Yuan, D.-K. Li, Exposure to bisphenol-A and reproductive hormones among male adults, Environ. Toxicol. Pharmacol. 39 (2015) 934–941. doi:10.1016/j.etap.2015.03.007.
[86] D. Guignard, V. Gayrard, M.Z. Lacroix, S. Puel, N. Picard-Hagen, C. Viguié, Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level representative of human exposure, Chemosphere. 182 (2017) 458–467. doi:10.1016/j.chemosphere.2017.05.028.
[87] R.T. Zoeller, R. Bansal, C. Parris, Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain, Endocrinology. 146 (2005) 607–612. doi:10.1210/en.2004-1018.
[88] K.J. Oliveira, M.I. Chiamolera, G. Giannocco, C.C. Pazos-Moura, T.M. Ortiga-Carvalho, Thyroid Function Disruptors: from nature to chemicals, J. Mol. Endocrinol. (2018). doi:10.1530/JME-18-0081.
[89] J.R. Rochester, Bisphenol A and human health: A review of the literature, Reprod. Toxicol. 42 (2013) 132–155. doi:10.1016/j.reprotox.2013.08.008.
[90] C.V. Sartain, P.A. Hunt, An old culprit but a new story: bisphenol A and "NextGen" bisphenols, Fertil. Steril. 106 (2016) 820–826. doi:10.1016/j.fertnstert.2016.07.1114.
[91] X.D. Andrianou, S. Gängler, A. Piciu, P. Charisiadis, C. Zira, K. Aristidou, D. Piciu, R. Hauser, K.C. Makris, Human Exposures to Bisphenol A, Bisphenol F and Chlorinated Bisphenol A Derivatives and Thyroid Function, PLOS ONE. 11 (2016) e0155237. doi:10.1371/journal.pone.0155237.
[92] P.Y. Kunz, K. Fent, Multiple hormonal activities of UV filters and comparison of in vivo and in vitro estrogenic activity of ethyl-4-aminobenzoate in fish, Aquat. Toxicol. 79 (2006) 305–324. doi:10.1016/j.aquatox.2006.06.016.

[93] R. Ma, B. Cotton, W. Lichtensteiger, M. Schlumpf, UV filters with antagonistic action at androgen receptors in the MDA-kb2 cell transcriptional-activation assay, Toxicol. Sci. Off. J. Soc. Toxicol. 74 (2003) 43–50. doi:10.1093/toxsci/kfg102.
[94] C. Schmutzler, I. Gotthardt, P.J. Hofmann, B. Radovic, G. Kovacs, L. Stemmler, I. Nobis, A. Bacinski, B. Mentrup, P. Ambrugger, A. Grüters, L.K. Malendowicz, J. Christoffel, H. Jarry, D. Seidlovà-Wuttke, W. Wuttke, J. Köhrle, Endocrine Disruptors and the Thyroid Gland—A Combined in Vitro and in Vivo Analysis of Potential New Biomarkers, Environ. Health Perspect. 115 (2007) 77–83. doi:10.1289/ehp.9369.
[95] R.H.M.M. Schreurs, E. Sonneveld, J.H.J. Jansen, W. Seinen, B. van der Burg, Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene bioassays, Toxicol. Sci. Off. J. Soc. Toxicol. 83 (2005) 264–272. doi:10.1093/toxsci/kfi035.
[96] I. Ozáez, M. Aquilino, G. Morcillo, J.-L. Martínez-Guitarte, UV filters induce transcriptional changes of different hormonal receptors in Chironomus riparius embryos and larvae, Environ. Pollut. Barking Essex 1987. 214 (2016) 239–247. doi:10.1016/j.envpol.2016.04.023.
[97] T.-H. Chen, Y.-T. Wu, W.-H. Ding, UV-filter benzophenone-3 inhibits agonistic behavior in male Siamese fighting fish (Betta splendens), Ecotoxicol. Lond. Engl. 25 (2016) 302–309. doi:10.1007/s10646-015-1588-4.
[98] C. Wang, L. Chen, S. Zhao, Y. Hu, Y. Zhou, Y. Gao, W. Wang, J. Zhang, Y. Tian, Impacts of prenatal triclosan exposure on fetal reproductive hormones and its potential mechanism, Environ. Int. 111 (2018) 279–286. doi:10.1016/j.envint.2017.11.007.
[99] K.M. Crofton, K.B. Paul, M.J. Devito, J.M. Hedge, Short-term in vivo exposure to the water contaminant triclosan: Evidence for disruption of thyroxine, Environ. Toxicol. Pharmacol. 24 (2007) 194–197. doi:10.1016/j.etap.2007.04.008.
[100] K.B. Paul, J.M. Hedge, M.J. Devito, K.M. Crofton, Developmental triclosan exposure decreases maternal and neonatal thyroxine in rats, Environ. Toxicol. Chem. 29 (2010) 2840–2844. doi:10.1002/etc.339.
[101] L.M. Zorrilla, E.K. Gibson, S.C. Jeffay, K.M. Crofton, W.R. Setzer, R.L. Cooper, T.E. Stoker, The effects of triclosan on puberty and thyroid hormones in male Wistar rats, Toxicol. Sci. Off. J. Soc. Toxicol. 107 (2009) 56–64. doi:10.1093/toxsci/kfn225.
[102] J. Chen, K.C. Ahn, N.A. Gee, M.I. Ahmed, A.J. Duleba, L. Zhao, S.J. Gee, B.D. Hammock, B.L. Lasley, Triclocarban enhances testosterone action: a new type of endocrine disruptor?, Endocrinology. 149 (2008) 1173–1179. doi:10.1210/en.2007-1057.
[103] S. El Hussein, P. Muret, M. Berard, S. Makki, P. Humbert, Assessment of principal parabens used in cosmetics after their passage through human epidermis-dermis layers (ex-vivo study), Exp. Dermatol. 16 (2007) 830–836. doi:10.1111/j.1600-0625.2007.00625.x.
[104] C. Taxvig, A.M. Vinggaard, U. Hass, M. Axelstad, J. Boberg, P.R. Hansen, H. Frederiksen, C. Nellemann, Do parabens have the ability to interfere with steroidogenesis?, Toxicol. Sci. Off. J. Soc. Toxicol. 106 (2008) 206–213. doi:10.1093/toxsci/kfn148.
[105] M. Kawaguchi, K. Morohoshi, J. Masuda, G. Watanabe, M. Morita, H. Imai, K. Taya, T. Himi, Maternal isobutyl-paraben exposure decreases the plasma corticosterone level in dams and sensitivity to estrogen in female offspring rats, J. Vet. Med. Sci. Jpn. Soc. Vet. Sci. 71 (2009) 1027–1033.

[106] T.T.B. Vo, Y.-M. Yoo, K.-C. Choi, E.-B. Jeung, Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model, Reprod. Toxicol. Elmsford N. 29 (2010) 306–316. doi:10.1016/j.reprotox.2010.01.013.
[107] L.E. Dodge, P.L. Williams, M.A. Williams, S.A. Missmer, T.L. Toth, A.M. Calafat, R. Hauser, Paternal Urinary Concentrations of Parabens and Other Phenols in Relation to Reproductive Outcomes among Couples from a Fertility Clinic, Environ. Health Perspect. (2015). doi:10.1289/ehp.1408605.
[108] K.-N. Kim, Y.-C. Hong, The exposome and the future of epidemiology: a vision and prospect, Environ. Health Toxicol. 32 (2017) e2017009. doi:10.5620/eht.e2017009.
[109] C.P. Wild, Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology, Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 14 (2005) 1847–1850. doi:10.1158/1055-9965.EPI-05-0456.
[110] H.M. Vesterinen, R. Morello-Frosch, S. Sen, L. Zeise, T.J. Woodruff, Cumulative effects of prenatal-exposure to exogenous chemicals and psychosocial stress on fetal growth: Systematic-review of the human and animal evidence, PLoS ONE. 12 (2017). doi:10.1371/journal.pone.0176331.
[111] M.R. Sable, D.S. Wilkinson, Impact of perceived stress, major life events and pregnancy attitudes on low birth weight, Fam. Plann. Perspect. 32 (2000) 288–294.
[112] G.A. Barbosa, The association of life events to gestational age at delivery among low-income, urban, African American women, J. Perinatol. Off. J. Calif. Perinat. Assoc. 20 (2000) 438–442.
[113] N. Dole, D.A. Savitz, I. Hertz-Picciotto, A.M. Siega-Riz, M.J. McMahon, P. Buekens, Maternal stress and preterm birth, Am. J. Epidemiol. 157 (2003) 14–24.
[114] T.P. Dominguez, C.D. Schetter, R. Mancuso, C.M. Rini, C. Hobel, Stress in African American pregnancies: testing the roles of various stress concepts in prediction of birth outcomes, Ann. Behav. Med. Publ. Soc. Behav. Med. 29 (2005) 12–21. doi:10.1207/s15324796abm2901_3.
[115] J.W. Rich-Edwards, T.A. Grizzard, Psychosocial stress and neuroendocrine mechanisms in preterm delivery, Am. J. Obstet. Gynecol. 192 (2005) S30-35. doi:10.1016/j.ajog.2005.01.072.
[116] D. Kingston, S. Tough, H. Whitfield, Prenatal and postpartum maternal psychological distress and infant development: a systematic review, Child Psychiatry Hum. Dev. 43 (2012) 683–714. doi:10.1007/s10578-012-0291-4.
[117] M. Weinstock, The long-term behavioural consequences of prenatal stress, Neurosci. Biobehav. Rev. 32 (2008) 1073–1086. doi:10.1016/j.neubiorev.2008.03.002.
[118] S.M. Smith, W.W. Vale, The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress, Dialogues Clin. Neurosci. 8 (2006) 383–395.
[119] E. Kajantie, D.I.W. Phillips, S. Andersson, D.J.P. Barker, L. Dunkel, T. Forsén, C. Osmond, J. Tuominen, P.J. Wood, J. Eriksson, Size at birth, gestational age and cortisol secretion in adult life: foetal programming of both hyper- and hypocortisolism?, Clin. Endocrinol. (Oxf.). 57 (2002) 635–641.
[120] A.M.V. Ward, H.E. Syddall, P.J. Wood, G.P. Chrousos, D.I.W. Phillips, Fetal programming of the hypothalamic-pituitary-adrenal (HPA) axis: low birth weight and central HPA regulation, J. Clin. Endocrinol. Metab. 89 (2004) 1227–1233. doi:10.1210/jc.2003-030978.

[121] D.I.W. Phillips, A. Jones, Fetal programming of autonomic and HPA function: do people who were small babies have enhanced stress responses?, J. Physiol. 572 (2006) 45–50. doi:10.1113/jphysiol.2005.104695.

CHAPTER II. Associations between Maternal Phenol and Paraben Urinary Biomarkers and Maternal Hormones during Pregnancy: A Repeated Measures Study (LifeCodes)

Abstract:

Background: A number of phenols and parabens are added to consumer products for a variety of functions, and have been found at detectable levels in the majority of the U.S. population. Among other functions, thyroid hormones are essential in fetal neurodevelopment, and could be impacted by the endocrine disrupting effects of phenols and parabens. The present study investigated the association between ten maternal urinary phenol and paraben biomarkers (bisphenol S, triclosan, triclocarban, benzophenone-3, 2,4-dichlorophenol, 2,5-dichlorophenol, and ethyl, butyl, methyl and propyl paraben) and four plasma thyroid hormones in 439 pregnant women in a case-control sample nested within a cohort study based in Boston, MA. Methods: Urine and blood samples were collected from up to four visits during pregnancy (median weeks of gestation at each visit: Visit 1: 9.64, Visit 2: 17.9, Visit 3: 26.0, Visit 4: 35.1). Linear mixed models were constructed to take into account the repeated measures jointly, followed by multivariate linear regression models stratified by gestational age to explore potential windows of susceptibility. Results: We observed decreased total triiodothyronine (T3) in relation to an IQR increase in benzophenone-3 (percent change [%Δ] = -2.07; 95% confidence interval [CI] = -4.16, 0.01), butyl paraben (%Δ = -2.76; 95% CI = -5.25, -0.26) and triclosan (%Δ = -2.53; 95% CI = -4.75, -0.30), and triclocarban at levels above the LOD (%Δ = -5.71; 95% CI = -10.45, -0.97). A 2.41 % increase in T3 was associated with an IQR increase in methyl paraben (95% CI = 0.58, 4.24). We also detected a negative association between free thyroxine (FT4) and propyl paraben (%Δ = -3.14; 95% CI = -6.12, -0.06), and a suggestive positive association between total

thyroxine (T4) and methyl paraben (%Δ = 1.19; 95% CI = -0.10, 2.47). Gestational age-specific multivariate regression analyses showed that the magnitude and direction of some of the observed associations were dependent on the timing of exposure. Conclusion: Certain phenols and parabens were associated with altered thyroid hormone levels during pregnancy, and the timing of exposure influenced the association between phenol and paraben, and hormone concentrations. These changes may contribute to downstream maternal and fetal health outcomes. Additional research is required to replicate the associations, and determine the potential biological mechanisms underlying the observed associations.

Introduction

There are thousands of chemicals found in personal care products (PCP) and household items to which humans could potentially be exposed [1,2]. Usage of PCPs continues during pregnancy, and this may have unique effects on the mother and/or her developing fetus [3,4].

Phenols and parabens are among the chemicals used in PCPs, and are regularly found at detectable levels in the U.S. population [5]. Phenols regularly detected in exposure biomonitoring studies include triclosan (TCS), triclocarban (TCC), benzophenone-3 (BP-3), bisphenol-A (BPA), bisphenol-S (BPS), 2,4-dichlorophenol (2,4-DCP) and 2,5-dichlorophenol (2,5-DCP). Parabens, TCS and TCC are used in PCPs such as soaps and makeup for their anti-microbial properties [5]. The phenol BP-3 is a UV-filter, and is used in sunscreen, cosmetics and some plastic products [5]. BPS is a common alternative to BPA, and is found in foods, plastics and paper products [6]. 2,4-DCP and 2,5-DCP are biomarkers of a compound used in mothballs and room deodorizers; 2,4-DCP is also a metabolite of a herbicide used as a weed killer [5].

Although results have been conflicting, in vitro, animal and human studies have linked a range of phenols and parabens with changes in thyroid hormones [7–14]. Studies have also linked these exposures to a series of adverse health effects that could potentially be mediated through the thyroid hormone system, including changes in pubertal development [15], adverse birth outcomes [16,17], male infertility [18], diminished female fecundity [19], increases in oxidative stress [20], and childhood adiposity [21], among other health effects.

Thyroid hormones, triiodothyronin (T3) and thyroxine (T4), are produced in the thyroid gland, and their levels are negatively controlled by thyroid stimulating hormone (TSH) from the

pituitary gland via the hypothalamic-pituitary-thyroid axis [22]. The fetal thyroid only begins to produce hormones at 10-12 weeks gestation, and is completely dependent on maternal thyroid hormones for neurodevelopment in its first weeks of life, particularly T4 [23–25]. Additionally, thyroid hormones (maternal and fetal) are essential to the development of fetal tissues, and fetal growth promotion [26]. Given this, and the complexity in the hypothalamic–pituitary–thyroid axis, even slight alterations in thyroid hormones could lead to adverse effects in the child [27,28]. In fact, subclinical maternal thyroid dysfunction has been associated with low birth weight, low Apgar scores, and neurological disabilities [29–31]. It is, therefore, important to understand the effects of in-utero exposure to chemicals such as phenols and parabens on maternal thyroid hormones.

Our group recently reported associations between phenols and parabens and maternal thyroid hormones in pregnancy in a small prospective cohort study in Puerto Rico [32]. The present study aimed to test these relationships in a larger study of pregnant women recruited in Boston, USA. We published a study on the effects of BPA on thyroid hormones in this same cohort since BPA was initially the only phenol measured in our samples [33]; the analytical method was recently expanded to include additional phenols and parabens, which are the focus of this study.

Methods

Study Population

The study population includes pregnant women who were participants in a case-control study nested within the longitudinal birth cohort study in Boston, MA, Lifecodes [34,35]. Lifecodes enrolled 1,600 women from 2006-2008, of whom 1,181 were followed until delivery to live singletons. Women ages ≥ 18 years old were recruited early in pregnancy (< 15 weeks of gestation) between 2006 and 2008 and were eligible for participation if they were carrying a singleton, non-anomalous fetus and planned to deliver at Brigham and Women's Hospital. Additional information regarding recruitment and eligibility criteria are described in detail elsewhere [35,36]. Women were followed through the duration of their pregnancy and relevant health information as well as urine and blood samples were collected at initial study visit (Visit 1: median 9.64 weeks of gestation [range = 5.43–19.1 weeks]) as well as three subsequent visits: Visit 2 (median = 17.9 weeks of gestation [range = 14.9–32.1 weeks]), Visit 3 (median = 26.0 weeks of gestation [range = 22.9–36.3 weeks]), and Visit 4 (median = 35.1 weeks of gestation

[range = 33.1–38.3]). The study protocols were approved by the ethics and research committees of the participating institutions, and all study participants provided written informed consent.

From the parent birth cohort, 130 women who delivered preterm (<37 weeks gestation) and 352 randomly selected women who delivered after a full term pregnancy (≥ 37 week gestation) were included in the case-control study. From these, we excluded participants who had pre-existing thyroid conditions such as thyroid cancer, Graves' disease, and hyper- or hypothyroidism (N=41), and excluded those who did not provide any blood samples at any visit (N=2). Our final study population (N=439) included 116 preterm birth cases and 323 controls.

Phenol and paraben measurement

Spot urine samples were collected at each of the four visits. After collection, spot urine samples were divided into aliquots and frozen at -80^{o}C until they were shipped overnight. The samples were analyzed at NSF International (Ann Arbor, MI) for six phenols (2,4-DCP, 2,5-DCP, BP-3, BPS, TCS, and TCC) and four parabens (ethyl- (EPB), methyl- (MPB), butyl- (BPB), and propyl- (PPB) paraben) using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS). The analytical method was a modification of a method developed by the Centers for Disease Control and Prevention (CDC), as described previously [37–39]. Samples below the limit of detection (LOD) were assigned a value of LOD/√2 [40]. Urinary specific gravity (SG) was used to account for urinary dilution, and was measured using a digital handheld refractometer (AtagoCo., Ltd., Tokyo, Japan). For descriptive, univariate data analysis, phenol and paraben concentrations were corrected for SG as follows: $Pc = M [(SGm - 1) / (SGi - 1)]$, where Pc is the SG-corrected exposure concentration (ng/mL), M is the measured exposure concentration, SGm is the study population median urinary specific gravity (1.0196), and SGi is the individual's urinary specific gravity. For bivariate and multivariate analysis, urinary specific gravity was included as a covariate in all models.

To minimize measurement error from the variability in urine dilution, we applied the method developed by [41]. In brief, we ran a linear mixed model (LMM) regressing SG on maternal age, extracted the predicted values of SG from that regression, calculated the ratio of SG and the predicted SG, and used this ratio to standardize the phenol and paraben measurements.

Thyroid hormone measurement

Blood samples were collected at each of the four visits. The samples were frozen at -80˚C until shipped overnight on dry ice to the analytical laboratory. Blood plasma was analyzed for free thyroxine (FT4), total thyroxine (T4), total triiodothyronine (T3), and thyroid stimulating hormone (TSH) at the Clinical Ligand Assay Service Satellite (CLASS) lab at the University of Michigan (Ann Arbor, MI). TSH, T3 and T4 were measured using an automated chemiluminescence immunoassay (Bayer ADVIA Centaur, Siemens Health Care Diagnostics, Inc.), and FT4 was measured using direct equilibrium dialysis followed by radioimmunoassay (IVD Technologies). Thyroid hormones were highly detected in the study population [42]. TSH samples below the LOD (N=5) were assigned a level at the LOD level (0.01 μIU/mL). Given that the LOD for FT4 is not biologically feasible, samples <LOD for FT4 were regarded as missing values in our statistical analyses. The inter-assay coefficients of variation (CV) for all hormones ranged from 2.3% (for T3) to 10.4% (for FT4). The intra-assay CVs ranged from 1.2% (for T3) to 12.3% (for FT4). Volume limitations in some of the samples resulted in differences in the number of samples.

Statistical analyses

We applied inverse probability weighting to all statistical analyses in order to account for the study's case-control design and over-representation of preterm birth cases in our sample. This ensures that the association observed between secondary outcomes (hormone levels) and biomarkers (phenols and parabens) in the present study population would be representative to the overall cohort population, and enhances the generalizability of our results [43]. We constructed the weights as the inverse of the selection probability for cases and controls from the set of eligible cases and controls in the entire cohort. Weighted distributions of key demographic characteristics were calculated. All biomarkers, and the hormones TSH and FT4, were positively-skewed, and were natural log-transformed. The distributions of T3 and T4 approximated normality and remained untransformed in all analyses. Geometric means and standard deviations were calculated for all SG-corrected biomarkers, hormones, and the ratio of T3/T4. The ratio between T3 and T4 provides an indication of thyroid homeostasis, specifically the peripheral conversion of T4 to T3 in tissues.

Two urinary biomarkers, BPS and TCC, were detected in less than 25% of the samples. Therefore, we categorized BPS and TCC into dichotomous variables for the statistical models, where 1 represented a detectable level (>LOD), and 0 was <LOD. BPS and TCC were analyzed as binary variables in all regression models, whereas all other biomarkers were analyzed continuously.

In our repeated measures analysis, we regressed one thyroid hormone on one urinary biomarker using Linear Mixed Models (LMM) with a subject-specific random intercept to account for intra-individual correlation of serial hormone measurements collected over time. The biomarker measures across time were treated as time varying variables in the model. Potential confounders were identified from the literature. They included socio-economic status, body mass index (BMI), alcohol and smoking during pregnancy, and age. The availability of private insurance was used as socioeconomic status indicator. Potential confounders that were found to change the main effect estimate by >10% were retained in the final models as covariates. Final models were adjusted for specific gravity, study visit, BMI at the first study visit, insurance provider, maternal age and gestational age at time of sample collection. To explore windows of susceptibility, we ran multiple linear regressions (MLR) stratified by gestational age, adjusted for the same covariates as those in LMMs. The gestational age groups were <15 weeks, 15-21 weeks, 21-30 weeks, and >30 weeks gestation. In addition to the visit-specific stratified analysis, to conduct a statistical test whether the associations between phenol and/or paraben biomarker varied by study visit of sample collection, we included in our repeated measures analysis interaction terms between urinary biomarkers and the study visit. We also re-ran our analyses in only term births status (>37 weeks gestation) as a sensitivity analysis. To make our results from these models including ln-transformed continuous biomarkers and/or outcomes more interpretable, we transformed regression coefficients to percent changes (and associated 95% confidence intervals) in hormone concentration in relation to the interquartile range (IQR) increase in urinary biomarker concentrations. Beta coefficients from models with categorical biomarkers (BPS and TCC) were transformed to percent changes (and associated 95% confidence intervals) in hormone concentration at biomarker levels above the LOD versus biomarker levels below the LOD. The alpha level was set at 0.05. All statistical analyses were conducted in R Version 3.2.2.

Results

The mean age of the study participants was 31.8 years, 66% attained at least some college education, 56% were White, and less than 20% depended on Medicaid (

Table II.1). The women also had low levels of smoking (7%) and alcohol use (4%) during pregnancy, and only 22% had a BMI level above 30 kg/m^2.

Table II.2 shows the results of the univariate analyses conducted on the urinary biomarkers and hormone levels. Little variation was observed in urinary biomarker concentrations across pregnancy, whereas all hormones showed varied levels across the four time points in pregnancy, as was described previously in greater detail [42,44]. MPB was detected at the highest levels among all biomarkers, followed by BP-3 (Table II.2). MPB was strongly correlated with PPB (Correlation coefficient= 0.83), whereas BPB and EPB (Correlation coefficient =0.57), and 2,4-DCP and 2,5-DCP (Correlation coefficient=0.59) were moderately correlated. No other biomarker concentrations strongly correlated with one another. T3 and FT4 were correlated with T3/T4 (Correlation coefficients=0.74, -0.44, respectively), but T4 was weakly correlated with T3/T4 (Correlation coefficient=0.2) (Figure II.2). T3 and T4 were also moderately correlated (Correlation coefficient=0.46).

Table II.3 shows the results from LMMs, which included measurements collected at up to four time points in pregnancy. 2,4-DCP and 2,5-DCP were not associated with any thyroid hormones, although an IQR increase in 2,4-DCP was suggestively associated with 1.4% decrease in the T3/T4 ratio (percent change [%Δ] = -1.44; 95% confidence interval [CI] = -3.11, 0.22). There were significant 2-3% decreases in T3 in relation to an IQR increase in BP-3 (%Δ = -2.07; 95% CI = -4.16, 0.01), and TCS (%Δ = -2.53; 95% CI = -4.75, -0.30). TCC at levels above the LOD were also negatively associated with T3 (%Δ = -5.71; 95% CI = -10.45, -0.97). While an IQR increase in TCS was associated with a 7.7% increase in TSH (95% CI = 0.01, 16.02), TCC levels above the LOD were associated with a suggestive 13% decrease in TSH (%Δ = -12.86; 95% CI = -25.6, 2.05).

There were no consistent associations among the parabens. There were 2.4% (%Δ = 2.41; 95% CI = 0.58, 4.24), and 1.2% (%Δ = 1.19; 95% CI = -0.10, 2.47) increases in T3 and T4, respectively, with an IQR increase in MPB. BPB was associated with a 2.8% (%Δ = -2.76; 95% CI = -5.25, -0.26), and 3.7% (%Δ = -3.70; 95% CI = -5.98) decrease in T3 and T3/T4 ratio. EPB

was also associated with a reduced T3/T4 ratio (%Δ = -1.87; 95% CI = -3.76, 0.02). PPB was inversely associated with FT4 (%Δ = -3.14; 95% CI = -6.12, -0.06).

To further understand these associations and explore the relationships at specific time points, multiple linear regressions were conducted stratifying by GA (Table II.5). 2,4-DCP was associated with a decrease in T3 (%Δ = -4.05; 95% CI = -7.49, -0.61), and a marginal increase in TSH (%Δ = 6.81; 95% CI = -0.74, 14.94) at 21-30 weeks. 2,4-DCP and 2,5-DCP were associated with decreases in TSH <21 weeks GA, and increases in TSH >21 weeks GA, although only the association between TSH and 2,5-DCP and >30 weeks GA reached statistical association (%Δ = 10.03; 95% CI = 0.74, 20.19). This change in the direction of the associations between 2,4-DCP and 2,5-DCP and TSH by timing in pregnancy was further reflected in the significant interaction terms between the phenol and visit in LMMs.

In contrast to LMM results, BP-3 was associated with all thyroid hormones at at least one time point in MLR models. BP-3 was associated with 5-7% decreases in T3 at all gestational ages except at 15-21 weeks GA, 3-4% decreases in T4 at all gestational ages except at >30 weeks GA, and 8-16% increases in TSH at 15-21 and 21-30 weeks GA. BP-3 was additionally associated with a 7.4% decrease in the T3/T4 ratio at >30 weeks GA (%Δ = -7.37; 95% CI = -12.70, -2.04).

BPS was associated with an increase in FT4 (%Δ = -9.63; 95% CI = -18.10, -0.33), and a marginal decrease in TSH (%Δ = 26.02; 95% CI = -3.53, 64.63) at <15 weeks GA, indicating a potential window of vulnerability during early pregnancy. TCS and TCC showed no significant associations with any thyroid hormone in MLR models stratified by GA.

Associations between parabens and thyroid hormones also varied by gestational age. EPB was not associated with any thyroid hormones, except for a marginal 4% decrease in the T3/T4 ratio at 15-21 GA weeks (%Δ = -4.29; 95% CI = -9.24, 0.65). Later time points in pregnancy may be a vulnerable time point for associations with BPB, given that BPB was associated with 6-7% decreases in T3 at 15-21 and >30 weeks GA, 5-6% decreases in the T3/T4 ratio at 15-21 and >30 weeks GA, and a 14.7% increase in TSH at >30 weeks GA. In contrast, MPB and PPB were associated with changes in thyroid hormones during early pregnancy. An IQR increase in PPB was associated with 5-6% decreases in FT4 at <15 weeks and 15-21 weeks GA [(%Δ = -4.60; 95% CI = -8.88, -0.12), (%Δ = -5.57; 95% CI = -10.74, -0.10), respectively]. At <15 weeks GA, an IQR increase in MPB was associated with a 4.2% decrease in FT4 (95% CI: -8.84, 0.69), a

3.7% increase in T3 (%Δ = 3.67; 95% CI = -0.33, 7.67), and a 2.7% increase in T3/T4 ratio (%Δ = 2.65; 95% CI = 0.09, 5.21). MPB was also associated with a marginal 7.0% decrease in TSH at 21-30 weeks GA (%Δ = -6.98; 95% CI = -13.91, 0.51). In a sensitivity analysis conducted only among term births only, most of the results remained consistent, with the exception of the associations involving TCS and TCC with T3 and T4 which were no longer statistically significant (Table II.5).

Discussion

In the present study, phenols and parabens were associated with changes in maternal thyroid hormone levels during pregnancy. In particular, T3 was most strongly and consistently related to phenols and parabens as compared to the other studied thyroid hormones in our analysis. In general, BP-3, TCS, TCC, and BPB were associated with a decrease in T3, while MPB were associated with an increase in T3. There was a general positive association between BPS, BP-3, TCS and BPB in relation to TSH, and a negative association between TCC in relation to TSH. Few associations between the urinary biomarkers and FT4 or T4 were observed, with the exception of MPB, PPB and BPS which were negatively associated with FT4 at <21 weeks GA, and the negative association between BP-3 and T4 at <30 weeks GA. Windows of vulnerability were identified. BPS, MPB and PPB were associated with thyroid hormone at earlier time points in pregnancy, whereas BPB was associated with thyroid hormone later in pregnancy.

An IQR increase in 2,5-DCP was associated with a suggestive 9% decrease in TSH at 15-21 weeks GA, and a 10% increase in TSH at >30 weeks GA. 2,4-DCP was associated with increases in T3/T4 ratio at 15-21 weeks GA and TSH at 21-30 weeks GA, and a decrease in T3 at 21-30 weeks GA. Few studies have looked into the effect of either phenol on thyroid hormones, and it is unclear why the associations between the dichlorophenols change direction from one time point to the next. No associations were observed between 2,4-DCP or 2,5-DCP and thyroid hormones in our previous small cohort study in Puerto Rico [32]. 2,5-DCP was associated with increased TSH and hypothyroidism in a subsample of adolescents in NHANES, and negatively associated with FT4 among a large Belgian adolescent population [45,46], while chlorophenols (including 2,5-DCP) were found to interfere with the T4 binding site of a T4 carrier in an in vitro study [47].

In the present study, there were consistent negative associations between BP-3 with T4 and T3, and positive associations with TSH. Given the concurrent negative associations between BP-3 and bound T4 and T3 hormones, it is possible that BP-3 could be reducing the thyroxine-binding globulin (TBG) level [48]. The lower levels in T4 and T3 alongside the elevated level of TSH in association with BP-3 could also indicate a situation of slight hypothyroidism, stimulating an increase in TSH. While BP-3 has shown anti-estrogenic and anti-androgenic effects in vitro [49], and caused cytotoxicity in yeast cells and developmental disorders in zebrafish [50], few studies have studied the effect of BP-3 on thyroid hormones, and study designs, hormones measured, and results have varied. Our previous study of a smaller pregnancy cohort in Puerto Rico found a significant negative association of BP-3 and free T3, but no significant associations with TSH [32]. After topical application of sunscreen containing BP-3, there were small changes in thyroid hormone levels in young men (n=15) and older women (n=17) after two weeks, but the authors concluded that this was a chance finding, and attributed no effect of sunscreen on thyroid hormone levels [51].

BPS, which is used as a replacement chemical for BPA, was associated with a suggestive increase in TSH, particularly <15 weeks GA, as well as a decrease in FT4 at <15 weeks GA. BPS was observed to induce uterine growth in rats [52], affect the feedback regulatory circuits in parental zebrafish hypothalamo-pituitary-gonadal axes, impair the development of offspring [53], and possibly play a role in gene expression on the reproductive neuroendocrine axis in zebrafish [54]. The parallel associations observed between BPS and TSH, and BPS and FT4 could be indicative of a change in the feedback regulatory circuits similar to the effects observed in zebrafish [53]. However, no previous human studies were identified that have investigated the effect of BPS on thyroid hormones. In the same LifeCodes cohort, BPA was associated with a decrease in TSH and an increase in FT4, particularly at later time points in pregnancy [33]. While BPS was also associated with TSH and FT4, the associations were in the opposite direction. BPA and BPS were not correlated in this study (Spearman correlation = 0.24), and structural variations among bisphenols may be sufficient to alter receptor-binding affinities [55]. This structural variation partly explains the differences observed in our results; however, the mechanism of action leading to the opposite directions in hormone associations is unclear. Furthermore, our detection level for BPS is very low (<25%), which may potentially introduce

some bias to our results. This makes the associations between BPS and BPA a little more difficult to interpret.

The impact of TCS on thyroid hormones has been the most commonly researched in the literature, primarily in animal studies. TCS is structurally similar to thyroid hormones [56], and a study in pregnant rats showed that the greatest accumulation of TCS was in the placenta as compared to six other rat tissues, indicating pregnancy may be a sensitive time period for TCS exposure [57]. In this study, LMMs provided statistical power to observe a negative association between TCS and T3, and a positive association with TSH. This association with T3 is consistent with animal studies that also showed linear decreases in T3 with increased exposure to TCS [56], including in pregnant rats [58] and pregnant mice [59]. A study in pregnant mice observed an effect of TCS on signal pathways involved in placental growth through the activation of thyroid hormone receptors that trigger this signaling pathway [60]. On the other hand, no previous studies have reported an association between TCS and TSH during pregnancy in humans [32,61], or in animals [62,63]. However, if TCS was indeed responsible for the decrease in T3, it would make biological sense that TSH levels would increase given the negative feedback loop. A short-term (n=12) and long-term (n=132) study of adult men and women found no effects on thyroid hormone levels following exposure to TCS-containing toothpaste [64,65]. Other studies point to a decrease in T4 levels in relation to TCS exposure, including animal dams [58,59,63,66–71]. In the present study we found a negative but not statistically significant association between TCS and T4 (%Δ = -1.04%; 95% CI = -2.55, 0.49; p value = 0.18).

We found numerous associations between urinary paraben concentrations and hormone levels in the present study, and the associations differed by gestational age. MPB was positively associated with T3 and T4 at <15 weeks GA, PPB was associated with a decrease in FT4 at <15 weeks GA, EPB was associated with a marginal decrease in the T3/T4 ratio at 15-21 weeks GA, and BPB was associated with a decrease in T3, T3/T4 ratio, and an increase in TSH during the second half of gestation. Few studies have looked into the effect of parabens on thyroid hormones in pregnant women. In our previous cohort study of pregnant women in Puerto Rico, BPB was positively associated with FT4 during pregnancy, which we did not observe in the present study [32]. In the earlier study, MPB was positively associated with FT4 early in pregnancy, and negatively associated with FT4 later in pregnancy [32]. In the present study MPB

was negatively associated with FT4 earlier in pregnancy, and non-significant with FT4 later in pregnancy. This could indicate that impacts on thyroid signaling in relation to exposure to parabens is dependent on the timing of the exposure, although chance findings cannot be ruled out. In other research, a study on NHANES women over the age of 20 reported that all measured parabens were negatively associated with FT4, and BPB and EPB were negatively associated with T4 [10]. [72] found that MPB and PPB significantly decreased T4 levels in prepubertal female rats. However, two small human studies in men found no effect on thyroid hormones [73,74]. It is unclear why the associations with hormones between parabens varied to this extent. This could be explained by the higher levels of MPB and PPB in our study, the differences in paraben concentrations by study visit, or true differences in the associations due to the structural differences between the parabens.

Our study had a few limitations. Even though we applied sampling weights in our analyses to account for the case-control study design, there may still be constraints to the generalizability of our results to other populations given our sampling methods. Additionally, our study population had older maternal ages, and high educational attainment as compared to the general public, which could reduce the generalizability of our results. Loss to follow up may introduce selection bias, although the sample size at study visit 4 was still relatively high as compared to the study population at study visit 1. Although our study design greatly improved upon the standard cross-sectional design by measuring urinary biomarkers at four separate time points, variation of phenols and parabens is relatively high, and the four time points may not be sufficient to eliminate potential bias stemming from random measurement error. Another limitation was the lack of data on iodine status of the women, given that deficiencies in these minerals could affect thyroid hormone function. However, controlling for iodine status could lead to bias, given that iodine may act as mechanistic intermediate between the exposure and thyroid hormone [75]. Further, iodine had no impact on the associations between phenols/parabens and thyroid hormones in our previous study of NHANES data [10]. We also did not measure thyroperoxidase antibodies and human chorionic gonadotropin (hCG). Thyroperoxidase antibodies may influence thyroid function in pregnancy [76], and hCG has similar homology to TSH, and may affect maternal thyroid function in pregnancy [22]. Finally, given the exploratory nature of the study, many comparisons were made, increasing the chance of Type I error.

Our study also had many strengths, including a robust sample size, sensitive and state-of-the-art biomarkers of exposure, and the collection of data at four time points during pregnancy to account for the chemicals' short lifespan in the body, and the varying levels of thyroid hormones throughout pregnancy. The repeated measures allow us to control for intra-individual variability and increase statistical power, as well as explore potential windows of susceptibility for these associations.

Conclusion

Our results provide suggestive human evidence for associations between phenols and parabens with thyroid hormones during pregnancy. The strength of our associations may vary across pregnancy. Further studies are required to confirm our findings, determine the biological mechanisms involved, and better understand if and how these hormone changes may affect downstream maternal and infant health outcomes.

Table II.1: Summary demographics of the 439 pregnant women in the sample

		N	% of cohort*
Age	18–24 years	54	12
	25–29 years	92	21
	30–34 years	176	40
	35+ years	117	27
Race/ethnicity	White	247	56
	African-American	75	17
	Other	117	27
Education	High school degree	67	16
	Technical school	76	18
	Junior college or some college	127	29
	College graduate	159	37
Health insurance provider	Private/HMO/Self-pay	344	81
	Medicaid/SSI/MassHealth	83	19
BMI at initial visit	< 25 kg/m^2	227	52
	25–29.9 kg/m^2	113	26
	≥ 30 kg/m^2	99	22
Tobacco use	Smoking during pregnancy	31	7
	No smoking during pregnancy	402	93
Alcohol use	Alcohol use during pregnancy	18	4

No alcohol use during pregnancy	412	96

*Weighted by case-control sampling probabilities to represent the general sampling population.

Table II.2: Weighted distributions of SG-corrected urinary phenols and parabens and thyroid hormones by study visit of sample collection in pregnancy (N = 439 subjects). Median gestational weeks: Visit 1: 10, Visit 2: 18, Visit 3: 26, Visit 4: 35.

		All	Visit 1	Visit 2	Visit 3	Visit 4
	% LOD (LOD)	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)	GM (GSD)
Urinary Biomarkers (µg/L)						
2,4-DCP	15.7 (0.2)	0.89 (3.51)	0.95 (3.85)	0.93 (3.50)*	0.81 (3.52)*	0.88 (3.14)*
2,5-DCP	3.1 (0.2)	5.11 (6.42)	5.35 (7.08)	5.63 (6.63)	4.69 (6.46)*	4.77 (5.47)*
BP-3	0.1 (0.4)	55.82 (8.48)	52.62 (8.16)	61.66 (8.11)	51.62 (8.76)	57.94 (9.02)
BPB	29.3 (0.2)	1.19 (7.14)	1.21 (7.53)	1.29 (6.71)	1.18 (7.39)	1.09 (6.96)
EPB	38.1 (1)	3.56 (5.82)	3.73 (6.20)	3.92 (6.05)	3.45 (5.67)*	3.15 (5.33)*
MPB	0.1 (1)	169.64 (4.31)	152.43 (4.14)	208.05 (4.07)	161.80 (4.55)	161.49 (4.45)
PPB	1 (0.2)	37.35 (5.71)	33.90 (5.67)	50.85 (5.04)*	34.36 (5.82)	32.63 (6.23)
TCS	17.4 (2)	16.63 (6.65)	16.82 (6.98)	17.44 (6.20)	14.91 (6.49)	17.35 (6.95)
TCC	85.9 (2)	<LOD	<LOD	<LOD	<LOD	<LOD
BPS	73.8 (0.4)	<LOD	<LOD	<LOD	<LOD	<LOD
Thyroid Hormones						
FT4 (ng/dL)	98.2 (0.1)	1.10 (1.52)	1.37 (1.48)	1.08 (1.49)*	0.99 (1.50)*	0.96 (1.45)*
TSH (µIU/mL)	99.5 (0.01)	1.13 (2.12)	0.77 (2.75)	1.29 (1.92)*	1.27 (1.68)*	1.31 (1.71)*
T3 (ng/mL)	100 (0.1)	1.56 (1.30)	1.35 (1.31)	1.61 (1.27)*	1.65 (1.27)*	1.66 (1.29)*
T4 (µg/dL)	100 (0.3)	10.22 (1.21)	9.98 (1.22)	10.61 (1.18)*	10.29 (1.20)*	10.01 (1.23)
T3/T4 ratio	-	0.15 (1.27)	0.14 (1.21)	0.15 (1.24)*	0.16 (1.27)*	0.17 (1.29)*

GM: Geometric mean

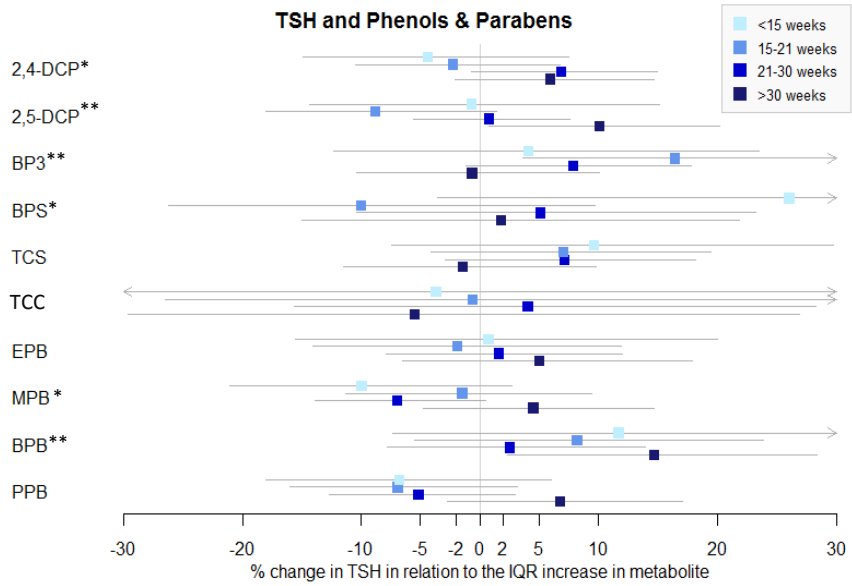
GSD: Geometric standard deviation

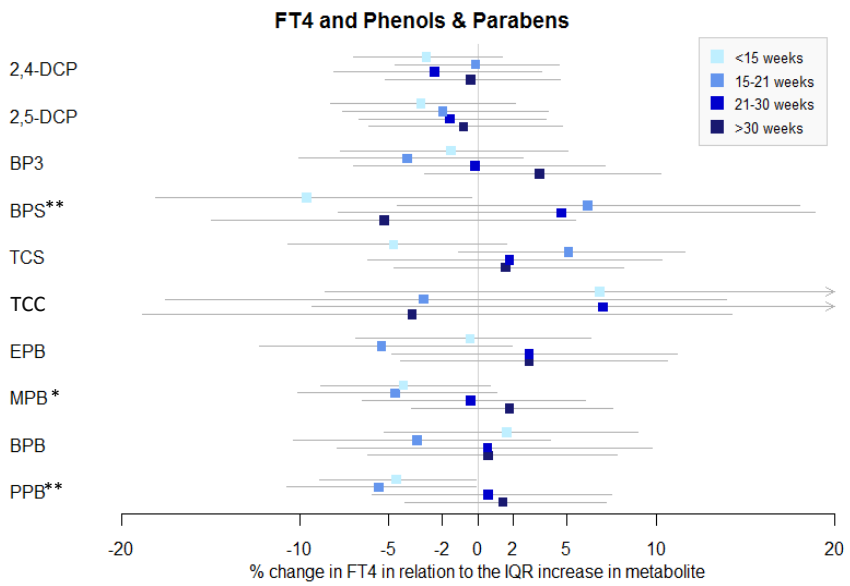
* Significant difference ($p<0.05$) in urinary biomarker metabolite or thyroid hormone compared to reference (visit 1) using linear mixed models with a random intercept

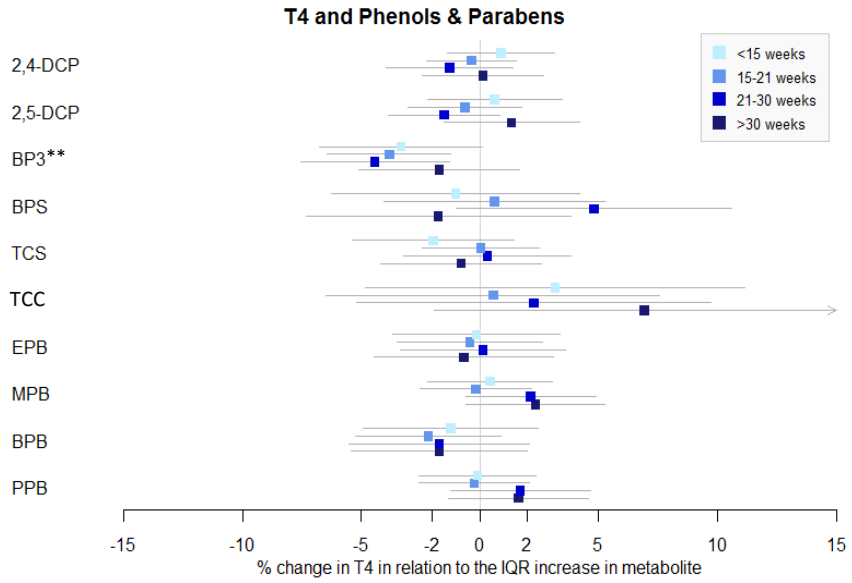
Table II.3: Results of the linear mixed models regressing thyroid hormones versus phenols and parabens: % change in thyroid hormone per IQR change in urinary biomarker concentration

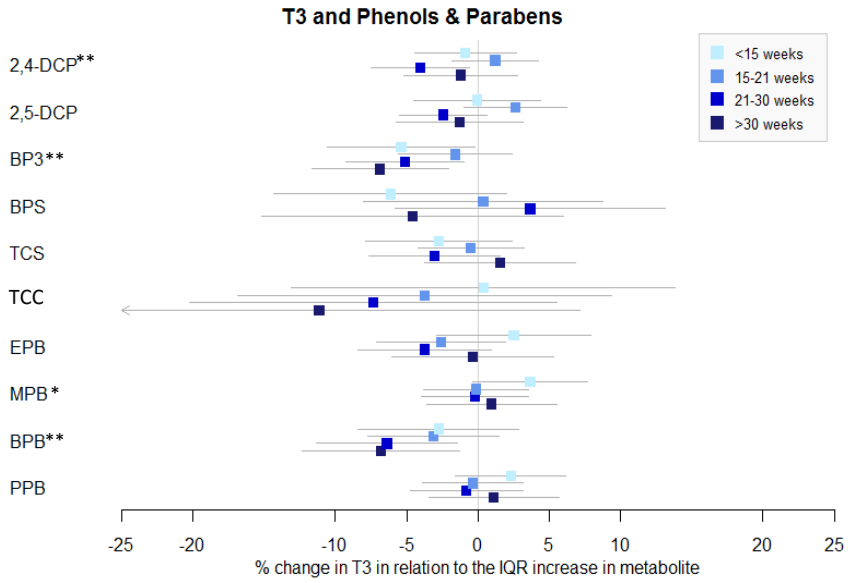
		TSH (N=1069)	FT4 (N=1240)	T4 (N=1230)	T3 (N=993)	T3/T4 Ratio (N=985)
2,4-DCP	% Δ/IQR (95% CI)	0.24 (-5.71, 6.57)	-0.30 (-3.46, 2.96)	0.31 (-0.96, 1.58)	-1.33 (-3.15, 0.49)	-1.44 (-3.11, 0.22)
	p value	0.94	0.85	0.63	0.15	0.09*
2,5-DCP	% Δ/IQR (95% CI)	-2.71 (-9.13, 4.16)	-1.64 (-5.05, 1.90)	-0.06 (-1.53, 1.40)	-0.52 (-2.60, 1.56)	-0.36 (-2.28, 1.55)
	p value	0.43	0.36	0.93	0.63	0.71
BP-3	% Δ/IQR (95% CI)	5.42 (-1.58, 12.9)	-0.62 (-4.11, 3.00)	-1.13 (-2.59, 0.34)	-2.07 (-4.16, 0.01)	-1.33 (-3.24, 0.59)
	p value	0.13	0.73	0.13	0.05*	0.18
BPS◊	% Δ (95% CI)	9.77 (-0.89, 21.6)	-1.11 (-6.27, 4.34)	-0.30 (-0.19, 0.20)	-0.68 (-3.68, 2.33)	-0.54 (-3.29, 2.21)
	p value	0.07*	0.68	0.77	0.66	0.70
TCS	% Δ/IQR (95% CI)	7.72 (0.01, 16.02)	1.22 (-2.59, 5.18)	-1.20 (-2.76, 0.35)	-2.53 (-4.75, -0.30)	-0.80 (-2.84, 1.25)
	p value	0.05**	0.54	0.13	0.03**	0.45
TCC◊	% Δ (95% CI)	-12.86 (-25.6, 2.05)	1.14 (-6.98, 9.96)	-0.95 (-0.35, 0.27)	-5.71 (-10.45, -0.97)	-3.99 (-8.30, 0.33)
	p value	0.09 *	0.79	0.56	0.02**	0.07*
EPB	% Δ/IQR (95% CI)	2.84 (-3.92, 10.1)	0.50 (-3.06, 4.18)	0.67 (-0.77, 2.11)	-0.86 (-2.92, 1.20)	-1.87 (-3.76, 0.02)
	p value	0.42	0.79	0.36	0.41	0.05*
MPB	% Δ/IQR (95% CI)	0.55 (-5.45, 6.93)	-2.13 (-5.24, 1.09)	1.19 (-0.10, 2.47)	2.41 (0.58, 4.24)	0.79 (-0.90, 2.48)
	p value	0.86	0.19	0.07*	0.01**	0.36
BPB	% Δ/IQR (95% CI)	6.85 (-1.67, 16.1)	0.43 (-3.86, 4.92)	0.76 (-0.98, 2.49)	-2.76 (-5.25, -0.26)	-3.70 (-5.98, -1.42)
	p value	0.12	0.85	0.39	0.03**	0.002**
PPB	% Δ/IQR (95% CI)	0.87 (-5.02, 7.13)	-3.14 (-6.12, -0.06)	0.99 (-0.25, 2.22)	1.41 (-0.36, 3.18)	0.26 (-1.36, 1.88)
	p value	0.78	0.05**	0.12	0.12	0.76

◊ Phenol metabolite was transformed into a dichotomous variable. * represents a p value below 0.10, and **represents a p value below 0.05.


TSH and Phenols & Parabens
<15 weeks
15-21 weeks
21-30 weeks
>30 weeks
2,4-DCP*
2,5-DCP**
BP3**
BPS*
TCS
TCC
EPB
MPB*
BPB**
PPB
-30 -20 -10 -5 -2 0 2 5 10 20 30
% change in TSH in relation to the IQR increase in metabolite


FT4 and Phenols & Parabens
<15 weeks
15-21 weeks
21-30 weeks
>30 weeks
2,4-DCP
2,5-DCP
BP3
BPS**
TCS
TCC
EPB
MPB*
BPB
PPB**
-20 -10 -5 -2 0 2 5 10 20
% change in FT4 in relation to the IQR increase in metabolite


T4 and Phenols & Parabens
<15 weeks
15-21 weeks
21-30 weeks
>30 weeks
2,4-DCP
2,5-DCP
BP3**
BPS
TCS
TCC
EPB
MPB
BPB
PPB
-15 -10 -5 -2 0 2 5 10 15
% change in T4 in relation to the IQR increase in metabolite


T3 and Phenols & Parabens
<15 weeks
15-21 weeks
21-30 weeks
>30 weeks
2,4-DCP**
2,5-DCP
BP3**
BPS
TCS
TCC
EPB
MPB*
BPB**
PPB
-25 -20 -10 -5 0 5 10 20 25
% change in T3 in relation to the IQR increase in metabolite

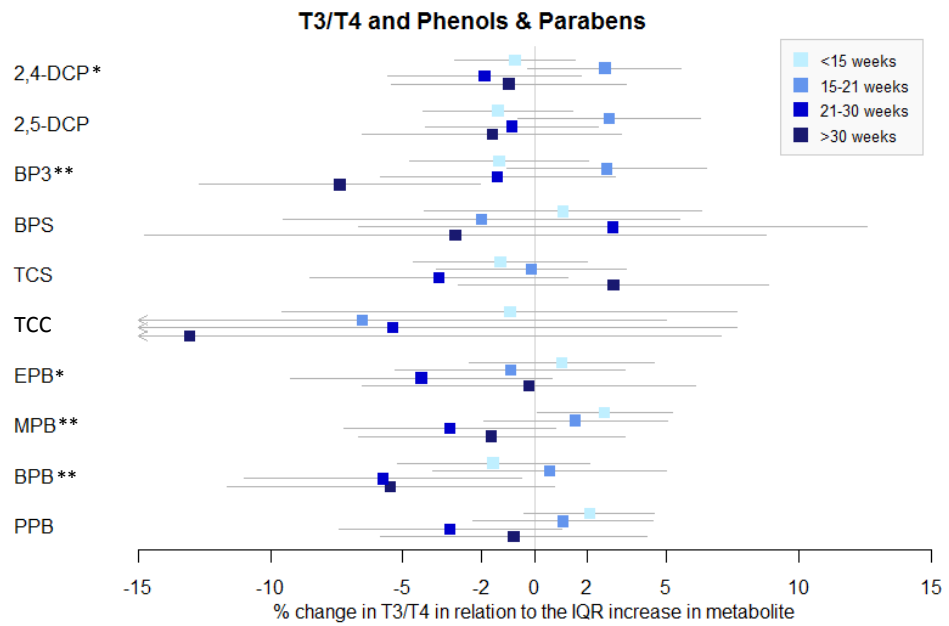


Figure II.1: Multiple linear regressions of thyroid hormone versus urinary concentrations of phenol/paraben stratified by gestational age. BPS and TCC are categorical variables. Associations with BPS and TCC are transformed to % changes in the hormone comparing urinary biomarkers above and below the LOD. * represents at least one marginal association between the urinary concentration and the hormone across the four time points. ** represents at least one significant association between the urinary concentration and the hormone across the four time points.

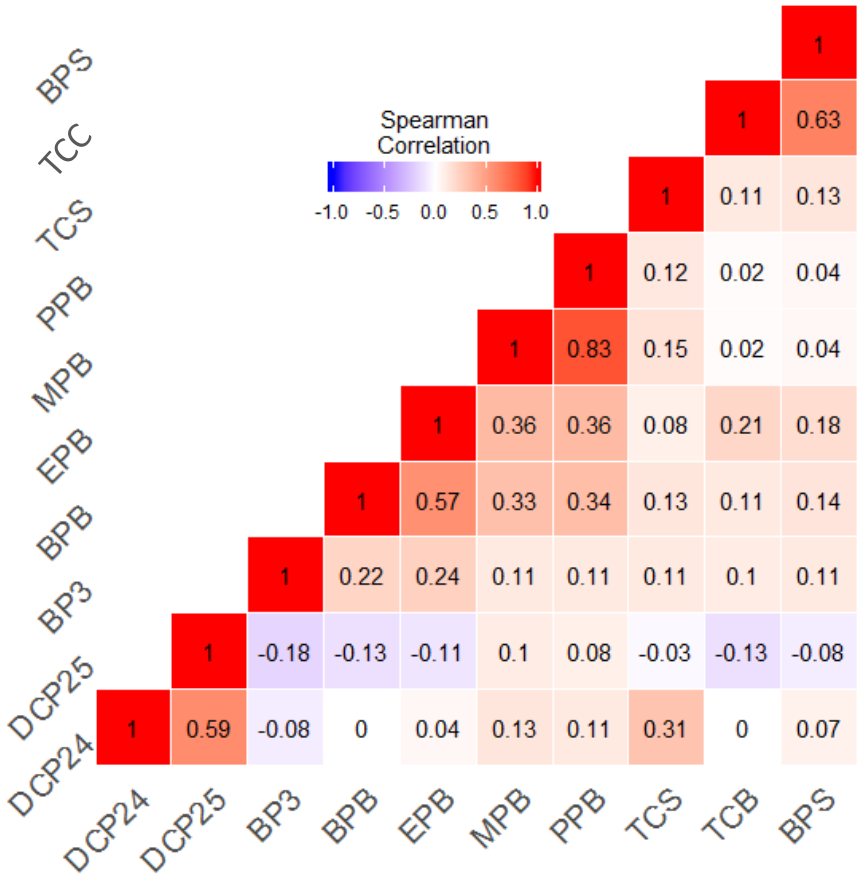


Figure II.2: Heat map of Spearman correlations between urinary phenol and paraben biomarkers. Biomarkers were adjusted for urinary dilution.

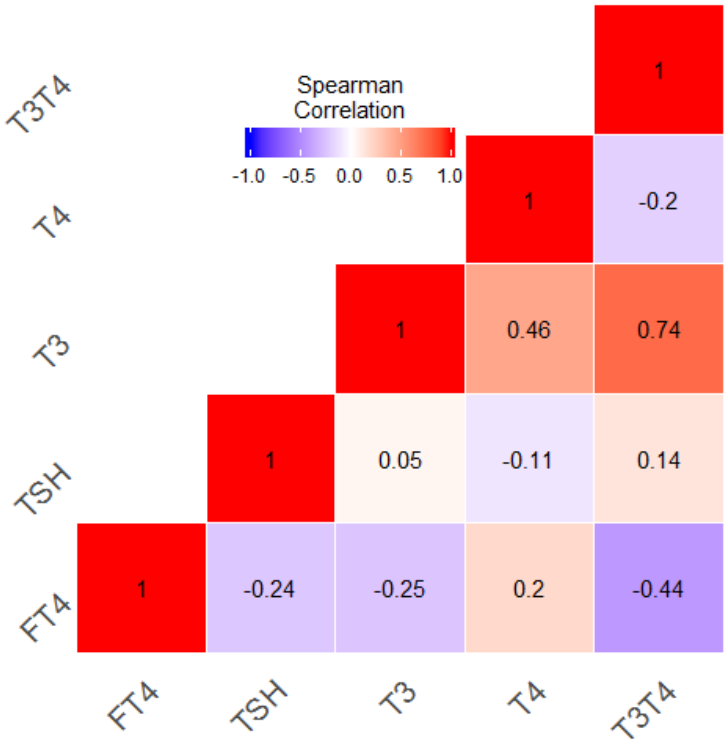


Figure II.3: Heat map of Spearman correlations between serum thyroid hormones.

Table II.4: Results of the multiple linear models regressing thyroid hormones versus phenols/parabens by gestational age.

	<15 GA weeks		15-21 GA weeks		21-30 GA weeks		>30 GA weeks	
FT4								
N	**334**		**323**		**288**		**295**	
2,4-DCP	-2.91 (-6.98, 1.35)	0.18	-0.15 (-4.66, 4.57)	0.95	-2.44 (-8.08, 3.55)	0.42	-0.40 (-5.18, 4.62)	0.87
2,5-DCP	-3.21 (-8.25, 2.1)	0.23	-2.00 (-7.6, 3.95)	0.50	-1.59 (-6.7, 3.8)	0.56	-0.83 (-6.13, 4.77)	0.77
BP-3	-1.53 (-7.7, 5.06)	0.64	-3.98 (-10.08, 2.54)	0.23	-0.18 (-7, 7.14)	0.96	3.44 (-2.97, 10.28)	0.30
BPS♦◊	-9.63 (-18.1, -0.33)	0.04**	6.17 (-4.53, 18.06)	0.27	4.69 (-7.82, 18.89)	0.48	-5.28 (-14.94, 5.48)	0.32
TCS	-4.73 (-10.67, 1.61)	0.14	5.07 (-1.06, 11.59)	0.11	1.75 (-6.18, 10.36)	0.67	1.54 (-4.69, 8.18)	0.64
TCC◊	6.84 (-8.56, 24.82)	0.41	-3.05 (-17.52, 13.94)	0.71	7.01 (-9.32, 26.28)	0.42	-3.69 (-18.83, 14.28)	0.67
EPB	-0.45 (-6.83, 6.36)	0.89	-5.42 (-12.26, 1.94)	0.15	2.88 (-4.81, 11.2)	0.47	2.88 (-4.35, 10.65)	0.45
MPB*	-4.19 (-8.84, 0.69)	0.09*	-4.66 (-10.09, 1.09)	0.11	-0.43 (-6.48, 6.01)	0.89	1.77 (-3.71, 7.57)	0.53
BPB	1.61 (-5.28, 8.99)	0.66	-3.42 (-10.34, 4.04)	0.36	0.53 (-7.92, 9.76)	0.91	0.58 (-6.18, 7.82)	0.87
PPB*	-4.60 (-8.88, -0.12)	0.05**	-5.57 (-10.74, -0.10)	0.05**	0.57 (-5.93, 7.52)	0.87	1.40 (-4.09, 7.2)	0.62
T3								
N	**273**		**268**		**240**		**212**	
2,4-DCP	-0.88 (-4.43, 2.67)	0.63	1.20 (-1.83, 4.24)	0.44	-4.05 (-7.49, -0.61)	0.02**	-1.20 (-5.18, 2.77)	0.55
2,5-DCP	-0.06 (-4.49, 4.38)	0.98	2.63 (-0.97, 6.24)	0.15	-2.44 (-5.52, 0.64)	0.12	-1.27 (-5.7, 3.16)	0.57
BP-3	-5.39 (-10.6, -0.24)	0.04**	-1.59 (-5.55, 8.77)	0.43	-5.14 (-9.29, -0.98)	0.02**	-6.87 (-11.65, -2.09)	0.01**
BPS♦◊	-6.15 (-14.3, 2.01)	0.14	0.35 (-8.07, 12.28)	0.93	3.67 (-5.84, 13.17)	0.45	-4.59 (-15.21, 6.03)	0.40
TCS	-2.73 (-7.86, 2.41)	0.30	-0.49 (-4.23, 3.24)	0.80	-3.04 (-7.64, 1.57)	0.20	1.54 (-3.76, 6.84)	0.57
TCC◊	0.38 (-13.09, 13.85)	0.96	-3.74 (-16.83, 9.35)	0.58	-7.35 (-20.24, 5.53)	0.26	-11.15 (-29.48, 7.18)	0.23
EPB	2.52 (-2.87, 7.91)	0.36	-2.62 (-7.16, 1.92)	0.26	-3.73 (-8.43, 0.96)	0.12	-0.37 (-6.05, 5.32)	0.90
MPB	3.67 (-0.33, 7.67)	0.07*	-0.14 (-3.79, 3.51)	0.94	-0.23 (-4, 3.55)	0.91	0.95 (-3.61, 5.51)	0.68
BPB	-2.77 (-8.41, 2.87)	0.34	-3.12 (-7.72, 1.49)	0.19	-6.38 (-11.34, -1.43)	0.01**	-6.80 (-12.34, -1.27)	0.02**
PPB	2.32 (-1.56, 6.19)	0.24	-0.34 (-3.87, 3.19)	0.85	-0.80 (-4.78, 3.17)	0.69	1.12 (-3.44, 5.68)	0.63
T3/T4								
N	**270**		**266**		**238**		**211**	
2,4-DCP	-0.76 (-3.02, 1.51)	0.51	2.65 (-0.25, 5.55)	0.07*	-1.90 (-5.58, 1.78)	0.31	-0.98 (-5.41, 3.45)	0.66
2,5-DCP	-1.40 (-4.23, 1.44)	0.34	2.82 (-0.64, 6.29)	0.11	-0.86 (-4.13, 2.40)	0.61	-1.61 (-6.51, 3.29)	0.52

BP-3♦	-1.35 (-4.71, 2.02)	0.43	2.73 (-1.05, 6.51)	0.16	-1.41 (-5.85, 3.03)	0.53	-7.37 (-12.7, -2.04)	0.01**
BPS◊	1.07 (-4.19, 6.32)	0.69	-2.01 (-9.52, 5.51)	0.60	2.94 (-6.68, 12.57)	0.55	-3.01 (-14.78, 8.76)	0.62
TCS	-1.30 (-4.6, 2.0)	0.44	-0.13 (-3.71, 3.44)	0.94	-3.62 (-8.48, 1.25)	0.15	2.98 (-2.87, 8.84)	0.32
TCC◊	-0.94 (-9.55, 7.67)	0.83	-6.53 (-18.05, 4.99)	0.27	-5.38 (-18.43, 7.66)	0.42	-13.06 (-33.16, 7.03)	0.20
EPB	1.03 (-2.45, 4.51)	0.56	-0.93 (-5.27, 3.42)	0.68	-4.29 (-9.24, 0.65)	0.09*	-0.23 (-6.54, 6.08)	0.94
MPB	2.65 (0.09, 5.21)	0.04**	1.55 (-1.95, 5.04)	0.39	-3.21 (-7.2, 0.78)	0.12	-1.65 (-6.69, 3.40)	0.52
BPB	-1.57 (-5.21, 2.06)	0.40	0.58 (-3.84, 5.0)	0.80	-5.74 (-10.98, -0.50)	0.03**	-5.46 (-11.65, 0.73)	0.09*
PPB	2.07 (-0.41, 4.54)	0.10	1.06 (-2.36, 4.48)	0.54	-3.20 (-7.41, 1.01)	0.14	-0.79 (-5.85, 4.26)	0.76

T4

N	**328**		**317**		**304**		**301**	
2,4-DCP	0.88 (-1.38, 3.14)	0.44	-0.35 (-2.25, 1.54)	0.71	-1.28 (-3.95, 1.39)	0.35	0.12 (-2.44, 2.68)	0.93
2,5-DCP	0.62 (-2.21, 3.45)	0.67	-0.63 (-3.03, 1.78)	0.61	-1.50 (-3.85, 0.84)	0.21	1.33 (-1.51, 4.18)	0.36
BP-3	-3.33 (-6.74, 0.08)	0.06*	-3.82 (-6.42, -1.22)	0.004**	-4.42 (-7.55, -1.30)	0.01**	-1.71 (-5.11, 1.68)	0.32
BPS◊	-1.03 (-6.26, 4.21)	0.70	0.61 (-4.05, 5.28)	0.80	4.79 (-1.0, 10.58)	0.11	-1.76 (-7.33, 3.81)	0.54
TCS	-1.97 (-5.36, 1.42)	0.26	0.04 (-2.42, 2.50)	0.97	0.32 (-3.21, 3.85)	0.86	-0.79 (-4.18, 2.61)	0.65
TCC◊	3.17 (-4.81, 11.15)	0.44	0.56 (-6.46, 7.57)	0.88	2.26 (-5.2, 9.73)	0.55	6.92 (-1.93, 15.77)	0.13
EPB	-0.15 (-3.66, 3.35)	0.93	-0.43 (-3.49, 2.62)	0.78	0.13 (-3.35, 3.61)	0.94	-0.68 (-4.47, 3.11)	0.73
MPB*	0.42 (-2.21, 3.05)	0.75	-0.18 (-2.54, 2.18)	0.88	2.13 (-0.60, 4.87)	0.13	2.33 (-0.60, 5.25)	0.12
BPB	-1.23 (-4.92, 2.46)	0.51	-2.18 (-5.24, 0.88)	0.16	-1.71 (-5.50, 2.07)	0.38	-1.71 (-5.41, 1.99)	0.37
PPB*	-0.10 (-2.57, 2.37)	0.94	-0.24 (-2.56, 2.09)	0.84	1.70 (-1.24, 4.64)	0.26	1.63 (-1.32, 4.58)	0.28

TSH

N	**286**		**283**		**258**		**242**	
2,4-DCP♦	-4.37 (-14.90, 7.47)	0.45	-2.28 (-10.52, 6.71)	0.61	6.81 (-0.74, 14.94)	0.08*	5.94 (-2.11, 14.65)	0.15
2,5-DCP♦	-0.69 (-14.3, 15.08)	0.93	-8.79 (-17.99, 1.44)	0.09*	0.74 (-5.61, 7.53)	0.82	10.03 (0.74, 20.19)	0.03**
BP-3♦	4.06 (-12.31, 23.48)	0.65	16.38 (3.63, 30.7)	0.01**	7.85 (-1.22, 17.75)	0.09*	-0.68 (-10.35, 10.03)	0.90
BPS♦◊	26.02 (-3.53, 64.63)	0.09*	-10.03 (-26.18, 9.65)	0.30	5.11 (-10.35, 23.23)	0.54	1.80 (-14.94, 21.83)	0.85
TCS	9.59 (-7.48, 29.81)	0.29	7.01 (-4.12, 19.43)	0.23	7.13 (-2.89, 18.18)	0.17	-1.45 (-11.51, 9.74)	0.79
TCC◊	-3.72 (-38.2, 49.90)	0.87	-0.65 (-26.52, 34.33)	0.97	4.02 (-15.65, 28.29)	0.71	-5.48 (-29.61, 26.91)	0.71
EPB	0.71 (-15.49, 20.0)	0.94	-1.93 (-14.05, 11.91)	0.77	1.56 (-7.91, 12)	0.76	4.97 (-6.51, 17.85)	0.41
MPB	-9.98 (-21.09, 2.69)	0.12	-1.50 (-11.32, 9.41)	0.78	-6.98 (-13.91, 0.51)	0.07*	4.49 (-4.80, 14.68)	0.36
BPB	11.66 (-7.36, 34.57)	0.25	8.18 (-5.51, 23.85)	0.26	2.51 (-7.79, 13.97)	0.65	14.64 (2.36, 28.39)	0.02**

PPB	-6.81 (-18.06, 5.97)	0.28	-6.93 (-16.02, 3.15)	0.17	-5.19 (-12.72, 2.99)	0.21	6.72 (-2.74, 17.09)	0.17

Beta coefficients are transformed into percent change of hormone in an IQR change in the biomarker. ◊ Phenol metabolite was transformed into a categorical variable. Beta coefficients and their 95% CI are displayed. ♦ represents a significant interaction term between the biomarker*visit in LMM models. ** p value <0.05; * 0.05< p value < 0.10.

Table II.5: Linear mixed models in term births only (defined by >37 weeks).

		TSH N=816	**FT4** N=949	**T4** N=933	**T3** N=754	**T3/T4** N=746
2,4-DCP	% Δ/IQR (95% CI)	2.86 (-2.41, 8.41)	-0.29 (-3.12, 2.63)	0.66 (-0.45, 1.76)	-0.44 (-1.96, 1.07)	-0.71 (-2.18, 0.76)
	p value	0.29	0.85	0.24	0.57	0.34
2,5-DCP	% Δ/IQR (95% CI)	0.01 (-6.74, 7.25)	-2.53 (-6.05, 1.13)	0.74 (-0.78, 2.26)	0.69 (-1.36, 2.74)	0.14 (-1.87, 2.15)
	p value	1.00	0.17	0.34	0.51	0.89
BP-3	% Δ/IQR (95% CI)	3.93 (-3.64, 12.1)	-0.13 (-4.2, 4.11)	-1.20 (-2.84, 0.44)	-2.76 (-4.94, -0.57)	-2.27 (-4.4, -0.13)
	p value	0.32	0.95	0.15	0.01**	0.04**
BPS◊	% Δ (95% CI)	11.77 (0.08, 24.8)	-0.88 (-6.92, 5.55)	-1.34 (-3.57, 0.88)	-1.18 (-4.31, 1.95)	-0.11 (-3.15, 2.94)
	p value	0.05**	0.78	0.24	0.46	0.94
TCS	% Δ/IQR (95% CI)	11.57 (3.66, 20.1)	-0.33 (-4.29, 3.79)	-0.62 (-2.18, 0.95)	-1.12 (-3.25, 1.01)	0.08 (-1.99, 2.15)
	p value	0.004**	0.87	0.44	0.30	0.94
TCC◊	% Δ (95% CI)	-14.49 (-27.9, 1.46)	0.00 (-9.27, 10.2)	1.31 (-2.17, 4.78)	-0.38 (-5.36, 4.61)	-1.63 (-6.42, 3.16)
	p value	0.07*	1.00	0.46	0.88	0.51
EPB	% Δ/IQR (95% CI)	2.99 (-4.51, 11.1)	2.24 (-2.06, 6.71)	1.15 (-0.47, 2.77)	-1.03 (-3.22, 1.16)	-2.02 (-4.14, 0.11)
	p value	0.45	0.31	0.16	0.36	0.06*
MPB	% Δ/IQR (95% CI)	2.75 (-3.18, 9.04)	-0.93 (-4.19, 2.44)	1.31 (0.06, 2.55)	2.17 (0.46, 3.87)	0.71 (-0.96, 2.37)
	p value	0.37	0.58	0.04**	0.01**	0.41
BPB	% Δ/IQR (95% CI)	11.27 (2.51, 20.8)	0.69 (-3.86, 5.45)	0.72 (-1.02, 2.46)	-3.09 (-5.47, -0.72)	-3.38 (-5.69, -1.08)
	p value	0.01	0.77	0.42	0.01**	0.004**
PPB	% Δ/IQR (95% CI)	2.98 (-3.24, 9.59)	-1.66 (-5.0, 1.79)	1.28 (-0.01, 2.57)	1.05 (-0.71, 2.81)	0.05 (-1.66, 1.76)
	p value	0.36	0.34	0.05*	0.24	0.95

Beta coefficients are transformed into percent change of hormone in an IQR change in the biomarker. ◊ Phenol metabolite was transformed into a categorical variable.
Beta coefficients and their 95% CI are displayed. ** p value <0.05; * 0.05< p value < 0.10.

References

[1] P.P. Egeghy, R. Judson, S. Gangwal, S. Mosher, D. Smith, J. Vail, E.A. Cohen Hubal, The exposure data landscape for manufactured chemicals, Sci. Total Environ. 414 (2012) 159–166. doi:10.1016/j.scitotenv.2011.10.046.

[2] Y. Guo, K. Kannan, A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure, Environ. Sci. Technol. 47 (2013) 14442–14449. doi:10.1021/es4042034.

[3] J.M. Braun, A.C. Just, P.L. Williams, K.W. Smith, A.M. Calafat, R. Hauser, Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic, J. Expo. Sci. Environ. Epidemiol. 24 (2014) 459–466. doi:10.1038/jes.2013.69.

[4] C. Lang, M. Fisher, A. Neisa, L. MacKinnon, S. Kuchta, S. MacPherson, A. Probert, T.E. Arbuckle, Personal Care Product Use in Pregnancy and the Postpartum Period: Implications for Exposure Assessment, Int. J. Environ. Res. Public. Health. 13 (2016) 105. doi:10.3390/ijerph13010105.

[5] Centers for Disease Control and Prevention, Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2017, Centers for Disease Control and Prevention (CDC), Atlanta, GA, 2017. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf (accessed May 9, 2017).

[6] J.R. Rochester, A.L. Bolden, Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes, Environ. Health Perspect. 123 (2015) 643–650. doi:10.1289/ehp.1408989.

[7] X.D. Andrianou, S. Gängler, A. Piciu, P. Charisiadis, C. Zira, K. Aristidou, D. Piciu, R. Hauser, K.C. Makris, Human Exposures to Bisphenol A, Bisphenol F and Chlorinated Bisphenol A Derivatives and Thyroid Function, PLOS ONE. 11 (2016) e0155237. doi:10.1371/journal.pone.0155237.

[8] D. Guignard, V. Gayrard, M.Z. Lacroix, S. Puel, N. Picard-Hagen, C. Viguié, Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level representative of human exposure, Chemosphere. 182 (2017) 458–467. doi:10.1016/j.chemosphere.2017.05.028.

[9] S. Kim, S. Kim, S. Won, K. Choi, Considering common sources of exposure in association studies - Urinary benzophenone-3 and DEHP metabolites are associated with altered thyroid hormone balance in the NHANES 2007–2008, Environ. Int. 107 (2017) 25–32. doi:10.1016/j.envint.2017.06.013.

[10] E.S. Koeppe, K.K. Ferguson, J.A. Colacino, J.D. Meeker, Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007-2008, Sci. Total Environ. 445–446 (2013) 299–305. doi:10.1016/j.scitotenv.2012.12.052.

[11] S. Lee, C. Kim, H. Youn, K. Choi, Thyroid hormone disrupting potentials of bisphenol A and its analogues - in vitro comparison study employing rat pituitary (GH3) and thyroid follicular (FRTL-5) cells, Toxicol. Vitro Int. J. Publ. Assoc. BIBRA. 40 (2017) 297–304. doi:10.1016/j.tiv.2017.02.004.

[12] H. Wang, Z. Ding, Q.-M. Shi, X. Ge, H.-X. Wang, M.-X. Li, G. Chen, Q. Wang, Q. Ju, J.-P. Zhang, M.-R. Zhang, L.-C. Xu, Anti-androgenic mechanisms of Bisphenol A

involve androgen receptor signaling pathway, Toxicology. 387 (2017) 10–16. doi:10.1016/j.tox.2017.06.007.
[13] X. Wang, F. Ouyang, L. Feng, X. Wang, Z. Liu, J. Zhang, Maternal Urinary Triclosan Concentration in Relation to Maternal and Neonatal Thyroid Hormone Levels: A Prospective Study, Environ. Health Perspect. 125 (2017) 067017. doi:10.1289/EHP500.
[14] D.-H. Zhang, E.-X. Zhou, Z.-L. Yang, Waterborne exposure to BPS causes thyroid endocrine disruption in zebrafish larvae, PloS One. 12 (2017) e0176927. doi:10.1371/journal.pone.0176927.
[15] M.S. Wolff, S.L. Teitelbaum, K. McGovern, S.M. Pinney, G.C. Windham, M. Galvez, A. Pajak, M. Rybak, A.M. Calafat, L.H. Kushi, F.M. Biro, Breast Cancer and Environment Research Program. Electronic address: http://www.bcerp.org/index.htm, Environmental phenols and pubertal development in girls, Environ. Int. 84 (2015) 174–180. doi:10.1016/j.envint.2015.08.008.
[16] C. Philippat, M. Mortamais, C. Chevrier, C. Petit, A.M. Calafat, X. Ye, M.J. Silva, C. Brambilla, I. Pin, M.-A. Charles, S. Cordier, R. Slama, Exposure to phthalates and phenols during pregnancy and offspring size at birth, Environ. Health Perspect. 120 (2012) 464–470. doi:10.1289/ehp.1103634.
[17] R. Tang, M.-J. Chen, G.-D. Ding, X.-J. Chen, X.-M. Han, K. Zhou, L.-M. Chen, Y.-K. Xia, Y. Tian, X.-R. Wang, Associations of prenatal exposure to phenols with birth outcomes, Environ. Pollut. Barking Essex 1987. 178 (2013) 115–120. doi:10.1016/j.envpol.2013.03.023.
[18] E. Den Hond, H. Tournaye, P. De Sutter, W. Ombelet, W. Baeyens, A. Covaci, B. Cox, T.S. Nawrot, N. Van Larebeke, T. D'Hooghe, Human exposure to endocrine disrupting chemicals and fertility: A case-control study in male subfertility patients, Environ. Int. 84 (2015) 154–160. doi:10.1016/j.envint.2015.07.017.
[19] M.P. Vélez, T.E. Arbuckle, W.D. Fraser, Female exposure to phenols and phthalates and time to pregnancy: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, Fertil. Steril. 103 (2015) 1011–1020.e2. doi:10.1016/j.fertnstert.2015.01.005.
[20] D.J. Watkins, K.K. Ferguson, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico, Int. J. Hyg. Environ. Health. 218 (2015) 212–219. doi:10.1016/j.ijheh.2014.11.001.
[21] J.P. Buckley, A.H. Herring, M.S. Wolff, A.M. Calafat, S.M. Engel, Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study, Environ. Int. 91 (2016) 350–356. doi:10.1016/j.envint.2016.03.019.
[22] E. Tingi, A.A. Syed, A. Kyriacou, G. Mastorakos, A. Kyriacou, Benign thyroid disease in pregnancy: A state of the art review, J. Clin. Transl. Endocrinol. 6 (2016) 37–49. doi:10.1016/j.jcte.2016.11.001.
[23] G. Mastorakos, E.I. Karoutsou, M. Mizamtsidi, G. Creatsas, The menace of endocrine disruptors on thyroid hormone physiology and their impact on intrauterine development, Endocrine. 31 (2007) 219–237.
[24] G.R. Williams, Neurodevelopmental and Neurophysiological Actions of Thyroid Hormone, J. Neuroendocrinol. 20 (2008) 784–794. doi:10.1111/j.1365-2826.2008.01733.x.

[25] T.R. Zoeller, A.L.S. Dowling, C.T.A. Herzig, E.A. Iannacone, K.J. Gauger, R. Bansal, Thyroid hormone, brain development, and the environment, Environ. Health Perspect. 110 Suppl 3 (2002) 355–361.
[26] A.J. Forhead, A.L. Fowden, Thyroid hormones in fetal growth and prepartum maturation, J. Endocrinol. 221 (2014) R87–R103. doi:10.1530/JOE-14-0025.
[27] A. Alemu, B. Terefe, M. Abebe, B. Biadgo, Thyroid hormone dysfunction during pregnancy: A review, Int. J. Reprod. Biomed. 14 (2016) 677–686.
[28] M.N. Mead, Children's Health: Mother's Thyroid, Baby's Health, Environ. Health Perspect. 112 (2004) A612.
[29] J.M. Braun, Early-life exposure to EDCs: role in childhood obesity and neurodevelopment, Nat. Rev. Endocrinol. 13 (2017) 161–173. doi:10.1038/nrendo.2016.186.
[30] L.-M. Chen, W.-J. Du, J. Dai, Q. Zhang, G.-X. Si, H. Yang, E.-L. Ye, Q.-S. Chen, L.-C. Yu, C. Zhang, X.-M. Lu, Effects of subclinical hypothyroidism on maternal and perinatal outcomes during pregnancy: a single-center cohort study of a Chinese population, PloS One. 9 (2014) e109364. doi:10.1371/journal.pone.0109364.
[31] F. Saki, M.H. Dabbaghmanesh, S.Z. Ghaemi, S. Forouhari, G. Ranjbar Omrani, M. Bakhshayeshkaram, Thyroid function in pregnancy and its influences on maternal and fetal outcomes, Int. J. Endocrinol. Metab. 12 (2014) e19378. doi:10.5812/ijem.19378.
[32] A.M. Aker, D.J. Watkins, L.E. Johns, K.K. Ferguson, O.P. Soldin, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women, Environ. Res. 151 (2016) 30–37. doi:10.1016/j.envres.2016.07.002.
[33] M.T. Aung, L.E. Johns, K.K. Ferguson, B. Mukherjee, T.F. McElrath, J.D. Meeker, Thyroid hormone parameters during pregnancy in relation to urinary bisphenol A concentrations: A repeated measures study, Environ. Int. 104 (2017) 33–40. doi:10.1016/j.envint.2017.04.001.
[34] K.K. Ferguson, T.F. McElrath, Y.-H. Chen, B. Mukherjee, J.D. Meeker, Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis, Environ. Health Perspect. 123 (2015) 210–216. doi:10.1289/ehp.1307996.
[35] K.K. Ferguson, T.F. McElrath, Y.-A. Ko, B. Mukherjee, J.D. Meeker, Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth, Environ. Int. 70 (2014) 118–124. doi:10.1016/j.envint.2014.05.016.
[36] T.F. McElrath, K.-H. Lim, E. Pare, J. Rich-Edwards, D. Pucci, R. Troisi, S. Parry, Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy, Am. J. Obstet. Gynecol. 207 (2012) 407.e1-407.e7. doi:10.1016/j.ajog.2012.08.010.
[37] R.C. Lewis, J.D. Meeker, K.E. Peterson, J.M. Lee, G.G. Pace, A. Cantoral, M.M. Téllez-Rojo, Predictors of urinary bisphenol A and phthalate metabolite concentrations in Mexican children, Chemosphere. 93 (2013) 2390–2398. doi:10.1016/j.chemosphere.2013.08.038.
[38] X. Ye, Z. Kuklenyik, A.M. Bishop, L.L. Needham, A.M. Calafat, Quantification of the urinary concentrations of parabens in humans by on-line solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry, J.

Chromatogr. B Analyt. Technol. Biomed. Life. Sci. 844 (2006) 53–59. doi:10.1016/j.jchromb.2006.06.037.
[39] X. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 383 (2005) 638–644. doi:10.1007/s00216-005-0019-4.
[40] R.W. Hornung, L.D. Reed, Estimation of Average Concentration in the Presence of Nondetectable Values, Appl. Occup. Environ. Hyg. 5 (1990) 46–51. doi:10.1080/1047322X.1990.10389587.
[41] K.M. O'Brien, K. Upson, N.R. Cook, C.R. Weinberg, Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment, Environ. Health Perspect. 124 (2016) 220–227. doi:10.1289/ehp.1509693.
[42] L.E. Johns, K.K. Ferguson, T.F. McElrath, B. Mukherjee, E.W. Seely, J.D. Meeker, Longitudinal Profiles of Thyroid Hormone Parameters in Pregnancy and Associations with Preterm Birth, PLOS ONE. 12 (2017) e0169542. doi:10.1371/journal.pone.0169542.
[43] D.B. Richardson, P. Rzehak, J. Klenk, S.K. Weiland, Analyses of Case-Control Data for Additional Outcomes, Epidemiology. 18 (2007) 441–445. doi:10.2307/20486400.
[44] L.E. Johns, K.K. Ferguson, T.F. McElrath, B. Mukherjee, J.D. Meeker, Associations between Repeated Measures of Maternal Urinary Phthalate Metabolites and Thyroid Hormone Parameters during Pregnancy, Environ. Health Perspect. 124 (2016). doi:10.1289/EHP170.
[45] K. Croes, E. Den Hond, L. Bruckers, E. Govarts, G. Schoeters, A. Covaci, I. Loots, B. Morrens, V. Nelen, I. Sioen, N. Van Larebeke, W. Baeyens, Endocrine actions of pesticides measured in the Flemish environment and health studies (FLEHS I and II), Environ. Sci. Pollut. Res. Int. 22 (2015) 14589–14599. doi:10.1007/s11356-014-3437-z.
[46] Y. Wei, J. Zhu, Para-Dichlorobenzene Exposure Is Associated with Thyroid Dysfunction in US Adolescents, J. Pediatr. 177 (2016) 238–243. doi:10.1016/j.jpeds.2016.06.085.
[47] K.J. van den Berg, Interaction of chlorinated phenols with thyroxine binding sites of human transthyretin, albumin and thyroid binding globulin, Chem. Biol. Interact. 76 (1990) 63–75. doi:10.1016/0009-2797(90)90034-K.
[48] D. Glinoer, The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology, Endocr. Rev. 18 (1997) 404–433. doi:10.1210/edrv.18.3.0300.
[49] P.Y. Kunz, K. Fent, Multiple hormonal activities of UV filters and comparison of in vivo and in vitro estrogenic activity of ethyl-4-aminobenzoate in fish, Aquat. Toxicol. 79 (2006) 305–324. doi:10.1016/j.aquatox.2006.06.016.
[50] A. Balázs, C. Krifaton, I. Orosz, S. Szoboszlay, R. Kovács, Z. Csenki, B. Urbányi, B. Kriszt, Hormonal activity, cytotoxicity and developmental toxicity of UV filters, Ecotoxicol. Environ. Saf. 131 (2016) 45–53. doi:10.1016/j.ecoenv.2016.04.037.
[51] N. r. Janjua, B. Kongshoj, J. h. Petersen, H. c. Wulf, Sunscreens and thyroid function in humans after short-term whole-body topical application: a single-blinded study, Br. J. Dermatol. 156 (2007) 1080–1082. doi:10.1111/j.1365-2133.2007.07803.x.
[52] S. Rattan, C. Zhou, C. Chiang, S. Mahalingam, E. Brehm, J. Flaws, Exposure to endocrine disruptors during adulthood: consequences for female fertility, J. Endocrinol. (2017). doi:10.1530/JOE-17-0023.

[53] K. Ji, S. Hong, Y. Kho, K. Choi, Effects of bisphenol s exposure on endocrine functions and reproduction of zebrafish, Environ. Sci. Technol. 47 (2013) 8793–8800. doi:10.1021/es400329t.
[54] W. Qiu, Y. Zhao, M. Yang, M. Farajzadeh, C. Pan, N.L. Wayne, Actions of Bisphenol A and Bisphenol S on the Reproductive Neuroendocrine System During Early Development in Zebrafish, Endocrinology. 157 (2016) 636–647. doi:10.1210/en.2015-1785.
[55] C.V. Sartain, P.A. Hunt, An old culprit but a new story: bisphenol A and "NextGen" bisphenols, Fertil. Steril. 106 (2016) 820–826. doi:10.1016/j.fertnstert.2016.07.1114.
[56] J.A. Ruszkiewicz, S. Li, M.B. Rodriguez, M. Aschner, Is Triclosan a neurotoxic agent?, J. Toxicol. Environ. Health B Crit. Rev. 20 (2017) 104–117. doi:10.1080/10937404.2017.1281181.
[57] Y. Feng, P. Zhang, Z. Zhang, J. Shi, Z. Jiao, B. Shao, Endocrine Disrupting Effects of Triclosan on the Placenta in Pregnant Rats, PloS One. 11 (2016) e0154758. doi:10.1371/journal.pone.0154758.
[58] P.E.A. Rodríguez, M.S. Sanchez, Maternal exposure to triclosan impairs thyroid homeostasis and female pubertal development in Wistar rat offspring, J. Toxicol. Environ. Health A. 73 (2010) 1678–1688. doi:10.1080/15287394.2010.516241.
[59] X. Wang, X. Chen, X. Feng, F. Chang, M. Chen, Y. Xia, L. Chen, Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice, Sci. Rep. 5 (2015) 18252. doi:10.1038/srep18252.
[60] X. Cao, X. Hua, X. Wang, L. Chen, Exposure of pregnant mice to triclosan impairs placental development and nutrient transport, Sci. Rep. 7 (2017) 44803. doi:10.1038/srep44803.
[61] R.J. Witorsch, Critical analysis of endocrine disruptive activity of triclosan and its relevance to human exposure through the use of personal care products, Crit. Rev. Toxicol. 44 (2014) 535–555. doi:10.3109/10408444.2014.910754.
[62] K.B. Paul, J.M. Hedge, R. Bansal, R.T. Zoeller, R. Peter, M.J. DeVito, K.M. Crofton, Developmental triclosan exposure decreases maternal, fetal, and early neonatal thyroxine: a dynamic and kinetic evaluation of a putative mode-of-action, Toxicology. 300 (2012) 31–45. doi:10.1016/j.tox.2012.05.023.
[63] L.M. Zorrilla, E.K. Gibson, S.C. Jeffay, K.M. Crofton, W.R. Setzer, R.L. Cooper, T.E. Stoker, The effects of triclosan on puberty and thyroid hormones in male Wistar rats, Toxicol. Sci. Off. J. Soc. Toxicol. 107 (2009) 56–64. doi:10.1093/toxsci/kfn225.
[64] M. Allmyr, G. Panagiotidis, E. Sparve, U. Diczfalusy, G. Sandborgh-Englund, Human exposure to triclosan via toothpaste does not change CYP3A4 activity or plasma concentrations of thyroid hormones, Basic Clin. Pharmacol. Toxicol. 105 (2009) 339–344. doi:10.1111/j.1742-7843.2009.00455.x.
[65] M.P. Cullinan, J.E. Palmer, A.D. Carle, M.J. West, G.J. Seymour, Long term use of triclosan toothpaste and thyroid function, Sci. Total Environ. 416 (2012) 75–79. doi:10.1016/j.scitotenv.2011.11.063.
[66] M. Axelstad, J. Boberg, A.M. Vinggaard, S. Christiansen, U. Hass, Triclosan exposure reduces thyroxine levels in pregnant and lactating rat dams and in directly exposed offspring, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 59 (2013) 534–540. doi:10.1016/j.fct.2013.06.050.

[67] K.M. Crofton, K.B. Paul, M.J. Devito, J.M. Hedge, Short-term in vivo exposure to the water contaminant triclosan: Evidence for disruption of thyroxine, Environ. Toxicol. Pharmacol. 24 (2007) 194–197. doi:10.1016/j.etap.2007.04.008.
[68] P.I. Johnson, E. Koustas, H.M. Vesterinen, P. Sutton, D.S. Atchley, A.N. Kim, M. Campbell, J.M. Donald, S. Sen, L. Bero, L. Zeise, T.J. Woodruff, Application of the Navigation Guide systematic review methodology to the evidence for developmental and reproductive toxicity of triclosan, Environ. Int. 92–93 (2016) 716–728. doi:10.1016/j.envint.2016.03.009.
[69] K.B. Paul, J.T. Thompson, S.O. Simmons, J.P. Vanden Heuvel, K.M. Crofton, Evidence for triclosan-induced activation of human and rodent xenobiotic nuclear receptors, Toxicol. In Vitro. 27 (2013) 2049–2060. doi:10.1016/j.tiv.2013.07.008.
[70] K.B. Paul, J.M. Hedge, M.J. Devito, K.M. Crofton, Developmental triclosan exposure decreases maternal and neonatal thyroxine in rats, Environ. Toxicol. Chem. 29 (2010) 2840–2844. doi:10.1002/etc.339.
[71] K.B. Paul, J.M. Hedge, M.J. DeVito, K.M. Crofton, Short-term exposure to triclosan decreases thyroxine in vivo via upregulation of hepatic catabolism in Young Long-Evans rats, Toxicol. Sci. Off. J. Soc. Toxicol. 113 (2010) 367–379. doi:10.1093/toxsci/kfp271.
[72] T.T.B. Vo, Y.-M. Yoo, K.-C. Choi, E.-B. Jeung, Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model, Reprod. Toxicol. Elmsford N. 29 (2010) 306–316. doi:10.1016/j.reprotox.2010.01.013.
[73] N.R. Janjua, G.K. Mortensen, A.-M. Andersson, B. Kongshoj, N.E. Skakkebaek, H.C. Wulf, Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans, Environ. Sci. Technol. 41 (2007) 5564–5570.
[74] J.D. Meeker, T. Yang, X. Ye, A.M. Calafat, R. Hauser, Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage, Environ. Health Perspect. 119 (2011) 252–257. doi:10.1289/ehp.1002238.
[75] B. Rousset, Antithyroid effect of a food or drug preservative: 4-hydroxybenzoic acid methyl ester, Experientia. 37 (1981) 177–178.
[76] E. van den Boogaard, R. Vissenberg, J.A. Land, M. van Wely, J.A.M. van der Post, M. Goddijn, P.H. Bisschop, Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review, Hum. Reprod. Update. 17 (2011) 605–619. doi:10.1093/humupd/dmr024.

CHAPTER III. A Repeated Measures Study of Phenol, Paraben and Triclocarban Urinary Biomarkers and Circulating Maternal Hormones during Gestation (PROTECT)

Abstract:

Introduction: Prenatal exposure to some phenols and parabens has been associated with adverse birth outcomes. Hormones may play an intermediate role between phenols and adverse outcomes. We examined the associations of phenol and paraben exposures with maternal reproductive and thyroid hormones in 602 pregnant women in Puerto Rico. Urinary triclocarban, phenol and paraben biomarkers, and serum hormones (estriol, progesterone, testosterone, sex-hormone-binding globulin (SHBG), corticotropin-releasing hormone (CRH), total triiodothyronine (T3), total thyroxine (T4), free thyroxine (FT4) and thyroid-stimulating hormone (TSH)) were measured at two visits during pregnancy. Methods: Linear mixed models with a random intercept were constructed to examine the associations between hormones and urinary biomarkers. Results were additionally stratified by study visit. Results were transformed to hormone percent changes for an inter-quartile-range difference in exposure biomarker concentrations (%Δ). Results: Bisphenol-S was associated with a decrease in CRH [(%Δ -11.35; 95% CI: -18.71, -3.33), and bisphenol-F was associated with an increase in FT4 (%Δ: 2.76; 95% CI: 0.29, 5.22). Butyl-, methyl- and propylparaben were associated with decreases in SHBG [(%Δ: -5.27; 95% CI: -9.4, -1.14); (%Δ: -3.53; 95% CI: -7.37, 0.31); (%Δ: -3.74; 95% CI: -7.76, 0.27)]. Triclocarban was positively associated with T3 (%Δ: 4.08; 95% CI: 1.18, 6.98) and T3/T4 ratio (%Δ: 4.67; 95% CI: -1.37, 6.65), and suggestively negatively associated with TSH (%Δ: -10.12; 95% CI: -19.47, 0.32). There was evidence of susceptible windows of vulnerability

for some associations. At 24-28 weeks gestation, there was a positive association between 2,4-dichlorophenol and CRH (%Δ: 9.66; 95% CI: 0.67, 19.45) and between triclosan and estriol (%Δ: 13.17; 95% CI: 2.34, 25.2); and a negative association between triclocarban and SHBG (%Δ: -9.71; 95% CI:-19.1, -0.27) and between bisphenol A and testosterone (%Δ: -17.37; 95% CI: -26.7, -6.87). Conclusion: Phenols and parabens are associated with hormone levels during pregnancy. Further studies are required to substantiate these findings.

Introduction

Exposure to phenols and parabens has been linked to various adverse health effects, including ovarian toxicity, cancer, and adverse neurodevelopmental outcomes [1–4]. Prenatal exposure to these chemicals, in particular, may have a long lasting effect on fetal health into adulthood. For example, prenatal exposure to phenols and parabens has been linked to adverse birth outcomes [5,6], respiratory health effects in children [7], and cardiometabolic risk [8]. The exact mechanisms at play are still not fully understood; however, endocrine disruption is hypothesized to be one of the main toxicity pathways [3,9–11].

Reproductive and thyroid hormones play an essential role in the maintenance of pregnancy and the development of the fetus [12–16], therefore pregnancy is a vulnerable window for endocrine disruption due to the varying levels of hormones involved in the growing organism [17]. Endocrine disrupting chemicals could act through several pathways, including hormone synthesis, regulation, transport and metabolism, and/or interference with receptors. Phenols and parabens have estrogenic and androgenic properties [1,18–21], but few human studies have looked into the effect of these chemicals on maternal hormones during pregnancy. Most existing studies in this area use smaller study populations or only examined a single time point in pregnancy, which do not capture the changing hormone levels and high variability of phenols and paraben exposure during pregnancy. Furthermore, no or few studies explored the associations between these chemicals and maternal testosterone, corticotropin-releasing hormone (CRH), sex hormone-binding globulin (SHBG) and estriol, all of which play essential roles in maintaining healthy pregnancies.

Given the growing evidence of the endocrine disrupting effects of phenols and parabens [19,22–26], our aim was to study the relationships between phenols and parabens on reproductive and thyroid hormones in our ongoing cohort of pregnant women in Puerto Rico. The study follows the women over multiple time points during pregnancy, providing more power than previous studies, and allows for the identification of potential windows of susceptibility. We previously reported early preliminary results on associations between select phenols and parabens with hormones in this Puerto Rican cohort [27]. This manuscript is an update of our previous results that utilizes a much larger sample size, includes additional hormones (estriol, testosterone, total triiodothyronine, and total thyroxine), as well as additional exposure biomarkers yet to be studied in detail (ethylparaben, BPS, BPF and triclocarban). Due to the lack of human health data, this study was exploratory in nature, with the exception of BPA, triclosan, methylparaben and propylparaben. We hypothesized a decrease in serum thyroid hormone levels in association with triclosan, methyl- and propyl-paraben, and an increase in serum thyroid hormones with BPA concentrations.

Methods

Study Participants

Participants for the present study were from an ongoing prospective cohort of pregnant women in Puerto Rico, named the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort. Details on the recruitment and inclusion criteria have been described previously [28,29]. The study participants included in the present analysis were recruited from 2012-2017 at 14 ± 2 weeks gestation from two hospitals and five affiliated prenatal clinics in Northern Puerto Rico. They were aged between 18-40 years. The exclusion criteria included women who lived outside the region, had multiple gestations, used oral contraceptives within three months prior to getting pregnant, got pregnant using in vitro fertilization, or had known medical health conditions (diabetes, hypertension, etc.). Three visits were conducted with the study participants to coincide with periods of rapid fetal growth and routine clinical visits (Visit 1: 16-20; Visit 2: 20-24; Visit 3: 24-28 gestation weeks). Demographic information was collected via questionnaires at the initial study visit. Spot urine samples were collected at the three study visits, whereas blood samples were collected during the first and third visits.

The present analysis includes 602 women recruited into the study (of the total 1311 women enrolled in the cohort to date) for whom both total phenol and paraben concentrations and hormone measurements from at least one study visit were available. This study was approved by the research and ethics committees of the University Of Michigan School Of Public Health, University of Puerto Rico, Northeastern University, and the University of Georgia. All study participants provided full informed consent prior to participation. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research.

Quantification of urinary biomarkers

After collection, spot urine samples were divided into aliquots and frozen at -80^{o}C until they were shipped overnight with dry ice to the CDC for analysis. Urine samples were analyzed for seven phenols (2,4-dichlorophenol, 2,5-dichlorophenol, BPA, BPS, BPF, benzophenone-3, triclosan), triclocarban, and four parabens [ethylparaben, methylparaben, butylparaben, propylparaben] using online solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry [30–32]. Biomarker concentrations below the limit of detection (LOD) were assigned a value of the LOD divided by √2 [33]. The LODs were as follows: 0.1 µg/L (2,4-dichlorphenol, 2,5-dichlorophenol, BPS, triclocarban, butylparaben, propylparaben); 0.2 µg/L (BPA, BPF); 0.4 µg/L (benzophenone-3); 1 µg/L (methylparaben, ethylparaben); and 1.7 µg/L (triclosan). Urinary dilution was accounted for by using urinary specific gravity (SG), and was measured using a digital handheld refractometer (AtagoCo., Ltd., Tokyo, Japan). For preliminary data analysis, urinary biomarker concentrations were corrected for SG using the following formula:

$P_c = M [(SG_m – 1) / (SG_i – 1)]$ where P_c is the SG-corrected concentration (µg/L), M is the measured concentration, SG_m is the study population median urinary specific gravity (1.0196), and SG_i is the individual's urinary specific gravity. The sample size for BPF, BPS, triclocarban and ethylparaben was smaller than the rest of the biomarkers because they were only quantified in a later sub-sample of the cohort.

Hormone measurement

Serum samples were collected during visits 1 and 3. Volume limitations resulted in differences in the number of samples analyzed by hormone. All hormone analyses were conducted at the

Central Ligand Assay Satellite Services (CLASS) laboratory, Department of Epidemiology, School of Public Health, University of Michigan. Progesterone, SHBG, testosterone, total triiodothyronine (T3), total thyroxine (T4), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were measured in serum using a chemiluminescence immunoassay (ADVIA Centaur® CP Immunoassay System, Seimens Healthineers). Estriol and CRH were measured in serum using an enzyme immunoassay (Estriol ELISA Kit, ALPCO; CRH ELISA Kit, LifeSpan BioSciences). In addition to measured hormones, the ratio of progesterone to estriol (Prog/Estriol Ratio), and the ratio of T3 and T4 (T3/T4 ratio) were calculated for the purposes of this analysis. Hormone ratios may be a better indicator of adverse pregnancy outcomes (such as preterm birth) than the individual hormones alone [34–36]. Two samples had a TSH level below the LOD. Because this result was not biologically plausible, these two values were dropped from the analyses.

Statistical Analyses

Distributions of key demographic characteristics were calculated. All urinary exposure biomarkers, and the serum hormones progesterone, estriol, CRH, TSH and progesterone/estriol ratio were positively-skewed, and were natural log-transformed. The distributions of SHBG, FT4, T3, T4 and T3/T4 ratio approximated normality and remained untransformed in all analyses. Geometric means and standard deviations were calculated for all SG-corrected exposure biomarkers, hormones, and the ratios of progesterone/estriol and T3/T4. We examined urinary exposure biomarkers concentrations and serum hormone levels by study visit, and calculated Spearman correlations between unlogged average SG-corrected exposure biomarkers. To assess differences in exposure biomarkers and hormones across study visits, we ran Linear Mixed Models (LMM) with a subject-specific random intercept regressing the biomarker or hormone against the study visit. Specific gravity was used as a covariate in the model instead of using the SG-corrected biomarker concentrations. The selection of a random intercept and slope was determined using BIC values. BPF and ethylparaben were detected in less than 50% of the samples. Therefore, we transformed BPF and ethylparaben into dichotomous variables, where 0 represented concentrations below the LOD, and 1 represented detectable concentrations. These categorical BPF and ethylparaben variables were used in all of the following regression analyses.

In our repeated measures analysis, we regressed one hormone or hormone ratio on one urinary biomarker using LMM, with a subject-specific random intercept for each model to account for intra-individual correlation of serial hormone measurements collected over the two study visits. The urinary biomarker concentrations at the two visits were treated as time-varying variables in the LMM models. Crude models included specific gravity and study visit as covariates. Potential confounders were selected a priori from the existing literature, and included as covariates if they were found to change the main effect estimate by >10%. Final models were adjusted for specific gravity, study visit, body mass index (BMI) at the first study visit, maternal age, the number of hours of second-hand smoking exposure per day, and a socio-economic variable. All covariates, except for maternal age and specific gravity, were categorical. The socio-economic variable used in the model differed by the hormone regressed. Maternal education was a strong confounder for models regressing progesterone, estriol, and progesterone/estriol ratio against urinary biomarkers concentrations, and was used as the socio-economic index for those models. All other models used insurance type as the socio-economic status index. The selection of the socio-economic variable was based on the percent change in the main effect estimate, and the p value of the socio-economic variable in final models. In an effort to compare our exposure distributions to our previous study discussed in CHAPTER II, we plotted density plots of each exposure biomarker from the PROTECT and LifeCodes cohorts.

To assess windows of vulnerability, we ran two more analyses. First, we ran the same LMMs regressing hormones and urinary biomarkers concentrations with an interaction term between the urinary biomarker and the study visit. Second, we ran multiple linear regressions (MLR) stratified by study visit of sample collection. The MLR models were adjusted for the same covariates as those in the LMMs.

To increase interpretability of our results, we transformed regression coefficients to percent changes (and associated 95% confidence intervals, CIs) in hormone concentration in relation to the interquartile range (IQR) increase in urinary biomarker concentrations. Beta coefficients from models with categorical biomarkers (BPF and ethylparaben) were transformed to percent changes (and associated 95% confidence intervals) in hormone concentration at detectable vs non-detectable biomarker concentrations. The alpha level was set at 0.05. All statistical analyses were conducted in R Version 3.4.2.

As a sensitivity analysis, all models were re-run using specific gravity as a covariate in combination with exposure biomarkers corrected for specific gravity as was described by O'Brien et al [37]. We observed no differences in our results, and therefore, retained our original models using un-corrected exposure biomarkers with specific gravity included as a covariate. We observed no differences in our results, and therefore, retained our original models using un-corrected exposure biomarkers with SG included as a covariate. In an effort to compare our exposure distributions to our previous study discussed in CHAPTER II, we plotted density plots of each exposure biomarker from the PROTECT and LifeCodes cohorts.

Results

The 602 study participants had a mean age of 26.4 and approximately 60% had BMI levels below 30 kg/m^2 (Table III.1). Although the majority of women reported never smoking (75%), 4% reported currently smoking, and 7% reported exposure to second-hand smoking for more than an hour per day. Six percent reported consuming alcohol in the last few months. A quarter of the study participants reported a household income of less than $10,000, and only 11% reported a household income >$50,000. A quarter of the participants did not report their incomes. As compared to the overall PROTECT cohort, the study participants included in the present analysis had higher rates of smoking, and had overall lower household income and education levels.

The exposure biomarkers included in this analysis were highly detected in the study population, with the exception of ethylparaben and BPF (Table III.2). BPF was detected in between 50%-60% of the study sample; ethylparaben was detected in between 42%-54% of the sample, depending on study visit. Concentrations of urinary biomarkers remained relatively consistent across the two study visits, with the exception of a decrease in BPA ($p < 0.001$) and butylparaben ($p = 0.04$). There was an increase in most hormones across the two study visits, particularly progesterone, estriol, SHBG and CRH. T4 levels remained consistent from 16-20 and 24-28 weeks gestation.

Methylparaben and propylparaben were strongly correlated [Spearman correlation of 0.8 ($p<0.001$)] (Figure III.1). Ethylparaben and butylparaben showed moderate correlation with methylparaben and propylparaben with Spearman correlations between 0.33-0.47 (p values < 0.001). 2,4-Dichlorophenol and 2,5-dichlorophenol showed a fairly strong correlation (Spearman

r = 0.6, p<0.001). Triclosan was moderately correlated with 2,4-dichlorophenol (Spearman r = 0.5, p<0.001), but not with 2,5-dichlorophenol (Spearman r = -0.03). BPA, BPS and BPF showed low correlation (Spearman r = 0.11-0.21, p<0.001).

As compared to the exposure biomarkers in the LifeCodes nested case control, PROTECT women had higher concentrations of 2,4-dichlorophenol, 2,5-dichlorophenol, BPA, BPS and triclocarban **Error! Reference source not found.**Figure III.5). Women in the LifeCodes cohort had higher concentrations of butylparaben and propylparaben. However, the LOD's from both cohorts differed. PROTECT had lower LOD's for a number of exposure biomarkers, including 2,4-dichlorophenol, 2,5-dichlorophenol, BPA, BPS, triclocarban, butylparaben and propylparaben. The lower LOD's in PROTECT indicate that the differences between the two populations may be even larger than observed in the plots.

Results from LMMs and MLRs are described in detail below by biomarker (Table III.3, Table III.4, and Table III.5). There were few differences between most adjusted and unadjusted models, with the exception of associations with CRH. MPB and PPB were associated with CRH in our unadjusted models, but in the adjusted models, these associations disappeared, and CRH was associated with BPS and TCS. A further analysis of CRH concentrations across the covariate levels did not reveal any large differences to report.

There were no associations between 2,4-dichlorophenol and 2,5-dichlorophenol with hormones in LMMs. An IQR increase in 2,4-dichlorophenol was associated with a 10% increase in CRH at 24-28 weeks [9.66 percent change in hormone per IQR change in the biomarker/ percent change in hormone at detectable biomarker concentrations (%Δ); 95% CI: 0.67, 19.45], and a suggestive 2% decrease in T3 at 16-20 weeks (%Δ -2.22 95% CI -4.55, 0.10).

Associations across the bisphenols differed, and BPS had the strongest associations in LMM models. BPS was associated with an 11% decrease in CRH (%Δ -11.35; 95% CI: -18.71, -3.33), and this association was stronger at 16-20 weeks gestation. At this time point, BPS was additionally associated with a 12% decrease in TSH (%Δ -11.93; 95% CI: -22.49, 0.07). BPF was associated with a 3% increase in FT4 (%Δ 2.76; 95% CI: 0.29, 5.22), and this association was also stronger at 16-20 weeks. BPA, on the other hand, had stronger associations at 24-28 weeks gestation. BPA was associated with a 17% decrease in testosterone, and 2-4% increases in FT4 and T3 at 24-28 weeks [(%Δ -17.37; 95% CI: -26.7, -6.87); (%Δ 2.38; 95% CI: 0.04, 4.72);

(%Δ4.33, 95% CI: 0.11, 8.55), respectively]. The increase in FT4 and T3 in relation to BPA was in line with our a priori hypothesis

Benzophenone-3 was not significantly associated with any hormones.

Triclocarban was associated with a number of thyroid hormones and SHBG. An IQR increase in triclocarban is associated with a 4% increase in T3 (%Δ 4.08; 95% CI: 1.18, 6.98), a 5% increase in the T3/T4 ratio (%Δ 4.67; 95% CI: 1.24, 10.10), a suggestive 10% decrease in TSH (%Δ -10.12; 95% CI: -19.47, 0.32), and a 10% decrease in SHBG at 24-28 weeks (%Δ -9.71; 95% CI: -19.1, -0.27).

Triclosan was associated with an increase in a number of reproductive hormones, however most were only suggestive with p values between 0.05 and 0.10. This includes a 9% increase in CRH (%Δ 9.20; 95% CI: -0.97, 20.42), a 7% increase in testosterone (%Δ 7.13; 95% CI: -0.60, 15.5), and 10-13% increases in progesterone and estriol at 24-28 weeks [(%Δ 9.72, 95% CI: -1.27, 21.9); (%Δ 13.2; 95% CI: 2.34, 25.2), respectively]. In addition, triclosan was associated with a 5.8% decrease in T3 at 24-28 weeks; this finding was in line with our a priori hypothesis.

IQR increases in butylparaben, methylparaben and propylparaben were associated with a decrease in SHBG [(%Δ -5.27; 95% CI:-9.40, -1.14); (%Δ -3.53; 95% CI: -7.37, 0.31); (%Δ -3.74; 95% CI: -7.76, 0.27), respectively]. Methylparaben was also associated with decreases in reproductive hormones, including an 8% decrease in estriol, a suggestive 3% increase in the progesterone/estriol ratio, and a suggestive decrease in testosterone at 16-20 weeks [(%Δ -7.76; 95% CI: -15.4, 0.61); (%Δ 3.14; 95% CI: -2.95, 9.61); (%Δ -6.77; 95% CI: -13.13, 0.29), respectively]. Conversely, an IQR increase in propylparaben was associated with a 9-10% *increase* in progesterone and estriol at 24-28 weeks [(%Δ 9.67; 95% CI: -1.30, 21.85); (%Δ 8.92; 95% CI: -1.56, 20.52)]. Interaction terms between study visit*methylparaben and propylparaben had p values <0.05 in models regressed against estriol. We expected to see a decrease in thyroid hormones in relation to methyl- and propyl- paraben, but only observed a decrease in TSH in association with methylparaben, particularly at 16-20 weeks (%Δ -11.69; 95% CI: -21.97, -0.06). The decrease in TSH could indicate an increase in circulating thyroid hormones, in contrast to our hypothesis.

Discussion

Associations differed by exposure biomarker and hormone, and there was little consistency within chemical classes with the exception of some parabens. There was evidence of a decrease up to 6% in T3 in association with 2,4-dichlorophenol, BPA and triclosan, whereas triclocarban was associated with a 4% increase in T3. In the case of bisphenols, BPS was more strongly related to decreases in hormones at 16-20 weeks, and BPA had stronger negative relationships at 24-28 weeks. Triclosan was associated with general increases in reproductive hormones of approximately 10%, and triclocarban was associated with 5-10% changes in thyroid hormones. Parabens were associated with a decreased level of SHBG.

While there may be structural similarities between BPA, BPS and BPF, the structural variations may be sufficient to alter receptor-binding affinities across the bisphenols [38]; therefore, the biological effects may vary among the bisphenols. To this, we found that the earlier time point (16-20 weeks gestation) may be a more vulnerable time of exposure to BPS and BPF, in contrast to the stronger relationships observed at the 24-28 weeks with respect to BPA. Our results were somewhat consistent with results from previous studies. BPA has been suspected to interfere with thyroid hormones, as evidenced by several epidemiological studies. We observed an increase in FT4 and T3, which was consistent with two previous studies our group conducted in a preliminary analysis in the PROTECT cohort, and a cohort of pregnant women in Boston, MA with four repeated measures during pregnancy [39,40]. Two cross-sectional studies in the United States (N=249 and 476 women) also looked at the association between maternal BPA and thyroid hormones during gestation [41,42]. The only significant association reported was between maternal urinary BPA and a decrease in T4 [41], which we did not observe in the present study. A decrease in T4 could be indicative of an increase in FT4, in the case of thyroxine becoming less bound to thyroxine-binding globulin, however, the associations between BPA and T4 in the current study had p values ranging from 0.51-0.93. Furthermore, we did not observe a relationship between BPA and TSH that was reported in the Boston cohort study [43], and among adults from the Korean National Environmental Health Survey [44].

One of the strongest associations we observed was the 17% decrease in testosterone in relation to BPA. This is the first study that explores this association in pregnant women, and there is little correlation between maternal and fetal testosterone levels [45]. However, a decrease in

testosterone was identified in an in vitro study on TM3 murine Leydig with BPA exposure [46], in the F2 generation after in-utero BPA exposure in mice [47], and in-utero BPA concentrations in young boys aged 8-14 [48]. These associations provide further evidence in support of our finding. Although the role of maternal testosterone in gestation is still unclear, evidence points to androgens playing an essential role in myometrial relaxation, cervical ripening and initiating parturition [49]. Therefore, BPA, via reduced testosterone, could increase gestational age, which we previously observed in this cohort [50]. Additionally, maternal testosterone has a role in gender role behaviors [51], indicating that maternal testosterone may impact fetal development.

No human studies have previously investigated the associations between triclocarban, phenols and parabens on CRH during pregnancy; however, CRH plays an important role in gestation. Maternal CRH levels during pregnancy largely originate from gestational tissues [52]. Evidence suggests CRH inhibits immune rejection processes by killing activated T cells [53], plays an important role in determining time of parturition, and an increase in CRH has been associated with the onset of miscarriage and preeclampsia [54–58]. CRH receptor expression is regulated by estrogen, and CRH gene expression in the placenta is mediated by ER-α [59,60]. Given the endocrine disrupting potential of bisphenols via estrogen receptors [61], associations between CRH and bisphenols (and potentially other phenols and parabens) could be important to consider in pregnancy studies. Animal and in vitro studies showed an increase in CRH with exposure to BPA and BPS, contrary to our results of an inverse relationship between CRH and BPS. BPA increased plasma concentrations of CRH in pregnant mice [62] and CRH levels in human placenta primary trophoblast cells [63]. The differences in our results could be in part due to the unique role CRH plays in human pregnancies, as compared to animals [64].

Triclosan was suggestively associated with select hormones, but none reached statistical significance, including an increase in testosterone, an increase in CRH at 16-20 weeks gestation, and a decrease in T3 at 24-28 weeks gestation. There was a similar decrease in T3 with increased urinary triclosan concentrations in the Boston cohort, albeit the associations were stronger earlier in pregnancy, in contrast to our stronger associations at the later visit in the current study [40]. While larger human studies with more statistical power may be needed, the decrease in T3 in association with triclosan is consistent with animal studies [65], including in pregnant rats [66] and pregnant mice [67,68], perhaps due to triclosan's structural similarities to thyroid hormones

[65]. Animal studies also report a decrease in T4 with triclosan exposure, including rat and mice dams [66–76], but we did not find evidence of this in humans. Other population studies found no associations between triclosan and thyroid hormones [77–79], although there was evidence of vulnerable time points during gestation [77,78]. Interestingly, a study in pregnant rats showed that the greatest accumulation of triclosan was in the placenta, indicating that pregnancy may be a sensitive time period for triclosan exposure [80]. Alternatively, maternal serum TSH and FT4 levels at >28 weeks gestation (obtained from medical records) were negatively associated with urinary triclosan at 38 weeks gestation [81]. The differences in our results could be explained by the differences in the study population, exposure biomarker concentrations, and differences in the pregnancy time points examined.

No studies have looked at the effect of triclosan on maternal testosterone and CRH during pregnancy in humans. However, in contrast to our results, triclosan was found to reduce testosterone levels in male rats[82], and in pregnant rats [80]. An excess of maternal testosterone has been associated with restricted fetal growth [83], as well as an increased chance of developing Alzheimer disease [84] and anxiety like symptoms in the offspring.

Triclocarban was associated with thyroid hormone changes. We observed an increase in T3 and a decrease in TSH in association with triclocarban, which is in line with the negative feedback loop in maintaining thyroid hormone homeostasis. We also observed a decrease in SHBG. SHBG levels tend to rise with thyroid hormones, so this observed pattern was unexpected. This could be due to factors influencing the relationship between thyroid hormone and SHBG levels that have not been accounted for in the present study. Our previous Boston study also reported a negative association between triclocarban and TSH, but a negative association with T3. Triclocarban concentrations in this cohort were much higher than the exposure levels found in the Boston cohort. In fact, the triclocarban concentrations observed in PROTECT are 37 times larger than the concentration observed in NHANES women of reproductive age [85]. This difference in exposure levels may explain the differences in the associations observed.

All parabens were generally negatively associated with SHBG. In contrast to our current findings, our previous preliminary analysis in the PROTECT cohort showed that methylparaben was positively associated with SHBG [27]. However, the current study has a much larger sample size. Associations between parabens and some hormones appeared to be dependent on the

timing of exposure. Associations between methylparaben and propylparaben and estriol changed direction from a negative association at 16-20 weeks to a positive association at 24-28 weeks gestation. We observed a similar change in direction in our preliminary analyses between methylparaben and propylparaben with estradiol [27]. Although not statistically significant, associations between methylparaben and propylparaben with progesterone followed a similar pattern to that of estriol. Given that the population urinary levels of methylparaben and propylparaben remained consistent between the two time points, the similar change of direction observed in associations with methylparaben and propylparaben in both of our previous analyses, and the significant interaction term between these parabens and visit in association with estriol, this lends confidence that these observations may not be occurring by chance and may be detected in future larger studies. The strong correlation between propyl- and methylparaben could indicate that their associations with estriol are being driven by only one of the parabens. However, given the differences in the associations between these two parabens and all hormones, there do seem to be unique relationships between the exposure and hormone levels. No previous studies have looked at the effect of parabens on estriol, SHBG or CRH; however, evidence suggests parabens have ER-β agonistic activity [86], and stimulate progesterone mRNA expression via ER-α signaling [87,88]. This could suggest a potential mechanism by which reproductive hormone levels could be directly or indirectly altered in response to paraben exposure.

The present study also showed a general decrease in TSH in association with parabens, but only methylparaben reached a significant association with TSH. Additionally, methylparaben and propylparaben were associated with a decrease in the T3/T4 ratio, particularly at 24-28 weeks gestation. Results from our Boston cohort also showed a decrease in T3/T4 ratio, as well as T3, at median 26 weeks gestation [89]. In other research, human and animal studies reported a decrease in T4 and FT4 with paraben exposure in females [79,90], and two small studies in men found no associations between parabens and thyroid hormones [91,92]. The difference in the results is likely due to the different study populations; none of those studies looked specifically at prenatal exposure.

Our study had several limitations. We did not have data on the iodine status of the women; deficiency in this element could affect thyroid hormone function. However, iodine may act as

mechanistic intermediate exposure between the exposure and thyroid hormone, and controlling for iodine status could lead to bias [93]. Furthermore, iodine had no effect on the associations between phenols and thyroid hormones in our previous study of NHANES data [79]. We also did not have data on thyroperoxidase antibodies nor human chorionic gonadotropin (hCG), which could potentially affect thyroid function as well [94,95]. While data at two time points is a great improvement from the more common cross-sectional study design, the two time points may not be sufficient to understand the potential influence of these biomarkers on maternal hormones. The relatively high variation in urinary concentrations of the target biomarkers (particularly BPA) over time may also introduce potential bias stemming from random measurement error. Given the multiple comparisons conducted, there is a chance of Type I error, and caution must be used when interpreting our findings. Finally, although one of the strengths of the present study is our ability to investigate the relationships between these chemicals and hormone levels in a vulnerable population, our study population was based in a population in Puerto Rico of lower income who also had higher urinary concentrations of some of the exposure biomarkers; therefore, the results may not be fully generalizable to other populations.

Our study also had many strengths. Our robust sample size, and the collection of exposure biomarkers and hormone data at two time points during pregnancy helps account for the biomarkers' short lifespan in the body, and the varying levels of hormones throughout pregnancy. The repeated measures allow for the control of intra-individual variability, and increases statistical power. We were also able to explore potential windows of susceptibility for these associations.

Additionally, we were able to compare our results from this analysis to our own analyses that employed similar statistical methods in two other data sets, namely LMMs to capture biomarkers at various time points and allow subject-specific intercepts. While there were many similarities in the results across the three analyses, the differences in results may point to the importance of outside factors that may not be captured in our models that alter the associations between these chemicals and endocrine disruption through interaction with the chemicals. These outside factors could include other endocrine-altering variables, such as exposure to other unaccounted for chemicals, maternal stress, genetic, epigenetic, or other differences. It is imperative that future

studies look beyond the association between a single chemical and singe hormone, and explore potential interactions with chemical exposure.

Conclusion

Our results provide suggestive human evidence for associations between select biomarkers with maternal thyroid and reproductive hormones during gestation. Of note, we report negative associations between parabens and SHBG, a negative association between BPS and CRH, and associations between triclocarban and triclosan with reproductive and thyroid hormones. Our stratified analyses show that some associations may be stronger at certain time points during pregnancy. Further studies in larger populations and with more repeated measures across pregnancy to will be useful to confirm our findings, and better understand if and how these hormone changes may affect downstream maternal and infant health outcomes.

Table III.1: Summary demographics of the 602 pregnant women in the study population

	Included	**Not Included**	**p**
Total N	602	709	
Age (mean [SD])	26.51 (5.66)	26.94 (5.34)	0.25
BMI in kg/m^2 (%)			0.99
<25	245 (40.7)	192 (27.1)	
25-30	114 (18.9)	87 (12.3)	
>30	73 (12.1)	56 (7.9)	
Missing	170 (28.2)	374 (52.8)	
Current Smoker (%)			0.03
Never	440 (73.1)	323 (45.6)	
Ever	63 (10.5)	57 (8.0)	
Current	23 (3.8)	6 (0.8)	
Missing	76 (12.6)	323 (45.6)	
Exposure to Second-Hand Smoking per Day (%)			0.16
Up to half an hour	443 (73.6)	338 (47.7)	
Up to an hour	25 (4.2)	19 (2.7)	
More than an hour	41 (6.8)	18 (2.5)	
Missing	93 (15.4)	334 (47.1)	
Alcohol Consumption (%)			0.61
No	273 (45.3)	190 (26.8)	
Before pregnancy	215 (35.7)	170 (24.0)	

Yes within the last few months	36 (6.0)	24 (3.4)	
Missing	78 (13.0)	325 (45.8)	
Household Income in U.S. $ (%)			0.03
<10,000	152 (25.2)	82 (11.6)	
10,000 - 30,000	132 (21.9)	114 (16.1)	
30,000 - 50,000	101 (16.8)	83 (11.7)	
>50,000	64 (10.6)	59 (8.3)	
Missing	153 (25.4)	371 (52.3)	
Maternal Education (%)			0.02
< High School	123 (20.4)	64 (9.0)	
Some college	194 (32.2)	137 (19.3)	
College graduate	210 (34.9)	182 (25.7)	
Missing	75 (12.5)	326 (46.0)	
Insurance Type (%)			0.001
Public (Mi Salud)	318 (52.8)	340 (48.0)	
Private	222 (36.9)	153 (21.6)	
Missing	62 (10.3)	216 (30.5)	

Table III.2: SG-corrected urinary biomarker concentrations and hormones by study visit of sample collection in pregnancy.

	16-20 weeks (N=389)						24-28 weeks (N=262)						
Biomarkers[a]	**GM (GSD)**	**% <LOD**	**25%**	**50%**	**75%**	**95%**	**GM (GSD)**	**% <LOD**	**25%**	**50%**	**75%**	**95%**	**p-value**
2,4-DCP	1.17 (3.24)	0.5	0.52	0.93	2.0	10.7	1.13 (9.8)	2.3	0.46	0.86	2.19	12.9	0.65
2,5-DCP	14.03 (5.14)	0.3	4.57	10.4	30.2	432.6	13.63 (360.3)	0	4.63	9.61	26.53	429.6	0.70
BPA	2.31 (2.25)	0.3	1.33	2.14	3.36	9.56	1.88 (2.4)	0.8	1.14	1.83	3.0	6.18	<0.001*
BPS[i]	0.54 (3.15)	3.4	0.23	0.50	1.07	4.01	0.54 (5.2)	8.6	0.23	0.47	1.06	4.23	0.95
BPF[i]	0.35 (3.18)	51.9	<LOD	0.25	0.56	2.88	0.31 (2.4)	59.9	<LOD	0.24	0.46	2.09	0.22
BP-3	38.34 (6.49)	0.5	10.6	22.4	110.8	1547	44.27 (2500.4)	0.8	11.9	25.3	160.7	1913.5	0.69
TCS	21.78 (8.72)	11.1	3.70	13.1	146.8	877.4	25.03 (327.4)	6.1	4.73	17.9	118.6	960.2	0.64
TCC[i]	4.34 (10.27)	5.8	0.70	3.36	33.6	157.7	4.86 (56.2)	5.6	0.78	4.78	32.76	168.6	0.46
EPB[i]	3.42 (7.73)	42.4	<LOD	1.66	15.4	177.5	2.55 (62.1)	54	<LOD	<LOD	7.6	76.1	0.12
MPB	80.72 (5.06)	0.3	25.08	116.5	274.8	846.1	92.5 (359)	0.8	30.2	111	314.5	1054.9	0.18
BPB	0.55 (8.12)	23.8	0.10	0.25	2.17	39.1	0.42 (12.2)	33.5	0.10	0.2	0.91	32.6	0.04*
PPB	17.51 (7.19)	0	3.59	21.1	80.5	262.4	17.61 (111.4)	0.4	4.0	25.17	83.65	253.8	0.99
	16-20 weeks (N=483)						**24-28 weeks (N=389)**						
Hormones	**GM (GSD)**	**% <LOD**	**25%**	**50%**	**75%**	**95%**	**GM (GSD)**	**% <LOD**	**25%**	**50%**	**75%**	**95%**	**p-value**
Progesterone[b]	49.63 (1.49)	0	37.2	48.5	61.6	98.2	95.94 (1.65)	0	70.9	90.9	129.2	222.7	<0.001*
Estriol[b]	18.85 (1.76)	0	13.2	17.6	27.5	50.5	44.63 (1.59)	0	33.8	44.9	57.5	97.3	<0.001*
SHBG[c]	575.22 (1.4)	0	482.4	588.6	703.8	907.9	670.19 (1.35)	0	536.3	672.9	832.6	1097.7	<0.001*
Prog/Estriol	2.64 (1.69)	-	1.97	2.66	3.70	5.83	2.15 (1.62)	-	1.58	2.12	2.86	4.48	<0.001*
CRH[d]	76.67 (1.70)	0	54.8	80.6	111.8	171.9	78.01 (1.76)	0	55.2	82.8	114.3	178.1	<0.001*
Testosterone[f]	50.55 (1.81)	1.8	37.5	51.5	73.5	124.4	60.22 (1.75)	0.9	45.1	60.7	88.1	131.6	<0.001*
TSH[e]	1.29 (2.14)	0	0.93	1.38	2.06	3.28	1.45 (1.79)	0	1.08	1.51	2.05	3.64	0.04*
FT4[f]	1.10 (1.13)	0	1.02	1.10	1.19	1.35	1.06 (1.13)	0	0.98	1.06	1.15	1.29	<0.001*
T3[b]	1.94 (1.22)	0	1.71	1.97	2.22	2.59	1.95 (1.21)	0	1.72	1.99	2.24	2.67	0.03*
T4[g]	11.90 (1.20)	0	10.7	11.95	13.3	15.7	11.71 (1.20)	0	10.5	11.7	13.2	15.5	0.27
T3/T4	0.16 (1.21)	-	0.14	0.16	0.19	0.23	0.17 (1.22)	-	0.15	0.17	0.19	0.23	<0.001*

GM: Geometric mean; GSD: Geometric standard deviation

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben
Range of gestational weeks: 16-20 weeks: 16-20 weeks gestation, 24-28 weeks: 24-28 weeks gestation.
* Significant difference ($p<0.05$) in urinary biomarker or hormone compared to reference (16-20 weeks) using linear mixed models with a random intercept
[a] Units: µg/L. [b] Units: ng/mL. [c] Units: nmol/L. [d] Units: pg/mL. [e] Units: uIU/mL. [f] Units: ng/dL. [g] Units: µg/dL.
[I] BPS, BPF, TCC and EPB had the lowest sample sizes because they were added to the biomarker assay at mid-study. At 16-20 weeks, these four urinary biomarkers had N= 295. At 24-28 weeks, these four urinary biomarkers had N= 198.

Table III.3: Results of the LMMs regressing hormones versus exposure biomarkers.

		CRH	SHBG	Testosterone	Progesterone	Estriol	Progesterone/ Estriol Ratio
2,4-DCP	% Δ/IQR	5.30 (-2.81, 14.08)	2.06 (-1.49, 5.61)	3.26 (-3.01, 9.94)	1.60 (-3.42, 6.87)	-1.93 (-7.54, 4.01)	3.58 (-1.85, 10.16)
	p	0.21	0.26	0.32	0.54	0.52	0.24
2,5-DCP	% Δ/IQR	3.89 (-3.29, 11.61)	0.96 (-2.25, 4.17)	1.75 (-3.85, 7.69)[a]	-0.46 (-4.79, 4.07)	-2.21 (-7.12, 2.96)	1.35 (-3.46, 6.82)
	p	0.30	0.56	0.55	0.84	0.40	0.61
BPA	% Δ/IQR	3.68 (-4.21, 12.22)	-0.22 (-3.60, 3.15)	-4.19 (-9.64, 1.59)[a]	-3.50 (-8.16, 1.39)	-2.18 (-7.78, 3.78)	-1.55 (-6.18, 5.01)
	p	0.37	0.90	0.15	0.16	0.47	0.60
BPF[b]	% Δ/IQR	3.90 (-9.72, 19.57)	-2.97 (-8.04, 2.11)	0.33 (-8.89, 10.49)	-1.33 (-23.9, 13.78)	3.84 (-6.40, 15.21)	-4.65 (-13.44, 5.05)
	p	0.60	0.26	0.95	0.76	0.48	0.34
BPS	% Δ/IQR	-11.35 (-18.71, -3.33)	-0.56 (-4.37, 3.25)	2.54 (-3.5, 8.97)	-4.38 (-9.49, 1.02)	-2.05 (-8.16, 4.47)	-2.96 (-7.85, 3.85)
	p	0.008**	0.77	0.42	0.11	0.53	0.34
BP-3	% Δ/IQR	-0.04 (-7.96, 8.57)	1.10 (-2.61, 4.82)	-0.51 (-6.8, 6.21)	0.46 (-4.62, 5.81)	-0.91 (-6.66, 5.18)	1.81 (-3.60, 8.42)
	p	0.99	0.56	0.88	0.86	0.76	0.56
TCC	% Δ/IQR	-3.69 (-14.5, 8.50)	-4.54 (-10.03, 0.94)	5.18 (-3.4, 14.51)	-3.22 (-10.13, 4.21)	0.36 (-7.93, 9.39)	-3.75 (-8.64, 7.7)
	p	0.54	0.11	0.25	0.39	0.94	0.39
TCS	% Δ/IQR	9.20 (-0.97, 20.42)	2.81 (-1.46, 7.08)	7.13 (-0.60, 15.5)	2.84 (-3.2, 9.25)[a]	4.16 (-3.07, 11.93)[a]	0.31 (-5.8, 8.4)
	p	0.08*	0.20	0.07*	0.37	0.27	0.93
EPB[b]	% Δ/IQR	1.52 (-11.55, 16.52)	-1.93 (-8.14, 4.29)	5.11 (-4.64, 15.86) [a]	-2.41 (-10.62, 6.56)	-1.92 (-11.4, 8.58)	-0.77 (-10.22, 9.67)
	p	0.83	0.55	0.32	0.59	0.71	0.88
BPB	% Δ/IQR	-1.86 (-10.64, 7.8)	-5.27 (-9.4, -1.14)	-6.77 (-13.3, 0.29)	-3.65 (-9.11, 2.14)	-5.18 (-11.45, 1.52)	1.96 (-4.9, 8.52)
	p	0.70	0.01**	0.06*	0.21	0.13	0.58
MPB	% Δ/IQR	5.88 (-3.0, 15.59)	-3.53 (-7.37, 0.31)	-4.41 (-10.68, 2.3)	0.03 (-5.29, 5.64)	-2.50 (-8.6, 4.01)[a]	2.64 (-3.06, 9.74)
	p	0.20	0.07*	0.19	0.99	0.44	0.43
PPB	% Δ/IQR	4.82 (-4.48, 15.02)	-3.74 (-7.76, 0.27)	-3.54 (-10.14, 3.54)	2.35 (-3.55, 8.6)	-0.63 (-7.36, 6.58)[a]	3.65 (-2.36, 11.66)
	p	0.32	0.07*	0.32	0.44	0.86	0.31

		TSH	FT4	T3	T4	T3/T4 ratio
2,4-DCP	% Δ/IQR	4.80 (-2.58, 12.74)	0.21 (-1.19, 1.60)	-1.58 (-3.58, 0.42)	-0.79 (-2.71, 1.13)	-1.16 (-4.86, 1.33)
	p	0.21	0.77	0.12	0.42	0.31
2,5-DCP	% Δ/IQR	4.63 (-2.08, 11.79)	0.82 (-0.43, 2.07)	-0.55 (-2.36, 1.26)	0.51 (-1.22, 2.24)	-1.38 (-4.64, 1.06)

	p	0.18	0.2	0.56	0.57	0.18
BPA	% Δ/IQR	-0.28 (-6.99, 6.91)	0.00 (-1.36, 1.36)	2.10 (0.22, 3.99)	0.69 (-1.13, 2.51)	1.46 (-2.23, 3.52)
	p	0.94	1	0.03**	0.46	0.19
BPF[b]	% Δ/IQR	7.29 (-4.59, 20.64)	2.76 (0.29, 5.22) [a]	-1.22 (-4.34, 1.90)	1.84 (-1.37, 5.04)	-2.50 (-6.47, 1.47)
	p	0.24	0.03**	0.45	0.26	0.22
BPS	% Δ/IQR	-1.01 (-8.12, 6.66)	-0.07 (-1.6, 1.46)	0.14 (-1.89, 2.18)	0.04 (-1.97, 2.05)	0.58 (-2.47, 2.9)
	p	0.79	0.93	0.89	0.97	0.64
BP-3	% Δ/IQR	-5.89 (-12.87, 1.65)	-0.40 (-1.85, 1.04)	-1.47 (-3.56, 0.63)	-1.37 (-3.35, 0.62)	-0.21 (-5.33, 1.26)
	p	0.13	0.59	0.17	0.18	0.86
TCC	% Δ/IQR	-10.12 (-19.47, 0.32)	-0.61 (-2.76, 1.55)	4.08 (1.18, 6.98)	-0.65 (-3.53, 2.23)	4.67 (-1.37, 6.65)
	p	0.06*	0.58	0.007**	0.66	0.01**
TCS	% Δ/IQR	0.57 (-7.74, 9.63)	-0.74 (-2.45, 0.96)	-1.97 (-4.36, 0.41)	-1.60 (-3.9, 0.69)	-0.15 (-2.69, 4.55)
	p	0.9	0.39	0.11	0.17	0.91
EPB[b]	% Δ/IQR	-6.78 (-17.6, 5.46)	-0.45 (-2.9, 2.0)	-0.76 (-4.10, 2.58)	-0.03 (-3.29, 3.24)	-1.68 (-5.66, 2.30)
	p	0.27	0.72	0.66	0.99	0.41
BPB	% Δ/IQR	-4.88 (-12.62, 3.54)	1.10 (-0.54, 2.74)	0.70 (-1.61, 3.02)	1.74 (-0.49, 3.96)	-1.56 (-7.01, -0.26)
	p	0.25	0.19	0.55	0.13	0.24
MPB	% Δ/IQR	-6.92 (-13.91, 0.64)[a]	0.77 (-0.76, 2.29)	-0.39 (-2.53, 1.76)	1.02 (-1.04, 3.09)	-1.78 (-4.64, 1.53)
	p	0.07*	0.33	0.73	0.33	0.15
PPB	% Δ/IQR	-6.29 (-13.6, 1.64)	0.81 (-0.8, 2.42)	0.21 (-2.02, 2.45)	0.65 (-1.52, 2.81)	-0.67 (-3.89, 2.63)
	p	0.12	0.32	0.85	0.56	0.60

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

Results converted to % change in hormone per IQR change in biomarker concentration.

* represents a p value below 0.10, and **represents a p value below 0.05; a Significant interaction ($p<0.05$) between urinary biomarker*visit; b Dichotomous variable

Table III.4: Results of the MLRs regressing reproductive hormones versus exposure biomarkers by visit.

		16-20 weeks gestation					
		CRH	**SHBG**	**Testosterone**	**Progesterone**	**Estriol**	**Progesterone/ Estriol Ratio**
2,4-DCP	% Δ/IQR	2.93 (-6.84, 13.73)	0.18 (-3.39, 3.74)	3.66 (-3.47, 11.32)	-0.33 (-4.75, 4.29)	-3.54 (-9.78, 3.12)	3.70 (-2.35, 11.15)
	p	0.57	0.92	0.32	0.92	0.29	0.89
2,5-DCP	% Δ/IQR	5.20 (-4.94, 16.43)	-0.16 (-3.79, 3.47)	2.11 (-5.04, 9.8)[a]	0.45 (-4.05, 5.17)	-0.92 (-7.4, 6.01)	1.48 (-5.03, 8.31)
	p	0.33	0.93	0.57	0.94	0.79	0.86
BPA	% Δ/IQR	6.35 (-4.08, 17.92)	0.09 (-3.64, 3.81)	2.77 (-4.58, 10.69)[a]	-2.11 (-6.36, 2.34)	0.17 (-6.4, 7.21)	-2.55 (-8.2, 4.6)
	p	0.24	0.96	0.47	0.45	0.96	0.38
BPF[b]	% Δ/IQR	10.11 (-11.4, 36.8)	-6.81 (-14.8, 1.16)	3.71 (-8.85, 18.01)	-1.45 (-27.8, 36.5)	7.50 (-7.52, 24.96)	-8.14 (-17.62, 2.43)
	p	0.39	0.1	0.58	0.97	0.35	0.79
BPS	% Δ/IQR	-16.15 (-26.6,-4.2)	-2.37 (-7.36, 2.61)	3.08 (-5.01, 11.86)	-4.51 (-9.77, 1.05)	-3.16 (-11.46, 5.9)	-1.48 (-8.62, 8.97)
	p	0.01**	0.35	0.47	0.35	0.48	0.13
BP-3	% Δ/IQR	-2.66 (-12.44, 8.2)	1.25 (-2.53, 5.04)	2.69 (-4.78, 10.75)	-0.60 (-5.27, 4.29)	-3.14 (-9.84, 4.06)	2.22 (-4.1, 9.93)
	p	0.62	0.52	0.49	0.72	0.38	0.82
TCC	% Δ/IQR	-6.95 (-21.9, 10.9)	-3.95 (-10.4, 2.5)	5.18 (-5.5, 17.07)	-3.93 (-10.9, 3.57)	1.68 (-9.36, 14.07)	-5.60 (-13.01, 8.6)
	p	0.42	0.23	0.36	0.62	0.78	0.32
TCS	% Δ/IQR	13.79 (-1.82, 31.9)	1.44 (-3.89, 6.76)	7.81 (-3, 19.83)	-0.65 (-6.78, 5.9)[a]	0.17 (-9.12, 10.4) [a]	0.12 (-6.88, 12.69)
	p	0.09*	0.60	0.16	0.63	0.97	0.85
EPB[b]	% Δ/IQR	3.40 (-15.66, 26.8)	-2.28 (-9.73, 5.18)	-0.25 (-11.9, 12.9)[a]	-0.31 (-49.9, 98.2)	4.83 (-8.72, 20.39)	-5.05 (-13.8, 4.58)
	p	0.75	0.55	0.97	0.32	0.5	0.95
BPB	% Δ/IQR	2.51 (-10.1, 16.9)	-3.47 (-8.18, 1.23)	-3.51 (-12.19, 6.04)	-1.80 (-7.08, 3.79)	-5.69 (-13.5, 2.78)	4.38 (-4.17, 12.69)
	p	0.71	0.15	0.46	0.71	0.18	0.56
MPB	% Δ/IQR	4.27 (-8.72, 19.1)	-5.94 (-10.7, -1.2)	-8.11 (-16.46, 1.09)	-5.33 (-10.6, 0.19)	-7.76 (-15.4, 0.6) [a]	3.14 (-2.95, 9.61)
	p	0.54	0.01**	0.08*	0.10	0.07*	0.08*
PPB	% Δ/IQR	3.55 (-10.07, 19.2)	-6.32 (-11.4, -1.3)	-7.02 (-15.95, 2.86)	-1.95 (-8.11, 4.62)	-6.35 (-14.9, 3.0) [a]	5.40 (-2.2, 17.62)

	p	0.63	0.01**	0.16	0.54	0.18	0.57
				24-28 weeks gestation			
		CRH	**SHBG**	**Testosterone**	**Progesterone**	**Estriol**	**Progesterone/ Estriol Ratio**
2,4-DCP	% Δ/IQR	9.66 (0.67, 19.45)	2.50 (-3.84, 8.83)	0.50 (-9.18, 11.22)	5.81 (-3, 15.42)	0.53 (-7.55, 9.31)	5.07 (-2.4, 14.89)
	p	0.04**	0.44	0.92	0.20	0.90	0.24
2,5-DCP	% Δ/IQR	3.92 (-3.66, 12.09)	0.22 (-5.35, 5.79)	-0.27 (-8.76, 9.01)[a]	-0.80 (-8.01, 6.97)	-4.58 (-11.2, 2.56)	3.74 (-2.69, 11.87)
	p	0.32	0.94	0.95	0.83	0.20	0.32
BPA	% Δ/IQR	-3.53 (-13.3, 7.32)	-0.48 (-8.26,7.31)	-17.37 (-26.7, -6.9)[a]	-3.65 (-13.4, 7.2)	-5.27 (-14.4, 4.87)	1.86 (-6.16, 14.1)
	p	0.51	0.90	0.002**	0.49	0.30	0.72
BPF[b]	% Δ/IQR	-1.64 (-16.7, 16.1)	-0.30 (-12.5,11.9)	-10.98 (-24.8, 5.41)	4.68 (-10.1, 21.88)	4.57 (-9.89, 21.35)	0.04 (-13.0, 15.07)
	p	0.84	0.96	0.18	0.56	0.56	1.00
BPS	% Δ/IQR	-7.61 (-16.3, 2.01)	-1.77 (-9.38, 5.8)	-6.26 (-15.49, 3.98)	-3.85 (-13.1, 6.4)	-2.05 (-10.75, 7.5)	-1.86 (-9.81, 7.49)
	p	0.12	0.65	0.22	0.45	0.66	0.69
BP-3	% Δ/IQR	1.71 (-7.23, 11.51)	1.80 (-5.09, 8.69)	-2.59 (-12.66, 8.64)	1.29 (-7.62, 11.1)	3.41 (-5.31, 12.94)	-2.09 (-9.89, 6.96)
	p	0.72	0.61	0.64	0.79	0.46	0.63
TCC	% Δ/IQR	-5.00 (-16.1, 7.57)	-9.71 (-19.1,-0.27)	10.25 (-3.14, 25.5)	-1.80 (-13.9, 12.0)	-1.94 (-13.1, 10.7)	0.14 (-7.77, 15.3)
	p	0.42	0.05**	0.14	0.79	0.75	0.98
TCS	% Δ/IQR	8.46 (-2.31, 20.43)	4.27 (-3.44, 11.98)	12.46 (-0.47, 27.08)	9.72 (-1.27, 21.9)[a]	13.17 (2.34, 25.2) [a]	-3.00 (-12.61, 6.43)
	p	0.13	0.28	0.06*	0.09*	0.02**	0.56
EPB[b]	% Δ/IQR	-3.51 (-17.06, 12.3)	-2.36 (-13.99, 9.3)	13.05 (-3.38, 32.28)[a]	-4.05 (-18.03, 12.3)	-11.47 (-23.3, 2.16)	8.43 (-5.77, 24.76)
	p	0.29	0.69	0.13	0.61	0.10	0.26
BPB	% Δ/IQR	-6.35 (-13.8, 1.71)	-3.77 (-9.86, 2.31)	-8.88 (-17.24, 0.32)	-2.20 (-10.1, 6.4)	-3.30 (-10.8, 4.87)	1.05 (-7.26, 8.5)
	p	0.12	0.23	0.06*	0.61	0.42	0.80
MPB	% Δ/IQR	8.20 (-1.67, 19.06)	1.25 (-5.82, 8.32)	-7.70 (-17.49, 3.25)	8.21 (-1.7, 19.11)	6.54 (-2.86, 16.84) [a]	1.05 (-7.83, 10.78)
	p	0.11	0.73	0.16	0.11	0.18	0.82
PPB	% Δ/IQR	8.87 (-1.95, 20.89)	-0.39 (-8.13, 7.35)	-2.21 (-13.55, 10.61)	9.67 (-1.3, 21.85)	8.92 (-1.56, 20.52) [a]	0.19 (-8.79, 10.8)
	p	0.11	0.92	0.72	0.09*	0.10	0.97

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

Beta coefficients are transformed into percent change of hormone in an IQR change in the exposure. Beta coefficients and their 95% CI are displayed. [a] represents a significant interaction term between the exposure*visit in LMM models ($p<0.05$). *p values < 0.1. ** p values < 0.05. [b] Dichotomous variable.

Table III.5: Results of the MLRs regressing thyroid hormones versus exposure biomarkers by visit.

		16-20 weeks gestation				
		TSH	**FT4**	**T3**	**T4**	**T3/T4 Ratio**
2,4-DCP	% Δ/IQR	6.72 (-2.7, 17.06)	0.21 (-1.4, 1.82)	-2.22 (-4.55, 0.1)	-0.85 (-3.07, 1.37)	-1.56 (-3.95, 0.84)
	p	0.17	0.80	0.06*	0.45	0.20
2,5-DCP	% Δ/IQR	3.01 (-6.3, 13.25)	0.78 (-0.86, 2.41)	-1.32 (-3.7, 1.06)	0.51 (-1.76, 2.77)	-2.12 (-4.55, 0.32)
	p	0.54	0.35	0.28	0.66	0.09*
BPA	% Δ/IQR	-4.08 (-12.94, 5.68)	-1.38 (-3.04, 0.29)	1.04 (-1.38, 3.47)	0.19 (-2.1, 2.48)	0.96 (-1.54, 3.46)
	p	0.40	0.11	0.40	0.87	0.45
BPF[b]	% Δ/IQR	15.44 (-5.92, 41.66)	6.94 (3.26, 10.62)[a]	0.26 (-4.94, 5.45)	3.00 (-1.87, 7.87)	-2.42 (-8.31, 3.47)
	p	0.17	0.0002**	0.92	0.23	0.42
BPS	% Δ/IQR	-11.93 (-22.49, 0.07)	0.32 (-1.99, 2.62)	2.50 (-0.56, 5.56)	0.38 (-2.57, 3.33)	2.69 (-0.74, 6.12)
	p	0.05*	0.79	0.11	0.80	0.13
BP-3	% Δ/IQR	-5.90 (-14.75, 3.87)	-1.37 (-3.07, 0.33)	-1.10 (-3.57, 1.38)	-1.74 (-4.06, 0.58)	0.90 (-4.88, 2.77)
	p	0.23	0.12	0.39	0.14	0.49
TCC	% Δ/IQR	-10.72 (-24.34, 5.34)	0.06 (-2.93, 3.04)	6.27 (2.38, 10.17)	1.00 (-2.85, 4.85)	5.67 (1.24, 10.10)
	p	0.18	0.97	0.002**	0.61	0.01**
TCS	% Δ/IQR	5.49 (-8.15, 21.16)	0.29 (-2.11, 2.7)	-0.81 (-4.32, 2.69)	-0.25 (-3.55, 3.06)	-0.28 (-3.86, 3.3)
	p	0.45	0.81	0.65	0.88	0.88
EPB[b]	% Δ/IQR	-2.50 (-19.56, 18.16)	-0.75 (-4.18, 2.69)	-1.47 (-6.11, 3.17)	-1.02 (-5.45, 3.41)	-1.12 (-6.34, 4.09)
	p	0.80	0.67	0.54	0.65	0.67
BPB	% Δ/IQR	-2.58 (-13.87, 10.2)	0.74 (-1.4, 2.88)	0.18 (-2.88, 3.25)	1.24 (-1.68, 4.17)	-1.46 (-4.62, 1.70)
	p	0.68	0.50	0.91	0.41	0.37
MPB	% Δ/IQR	-11.69 (-21.97, -0.06) [a]	1.00 (-1.16, 3.16)	0.33 (-2.8, 3.45)	1.22 (-1.76, 4.2)	-1.26 (-4.45, 1.94)
	p	0.05**	0.36	0.84	0.42	0.44

PPB	% Δ/IQR	-9.74 (-20.79, 2.85)	0.82 (-1.47, 3.11)	1.51 (-1.78, 4.8)	1.32 (-1.83, 4.47)	-0.06 (-3.44, 3.32)
	p	0.12	0.48	0.37	0.41	0.97

		24-28 weeks gestation				
		TSH	**FT4**	**T3**	**T4**	**T3/T4 Ratio**
2,4-DCP	% Δ/IQR	2.28 (-7.3, 12.84)	0.53 (-1.4, 2.46)	0.49 (-2.92, 3.89)	0.67 (-2.32, 3.66)	-0.73 (-6.4, 4.94)
	p	0.65	0.59	0.78	0.66	0.80
2,5-DCP	% Δ/IQR	0.50 (-7.81, 9.56)	1.01 (-0.68, 2.7)	2.12 (-0.86, 5.09)	0.83 (-1.8, 3.45)	-0.45 (-5.55, 4.65)
	p	0.91	0.24	0.16	0.54	0.86
BPA	% Δ/IQR	2.68 (-9.14, 16.03)	2.38 (0.04, 4.72)	4.33 (0.11, 8.55)	2.12 (-1.62, 5.85)	1.00 (-5.72, 7.72)
	p	0.67	0.05**	0.05**	0.27	0.77
BPF[b]	% Δ/IQR	1.23 (-15.77, 21.66)	-2.14 (-5.74, 1.45)[a]	-1.17 (-7.46, 5.12)	1.19 (-4.33, 6.70)	8.39 (-10.62, 27.39)
	p	0.90	0.24	0.72	0.67	0.39
BPS	% Δ/IQR	1.10 (-9.89, 13.43)	-1.40 (-3.75, 0.96)	-1.35 (-5.35, 2.66)	-1.01 (-4.66, 2.63)	-0.68 (-6.28, 4.92)
	p	0.85	0.25	0.51	0.59	0.81
BP-3	% Δ/IQR	-5.48 (-15.04, 5.17)	0.99 (-1.1, 3.08)	-3.11 (-6.78, 0.56)	-1.08 (-4.32, 2.17)	-3.46 (-9.22, 2.3)
	p	0.30	0.35	0.10	0.52	0.24
TCC	% Δ/IQR	-7.90 (-20.02, 6.06)	-0.81 (-3.75, 2.13)	5.64 (0.81, 10.46)	0.71 (-3.8, 5.22)	0.41 (-6.79, 7.61)
	p	0.25	0.59	0.02**	0.76	0.91
TCS	% Δ/IQR	5.46 (-6.44, 18.86)	-1.20 (-3.54, 1.14)	-5.81 (-9.9, -1.73)	-1.82 (-5.47, 1.82)	-1.33 (-8.32, 5.67)
	p	0.39	0.32	0.01**	0.33	0.71
EPB[b]	% Δ/IQR	-10.15 (-24.31, 6.66)	0.39 (-3.19, 3.97)	-1.08 (-7.07, 4.91)	2.78 (-2.72, 8.27)	12.88 (-18.25, 44.01)
	p	0.22	0.83	0.72	0.32	0.42
BPB	% Δ/IQR	-7.03 (-15.34, 2.11)	0.65 (-1.2, 2.5)	-0.74 (-4.01, 2.54)	1.32 (-1.56, 4.2)	-3.45 (-8.78, 1.89)
	p	0.13	0.49	0.66	0.37	0.21
MPB	% Δ/IQR	-7.22 (-16.8, 3.47) [a]	0.84 (-1.31, 2.98)	-2.96 (-6.74, 0.81)	0.95 (-2.4, 4.29)	-4.70 (-10.15, 0.76)
	p	0.18	0.45	0.13	0.58	0.09*
PPB	% Δ/IQR	-8.89 (-19.14, 2.65)	1.10 (-1.25, 3.44)	-1.72 (-5.87, 2.44)	0.76 (-2.9, 4.41)	-6.08 (-12.38, 0.23)
	p	0.13	0.36	0.42	0.69	0.06*

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

Beta coefficients are transformed into percent change of hormone in an IQR change in the exposure. Beta coefficients and their 95% CI are displayed. [a] represents a significant interaction term between the exposure*visit in LMM models ($p<0.05$). *p values < 0.1. **p values < 0.05. [b] Dichotomous variable.

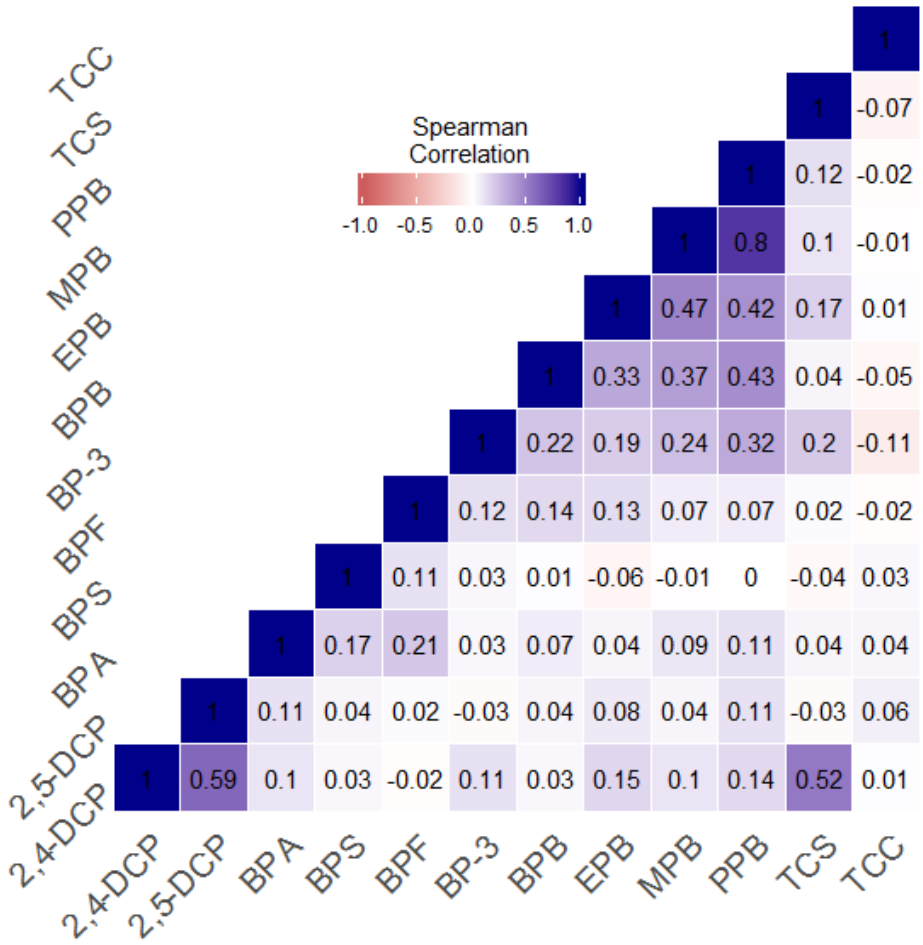
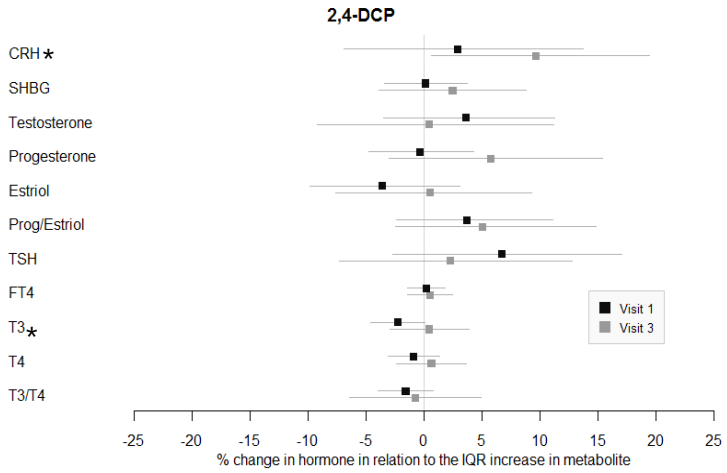
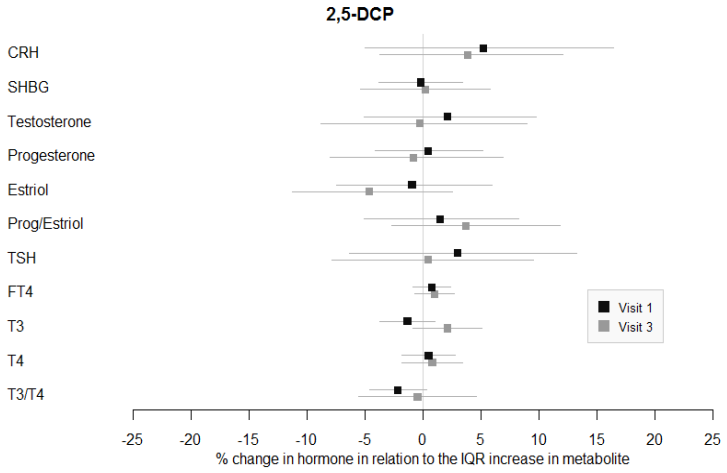
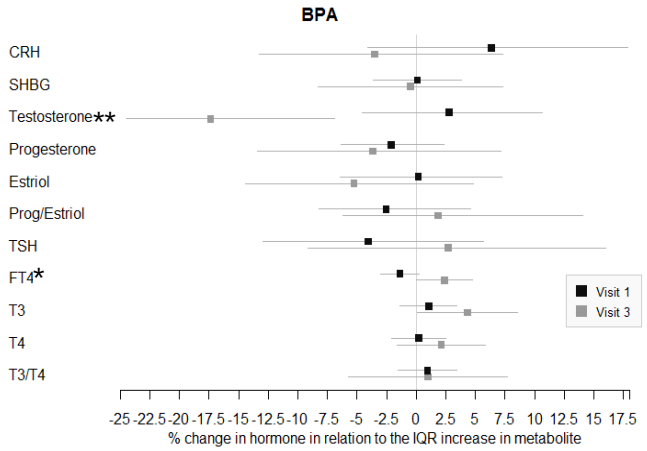
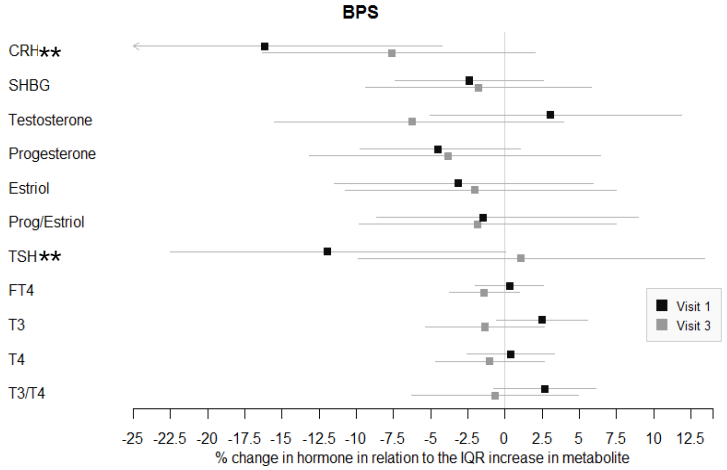


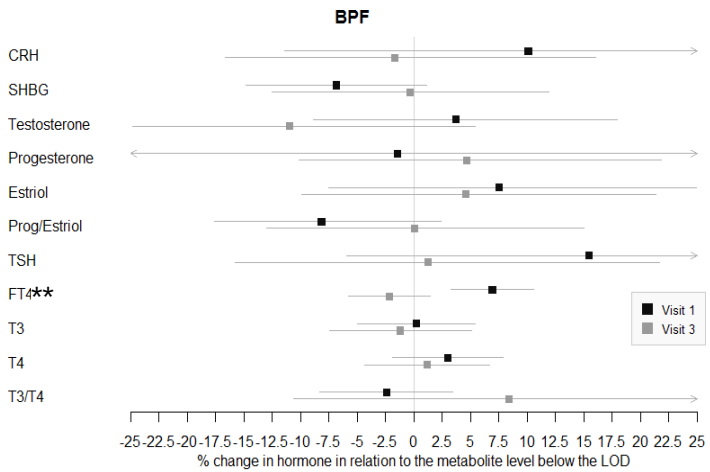
Figure III.1: Heat map of Spearman correlations between unlogged urinary triclocarban, phenols and parabens. Biomarkers concentrations were adjusted for urinary dilution.
2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

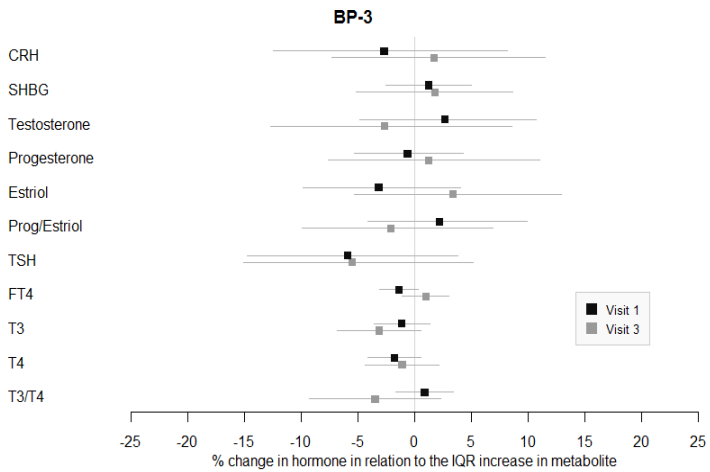

2,4-DCP
CRH*
SHBG
Testosterone
Progesterone
Estriol
Prog/Estriol
TSH
FT4
T3*
T4
T3/T4
Visit 1
Visit 3
-25 -20 -15 -10 -5 0 5 10 15 20 25
% change in hormone in relation to the IQR increase in metabolite

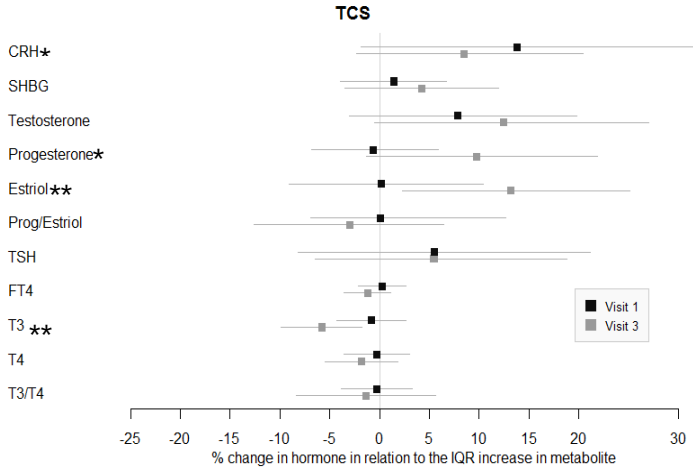

2,5-DCP
CRH
SHBG
Testosterone
Progesterone
Estriol
Prog/Estriol
TSH
FT4
T3
T4
T3/T4
Visit 1
Visit 3
-25 -20 -15 -10 -5 0 5 10 15 20 25
% change in hormone in relation to the IQR increase in metabolite

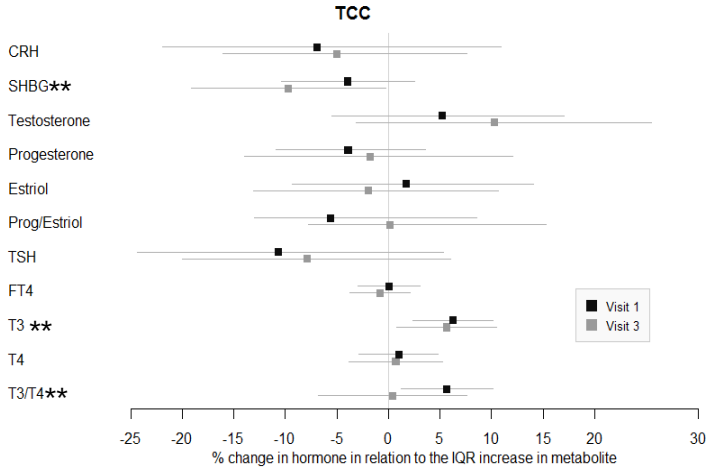

BPA
CRH
SHBG
Testosterone**
Progesterone
Estriol
Prog/Estriol
TSH
FT4*
T3
T4
T3/T4
Visit 1
Visit 3
-25 -22.5 -20 -17.5 -15 -12.5 -10 -7.5 -5 -2.5 0 2.5 5 7.5 10 12.5 15 17.5
% change in hormone in relation to the IQR increase in metabolite


BPS
CRH**
SHBG
Testosterone
Progesterone
Estriol
Prog/Estriol
TSH**
FT4
T3
T4
T3/T4
Visit 1
Visit 3
-25 -22.5 -20 -17.5 -15 -12.5 -10 -7.5 -5 -2.5 0 2.5 5 7.5 10 12.5
% change in hormone in relation to the IQR increase in metabolite


BPF
CRH
SHBG
Testosterone
Progesterone
Estriol
Prog/Estriol
TSH
FT4**
T3
T4
T3/T4
Visit 1
Visit 3
-25 -22.5 -20 -17.5 -15 -12.5 -10 -7.5 -5 -2.5 0 2.5 5 7.5 10 12.5 15 17.5 20 22.5 25
% change in hormone in relation to the metabolite level below the LOD


BP-3
CRH
SHBG
Testosterone
Progesterone
Estriol
Prog/Estriol
TSH
FT4
T3
T4
T3/T4
Visit 1
Visit 3
-25 -20 -15 -10 -5 0 5 10 15 20 25
% change in hormone in relation to the IQR increase in metabolite


TCS
CRH*
SHBG
Testosterone
Progesterone*
Estriol**
Prog/Estriol
TSH
FT4
T3 **
T4
T3/T4
Visit 1
Visit 3
-25 -20 -15 -10 -5 0 5 10 15 20 30
% change in hormone in relation to the IQR increase in metabolite


TCC
CRH
SHBG**
Testosterone
Progesterone
Estriol
Prog/Estriol
TSH
FT4
T3 **
T4
T3/T4**
Visit 1
Visit 3
-25 -20 -15 -10 -5 0 5 10 15 20 30
% change in hormone in relation to the IQR increase in metabolite

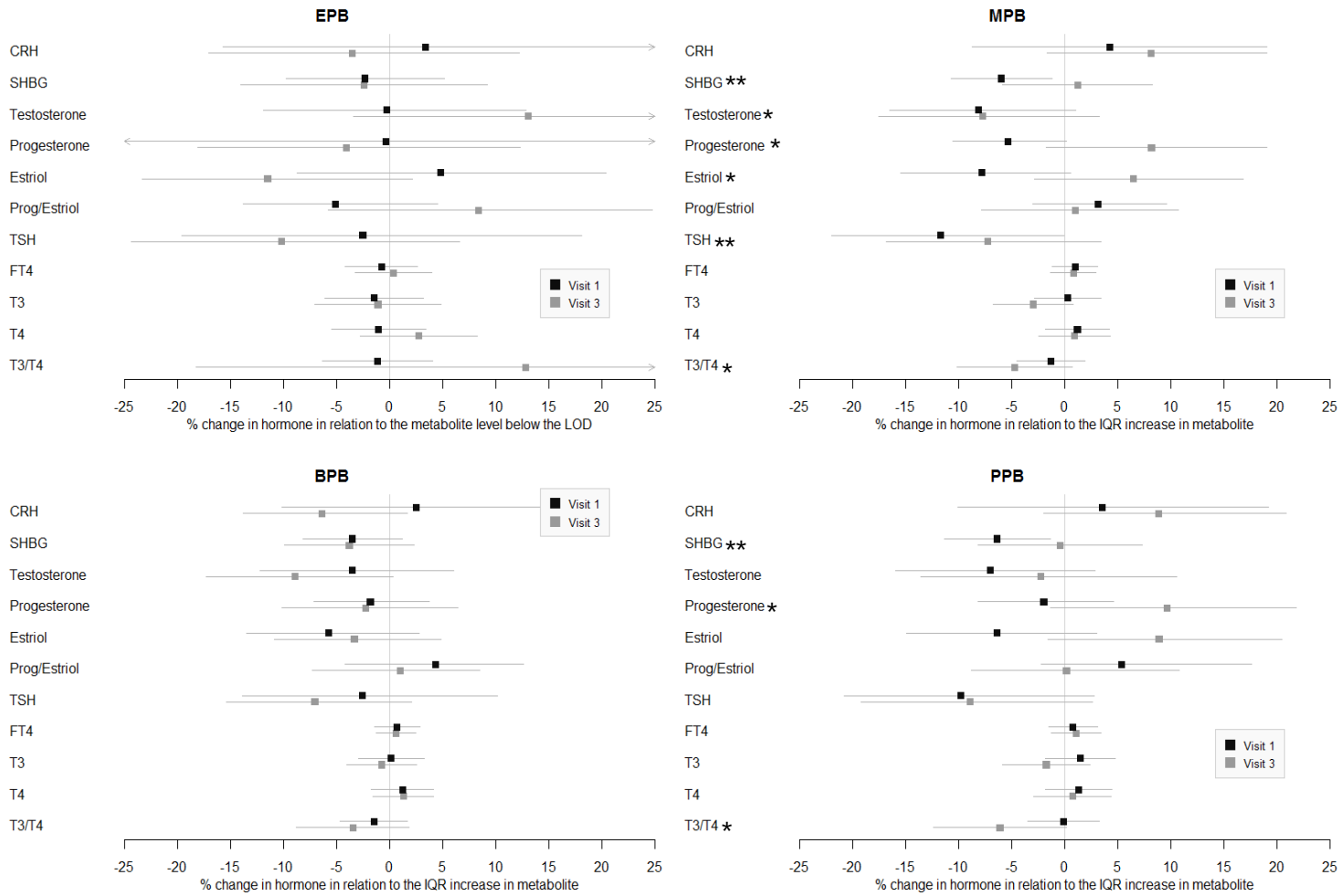


Figure III.2: Adjusted multiple linear regressions of hormones versus urinary concentrations of biomarkers stratified by study visit. Visit 1: 16-20 weeks; Visit 3: 24-28 weeks. EPB and BPF are categorical variables. * represents at least one marginal association between the urinary concentration and the hormone across the four time points. ** represents at least one significant association between the urinary biomarker concentration and the hormone across the four time points. BPF and EPB were dichotomous variables. 2,4-DCP: 2,4-dichlorophenol; 2,5-DCP:

2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

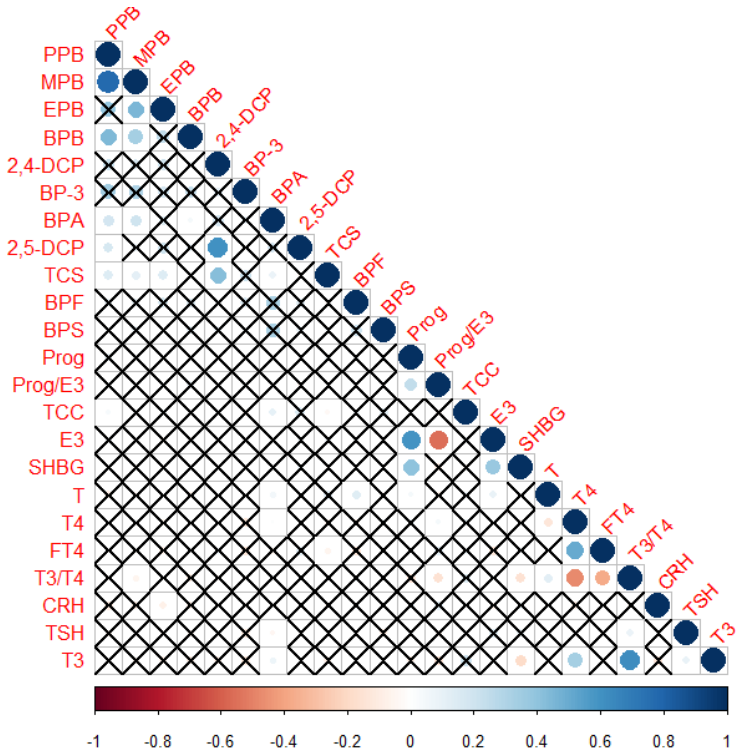


Figure III.3: Spearman correlation heat map of average phenols, parabens, triclocarban and hormones. All biomarkers are arranged by first principle component order. Color and size of circle depicts strength of association, correlations with p values > 0.05 displayed with X. BPF and EPB were dichotomous variables. 2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

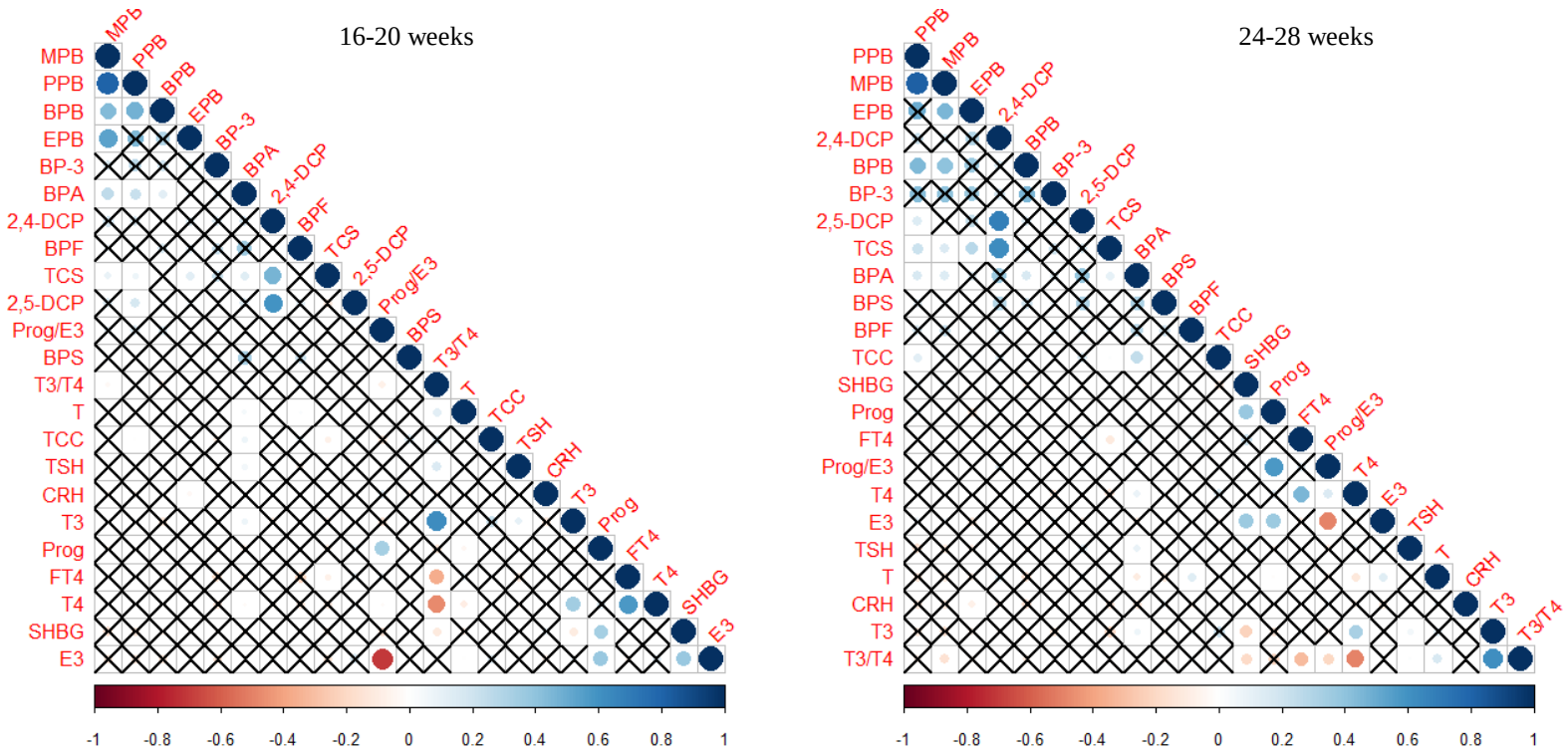
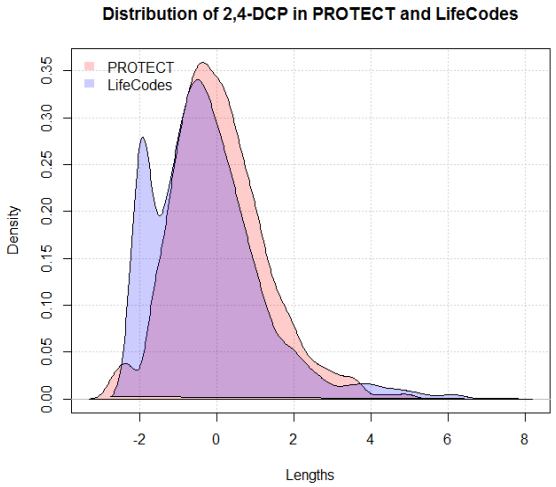
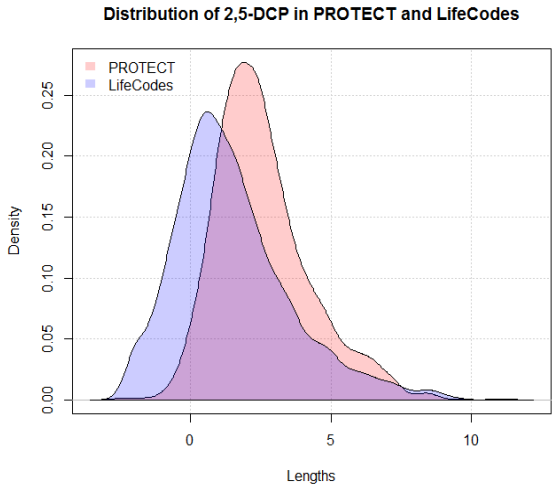
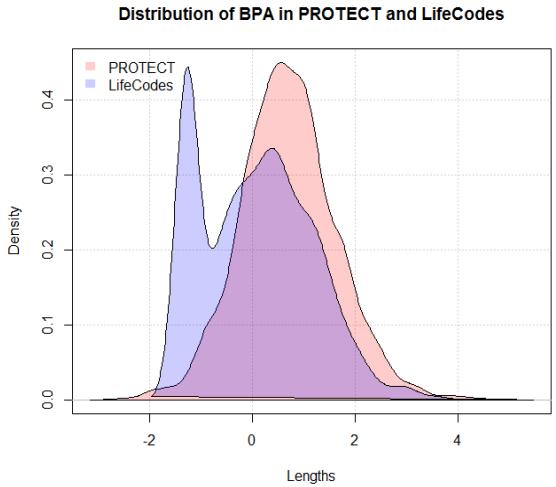
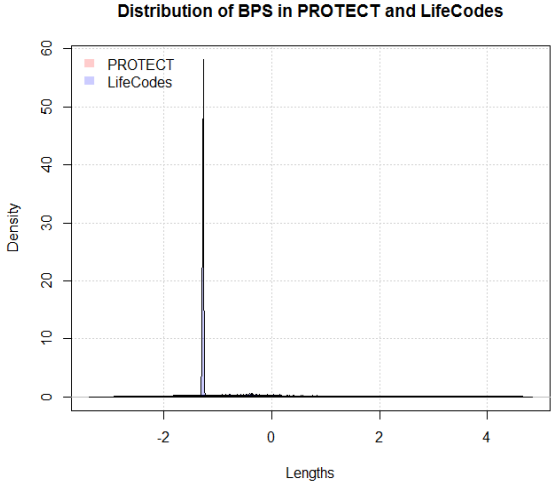


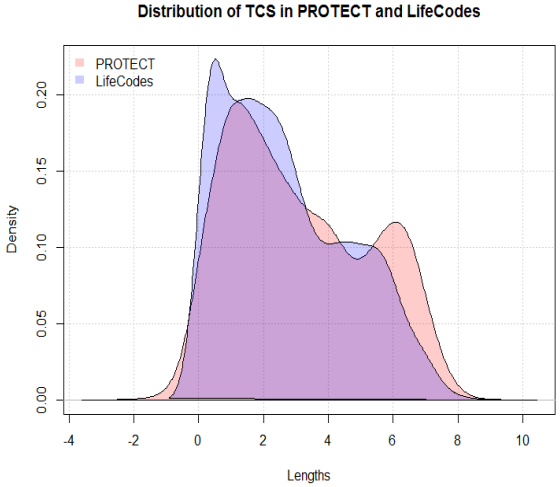
Figure III.4: Spearman correlation heat map of phenols, parabens, triclocarban and hormones at 16-20 weeks and 24-28 weeks gestation. All biomarkers are arranged by first principle component order. Color and size of circle depicts strength of association, correlations with p values > 0.05 displayed with X. BPF and EPB were dichotomous variables. 2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

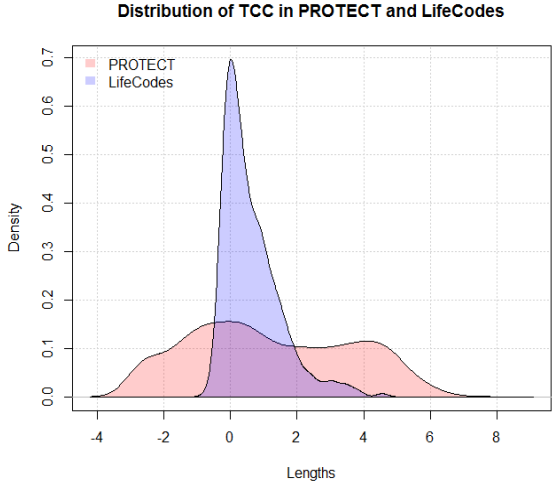

Distribution of 2,4-DCP in PROTECT and LifeCodes
PROTECT
LifeCodes
Density
Lengths


Distribution of 2,5-DCP in PROTECT and LifeCodes
PROTECT
LifeCodes
Density
Lengths


Distribution of BPA in PROTECT and LifeCodes
PROTECT
LifeCodes
Density
Lengths


Distribution of BPS in PROTECT and LifeCodes
PROTECT
LifeCodes
Density
Lengths


Distribution of TCS in PROTECT and LifeCodes
PROTECT
LifeCodes
Density
Lengths


Distribution of TCC in PROTECT and LifeCodes
PROTECT
LifeCodes
Density
Lengths

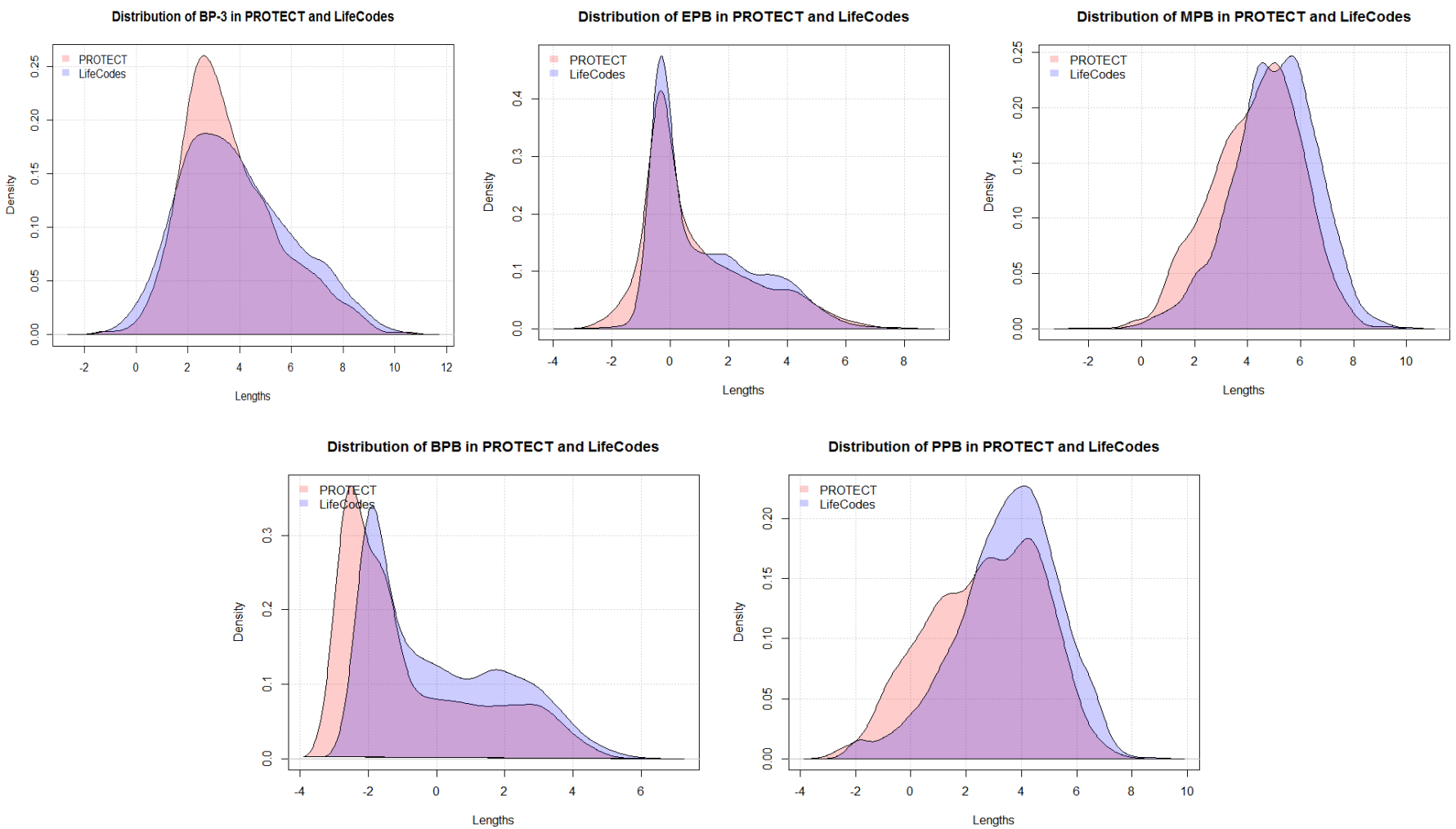


Figure III.5: Distribution of specific-gravity corrected logged exposure biomarkers comparing densities across PROTECT and LifeCodes cohorts. Note: The density plot for BPS in LifeCodes was not clear due to the large percentage of BPS concentrations below the LOD. 2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethylparaben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

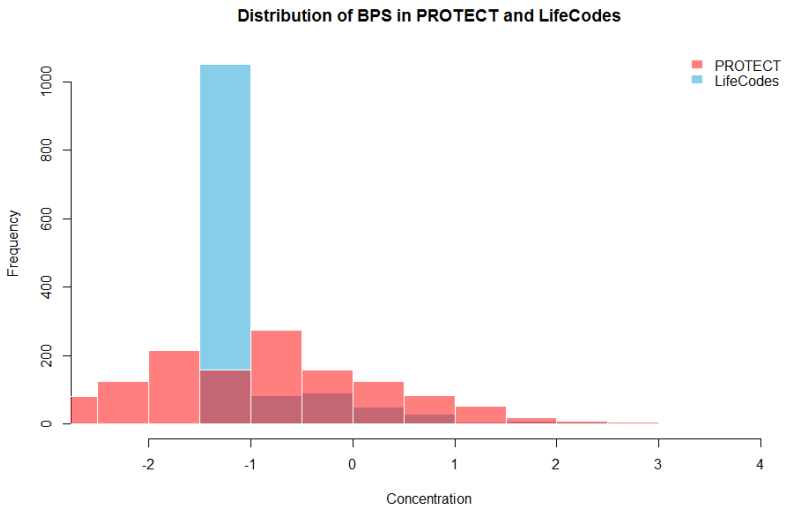


Figure III.6: Distribution of specific-gravity corrected logged BPS across PROTECT and LifeCodes cohorts. BPS: Bisphenol-S

Table III.6: Result comparison between the common exposure biomarkers and hormones from the preliminary analyses previously published* and the current analysis. Blue box shows similar associations; red boxes shows opposite directions of associations.

	Prog		SHBG		FT4		TSH	
	Prelim	**New**	**Prelim**	**New**	**Prelim**	**New**	**Prelim**	**New**
2,4-DCP								
2,5-DCP			1↓ 3↑					
BPA					↑	3↑		
BP-3			3↑					
TCS		(3↑)						
MPB			↑	1↓	↑			1↓
BPB				↓	↑			
PPB		(3↑)		1↓				

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

***** Previous analysis: Aker AM, Watkins DJ, Johns LE, Ferguson KK, Soldin OP, Anzalota Del Toro LV, et al. Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women. Environ Res. 2016;151:30–7.

Numbers in cell refer to a significant association observed in stated study visit only. Associations in brackets refer to suggestive associations with p values between 0.05 and 0.10.

Table III.7: Result comparison between the common exposure biomarkers and hormones from the LifeCodes case-control analysis previously published* and the current analysis. Blue box shows similar associations; red boxes shows opposite directions of associations.

	FT4		T4		T3		TSH	
	LifeCodes	**PROTECT**	**LifeCodes**	**PROTECT**	**LifeCodes**	**PROTECT**	**LifeCodes**	**PROTECT**
2,4-DCP					3↓	(1↓)	(3↑)	

2,5-DCP					4↑	
BPA	↑	3↑		↑	↓	
BPS	1↓				(1↑)	1↓
BP-3			1-3↓	↓	2&3↑	
TCS				↓	3↓	↑
TCC				↓	↑	(↓)	↓
EPB						
MPB	(1↓)		(↑)	↑	(3↓)	1↓
BPB				3&4↓	4↑	
PPB	1&2↓					

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

***** Previous analysis: Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int. 2018;113:341–9

Numbers in cell refer to a significant association observed in stated study visit only. Associations in brackets refer to suggestive associations with p values between 0.05 and 0.10.

References

[1] E. Karpuzoglu, S.D. Holladay, R.M. Gogal, Parabens: potential impact of low-affinity estrogen receptor binding chemicals on human health, J. Toxicol. Environ. Health B Crit. Rev. 16 (2013) 321–335. doi:10.1080/10937404.2013.809252.

[2] J. Peretz, L. Vrooman, W.A. Ricke, P.A. Hunt, S. Ehrlich, R. Hauser, V. Padmanabhan, H.S. Taylor, S.H. Swan, C.A. VandeVoort, J.A. Flaws, Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013, Environ. Health Perspect. 122 (2014) 775–786. doi:10.1289/ehp.1307728.

[3] J.R. Rochester, Bisphenol A and human health: A review of the literature, Reprod. Toxicol. 42 (2013) 132–155. doi:10.1016/j.reprotox.2013.08.008.

[4] J.R. Rochester, A.L. Bolden, Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes, Environ. Health Perspect. 123 (2015) 643–650. doi:10.1289/ehp.1408989.

[5] R. Tang, M.-J. Chen, G.-D. Ding, X.-J. Chen, X.-M. Han, K. Zhou, L.-M. Chen, Y.-K. Xia, Y. Tian, X.-R. Wang, Associations of prenatal exposure to phenols with birth outcomes, Environ. Pollut. Barking Essex 1987. 178 (2013) 115–120. doi:10.1016/j.envpol.2013.03.023.

[6] T.H. Lassen, H. Frederiksen, H.B. Kyhl, S.H. Swan, K.M. Main, A.-M. Andersson, D.V. Lind, S. Husby, C. Wohlfahrt-Veje, N.E. Skakkebæk, T.K. Jensen, Prenatal Triclosan Exposure and Anthropometric Measures including Anogenital Distance in Danish Infants, Environ. Health Perspect. (2016). doi:10.1289/ehp.1409637.

[7] C. Vernet, I. Pin, L. Giorgis-Allemand, C. Philippat, M. Benmerad, J. Quentin, A.M. Calafat, X. Ye, I. Annesi-Maesano, V. Siroux, R. Slama, In Utero Exposure to Select Phenols and Phthalates and Respiratory Health in Five-Year-Old Boys: A Prospective Study, Environ. Health Perspect. 125 (2017). doi:10.1289/EHP1015.

[8] D.S. Gutiérrez-Torres, A. Barraza-Villarreal, L. Hernandez-Cadena, C. Escamilla-Nuñez, I. Romieu, Prenatal Exposure to Endocrine Disruptors and Cardiometabolic Risk in Preschoolers: A Systematic Review Based on Cohort Studies, Ann. Glob. Health. 84 (2018) 239–249. doi:10.29024/aogh.911.

[9] Centers for Disease Control and Prevention, Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2017, Centers for Disease Control and Prevention (CDC), Atlanta, GA, 2017. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2017.pdf (accessed May 9, 2017).

[10] J. Boberg, C. Taxvig, S. Christiansen, U. Hass, Possible endocrine disrupting effects of parabens and their metabolites, Reprod. Toxicol. Elmsford N. 30 (2010) 301–312. doi:10.1016/j.reprotox.2010.03.011.

[11] R.E. Dodson, M. Nishioka, L.J. Standley, L.J. Perovich, J.G. Brody, R.A. Rudel, Endocrine disruptors and asthma-associated chemicals in consumer products, Environ. Health Perspect. 120 (2012) 935–943. doi:10.1289/ehp.1104052.

[12] V.D. Castracane, Endocrinology of preterm labor, Clin. Obstet. Gynecol. 43 (2000) 717–726.

[13] R. Smith, J.I. Smith, X. Shen, P.J. Engel, M.E. Bowman, S.A. McGrath, A.M. Bisits, P. McElduff, W.B. Giles, D.W. Smith, Patterns of plasma corticotropin-releasing hormone,

progesterone, estradiol, and estriol change and the onset of human labor, J. Clin. Endocrinol. Metab. 94 (2009) 2066–2074. doi:10.1210/jc.2008-2257.
[14] D. Glinoer, The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology, Endocr. Rev. 18 (1997) 404–433. doi:10.1210/edrv.18.3.0300.
[15] R. Smith, J. Paul, K. Maiti, J. Tolosa, G. Madsen, Recent advances in understanding the endocrinology of human birth, Trends Endocrinol. Metab. 23 (2012) 516–523. doi:10.1016/j.tem.2012.05.002.
[16] T.T. Schug, A.M. Blawas, K. Gray, J.J. Heindel, C.P. Lawler, Elucidating the Links between Endocrine Disruptors and Neurodevelopment, Endocrinology. (2015) en20141734. doi:10.1210/en.2014-1734.
[17] T.T. Schug, A. Janesick, B. Blumberg, J.J. Heindel, Endocrine Disrupting Chemicals and Disease Susceptibility, J. Steroid Biochem. Mol. Biol. 127 (2011) 204–215. doi:10.1016/j.jsbmb.2011.08.007.
[18] Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, Centers for Disease Control and Prevention (CDC), 2015.
[19] M. Krause, A. Klit, M. Blomberg Jensen, T. Søeborg, H. Frederiksen, M. Schlumpf, W. Lichtensteiger, N.E. Skakkebaek, K.T. Drzewiecki, Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters, Int. J. Androl. 35 (2012) 424–436. doi:10.1111/j.1365-2605.2012.01280.x.
[20] P.D. Darbre, P.W. Harvey, Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks, J. Appl. Toxicol. JAT. 28 (2008) 561–578. doi:10.1002/jat.1358.
[21] C.-F. Wang, Y. Tian, Reproductive endocrine-disrupting effects of triclosan: Population exposure, present evidence and potential mechanisms, Environ. Pollut. Barking Essex 1987. 206 (2015) 195–201. doi:10.1016/j.envpol.2015.07.001.
[22] M. Giulivo, M. Lopez de Alda, E. Capri, D. Barceló, Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review, Environ. Res. 151 (2016) 251–264. doi:10.1016/j.envres.2016.07.011.
[23] G.C. Di Renzo, J.A. Conry, J. Blake, M.S. DeFrancesco, N. DeNicola, J.N. Martin, K.A. McCue, D. Richmond, A. Shah, P. Sutton, T.J. Woodruff, S.Z. van der Poel, L.C. Giudice, International Federation of Gynecology and Obstetrics opinion on reproductive health impacts of exposure to toxic environmental chemicals, Int. J. Gynecol. Obstet. 131 (2015) 219–225. doi:10.1016/j.ijgo.2015.09.002.
[24] M. Mallozzi, G. Bordi, C. Garo, D. Caserta, The effect of maternal exposure to endocrine disrupting chemicals on fetal and neonatal development: A review on the major concerns, Birth Defects Res. Part C Embryo Today Rev. 108 (2016) 224–242. doi:10.1002/bdrc.21137.
[25] S. Rattan, C. Zhou, C. Chiang, S. Mahalingam, E. Brehm, J. Flaws, Exposure to endocrine disruptors during adulthood: consequences for female fertility, J. Endocrinol. (2017). doi:10.1530/JOE-17-0023.
[26] J.M. Braun, Early-life exposure to EDCs: role in childhood obesity and neurodevelopment, Nat. Rev. Endocrinol. 13 (2017) 161–173. doi:10.1038/nrendo.2016.186.

[27] A.M. Aker, D.J. Watkins, L.E. Johns, K.K. Ferguson, O.P. Soldin, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women, Environ. Res. 151 (2016) 30–37. doi:10.1016/j.envres.2016.07.002.
[28] D.E. Cantonwine, J.F. Cordero, L.O. Rivera-González, L.V. Anzalota Del Toro, K.K. Ferguson, B. Mukherjee, A.M. Calafat, N. Crespo, B. Jiménez-Vélez, I.Y. Padilla, A.N. Alshawabkeh, J.D. Meeker, Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: Distribution, temporal variability, and predictors, Environ. Int. 62 (2014) 1–11. doi:10.1016/j.envint.2013.09.014.
[29] J.D. Meeker, D.E. Cantonwine, L.O. Rivera-González, K.K. Ferguson, B. Mukherjee, A.M. Calafat, X. Ye, L.V. Anzalota Del Toro, N. Crespo-Hernández, B. Jiménez-Vélez, A.N. Alshawabkeh, J.F. Cordero, Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico, Environ. Sci. Technol. 47 (2013) 3439–3447. doi:10.1021/es400510g.
[30] X. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 383 (2005) 638–644. doi:10.1007/s00216-005-0019-4.
[31] X. Ye, A.M. Bishop, J.A. Reidy, L.L. Needham, A.M. Calafat, Parabens as Urinary Biomarkers of Exposure in Humans, Environ. Health Perspect. 114 (2006) 1843–1846. doi:10.1289/ehp.9413.
[32] D.J. Watkins, K.K. Ferguson, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico, Int. J. Hyg. Environ. Health. 218 (2015) 212–219. doi:10.1016/j.ijheh.2014.11.001.
[33] R.W. Hornung, L.D. Reed, Estimation of Average Concentration in the Presence of Nondetectable Values, Appl. Occup. Environ. Hyg. 5 (1990) 46–51. doi:10.1080/1047322X.1990.10389587.
[34] J.W. Dietrich, G. Landgrafe, E.H. Fotiadou, TSH and Thyrotropic Agonists: Key Actors in Thyroid Homeostasis, J. Thyroid Res. (2012). doi:10.1155/2012/351864.
[35] R. Romero, B. Scoccia, M. Mazor, Y.K. Wu, R. Benveniste, Evidence for a local change in the progesterone/estrogen ratio in human parturition at term, Am. J. Obstet. Gynecol. 159 (1988) 657–660.
[36] R.J. Ruiz, G.R. Saade, C.E.L. Brown, C. Nelson-Becker, A. Tan, S. Bishop, R. Bukowski, The effect of acculturation on progesterone/estriol ratios and preterm birth in Hispanics, Obstet. Gynecol. 111 (2008) 309–316. doi:10.1097/01.AOG.0000297896.00491.2c.
[37] K.M. O'Brien, K. Upson, N.R. Cook, C.R. Weinberg, Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment, Environ. Health Perspect. 124 (2016) 220–227. doi:10.1289/ehp.1509693.
[38] C.V. Sartain, P.A. Hunt, An old culprit but a new story: bisphenol A and "NextGen" bisphenols, Fertil. Steril. 106 (2016) 820–826. doi:10.1016/j.fertnstert.2016.07.1114.
[39] A.M. Aker, D.J. Watkins, L.E. Johns, K.K. Ferguson, O.P. Soldin, L.V.A. Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Phenols and Parabens in relation to

Reproductive and Thyroid Hormones in Pregnant Women, Environ. Res. 151 (2016) 30–37. doi:10.1016/j.envres.2016.07.002.
[40] A.M. Aker, L. Johns, T.F. McElrath, D.E. Cantonwine, B. Mukherjee, J.D. Meeker, Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study, Environ. Int. 113 (2018) 341–349. doi:10.1016/j.envint.2018.01.006.
[41] J. Chevrier, R.B. Gunier, A. Bradman, N.T. Holland, A.M. Calafat, B. Eskenazi, K.G. Harley, Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study, Environ. Health Perspect. 121 (2013) 138–144. doi:10.1289/ehp.1205092.
[42] M.E. Romano, G.M. Webster, A.M. Vuong, R. Thomas Zoeller, A. Chen, A.N. Hoofnagle, A.M. Calafat, M.R. Karagas, K. Yolton, B.P. Lanphear, J.M. Braun, Gestational urinary bisphenol A and maternal and newborn thyroid hormone concentrations: the HOME Study, Environ. Res. 138 (2015) 453–460. doi:10.1016/j.envres.2015.03.003.
[43] M.T. Aung, L.E. Johns, K.K. Ferguson, B. Mukherjee, T.F. McElrath, J.D. Meeker, Thyroid hormone parameters during pregnancy in relation to urinary bisphenol A concentrations: A repeated measures study, Environ. Int. 104 (2017) 33–40. doi:10.1016/j.envint.2017.04.001.
[44] C. Park, W. Choi, M. Hwang, Y. Lee, S. Kim, S. Yu, I. Lee, D. Paek, K. Choi, Associations between urinary phthalate metabolites and bisphenol A levels, and serum thyroid hormones among the Korean adult population - Korean National Environmental Health Survey (KoNEHS) 2012-2014, Sci. Total Environ. 584–585 (2017) 950–957. doi:10.1016/j.scitotenv.2017.01.144.
[45] C. van de Beek, J.H.H. Thijssen, P.T. Cohen-Kettenis, S.H.M. van Goozen, J.K. Buitelaar, Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: what is the best source of information to investigate the effects of fetal hormonal exposure?, Horm. Behav. 46 (2004) 663–669. doi:10.1016/j.yhbeh.2004.06.010.
[46] G.D. Gonçalves, S.C. Semprebon, B.I. Biazi, M.S. Mantovani, G.S.A. Fernandes, Bisphenol A reduces testosterone production in TM3 Leydig cells independently of its effects on cell death and mitochondrial membrane potential, Reprod. Toxicol. Elmsford N. 76 (2017) 26–34. doi:10.1016/j.reprotox.2017.12.002.
[47] S. Mahalingam, L. Ther, L. Gao, W. Wang, A. Ziv-Gal, J.A. Flaws, The effects of in utero bisphenol A exposure on ovarian follicle numbers and steroidogenesis in the F1 and F2 generations of mice, Reprod. Toxicol. Elmsford N. 74 (2017) 150–157. doi:10.1016/j.reprotox.2017.09.013.
[48] K.K. Ferguson, K.E. Peterson, J.M. Lee, A. Mercado-García, C. Blank-Goldenberg, M.M. Téllez-Rojo, J.D. Meeker, Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys, Reprod. Toxicol. Elmsford N. 47 (2014) 70–76. doi:10.1016/j.reprotox.2014.06.002.
[49] S. Makieva, P.T.K. Saunders, J.E. Norman, Androgens in pregnancy: roles in parturition, Hum. Reprod. Update. 20 (2014) 542–559. doi:10.1093/humupd/dmu008.
[50] A.M. Aker, K.K. Ferguson, Z.Y. Rosario, B. Mukherjee, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, The associations between prenatal exposure to triclocarban,

phenols and parabens with gestational age and birth weight in northern Puerto Rico, Environ. Res. 169 (2018) 41–51. doi:10.1016/j.envres.2018.10.030.
[51] M. Hines, S. Golombok, J. Rust, K.J. Johnston, J. Golding, T.A.L.S. of P. and C.S. Team, Testosterone during Pregnancy and Gender Role Behavior of Preschool Children: A Longitudinal, Population Study, Child Dev. 73 (2002) 1678–1687.
[52] M. Grino, G.P. Chrousos, A.N. Margioris, The corticotropin releasing hormone gene is expressed in human placenta, Biochem. Biophys. Res. Commun. 148 (1987) 1208–1214.
[53] A. Makrigiannakis, E. Zoumakis, S. Kalantaridou, C. Coutifaris, A.N. Margioris, G. Coukos, K.C. Rice, A. Gravanis, G.P. Chrousos, Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance, Nat. Immunol. 2 (2001) 1018–1024. doi:10.1038/ni719.
[54] M. Nezi, G. Mastorakos, Z. Mouslech, Corticotropin Releasing Hormone And The Immune/Inflammatory Response, in: L.J. De Groot, G. Chrousos, K. Dungan, K.R. Feingold, A. Grossman, J.M. Hershman, C. Koch, M. Korbonits, R. McLachlan, M. New, J. Purnell, R. Rebar, F. Singer, A. Vinik (Eds.), Endotext, MDText.com, Inc., South Dartmouth (MA), 2000. http://www.ncbi.nlm.nih.gov/books/NBK279017/.
[55] S.N. Kalantaridou, E. Zoumakis, A. Makrigiannakis, H. Godoy, G.P. Chrousos, The role of corticotropin-releasing hormone in blastocyst implantation and early fetal immunotolerance, Horm. Metab. Res. Horm. Stoffwechselforschung Horm. Metab. 39 (2007) 474–477. doi:10.1055/s-2007-980190.
[56] null McLean, null Smith, Corticotropin-releasing Hormone in Human Pregnancy and Parturition, Trends Endocrinol. Metab. TEM. 10 (1999) 174–178.
[57] B. Madhappan, D. Kempuraj, S. Christodoulou, S. Tsapikidis, W. Boucher, V. Karagiannis, A. Athanassiou, T.C. Theoharides, High Levels of Intrauterine Corticotropin-Releasing Hormone, Urocortin, Tryptase, and Interleukin-8 in Spontaneous Abortions, Endocrinology. 144 (2003) 2285–2290. doi:10.1210/en.2003-0063.
[58] P.C. Arck, M. Rücke, M. Rose, J. Szekeres-Bartho, A.J. Douglas, M. Pritsch, S.M. Blois, M.K. Pincus, N. Bärenstrauch, J.W. Dudenhausen, K. Nakamura, S. Sheps, B.F. Klapp, Early risk factors for miscarriage: a prospective cohort study in pregnant women, Reprod. Biomed. Online. 17 (2008) 101–113. doi:10.1016/S1472-6483(10)60300-8.
[59] F. Petraglia, A. Imperatore, J.R.G. Challis, Neuroendocrine Mechanisms in Pregnancy and Parturition, Endocr. Rev. 31 (2010) 783–816. doi:10.1210/er.2009-0019.
[60] X. Ni, Y. Hou, B.R. King, X. Tang, M.A. Read, R. Smith, R.C. Nicholson, Estrogen receptor-mediated down-regulation of corticotropin-releasing hormone gene expression is dependent on a cyclic adenosine 3’,5’-monophosphate regulatory element in human placental syncytiotrophoblast cells, J. Clin. Endocrinol. Metab. 89 (2004) 2312–2318. doi:10.1210/jc.2003-030948.
[61] D. Caserta, N. Di Segni, M. Mallozzi, V. Giovanale, A. Mantovani, R. Marci, M. Moscarini, Bisphenol a and the female reproductive tract: an overview of recent laboratory evidence and epidemiological studies, Reprod. Biol. Endocrinol. 12 (2014) 37. doi:10.1186/1477-7827-12-37.
[62] W. Tan, H. Huang, Y. Wang, T.Y. Wong, C.C. Wang, L.K. Leung, Bisphenol A differentially activates protein kinase C isoforms in murine placental tissue, Toxicol. Appl. Pharmacol. 269 (2013) 163–168. doi:10.1016/j.taap.2013.03.016.

[63] C. Rajakumar, H. Guan, D. Langlois, M. Cernea, K. Yang, Bisphenol A disrupts gene expression in human placental trophoblast cells, Reprod. Toxicol. Elmsford N. 53 (2015) 39–44. doi:10.1016/j.reprotox.2015.03.001.
[64] R. Smith, R.C. Nicholson, Corticotrophin releasing hormone and the timing of birth, Front. Biosci. J. Virtual Libr. 12 (2007) 912–918.
[65] J.A. Ruszkiewicz, S. Li, M.B. Rodriguez, M. Aschner, Is Triclosan a neurotoxic agent?, J. Toxicol. Environ. Health B Crit. Rev. 20 (2017) 104–117. doi:10.1080/10937404.2017.1281181.
[66] P.E.A. Rodríguez, M.S. Sanchez, Maternal exposure to triclosan impairs thyroid homeostasis and female pubertal development in Wistar rat offspring, J. Toxicol. Environ. Health A. 73 (2010) 1678–1688. doi:10.1080/15287394.2010.516241.
[67] X. Wang, X. Chen, X. Feng, F. Chang, M. Chen, Y. Xia, L. Chen, Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice, Sci. Rep. 5 (2015) 18252. doi:10.1038/srep18252.
[68] X. Cao, X. Hua, X. Wang, L. Chen, Exposure of pregnant mice to triclosan impairs placental development and nutrient transport, Sci. Rep. 7 (2017) 44803. doi:10.1038/srep44803.
[69] M. Axelstad, J. Boberg, A.M. Vinggaard, S. Christiansen, U. Hass, Triclosan exposure reduces thyroxine levels in pregnant and lactating rat dams and in directly exposed offspring, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 59 (2013) 534–540. doi:10.1016/j.fct.2013.06.050.
[70] K.M. Crofton, K.B. Paul, M.J. Devito, J.M. Hedge, Short-term in vivo exposure to the water contaminant triclosan: Evidence for disruption of thyroxine, Environ. Toxicol. Pharmacol. 24 (2007) 194–197. doi:10.1016/j.etap.2007.04.008.
[71] P.I. Johnson, E. Koustas, H.M. Vesterinen, P. Sutton, D.S. Atchley, A.N. Kim, M. Campbell, J.M. Donald, S. Sen, L. Bero, L. Zeise, T.J. Woodruff, Application of the Navigation Guide systematic review methodology to the evidence for developmental and reproductive toxicity of triclosan, Environ. Int. 92–93 (2016) 716–728. doi:10.1016/j.envint.2016.03.009.
[72] G.W. Louis, D.R. Hallinger, M.J. Braxton, A. Kamel, T.E. Stoker, Effects of chronic exposure to triclosan on reproductive and thyroid endpoints in the adult Wistar female rat, J. Toxicol. Environ. Health A. 80 (2017) 236–249. doi:10.1080/15287394.2017.1287029.
[73] K.B. Paul, J.T. Thompson, S.O. Simmons, J.P. Vanden Heuvel, K.M. Crofton, Evidence for triclosan-induced activation of human and rodent xenobiotic nuclear receptors, Toxicol. In Vitro. 27 (2013) 2049–2060. doi:10.1016/j.tiv.2013.07.008.
[74] K.B. Paul, J.M. Hedge, M.J. Devito, K.M. Crofton, Developmental triclosan exposure decreases maternal and neonatal thyroxine in rats, Environ. Toxicol. Chem. 29 (2010) 2840–2844. doi:10.1002/etc.339.
[75] K.B. Paul, J.M. Hedge, M.J. DeVito, K.M. Crofton, Short-term exposure to triclosan decreases thyroxine in vivo via upregulation of hepatic catabolism in Young Long-Evans rats, Toxicol. Sci. Off. J. Soc. Toxicol. 113 (2010) 367–379. doi:10.1093/toxsci/kfp271.
[76] L.M. Zorrilla, E.K. Gibson, S.C. Jeffay, K.M. Crofton, W.R. Setzer, R.L. Cooper, T.E. Stoker, The effects of triclosan on puberty and thyroid hormones in male Wistar rats, Toxicol. Sci. Off. J. Soc. Toxicol. 107 (2009) 56–64. doi:10.1093/toxsci/kfn225.

[77] J.M. Braun, A. Chen, A. Hoofnagle, G.D. Papandonatos, M. Jackson-Browne, R. Hauser, M.E. Romano, M.R. Karagas, K. Yolton, R. Thomas Zoeller, B.P. Lanphear, Associations of early life urinary triclosan concentrations with maternal, neonatal, and child thyroid hormone levels, Horm. Behav. 101 (2018) 77–84. doi:10.1016/j.yhbeh.2017.11.009.

[78] M.S. Jackson-Browne, G.D. Papandonatos, A. Chen, A.M. Calafat, K. Yolton, B.P. Lanphear, J.M. Braun, Identifying Vulnerable Periods of Neurotoxicity to Triclosan Exposure in Children, Environ. Health Perspect. 126 (2018) 057001. doi:10.1289/EHP2777.

[79] E.S. Koeppe, K.K. Ferguson, J.A. Colacino, J.D. Meeker, Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007-2008, Sci. Total Environ. 445–446 (2013) 299–305. doi:10.1016/j.scitotenv.2012.12.052.

[80] Y. Feng, P. Zhang, Z. Zhang, J. Shi, Z. Jiao, B. Shao, Endocrine Disrupting Effects of Triclosan on the Placenta in Pregnant Rats, PloS One. 11 (2016) e0154758. doi:10.1371/journal.pone.0154758.

[81] X. Wang, F. Ouyang, L. Feng, X. Wang, Z. Liu, J. Zhang, Maternal Urinary Triclosan Concentration in Relation to Maternal and Neonatal Thyroid Hormone Levels: A Prospective Study, Environ. Health Perspect. 125 (2017) 067017. doi:10.1289/EHP500.

[82] R.J. Witorsch, Critical analysis of endocrine disruptive activity of triclosan and its relevance to human exposure through the use of personal care products, Crit. Rev. Toxicol. 44 (2014) 535–555. doi:10.3109/10408444.2014.910754.

[83] S.M. Carlsen, G. Jacobsen, P. Romundstad, Maternal testosterone levels during pregnancy are associated with offspring size at birth, Eur. J. Endocrinol. 155 (2006) 365–370. doi:10.1530/eje.1.02200.

[84] M. Vladeanu, O. Giuffrida, V.J. Bourne, Prenatal sex hormone exposure and risk of Alzheimer disease: a pilot study using the 2D:4D digit length ratio, Cogn. Behav. Neurol. Off. J. Soc. Behav. Cogn. Neurol. 27 (2014) 102–106. doi:10.1097/WNN.0000000000000024.

[85] P. Ashrap, D.J. Watkins, A.M. Calafat, X. Ye, Z. Rosario, P. Brown, C.M. Vélez-Vega, A. Alshawabkeh, J.F. Cordero, J.D. Meeker, Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends, Environ. Int. (2018). doi:10.1016/j.envint.2018.08.020.

[86] Y. Watanabe, H. Kojima, S. Takeuchi, N. Uramaru, S. Ohta, S. Kitamura, Comparative study on transcriptional activity of 17 parabens mediated by estrogen receptor α and β and androgen receptor, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 57 (2013) 227–234. doi:10.1016/j.fct.2013.03.036.

[87] A.M. Wróbel, E.Ł. Gregoraszczuk, Actions of methyl-, propyl- and butylparaben on estrogen receptor-α and -β and the progesterone receptor in MCF-7 cancer cells and non-cancerous MCF-10A cells, Toxicol. Lett. 230 (2014) 375–381. doi:10.1016/j.toxlet.2014.08.012.

[88] T.T.B. Vo, E.-M. Jung, K.-C. Choi, F.H. Yu, E.-B. Jeung, Estrogen receptor α is involved in the induction of Calbindin-D(9k) and progesterone receptor by parabens in GH3 cells: a biomarker gene for screening xenoestrogens, Steroids. 76 (2011) 675–681. doi:10.1016/j.steroids.2011.03.006.

[89] A.M. Aker, L. Johns, T.F. McElrath, D.E. Cantonwine, B. Mukherjee, J.D. Meeker, Associations between maternal phenol and paraben urinary biomarkers and maternal

hormones during pregnancy: A repeated measures study, Environ. Int. (n.d.). doi:10.1016/j.envint.2018.01.006.
[90] T.T.B. Vo, Y.-M. Yoo, K.-C. Choi, E.-B. Jeung, Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model, Reprod. Toxicol. Elmsford N. 29 (2010) 306–316. doi:10.1016/j.reprotox.2010.01.013.
[91] N.R. Janjua, G.K. Mortensen, A.-M. Andersson, B. Kongshoj, N.E. Skakkebaek, H.C. Wulf, Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans, Environ. Sci. Technol. 41 (2007) 5564–5570.
[92] J.D. Meeker, T. Yang, X. Ye, A.M. Calafat, R. Hauser, Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage, Environ. Health Perspect. 119 (2011) 252–257. doi:10.1289/ehp.1002238.
[93] B. Rousset, Antithyroid effect of a food or drug preservative: 4-hydroxybenzoic acid methyl ester, Experientia. 37 (1981) 177–178.
[94] E. van den Boogaard, R. Vissenberg, J.A. Land, M. van Wely, J.A.M. van der Post, M. Goddijn, P.H. Bisschop, Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review, Hum. Reprod. Update. 17 (2011) 605–619. doi:10.1093/humupd/dmr024.
[95] E. Tingi, A.A. Syed, A. Kyriacou, G. Mastorakos, A. Kyriacou, Benign thyroid disease in pregnancy: A state of the art review, J. Clin. Transl. Endocrinol. 6 (2016) 37–49. doi:10.1016/j.jcte.2016.11.001.

CHAPTER IV. Associations between Prenatal Exposure to Triclocarban, Phenols and Parabens with Gestational Age and Birth Weight in Northern Puerto Rico

Abstract

Background: Prenatal exposure to certain xenobiotics has been associated with adverse birth outcomes. We examined the associations of triclocarban, phenols and parabens in a cohort of 922 pregnant women in Puerto Rico, the Puerto Rico Testsite for Exploring Contamination Threats Program (PROTECT). Methods: Urinary triclocarban, phenols and parabens were measured at three time points in pregnancy (visit 1: 16-20 weeks, visit 2: 20-24 weeks, visit 3: 24-28 weeks gestation). Multiple linear regression (MLR) models were conducted to regress gestational length and birthweight z-scores against each woman's log average concentrations of exposure biomarkers. Logistic regression models were conducted to calculate odds of preterm birth, small or large for gestational age (SGA and LGA) in association with each of the exposure biomarkers. An interaction term between the average urinary biomarker concentration and infant sex was included in models to identify effect modification. The results were additionally stratified by study visit to look for windows of vulnerability. Results were transformed into the change in the birth outcome for an inter-quartile-range difference in biomarker concentration (Δ). Results: Average benzophenone-3, methyl- and propyl-paraben concentrations were associated with an increase in gestational length [(Δ 1.90 days; 95% CI: 0.54, 3.26); (Δ 1.63; 95% CI: 0.37, 2.89); (Δ 2.06; 95% CI: 0.63, 3.48), respectively]. Triclocarban was associated with a suggestive 2-day decrease in gestational length (Δ -1.96; 95% CI: -4.11, 0.19). Bisphenol A measured at visit 1 was associated with a suggestive increase in gestational length (Δ 1.37; 95% CI: -0.05, 2.79). Triclosan was positively associated with gestational length among males, and negatively

associated with gestational length among females. Methyl-, butyl- and propyl-paraben were associated with significant 0.50-0.66 decreased odds of SGA. BPS was associated with an increase in the odds of SGA at visit 3, and a suggestive increase in the odds of LGA at visit 1. Conclusion: Benzophenone-3, methyl-paraben and propyl-paraben were associated with an increase in gestational length. Concentrations of triclocarban, which were much higher than reported in other populations, were associated with a suggestive decrease in gestational length. The direction of the association between triclosan and gestational length differed by infant sex. Parabens were associated with a decrease in SGA, and BPS was associated with both SGA and LGA depending on the study visit. Further studies are required to substantiate these findings.

Introduction

Phenols and parabens are a group of chemicals commonly found in personal care products and household items, and have been associated with a number of growth parameters at birth, including birth weight, birth length and gestational length [1–5]. We previously reported on associations between bisphenols, parabens and triclocarban with various birth outcomes in a cohort of pregnant women in Puerto Rico [6].

Maternal stress has also been associated with adverse birth outcomes. A population-based case control study reported a 60% increased odds of a very low birth weight among women who reported always feeling stressed as compared to women reporting no stress [7]. Likewise, other studies have reported a shortened gestational length with maternal stress or a larger number of negative life events [8–11]. The effects of maternal stress on the fetus could also extend beyond pregnancy, and potentially cause long-term adverse effects on the cognitive, behavioral and psychomotor development of the child [12,13].

There is a growing interest in looking at the combined effect of stressors in the environment on human health. In addition to looking at the effect of chemical mixtures on health, interactions between chemical and non-chemical stressors are important to consider in deepening our understanding on how the environment impacts humans. Although this area of research is relatively new and limited, evidence supports the notion of an increased risk of adverse effects with exposure to high concentrations of environmental chemicals and the modifying effect of high psychosocial stress levels. For example, a systematic review found the combined effect of chemical exposures such as smoking and traffic pollution in combination with high stress or

socio-economic stressors was associated with worse fetal growth parameters than with either chemical or socio-economic stressors on their own [14].

As a follow up to our previous study, we were interested in examining the potential modifying effect of maternal stress on our associations between some exposure biomarkers and gestational length. This study is part of the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) project, a multi-disciplinary center in Northern Puerto Rico that established a pregnancy cohort to investigate the role of environmental contamination in adverse birth outcomes. The analysis explores relationships between gestational length and the interaction between urinary concentrations of triclocarban, phenols (benzophenone-3, BPA and BPS) and parabens (methyl- and propyl-paraben) and psychosocial factors (depression, perceived stress, social support and life events).

Methods

Study Population

Participants for this study were from the PROTECT cohort, an ongoing prospective cohort of pregnant women based in Northern Puerto Rico. Details on the recruitment and inclusion criteria have been described previously [31,32]. The 922 study participants in the present analysis were recruited from 2011-2017 at 18 ± 2 weeks gestation from five affiliated health clinics whose patients deliver at two collaborating hospitals were older than 18 years old, and indicated that they were planning to deliver at one of the collaborating hospitals. The exclusion criteria included: 1) women who lived outside the region, 2) multiple gestations, 3) use of oral contraceptives within three months prior to getting pregnant, 4) pregnancy through in vitro fertilization, and 5) any known medical health complications (including diabetes, hypertension, etc.). Each woman participated in a total of up to three study visits which coincided with routine clinical visits and rapid fetal growth. Visit 1 was targeted at 16-20 weeks gestation; visit 2 was targeted at 20-24 weeks gestation; and, visit 3 was targeted at 24-28 weeks gestation.

Demographic information was collected via questionnaire at visit 1. Spot urine samples were collected at each of the study visits, and blood samples were collected at the first and third visit. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Puerto. Rico [33].

This study was approved by the research and ethics committees of the University Of Michigan School Of Public Health, University of Puerto Rico, Northeastern University, and the University of Georgia. All study participants provided full informed consent prior to participation.

Urinary biomarker measurement

Spot urine samples collected during the three study visits were divided into aliquots and frozen at -80 ^{o}C until they were shipped overnight on dry ice to the Centers for Disease Control and Prevention for analysis. Samples were analyzed for triclocarban, seven phenols (2,4-dichlorophenol, 2,5-dichlorophenol, BPA, BPS, BPF, benzophenone-3, triclosan), and four parabens (ethyl-paraben, methyl-paraben, butyl-paraben, propyl-paraben) using online solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry. Biomarker concentrations below the limit of detection (LOD) were assigned a value of the LOD divided by √2 [36]. Urinary dilution was accounted for using urinary specific gravity (SG), and was measured using a digital handheld refractometer (AtagoCo., Ltd., Tokyo, Japan) at the time of the urine collection. Urinary biomarkers were corrected for SG in preliminary analyses using the following formula:

$$P_c = M\ [\ (SG_m - 1) / (SG_i - 1)\ ]$$

Equation 1: Specific gravity correction of exposure biomarkers

where P_c is the SG-corrected biomarker concentration (ng/mL), M is the measured biomarker concentration, SG_m is the study population median urinary specific gravity (1.0196), and SG_i is the individual's urinary specific gravity. The sample size for BPF, BPS, triclocarban and ethyl-paraben was smaller than for the other urinary biomarkers because they were added to the analytical panel mid-way through the study, and thus, only available on a subset of the cohort.

Gestational Age Calculation

The gestational length for complete pregnancies was calculated according to the American Congress of Gynecologists (ACOG) recommendations [37], and it is described as the best obstetrical estimate. In brief, gestational length determination utilizes both the last menstrual cycle date (LMP) as reported by the mother, and the gestational age from the first ultrasound obtained before 20 weeks gestation. The selection between the gestational age from the LMP or ultrasound depends on two criteria: 1) gestational age at which the ultrasound was conducted,

and 2) whether the difference in the estimated date of delivery (EDD) calculated from the LMP and from the ultrasound is less or more than seven days. Further details on this calculation can be found in the ACOG Committee Opinion document (Committee on Obstetric Practice, the American Institute of Ultrasound in Medicine, and the Society for Maternal-Fetal Medicine, 2017). A PTB is defined as giving birth prior to 37 gestational weeks.

Birthweight Calculation

Birthweight values extracted from medical records were converted to gestational length and sex specific z-scores, calculated according to the INTERGROWTH-21st standards [38].

Infants were considered small for gestational age (SGA) if they fell below the 10th percentile of birthweight z-scores. Infants were considered large for gestational age (LGA) if they fell above the 90% percentile of birthweight z-scores.

Statistical Analyses

Distributions of key demographic characteristics were calculated. Spearman correlations were calculated to examine correlations between specific gravity-corrected average biomarker concentrations. Distributions of subject-specific average biomarker concentrations were examined using select percentiles. Distributions were also examined by study visit. Ethyl-paraben and BPF were detected in less than 50% of the samples. Therefore, we transformed BPF and ethyl-paraben into dichotomous variables, where 0 represents concentrations below the LOD, and 1 represents concentrations above the LOD (i.e. detected versus non-detected). These categorical BPF and ethyl-paraben variables were used for all regression analyses. The remaining urinary biomarkers were all positively-skewed, so they were log-transformed for the remainder of the analyses.

Models were constructed to regress each of the four birth outcome variables against each of the exposure biomarker variables. Multiple linear regressions (MLR) were conducted to examine associations between gestational length and one average exposure biomarker, as well as birthweight z-scores and one average exposure biomarker. Logistic regression models were conducted to examine associations between PTB, SGA or LGA and one average exposure biomarker. Due to the similar complications SGA and LGA may incur, SGA models did not include LGA births, and LGA models did not include SGA births. The average urinary exposure biomarker variables are the average urinary exposure biomarker concentrations across the three

study visits. In the case of a missing value at one of the study visits, the average was taken of the two remaining concentrations. In the case of two missing concentrations, the "average" phenol or paraben concentration was equal to the single available concentration. Crude analyses included only the average urinary biomarker and the average specific gravity. Additional covariates were included in the model in a step-wise fashion. A covariate was maintained in the model if it changed the main effect estimate by >10%. Finals models were controlled for the following covariates: specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking. Other variables considered include parity, smoking, maternal education, marital status, number of children, and BMI. To increase interpretability of our results, we transformed regression coefficients from MLRs and odds ratios from logistic models to the change in the birth outcome in relation to the interquartile range (IQR) increase in urinary biomarker concentrations. The alpha level was set at 0.05. All statistical analyses were conducted in R Version 3.4.2.

We performed two sensitivity analyses. First, we ran the same MLR and logistic regression models regressing the birth outcome measure against biomarkers concentrations stratified by study visit of sample collection. Second, we examined an interaction term between the average urinary biomarker concentration and infant sex in the models to examine any potential effect modification by infant sex, and then stratified the models by infant sex if the p value exceeded 0.05.

Given the right-skewed distribution of gestational length, we ran sensitivity analyses to test the robustness of our results. We created a new variable by subtracting the individual gestational length from the maximum gestational length, and then took the log of the result. The resulting variable had a normal distribution. We re-ran the MLR models using this new gestational length variable. There was no difference in the interpretation of the results; therefore, we maintained our original models. We also ran multinomial logistic regression models to examine associations between the chemicals and size at birth (SGA, LGA and 10-90% percentile at birth). There were no differences in the results between the multinomial logistic regression models and the original logistic regression modes; therefore, we maintained our original models.

Results

The mean age of the 922 study participants was 26.7 years (Table IV.1). Almost half of the participants had a body mass index (BMI) <25 kg/m^2, and 14% had a BMI > 29.9 kg/m^2, but 17% of the BMI values were missing. Sixty percent of the study population was dependent on government health insurance, suggesting a low socio-economic status (SES), and 50% of the population had household incomes up to $30,000. This is representative of the general Puerto Rican population [39]. Only 13% had a household income of >$50,000, though 43% of the women attended college or technical school. Three percent of the women smoked during pregnancy (the questionnaire was administered at 16-20 weeks gestation), and 6% reported over one hour of second-hand smoking exposure per day. Six and a half percent reported drinking alcohol within the last few months. Almost 80% of the women reported being married or living with their partner, while 20% reported being single. Nearly half of the women had no other children at home.

Up to 867 women had available data on urinary exposure biomarker concentrations (Table IV.2). The target biomarkers were highly detected in the study population, with the exception of BPF and ethyl-paraben. Fifty-seven and 43 percent of BPF and ethyl-paraben concentrations were below the LOD. Biomarker concentrations remained relatively consistent across the three study visits, with the exception of 2,5-dichlorophenol (p value=0.02) and butyl-paraben (p value=0.004) that had higher concentrations during visit 1 versus visits 2 and 3. Average methyl- and propyl-paraben concentrations were strongly correlated (Spearman correlation=0.78, p value<0.001) (Figure IV.2). Propyl-paraben was also moderately correlated with butyl-paraben and ethyl-paraben (Spearman correlation=0.42, p value<0.001). 2,4-Dichlorophenol and 2,5-dichlorophenol were strongly correlated (Spearman correlation=0.67, p value<0.001), and 2,4-dichlorophenol was also moderately correlated with triclosan (Spearman correlation=0.51, p value<0.001).

The sensitivity analysis showed no difference in the interpretation of results between the transformed and untransformed gestational length variables (Table IV.7); therefore, the remainder of the results are retrieved from MLR models using the untransformed gestational length variable.

Benzophenone-3, methyl-paraben and propyl-paraben were associated with an increase in gestational length [(1.90 change in gestational length days per IQR increase in biomarker concentration (Δ/IQR); 95% CI: 0.54, 3.26); (Δ/IQR 1.63; 95% CI: 0.37, 2.89); (Δ/IQR 2.06; 95% CI: 0.63, 3.48), respectively] (Table IV.3). Conversely, triclocarban was associated with a suggestive decrease in gestational length (Δ/IQR -1.96; 95% CI: -4.11, 0.19).

After stratification by study visit, visit 1 (16-20 weeks gestational length) showed stronger associations for a number of exposure biomarkers (

Table IV.4). At visit 1, BPA was suggestively associated with an increase in gestational length (Δ/IQR 1.37; 95% CI:-0.05, 2.79). Additionally, associations between gestational length and triclocarban, methyl-paraben and propyl-paraben were strongest (largest change in days and lowest p values) at visit 1. Propyl-paraben was also associated with a significant increase in gestational length at 24-28 weeks gestation (Δ/IQR 1.84; 95% CI: 0.02, 3.65), but not at 20-24 weeks gestation. Benzophenone-3 was consistently associated with an approximate 1.5 day increase in gestational length at all three study visits.

Sex differences were then explored by including an interaction term between the urinary biomarkers and infant sex (Table IV.9). The infant sex*biomarker interaction term was significant in the model for gestational length and triclosan (p value=0.006). Urinary triclosan concentration was associated with a suggestive decrease in gestational length among females (Δ/IQR -2.31; 95% CI:-4.86, 0.24; p value = 0.08), and associated with an increase in gestational length among males (Δ/IQR 2.96; 95% CI: 0.41, 5.51; p value = 0.02). Figure IV.1 is a graph of the interaction effect of infant sex on the association between the average triclosan concentration and gestational length. Figure IV.3 shows the conditional effect of the average triclosan concentration on gestational length stratified by infant sex to better view the difference in the direction of association between male and female infants.

Results from these models regressing PTB resembled the results observed in models regressing gestational length against the exposure biomarkers. Average BP-3, MPB and PPB were associated with a decreased odds of PTB [(OR/IQR 0.54; 95% CI: 0.36, 0.83); (OR/IQR 0.70; 95% CI: 0.49, 0.98); and (OR/IQR 0.64; 95% CI: 0.44, 0.94). The associations with BP-3 did not differ by study visit, but associations with MPB and PPB with PTB were stronger at study visit 1 [(OR/IQR 0.58; 95% CI: 0.38, 0.89) and (OR/IQR 0.51; 95% CI: 0.32, 0.80)]. BPA and TCC

were also associated with PTB at visit 1 only. BPA was associated with a suggestive decreased odds of PTB at 16-20 weeks (OR/IQR 0.69; 95% CI: 0.45, 1.05), while TCC was associated with a suggestive increased odds of PTB at 16-20 weeks (OR/IQR 1.71; 95% CI: 0.94, 3.10). Lastly, BPF was associated with a decreased odds of PTB at 24-28 weeks (OR/IQR 0.36; 95% CI: 0.15, 0.84).

No averaged exposure biomarkers were associated with the birthweight z-score (Table IV.5). After stratification by study visit, butyl-paraben was associated with a suggestive increase of 0.12 in the birthweight z-score at visit 2 (z-score Δ/IQR 0.12; 95% CI: -0.02, 0.26). Fetal sex did not modify any of the associations with birthweight z-scores; interaction terms between biomarkers and fetal sex were all above 0.10.

Average triclosan was associated with a 70% increased odds of SGA (OR/IQR 1.70; 95% CI: 1.06, 2.73); average 2,4-dichlorophenol was associated with a suggestive 32% increased odds of SGA (OR/IQR 1.32; 95% CI: 0.95, 1.84). Methyl-, butyl- and propyl-paraben were all associated with a 34-50% decrease in the odds of SGA [(OR/IQR 0.66; 95% CI: 0.47, 0.93); (OR/IQR 0.50; 95% CI: 0.28, 0.88); (OR/IQR 0.61; 95% CI: 0.41, 0.91), respectively].

After stratification of the results by study visit (Table IV.6), BPS was significantly associated with 82% increased odds of SGA at visit 3 (OR/IQR 1.82; 95% CI: 1.17, 2.84). Associations between parabens and SGA by study visit differed slightly by the type of paraben. There was no difference in association between SGA and methyl-paraben across the three time points. Associations between butyl-paraben and SGA was only significant at visit 2 and visit 3 [(OR/IQR 0.47; 95% CI: 0.26, 0.84) and (OR/IQR 0.51; 95% CI: 0.28, 0.91), respectively], while propyl-paraben was associated with a decrease in the odds of SGA at visit 1 and visit 3 [(OR/IQR 0.58; 95% CI: 0.38, 0.97); (OR/IQR 0.53; 95% CI: 0.32, 0.87), respectively]. Associations between triclosan and 2,4-dichlorophenol with SGA were significant at 20-24 weeks [(OR/IQR 1.51; 95% CI: 0.95, 2.42) and (OR/IQR 1.34; 95% CI: 0.96, 1.85), respectively]. Fetal sex did not modify any of the associations with SGA; interaction terms between biomarkers and fetal sex were all above 0.05.

Average BPS and BP-3 concentrations were associated with an increased odds of LGA [(OR/IQR 1.62; 95% CI: 1.06, 2.45) and (OR/IQR 1.38; 95% CI: 0.99, 1.93), respectively]. The association between BPS and SGA was strongest at study visit 1 (OR/IQR 1.49; 95% CI: 0.99,

2.25), whereas the association between BP-3 and LGA was strongest at study visit 2 (OR/IQR 1.52; 95% CI: 1.09, 2.13). Fetal sex did not modify any of the associations with LGA; interaction terms between biomarkers and fetal sex were all above 0.10.

Discussion

Urinary concentrations of benzophenone-3, methyl-paraben and propyl-paraben measured at up to 3 times during pregnancy were associated with an increase in gestational length. Urinary BPA concentrations at 16-20 weeks were also associated with a suggestive increase in gestational length. Triclocarban was associated with a suggestive decrease in gestational length, while the relationship between triclosan and gestational length differed by infant sex. PTB results mirrored gestational length results, with the addition of a decrease in the odds of PTB in association with BPF at 24-28 weeks gestation. No exposure biomarkers were associated with birth weight; however 2,4-dichlorophenol, triclosan and BPS were associated with an increase in the odds of SGA, while parabens were associated with a decrease in the odds of SGA. BP-3 and BPS were also associated with an increase in the odds of LGA.

Benzophenone-3 was consistently associated with an increase in gestational length at all study visits and in both sexes in this study. However, in contrast to our results, a previous study reported a decrease in gestational length in association with urinary benzophenone-3 concentrations at delivery in 567 infants [27]. We could not identify other studies that examined the effect of benzophenone-3 on gestational length, but did identify studies that reported associations between benzophenone-3 and other gestation growth parameters. Philippat et al. reported an increase in birth weight and head circumference in association with benzophenone-3 among 520 male infants in France [26,40], whereas Wolff et al. found an increase in birth weight among boys and a decrease in birth weight among girls in a cohort of 404 pregnant women in New York City [28]. Benzophenone-3 was also associated with a decrease in the abdominal circumference among boys, and a suggestive increase in abdominal circumference among females in a cohort of 476 women in Boston [24]. The reported increases in gestational growth parameters in association with benzophenone-3 are consistent with our finding of an increased odds of LGA with benzophenone-3.

The biological mechanisms involved in the observed associations between benzophenone-3 and birth outcomes are unclear. Benzophenone-3 exhibits estrogenic activity and can bind to estrogen

receptors α and β, as well as other estrogen-related receptors [41]. Therefore, an endocrine-mediated mechanism could potentially be leading to the increase in gestational length. Alternatively, BP-3 was associated with lower concentrations of pro-inflammatory markers in a large pregnancy cohort [42] which may also impact duration of pregnancy.

Methyl- and propyl-paraben were also associated with an increase in gestational length in this study, particularly at 16-20 weeks gestation, in addition to a decreased odds of SGA. One other study looked into the effect of parabens on gestational length. Geer et al. reported a decrease in gestational length in association with maternal urinary butyl-paraben at the third trimester, and a protective effect on PTB in association with cord blood propyl-paraben (but no effect on gestational length) in a small (N=185) immigrant population in New York [43]. Although we did not identify any associations between butyl-paraben and gestational length, we did observe a decrease in the odds of PTB with urinary propyl-paraben concentrations, which was similar to the results observed in the New York study. With regards to other growth parameters, parabens, including methyl-paraben and propyl-paraben, were associated with an increase in birth weight among boys [26,44]. A large Chinese cohort (N=1006) also reported an increase in birth length in association with methyl-paraben in boys [44]. We observed a similar association between butyl-paraben and an increase in birthweight, as well as a protective effect of parabens on SGA; however, the associations were not specific to infant sex. Parabens have demonstrated endocrine disrupting effects [45–47], and have been associated with changes in estriol, progesterone, sex-hormone binding globulin, and thyroid-stimulating hormone in this cohort (Aker el al., in press). These potential effects on hormone levels may play a role in the relationships between parabens and birth outcomes, though more research is needed to help clarify relevant biological mechanisms. Butyl-paraben has also been shown to activate PPARγ and increase adipogenesis, which could explain the decrease in the odds of SGA, although only butyl-paraben was included in this *in vitro* study [48].

In the current study, BPA was associated with a suggestive increase in pregnancy duration, particularly for urinary measures at 16-20 weeks gestation. One study did observe an increase in gestational length with maternal plasma BPA levels, particularly among female infants [49]. The plasma was analyzed during early pregnancy, which is reflective of the timing of our study visit 1; however it is difficult to compare BPA concentrations in plasma versus urine. In contrast,

other studies have found a decrease in gestational length in association with BPA. Urinary BPA at the third trimester was found to increase preterm birth in a nested case-control study conducted in Mexico [50]. Another nested case-control study found that maternal plasma BPA concentrations at time of delivery were associated with preterm birth, although interestingly, amniotic fluid BPA concentrations were not [51]. Three more cross-sectional studies looked at BPA levels at delivery, two of which reported a decrease in gestational length, particularly among male infants [27,52], and one that reported no association between BPA and gestation age among 496 mother-infant pairs [53]. It is important to note the difference in the timing of exposure assessment between past studies and our current study. Our study looked at earlier time points of BPA exposure, so it is possible that the differences in our results could be attributed to this difference in timing of the exposure measures, and possibly indicating a susceptible window of exposure. There are also noted differences in urine versus plasma BPA concentrations, with some possible bias in measuring plasma BPA concentrations as an exposure proxy given the high potential for contamination [54]. This difference in measurement could also explain the differences in our results.

BPS was associated with an increased odds of both SGA and LGA, depending on the timing of exposure. While BPS concentrations at 16-20 weeks were associated with LGA, BPS concentrations at 24-28 weeks were associated with SGA. We did not identify any other studies that reported associations between BPS and SGA or LGA; however, similar to our results, two cohort studies found no association between BPS and overall birth weight [24,55]. This difference in the potential effect on the size of the fetus could be attributed to the transient hormonal levels present throughout the different stages of pregnancy. For example, in a previous study by our group, BPS was associated with changes to thyroid-stimulating hormone and corticotropin-releasing hormone at different time points in pregnancy (Aker et al., in press). Although the increase in both SGA and LGA in association with BPS differed by time point, these results are difficult to explain. Therefore, we caution over-interpretation of these results until these association are studied in other cohorts, and experimental studies help to better understand the biological mechanisms by which BPS may impact birth outcomes.

We identified a relationship between triclosan and gestational length that differs by the sex of the infant, and an increase in the odds of SGA with urinary triclosan. There was an increase in

gestational length among boys, and a suggestive decrease in gestational length among females. In fact, four other studies observed associations between triclosan and birth outcomes among only boys [24–26,28]. The sex-specific effects observed could be attributed to sex hormone-specific targets of triclosan in the developing fetus. For example, triclosan was associated with an increase in maternal testosterone in this cohort (Aker et al., in press), which may impact the developing male fetus differently than the female fetus. Another birth cohort of 376 mother-infant pairs in Cincinnati collected urine at 16 and 26 weeks pregnancy [56]. Average triclosan concentrations were associated with a decrease in gestational length, but was not modified by infant sex. A large cohort study in China including over 1000 pregnant women found no association between urinary triclosan concentrations and gestational length after confounder adjustment [57]. There were also no differences by infant sex. Differences in our results could be due to the difference in the timing of triclosan exposure as the Chinese study measured urinary triclosan just prior to delivery. Differences may also be due to the lower urinary triclosan concentrations in comparison to our cohort. Several other studies also reported no association between triclosan and birth outcomes, including gestational length [28,40,43,53]. However, our triclosan concentrations were 3-20 times higher than the concentrations reported in these studies.

Similar to our results, no association was observed between triclosan and birth weight in two large prospective cohorts of pregnant Chinese women [53,57]. A study on a cohort of 378 mother-infant pairs in Ohio did observe a decrease in birth weight, birth length and head circumference with a 10-fold increase in urinary triclosan [56]. While we did not observe any effects in birth weight, results in the Ohio cohort could be consistent with an increase in SGA, as we observed in our cohort. Conversely, another large (N=620) Chinese cohort identified an increase in the birth weight of newborns among females [58]. However, that study modeled birth weight, and included gestational length as a covariate in the model, as opposed to our gestational age-adjusted z-scores. This difference in statistical methods may partially explain some the differences in our results.

A small study (N=185) reported no association between third trimester maternal urine triclosan concentrations and gestational length in an immigrant population in New York, but did find a significant decrease in gestational length with an increase in triclocarban neonatal cord blood plasma (N=34) [43]. This is similar to our finding of a suggestive decrease in gestational length

in association with average urinary triclocarban, where we also found stronger associations with triclocarban measured earlier in pregnancy. To our knowledge, no other studies reported associations between triclocarban and gestational length. Triclocarban concentrations in PROTECT were exceptionally high compared to other studies [59]. The median urinary concentrations of triclocarban in the U.S. National Health and Nutrition Examination Survey (NHANES) in 2013-2014 was < LOD (0.1 μg/L), and 0.2 μg/L was the 75th percentile [60]. Our cohort, on the other hand, had a median of 2.3 μg/L and a 75th percentile of 26.8 μg/L. Similarly, a recent review reported a relatively low detection (4-25%) of urinary triclocarban in Canada, Greece and Denmark [61]. This points to the variability of exposure to triclocarban worldwide. Our findings and previous studies suggest an association between triclocarban and birth outcomes [61]. Therefore, additional studies will be useful to better understand these associations and potential mechanisms of action.

While there was some consistency between our results and other studies, the differences with regards to observed associations, as well as the directions of association make the interpretations of results across studies difficult. However, this also may indicate the importance of 1) the timing of exposure on birth outcomes, 2) the methodological techniques used in analyzing exposure and outcome, and perhaps most importantly, 3) the undefined confounders and/or modifiers that may exist from one population to the other. Future studies should attempt to explore other factors that may interact with these chemicals that could explain the differences observed across cohorts.

Our study had a few limitations. While data at three time points are a great improvement from the more common cross-sectional study design, it may not be sufficient to understand the effects of these biomarkers on gestational length. The variation of concentrations of these exposure biomarkers over time may also introduce potential bias, stemming from random measurement error, but most likely towards the null. We also had a smaller sample size for some of the emerging phenols of concern, such as BPA replacement chemicals, as these were added to the analytical panel part-way through the study. Finally, given the multiple comparisons conducted, there is a possibility of chance findings due to Type I error.

Our study also had many strengths, including a robust sample size and the collection of exposure biomarkers data at three time points during pregnancy to help account for the chemicals' short

lifespan in the body and likely episodic nature of the exposures, as previously reported [59] The repeated measures also allowed us to explore potential windows of susceptibility for these associations. Additionally, our urinary biomarker panel included triclocarban, BPS and BPF, all of which have not been well studied in the current literature. Finally, the relatively high biomarkers concentrations in our cohort, particularly triclocarban, triclosan and BPF, provides as opportunity to study the potential effects of these chemicals in a vulnerable human population. The results of this study add evidence to the possibility of an influence by exposure to phenols and parabens on birth outcomes. Further studies are needed to better understand how they impact birth outcomes, but in the meantime advising parents on simple ways to reduce exposure to these chemicals is warranted.

Conclusion

Our study showed a significant association between triclocarban, and select phenols and parabens in association with gestational length and SGA. In particular, benzophenone-3, BPA, methyl- and propyl-paraben were associated with an increase in gestational length, while triclocarban was associated with a suggestive decrease in gestational length. Urinary biomarker concentrations at the earliest time point, 16-20 weeks gestation, may be a susceptible window of exposure for triclocarban and other chemicals. Triclosan, BPF and BPS were associated with an increase in SGA, whereas parabens were associated with a decrease in SGA. The association between triclosan and gestational length differed by infant sex, wherein triclosan was associated with an increase in gestational length among males, and a suggestive decrease in gestational length among females. These associations should be verified in further studies with repeated measures of exposure.

Table IV.1: Summary demographics of the 922 pregnant women in the study population

	Overall
Total N	922
Gestational Age	38.8 (2.0)
Birthweight (Mean (SD))	110.6oz (19.4)
Preterm Birth	10.3%
Small for Gestational Age	9.9%
Large for Gestational Age	9.1%
Maternal Age (Mean (SD))	26.70 (5.5)

BMI (%)	
<25 kg/m^2	437 (47.4)
25-29.9 kg/m^2	201 (21.8)
>29.9 kg/m^2	129 (14.0)
Missing	155 (16.8)
Insurance type (%)	
Public (Mi Salud)	555 (60.2)
Private	322 (34.9)
Missing	45 (4.9)
Household Income (%)	
<$10,000	234 (25.4)
$10,000-$30,000	246 (26.7)
$30,000-$50,000	184 (20.0)
>$50,000	123 (13.3)
Missing	135 (14.6)
Maternal Education (%)	
≤High School/GED	187 (20.3)
Some College or technical school	331 (35.9)
College graduate or technical school	392 (42.5)
Missing	12 (1.3)
Smoking (%)	
Never	763 (82.8)
Ever	120 (13.0)
Current	29 (3.1)
Missing	10 (1.1)
Exposure to second hand smoking (%)	
None	781 (84.7)
Up to 1 hour	44 (4.8)
More than 1 hour	59 (6.4)
Missing	38 (4.1)
Alcohol Consumption (%)	
None	463 (50.2)
Before pregnancy	385 (41.8)
Yes within the last few months	60 (6.5)
Missing	14 (1.5)
Marital Status (%)	
Single	180 (19.5)
Married	517 (56.1)
Divorced	11 (1.2)
Living together	205 (22.2)
Missing	9 (1.0)
Number of previous children (%)	

0	436 (47.3)
1	364 (39.5)
>1	111 (12.0)
Missing	11 (1.2)

Demographic variables collected during the second study visit (20-24 weeks).

Table IV.2: SG-corrected urinary biomarker average concentrations and by study visit of sample collection in pregnancy. Range of gestational weeks for sample collection: Visit 1: 16-20 weeks gestation, Visit 2: 20-24 weeks gestation Visit 3: 24-28 weeks gestation.

	All						Visit 1					
	% <LOD	**N**	**GM (GSD)**	**25%**	**50%**	**75%**	**% <LOD**	**N**	**GM (GSD)**	**25%**	**50%**	**75%**
2,4-Dichlorophenol	1.1	1871	1.09 (3.42)	0.50	0.99	2.17	0.7	674	1.16 (3.29)	0.50	1.01	2.10
2,5-Dichlorophenol[a]	0.1	1872	13.14 (5.40)	4.10	10.22	31.64	0.1	672	14.18 (5.42)	4.40	11.05	36.41
BPA	0.6	1868	2.02 (2.54)	1.10	1.98	3.49	0.1	667	2.16 (2.49)	1.21	2.09	3.68
BPS	6.1	1310	0.46 (3.16)	0.20	0.40	0.90	5.5	465	0.47 (3.32)	0.20	0.40	0.99
BPF	56.8	1144	0.27 (2.97)	0.14	0.14	0.40	54	416	0.30 (3.22)	0.14	0.14	0.50
Benzophenone-3	0.2	1869	39.45 (6.63)	10.36	26.50	124.85	0.4	671	42.06 (6.37)	11.35	29.27	126.61
Triclosan	1.6	1874	22.75 (8.65)	3.61	15.79	139.27	2.1	669	23.76 (8.85)	3.71	15.75	149.51
Triclocarban	6.4	1312	3.41 (10.90)	0.50	2.31	26.79	6.5	469	3.42 (10.79)	0.50	2.28	25.52
Ethyl-paraben	42.9	1318	2.56 (6.48)	0.71	1.24	7.83	39.7	470	3.10 (7.11)	0.71	1.55	10.52
Methyl-paraben	0.5	1876	74.74 (5.28)	23.56	88.54	249.93	0.4	670	77.67 (5.24)	25.50	97.51	262.15
Butyl-paraben[a]	26.3	1865	0.45 (7.61)	0.07	0.20	1.80	23.4	664	0.53 (8.43)	0.10	0.20	2.84
Propyl-paraben	0.3	1875	14.78 (7.60)	3.19	17.17	76.76	0	670	15.96 (7.43)	3.39	20.06	78.86
	Visit 2						**Visit 3**					
	% <LOD	**N**	**GM (GSD)**	**25%**	**50%**	**75%**	**% <LOD**	**N**	**GM (GSD)**	**25%**	**50%**	**75%**
2,4-Dichlorophenol	0.9	674	1.08 (3.42)	0.50	0.91	2.11	1.9	523	1.04 (3.58)	0.40	0.90	2.19
2,5-Dichlorophenol[a]	0	675	12.22 (5.33)	3.83	9.58	27.29	0.2	525	13.08 (5.47)	4.01	9.84	29.67
BPA	1.2	678	2.07 (2.69)	1.10	2.01	3.71	0.6	523	1.78 (2.40)	1.00	1.71	3.09
BPS	6.9	476	0.45 (3.02)	0.20	0.40	0.90	5.7	369	0.45 (3.16)	0.20	0.40	0.90
BPF	56.6	410	0.28 (3.05)	0.14	0.14	0.40	60.5	318	0.24 (2.52)	0.14	0.14	0.37
Benzophenone-3	0	675	39.83 (6.49)	10.35	26.62	128.77	0.2	523	35.88 (7.17)	8.87	23.67	112.91
Triclosan	1.2	676	22.56 (8.77)	3.57	16.18	147.99	1.5	529	21.76 (8.28)	3.61	15.72	121.40
Triclocarban	6.9	476	3.27 (11.06)	0.50	2.31	28.94	5.5	367	3.58 (10.88)	0.51	3.30	27.45
Ethyl-paraben	42	478	2.36 (5.98)	0.71	1.30	5.96	47.9	370	2.23 (6.25)	0.71	0.85	6.70
Methyl-paraben	0.6	678	69.43 (5.37)	21.48	78.36	236.91	0.6	528	78.24 (5.23)	23.99	94.39	251.31
Butyl-paraben[a]	26.8	675	0.41 (6.81)	0.07	0.20	1.39	29.4	526	0.43 (7.59)	0.07	0.20	1.49

Propyl-paraben	0.4	677	13.48 (7.50)	3.04	14.16	69.40	0.4	528	15.08 (7.92)	3.08	18.87	78.10

GM: Geometric mean
GSD: Geometric standard deviation
[a] Significant difference ($p<0.05$) in urinary exposure metabolite or hormone compared to reference (visit 1) using linear mixed models with a random intercept.
All biomarker units: µg/mL.
LODs for biomarkers: 2,4-dichlorphenol and 2,5-dichlorophenol, BPS, triclocarban, butylparaben, propylparaben: 0.1 µg/L. BPA and BPF: 0.2 µg/L. BPF: 0.4 µg/L. Methyl-paraben and ethyl-paraben: 1 µg/L. Triclosan: 1.7 µg/L.

Table IV.3: Adjusted[a] change in gestational length or odds ratios in relation to average exposure biomarker concentration across three time points during pregnancy. Beta coefficients were transformed to change or odds ratio for an IQR increase in exposure biomarker concentration.

		Gestational Age		**Preterm Birth**	
	N	**Change in days/IQR (95% CI)**	**p**	**OR/IQR change (95% CI)**	**p**
2,4-Dichlorophenol	748	0.07 (-1.20, 1.33)	0.92	1.04 (0.75, 1.43)	0.81
2,5-Dichlorophenol	749	-0.46 (-1.68, 0.76)	0.46	1.18 (0.87, 1.61)	0.29
BPA	748	0.98 (-0.41, 2.37)	0.17	0.72 (0.48, 1.07)	0.10
BPF[b]	500	0.06 (-0.28, 0.41)	0.72	0.60 (0.31, 1.17)	0.14
BPS	540	-0.52 (-2.14, 1.09)	0.53	1.08 (0.70, 1.66)	0.72
Benzophenone-3	749	1.90 (0.54, 3.26)	0.006**	0.54 (0.36, 0.83)	0.01**
Triclosan	749	0.12 (-1.64, 1.87)	0.90	0.94 (0.58, 1.51)	0.79
Triclocarban	544	-1.96 (-4.11, 0.19)	0.08*	1.50 (0.84, 2.67)	0.17
Ethyl-paraben[b]	550	-0.11 (-0.44, 0.23)	0.53	0.89 (0.47, 1.68)	0.71
Methyl-paraben	751	1.63 (0.37, 2.89)	0.01**	0.70 (0.49, 0.98)	0.04**
Butyl-paraben	746	0.60 (-1.23, 2.42)	0.52	0.90 (0.54, 1.49)	0.67
Propyl-paraben	752	2.06 (0.63, 3.48)	0.005**	0.64 (0.44, 0.94)	0.02**

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking.
[b] Categorical variable.
* $0.05 < p \text{ value} < 0.10$; ** $p \text{ value} < 0.05$

Table IV.4: Adjusted[a] change or odds ratio in relation to exposure biomarker concentration, stratified by visit during pregnancy. Beta coefficients were transformed to the change in the number of gestational days or odds ratio per IQR change in exposure biomarker concentration.

Gestational Age

	Visit 1 (16-20 weeks)			Visit 2 (20-24 weeks)			Visit 3 (24-28 weeks)		
	N	**Change in days (95% CI)**	**p**	**N**	**Change in days (95% CI)**	**p**	**N**	**Change in days (95% CI)**	**p**
2,4-DCP	613	0.05 (-1.21, 1.31)	0.94	624	0.65 (-0.59, 1.89)	0.30	470	-0.28 (-1.87, 1.31)	0.73
2,5-DCP	612	-0.50 (-1.78, 0.78)	0.45	624	0.29 (-0.95, 1.52)	0.65	472	-0.92 (-2.30, 0.45)	0.19
BPA	607	1.37 (-0.05, 2.79)	0.06*	626	1.13 (-0.26, 2.53)	0.11	470	0.90 (-0.81, 2.62)	0.30
BPF[b]	381	0.12 (-0.27, 0.51)	0.55	381	-0.22 (-0.62, 0.18)	0.28	291	0.24 (-0.19, 0.68)	0.27
BPS	422	-0.58 (-2.27, 1.11)	0.5	438	-0.27 (-2.02, 1.47)	0.76	329	0.44 (-1.34, 2.23)	0.63
BP-3	611	1.56 (0.18, 2.93)	0.03**	623	1.44 (0.05, 2.83)	0.04**	470	1.63 (0.15, 3.12)	0.03**
TCS	609	0.21 (-1.49, 1.91)	0.81	625	0.41 (-1.28, 2.09)	0.63	476	0.85 (-1.07, 2.76)	0.39
TCC	426	-2.21 (-4.43, 0.01)	0.05*	437	-2.10 (-4.33, 0.14)	0.07*	328	-1.33 (-3.62, 0.96)	0.26
EPB[b]	428	-0.20 (-0.55, 0.16)	0.28	440	0.06 (-0.30, 0.41)	0.75	330	-0.12 (-0.51, 0.26)	0.53
MPB	610	2.24 (0.75, 3.73)	0.003**	625	0.56 (-0.91, 2.03)	0.45	475	1.03 (-0.59, 2.65)	0.21
BPB	604	1.17 (-0.44, 2.79)	0.15	622	0.15 (-1.41, 1.72)	0.85	474	-0.13 (-1.91, 1.65)	0.89
PPB	610	2.59 (0.97, 4.21)	0.002**	625	0.90 (-0.67, 2.46)	0.26	475	1.84 (0.02, 3.65)	0.05**

Preterm Birth

	Visit 1 (16-20 weeks)			Visit 2 (20-24 weeks)			Visit 3 (24-28 weeks)		
	N	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**
2,4-DCP	613	1.16 (0.83, 1.61)	0.38	624	0.85 (0.60, 1.21)	0.37	470	1.12 (0.74, 1.72)	0.59
2,5-DCP	612	1.29 (0.92, 1.80)	0.14	624	0.99 (0.70, 1.39)	0.95	472	1.34 (0.93, 1.93)	0.11
BPA	607	0.69 (0.45, 1.05)	0.09*	626	0.77 (0.51, 1.15)	0.20	470	0.98 (0.60, 1.60)	0.94
BPF[a]	381	0.83 (0.41, 1.70)	0.62	381	1.30 (0.63, 2.67)	0.48	291	0.36 (0.15, 0.84)	0.02**
BPS	422	1.07 (0.69, 1.66)	0.77	438	1.11 (0.71, 1.75)	0.64	329	0.98 (0.57, 1.69)	0.95
BP-3	611	0.64 (0.42, 0.98)	0.04**	623	0.66 (0.43, 1.02)	0.06*	470	0.67 (0.41, 1.07)	0.10*
TCS	609	0.98 (0.60, 1.58)	0.92	625	0.76 (0.46, 1.26)	0.29	476	0.77 (0.44, 1.36)	0.37
TCC	426	1.71 (0.94, 3.10)	0.08*	437	1.61 (0.88, 2.94)	0.12	328	1.04 (0.51, 2.10)	0.92

EPB[a]	428	1.35 (0.69, 2.62)	0.38	440	0.79 (0.40, 1.54)	0.49	330	1.04 (0.46, 2.34)	0.93
MPB	610	0.58 (0.38, 0.89)	0.01**	625	0.81 (0.53, 1.24)	0.34	475	0.75 (0.47, 1.19)	0.22
BPB	604	0.75 (0.46, 1.21)	0.24	622	1.02 (0.65, 1.60)	0.92	474	0.95 (0.57, 1.59)	0.85
PPB	610	0.51 (0.32, 0.80)	0.004**	625	0.76 (0.49, 1.18)	0.22	475	0.69 (0.42, 1.14)	0.15

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: Ethyl-paraben; MPB: Methyl-paraben;
BPB: Butyl-paraben; PPB: Propyl-paraben

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking.

[b] Categorical variable.

[*] 0.05 < p value < 0.10; [**] p value < 0.05

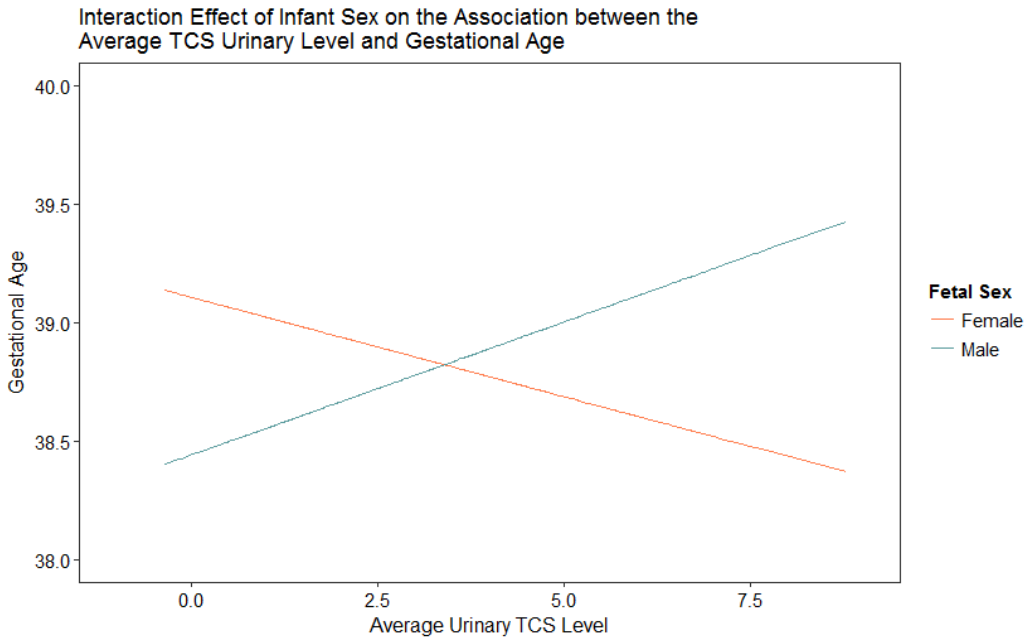


Figure IV.1: Interaction effect of infant sex on the association between the average triclosan urinary concentration and gestational length. Female p value = 0.06. Male p value = 0.03.

Table IV.5: Adjusted[a] change in birth weight z-scores or odds ratios associated with average exposure biomarker concentration across three time points during pregnancy. Effect estimates presented as change or odds ratio for IQR increase in exposure biomarker concentration.

	Birthweight z-scores			SGA			LGA		
	N	**Change in z-score (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**
2,4-DCP	736	-0.02 (-0.13, 0.09)	0.70	662	1.32 (0.95, 1.84)	0.10	667	1.04 (0.74, 1.47)	0.82
2,5-DCP	737	0.00 (-0.11, 0.10)	0.97	663	1.16 (0.84, 1.60)	0.36	668	1.11 (0.81, 1.54)	0.51
BPA	736	-0.01 (-0.13, 0.11)	0.90	662	1.09 (0.75, 1.58)	0.66	667	0.94 (0.65, 1.36)	0.74
BPF[b]	496	-0.09 (-0.30, 0.12)	0.39	444	1.00 (0.53, 1.89)	0.99	446	0.69 (0.36, 1.32)	0.26
BPS	535	0.05 (-0.09, 0.20)	0.46	479	1.49 (0.97, 2.29)	0.07*	482	1.62 (1.06, 2.45)	0.02**
BP-3	737	0.08 (-0.04, 0.20)	0.17	664	0.91 (0.62, 1.33)	0.63	668	1.38 (0.99, 1.93)	0.06*
TCS	737	-0.09 (-0.24, 0.06)	0.26	663	1.70 (1.06, 2.73)	0.03**	668	0.91 (0.57, 1.46)	0.70
TCC	539	-0.10 (-0.29, 0.09)	0.31	483	0.91 (0.50, 1.67)	0.77	486	1.04 (0.60, 1.81)	0.89
EPB[b]	545	-0.06 (-0.26, 0.15)	0.58	489	1.57 (0.86, 2.89)	0.15	492	1.25 (0.68, 2.30)	0.47
MPB	739	-0.01 (-0.12, 0.10)	0.88	665	0.66 (0.47, 0.93)	0.02**	670	0.92 (0.66, 1.30)	0.65
BPB	734	0.11 (-0.05, 0.27)	0.16	660	0.50 (0.28, 0.88)	0.02**	666	1.02 (0.63, 1.65)	0.93
PPB	740	0.01 (-0.11, 0.14)	0.85	666	0.61 (0.41, 0.91)	0.01**	671	0.96 (0.65, 1.41)	0.82

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: Ethyl-paraben; MPB: Methyl-paraben; BPB: Butyl-paraben; PPB: Propyl-paraben

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking.

[b] Categorical variable.

Table IV.6: Adjusted[a] change in birth weight z-scores or odds in relation to exposure biomarker concentration, stratified by visit during pregnancy. Effect estimates presented as change or odds ratio for IQR increase in exposure biomarker concentration.

Birthweight

	Visit 1 (16-20 weeks)			Visit 2 (20-24 weeks)			Visit 3 (24-28 weeks)		
	N	**Z-score change (95% CI)**	**p**	**N**	**Z-score change (95% CI)**	**p**	**N**	**Z-score change (95% CI)**	**p**
2,4-DCP	603	-0.04 (-0.15, 0.08)	0.52	613	-0.01 (-0.12, 0.10)	0.88	462	-0.04 (-0.18, 0.10)	0.59
2,5-DCP	602	-0.04 (-0.15, 0.08)	0.53	613	0.02 (-0.09, 0.13)	0.73	464	0.00 (-0.12, 0.13)	0.94
BPA	597	0.01 (-0.12, 0.13)	0.92	615	0.05 (-0.07, 0.18)	0.41	462	-0.05 (-0.20, 0.10)	0.51
BPF[a]	379	0.04 (-0.20, 0.28)	0.73	379	-0.20 (-0.43, 0.04)	0.10	289	-0.14 (-0.42, 0.14)	0.33
BPS	418	0.08 (-0.07, 0.24)	0.28	433	0.03 (-0.13, 0.18)	0.73	325	0.02 (-0.15, 0.19)	0.80
BP-3	601	0.06 (-0.06, 0.18)	0.31	612	0.10 (-0.02, 0.22)	0.12	462	0.06 (-0.07, 0.20)	0.35
TCS	599	-0.02 (-0.17, 0.13)	0.79	614	-0.11 (-0.26, 0.04)	0.15	468	-0.03 (-0.20, 0.14)	0.76
TCC	422	-0.11 (-0.31, 0.09)	0.28	432	-0.07 (-0.26, 0.13)	0.49	324	-0.02 (-0.23, 0.20)	0.88
EPB[a]	424	-0.05 (-0.28, 0.17)	0.64	435	-0.08 (-0.30, 0.14)	0.46	326	-0.20 (-0.45, 0.05)	0.12
MPB	600	0.03 (-0.10, 0.16)	0.67	614	-0.01 (-0.13, 0.12)	0.93	467	0.03 (-0.11, 0.18)	0.65
BPB	594	-0.04 (-0.18, 0.11)	0.62	611	0.12 (-0.02, 0.26)	0.08*	466	0.11 (-0.04, 0.27)	0.15
PPB	600	0.04 (-0.11, 0.18)	0.60	614	0.00 (-0.13, 0.14)	0.96	467	0.04 (-0.12, 0.20)	0.63

SGA

	Visit 1 (16-20 weeks)			Visit 2 (20-24 weeks)			Visit 3 (24-28 weeks)		
	N	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**
2,4-DCP	530	1.15 (0.82, 1.60)	0.41	547	1.34 (0.96, 1.85)	0.08*	431	1.36 (0.90, 2.05)	0.14
2,5-DCP	529	1.03 (0.73, 1.46)	0.87	547	1.24 (0.89, 1.72)	0.20	433	1.23 (0.86, 1.75)	0.25
BPA	524	1.02 (0.71, 1.47)	0.90	549	1.07 (0.73, 1.55)	0.74	431	1.12 (0.74, 1.69)	0.59
BPF[a]	444	1.00 (0.53, 1.89)	0.99	444	1.00 (0.53, 1.89)	0.99	444	1.00 (0.53, 1.89)	0.99
BPS	369	0.91 (0.57, 1.46)	0.70	389	1.40 (0.89, 2.22)	0.14	304	1.82 (1.17, 2.84)	0.01**
BP-3	528	1.07 (0.74, 1.55)	0.70	547	0.99 (0.67, 1.49)	0.98	431	0.69 (0.44, 1.08)	0.11
TCS	526	1.44 (0.92, 2.26)	0.11	548	1.51 (0.95, 2.42)	0.08*	435	1.15 (0.69, 1.91)	0.60
TCC	373	0.85 (0.46, 1.56)	0.59	389	1.06 (0.58, 1.94)	0.85	304	0.81 (0.43, 1.52)	0.51
EPB[a]	489	1.57 (0.86, 2.89)	0.15	489	1.57 (0.86, 2.89)	0.15	489	1.57 (0.86, 2.89)	0.15

MPB	527	0.61 (0.42, 0.90)	0.01**	549	0.68 (0.45, 1.02)	0.06*	435	0.66 (0.43, 1.01)	0.06*
BPB	522	0.76 (0.48, 1.20)	0.24	547	0.47 (0.26, 0.84)	0.01**	433	0.51 (0.28, 0.91)	0.02**
PPB	527	0.58 (0.38, 0.91)	0.02**	548	0.81 (0.52, 1.27)	0.37	435	0.53 (0.32, 0.87)	0.01**

LGA

	Visit 1 (16-20 weeks)			Visit 2 (20-24 weeks)			Visit 3 (24-28 weeks)		
	N	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**	**N**	**OR/IQR change (95% CI)**	**p**
2,4-DCP	535	1.06 (0.75, 1.50)	0.73	556	1.05 (0.76, 1.47)	0.76	425	1.07 (0.68, 1.68)	0.76
2,5-DCP	534	1.02 (0.72, 1.45)	0.90	556	1.15 (0.83, 1.60)	0.39	427	1.06 (0.72, 1.56)	0.78
BPA	529	0.85 (0.59, 1.24)	0.40	558	1.22 (0.86, 1.72)	0.26	425	0.88 (0.56, 1.40)	0.60
BPF[a]	446	0.69 (0.36, 1.32)	0.26	446	0.69 (0.36, 1.32)	0.26	446	0.69 (0.36, 1.32)	0.26
BPS	370	1.49 (0.99, 2.25)	0.06*	396	1.33 (0.88, 2.01)	0.18	299	1.46 (0.86, 2.49)	0.16
BP-3	533	1.34 (0.96, 1.89)	0.09*	555	1.52 (1.09, 2.13)	0.01**	425	1.00 (0.67, 1.51)	0.98
TCS	531	1.26 (0.81, 1.96)	0.31	557	0.77 (0.49, 1.22)	0.27	430	1.05 (0.61, 1.82)	0.85
TCC	374	0.99 (0.55, 1.76)	0.97	395	1.10 (0.63, 1.91)	0.73	299	0.73 (0.37, 1.43)	0.36
EPB[a]	492	1.25 (0.68, 2.30)	0.47	492	1.25 (0.68, 2.30)	0.47	492	1.25 (0.68, 2.30)	0.47
MPB	532	1.02 (0.69, 1.50)	0.94	557	1.03 (0.69, 1.54)	0.88	430	0.99 (0.62, 1.56)	0.95
BPB	527	0.88 (0.58, 1.35)	0.56	557	1.08 (0.72, 1.61)	0.72	428	1.21 (0.75, 1.95)	0.43
PPB	532	1.09 (0.70, 1.70)	0.69	557	1.22 (0.81, 1.86)	0.34	430	0.91 (0.53, 1.55)	0.73

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: Ethyl-paraben; MPB: Methyl-paraben; BPB: Butyl-paraben; PPB: Propyl-paraben

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking.

[b] Categorical variable.

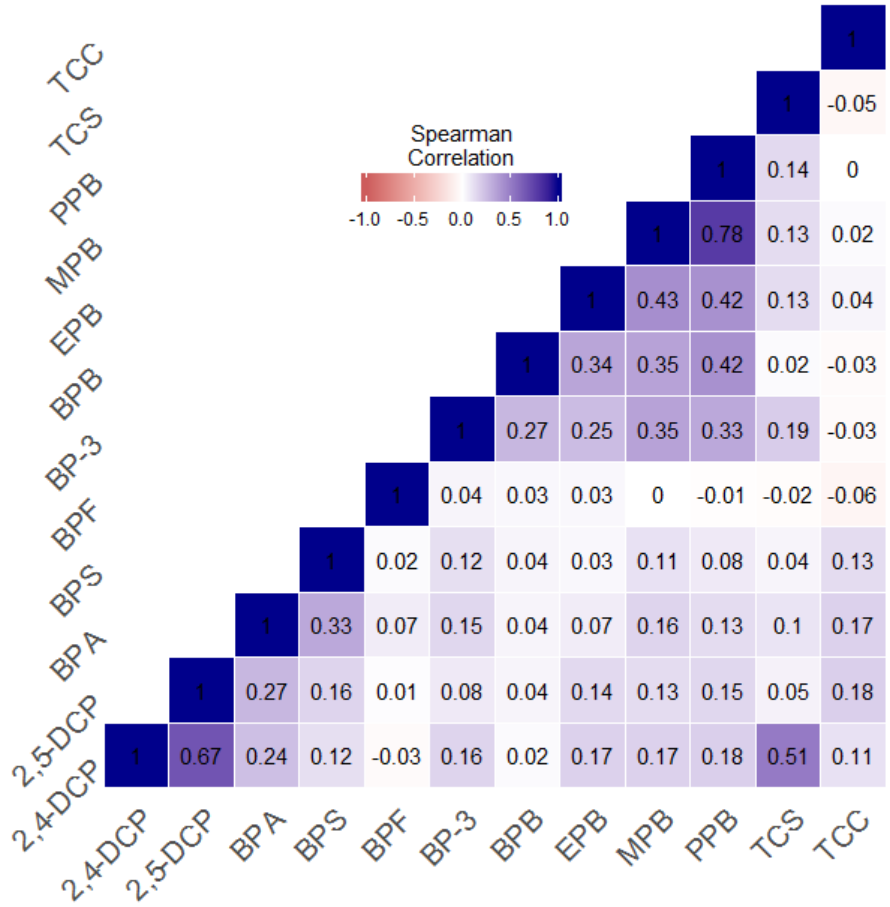


Figure IV.2: Heat map of Spearman correlation coefficients of specific gravity corrected average triclocarban, phenol and paraben urinary biomarkers

Table IV.7: Comparison of main effect estimates and p values of the two gestational length variables calculated as a sensitivity analysis.

	Untransformed GA	**Transformed GA**

	N	β	p-value	β	p-value
2,4-dichlorophenol	748	0.01	0.92	-0.004	0.71
2,5-dichlorophenol	749	-0.03	0.46	0.001	0.87
BPA	748	0.14	0.17	-0.021	0.23
BPS	540	-0.06	0.53	0.008	0.62
BPF	500	-0.14	0.15	0.021	0.20
BP-3	749	0.11	0.01**	-0.018	0.01**
Triclosan	749	0.0005	0.90	0.001	0.85
Triclocarban	544	-0.07	0.08*	0.011	0.09*
Ethyl-paraben	550	-0.01	0.82	0.003	0.70
Methyl-paraben	751	0.12	0.01**	-0.015	0.06*
Butyl-paraben	746	0.02	0.52	-0.002	0.77
Propyl-paraben	752	0.11	0.01**	-0.015	0.03**

Table IV.8: Comparing MLR results using continuous BPF and ethyl-paraben variables versus categorical BPF and ethyl-paraben variables

		Continuous Variable		**Categorical Variable**	
BPF	**Average**	-1.19 (-2.80, 0.42)	0.15	0.06 (-0.28, 0.41)	0.72
	Visit 1	-1.20 (-2.61, 0.20)	0.09	0.12 (-0.27, 0.51)	0.55
	Visit 2	0.42 (-0.84, 1.69)	0.51	-0.22 (-0.62, 0.18)	0.28
	Visit 3	-1.18 (-2.75, 0.40)	0.14	0.24 (-0.19, 0.68)	0.27
Ethyl-paraben	**Average**	-0.23 (-2.15, 1.69)	0.82	-0.11 (-0.44, 0.23)	0.53
	Visit 1	0.61 (-1.20, 2.42)	0.51	-0.20 (-0.55, 0.16)	0.28
	Visit 2	-0.86 (-2.40, 0.68)	0.28	0.06 (-0.3, 0.41)	0.75
	Visit 3	0.32 (-1.31, 1.95)	0.70	-0.12 (-0.51, 0.26)	0.53

Table IV.9: Beta coefficients of MLR and logistic regression models regressing birth outcomes against urinary exposure biomarkers with the addition of an interaction term between fetal sex and the urinary exposure biomarker

Gestational Age

	N	Main effect estimate	p	Fetal sex effect estimate	p	Interaction effect estimate	p
2,4-DCP	687	-0.058	0.54	-0.09	0.51	0.172	0.18
2,5-DCP	688	-0.019	0.78	-0.104	0.70	0.012	0.90
BPA	687	0.21	0.15	-0.026	0.89	-0.045	0.80
BPF[a]	451	-0.112	0.44	-0.047	0.88	0.05	0.80
BPS	488	0.059	0.68	-0.255	0.26	-0.133	0.49
BP-3	688	0.075	0.19	-0.141	0.66	0.025	0.75
TCS	688	-0.079	0.12	-0.669	0.01	0.194	0.006**
TCC	492	-0.034	0.57	-0.116	0.54	-0.044	0.56
EPB[a]	498	-0.072	0.34	-0.267	0.16	0.102	0.30
MPB	690	0.126	0.07	-0.04	0.93	-0.006	0.95
BPB	685	0.048	0.41	-0.091	0.55	-0.038	0.63
PPB	691	0.124	0.03	-0.058	0.82	-0.006	0.94

Birth Weight

	N	Main effect estimate	p	Fetal sex effect estimate	p	Interaction effect estimate	p
2,4-DCP	683	0.032	0.57	0.016	0.85	-0.080	0.29
2,5-DCP	684	0.051	0.21	0.193	0.24	-0.073	0.18
BPA	683	0.018	0.84	-0.004	0.97	0.007	0.95
BPF[a]	449	-0.140	0.39	-0.030	0.82	0.104	0.63
BPS	486	0.069	0.43	0.044	0.75	0.012	0.92
BP-3	684	0.053	0.13	0.122	0.53	-0.033	0.48
TCS	684	-0.055	0.07	-0.138	0.38	0.048	0.26
TCC	490	0.007	0.85	0.034	0.77	0.000	1.00
EPB[a]	496	-0.219	0.18	-0.070	0.55	0.302	0.16
MPB	686	-0.009	0.84	0.060	0.82	-0.014	0.80
BPB	681	0.014	0.69	0.018	0.84	0.016	0.74
PPB	687	-0.006	0.87	-0.017	0.91	0.006	0.90

Small for Gestational Age

	N	Main effect estimate	p	Fetal sex effect estimate	p	Interaction effect estimate	p
2,4-DCP	683	0.052	0.78	0.037	0.89	0.235	0.33
2,5-DCP	684	-0.118	0.42	-0.631	0.23	0.285	0.11
BPA	683	0.045	0.87	0.205	0.57	-0.106	0.76
BPF[a]	449	0.345	0.50	0.243	0.57	-0.388	0.56
BPS	486	0.124	0.65	0.060	0.89	0.100	0.78
BP-3	684	-0.097	0.42	-0.239	0.70	0.107	0.50
TCS	684	0.243	0.01	0.636	0.27	-0.141	0.30
TCC	490	-0.117	0.32	-0.099	0.77	0.069	0.64
EPB[a]	496	1.069	0.03	0.409	0.32	-1.092	0.10
MPB	686	-0.108	0.44	0.643	0.41	-0.125	0.49
BPB	681	-0.341	0.02	0.401	0.27	0.224	0.22
PPB	687	-0.114	0.33	0.290	0.52	-0.062	0.68

Large for Gestational Age

	N	Main effect estimate	p	Fetal sex effect estimate	p	Interaction effect estimate	p
2,4-DCP	683	0.032	0.85	-0.193	0.46	-0.067	0.78
2,5-DCP	684	0.108	0.36	0.093	0.86	-0.111	0.50
BPA	683	-0.292	0.28	-0.449	0.19	0.412	0.23
BPF[a]	449	0.100	0.83	0.050	0.90	-0.622	0.35
BPS	486	0.389	0.12	-0.175	0.64	-0.092	0.79
BP-3	684	0.159	0.10	-0.190	0.76	-0.006	0.96
TCS	684	-0.082	0.38	-0.320	0.51	0.049	0.72
TCC	490	0.085	0.41	-0.036	0.92	-0.089	0.51
EPB[a]	496	0.315	0.49	-0.004	0.99	-0.342	0.59
MPB	686	-0.038	0.77	-0.449	0.59	0.061	0.74
BPB	681	-0.107	0.34	-0.039	0.89	0.182	0.21
PPB	687	-0.033	0.76	-0.525	0.29	0.121	0.42

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: ethyl-paraben; MPB: Methyl-paraben; BPB: Butyl-paraben; PPB: Propyl-paraben

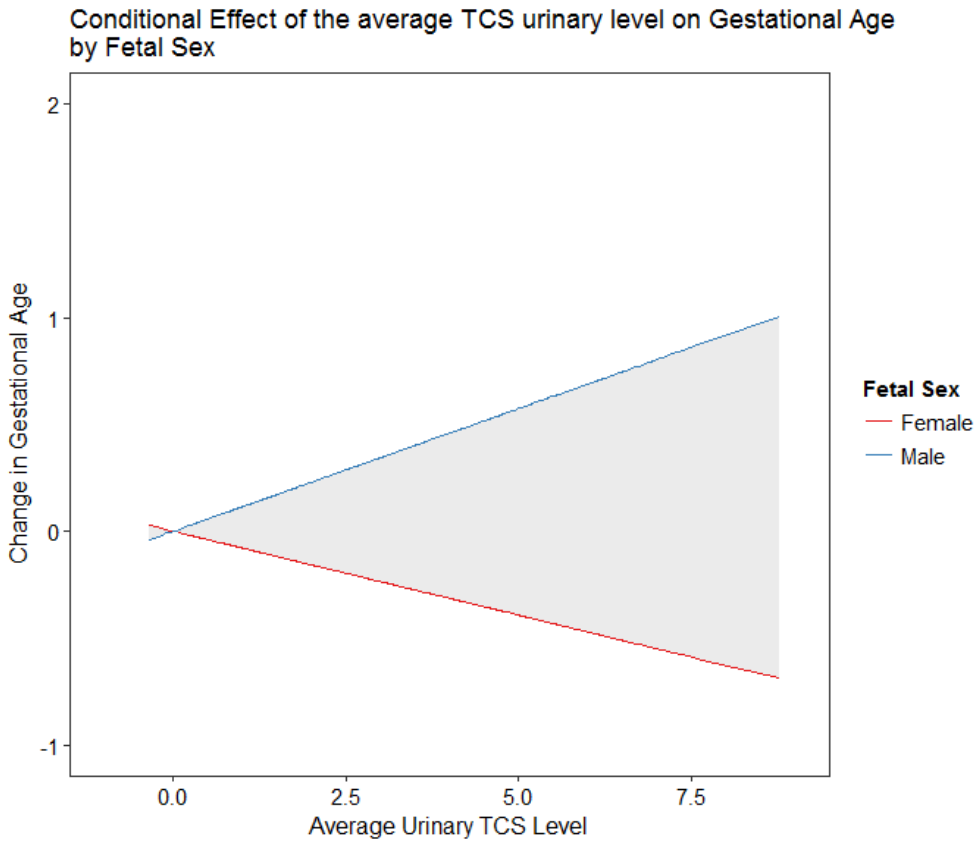


Figure IV.3: Conditional effect of the average triclosan concentration across the three study visits on gestational length, stratified by fetal sex.

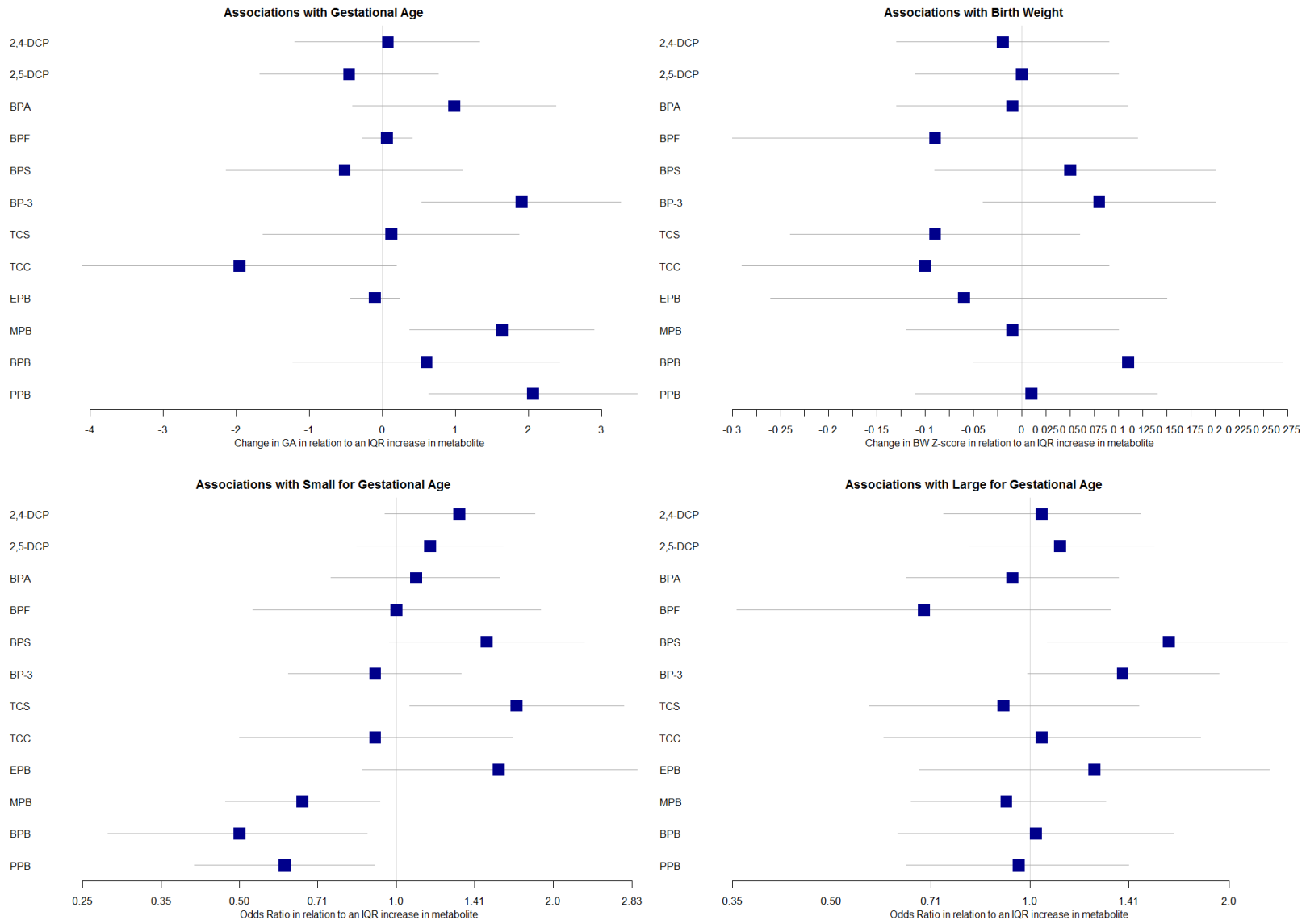


Figure IV.4: Summary results of models regressing birth outcomes against average phenol, paraben and triclocarban.

References

[1] L. Liu, H.L. Johnson, S. Cousens, J. Perin, S. Scott, J.E. Lawn, I. Rudan, H. Campbell, R. Cibulskis, M. Li, C. Mathers, R.E. Black, Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000, The Lancet. 379 (2012) 2151–2161. doi:10.1016/S0140-6736(12)60560-1.

[2] H. Blencowe, S. Cousens, D. Chou, M. Oestergaard, L. Say, A.-B. Moller, M. Kinney, J. Lawn, Born Too Soon: The global epidemiology of 15 million preterm births, Reprod. Health. 10 (2013) S2. doi:10.1186/1742-4755-10-S1-S2.

[3] Centers for Disease Control and Prevention, Preterm Birth | Maternal and Infant Health | Reproductive Health | CDC, Preterm Birth. (2015). http://www.cdc.gov/reproductivehealth/maternalinfanthealth/pretermbirth.htm (accessed February 8, 2016).

[4] T.M. Luu, S.L. Katz, P. Leeson, B. Thébaud, A.-M. Nuyt, Preterm birth: risk factor for early-onset chronic diseases, CMAJ Can. Med. Assoc. J. J. Assoc. Medicale Can. (2015). doi:10.1503/cmaj.150450.

[5] N. Marlow, D. Wolke, M.A. Bracewell, M. Samara, Neurologic and Developmental Disability at Six Years of Age after Extremely Preterm Birth, N. Engl. J. Med. 352 (2005) 9–19. doi:10.1056/NEJMoa041367.

[6] M.C. McCormick, The contribution of low birth weight to infant mortality and childhood morbidity, N. Engl. J. Med. 312 (1985) 82–90. doi:10.1056/NEJM198501103120204.

[7] R. Valdez, M.A. Athens, G.H. Thompson, B.S. Bradshaw, M.P. Stern, Birthweight and adult health outcomes in a biethnic population in the USA, Diabetologia. 37 (1994) 624–631.

[8] C.N. Schoen, S. Tabbah, J.D. Iams, A.B. Caughey, V. Berghella, Why the United States preterm birth rate is declining, Am. J. Obstet. Gynecol. 213 (2015) 175–180. doi:10.1016/j.ajog.2014.12.011.

[9] B.E. Hamilton, J.A. Martin, M.J.K. Osterman, S.C. Curtin, T.J. Matthews, Births: Final Data for 2014, Natl. Vital Stat. Rep. Cent. Dis. Control Prev. Natl. Cent. Health Stat. Natl. Vital Stat. Syst. 64 (2015) 1–64.

[10] A.B. Caughey, N.E. Stotland, A.E. Washington, G.J. Escobar, Maternal and obstetric complications of pregnancy are associated with increasing gestational age at term, Am. J. Obstet. Gynecol. 196 (2007) 155.e1-6. doi:10.1016/j.ajog.2006.08.040.

[11] M. Galal, I. Symonds, H. Murray, F. Petraglia, R. Smith, Postterm pregnancy, Facts Views Vis. ObGyn. 4 (2012) 175–187.

[12] J.A. Martin, B.E. Hamilton, P.D. Sutton, S.J. Ventura, F. Menacker, S. Kirmeyer, M.L. Munson, Centers for Disease Control and Prevention National Center for Health Statistics National Vital Statistics System, Births: final data for 2005, Natl. Vital Stat. Rep. Cent. Dis. Control Prev. Natl. Cent. Health Stat. Natl. Vital Stat. Syst. 56 (2007) 1–103.

[13] B.M. Casey, Subclinical Hypothyroidism and Pregnancy:, Obstet. Gynecol. Surv. 61 (2006) 415–420. doi:10.1097/01.ogx.0000223331.51424.9b.

[14] V.D. Castracane, Endocrinology of preterm labor, Clin. Obstet. Gynecol. 43 (2000) 717–726.

[15] M.W. Kemp, Preterm Birth, Intrauterine Infection, and Fetal Inflammation, Front. Immunol. 5 (2014). doi:10.3389/fimmu.2014.00574.

[16] P. Kumar, N. Magon, Hormones in pregnancy, Niger. Med. J. J. Niger. Med. Assoc. 53 (2012) 179. doi:10.4103/0300-1652.107549.
[17] R. Smith, J.I. Smith, X. Shen, P.J. Engel, M.E. Bowman, S.A. McGrath, A.M. Bisits, P. McElduff, W.B. Giles, D.W. Smith, Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and the onset of human labor, J. Clin. Endocrinol. Metab. 94 (2009) 2066–2074. doi:10.1210/jc.2008-2257.
[18] A.M. Aker, D.J. Watkins, L.E. Johns, K.K. Ferguson, O.P. Soldin, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women, Environ. Res. 151 (2016) 30–37. doi:10.1016/j.envres.2016.07.002.
[19] M. Boas, K.M. Main, U. Feldt-Rasmussen, Environmental chemicals and thyroid function: an update, Curr. Opin. Endocrinol. Diabetes Obes. 16 (2009) 385–391. doi:10.1097/MED.0b013e3283305af7.
[20] L. Kolatorova, J. Vitku, R. Hampl, K. Adamcova, T. Skodova, M. Simkova, A. Parizek, L. Starka, M. Duskova, Exposure to bisphenols and parabens during pregnancy and relations to steroid changes, Environ. Res. 163 (2018) 115–122. doi:10.1016/j.envres.2018.01.031.
[21] K. Neier, E.H. Marchlewicz, D.C. Dolinoy, V. Padmanabhan, Assessing Human Health Risk to Endocrine Disrupting Chemicals: a Focus on Prenatal Exposures and Oxidative Stress, Endocr. Disruptors Austin Tex. 3 (2015). doi:10.1080/23273747.2015.1069916.
[22] D.J. Watkins, K.K. Ferguson, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico, Int. J. Hyg. Environ. Health. 218 (2015) 212–219. doi:10.1016/j.ijheh.2014.11.001.
[23] E.S. Koeppe, K.K. Ferguson, J.A. Colacino, J.D. Meeker, Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007-2008, Sci. Total Environ. 445–446 (2013) 299–305. doi:10.1016/j.scitotenv.2012.12.052.
[24] K.K. Ferguson, J.D. Meeker, D.E. Cantonwine, B. Mukherjee, G.G. Pace, D. Weller, T.F. McElrath, Environmental phenol associations with ultrasound and delivery measures of fetal growth, Environ. Int. 112 (2018) 243–250. doi:10.1016/j.envint.2017.12.011.
[25] T.H. Lassen, H. Frederiksen, H.B. Kyhl, S.H. Swan, K.M. Main, A.-M. Andersson, D.V. Lind, S. Husby, C. Wohlfahrt-Veje, N.E. Skakkebæk, T.K. Jensen, Prenatal Triclosan Exposure and Anthropometric Measures including Anogenital Distance in Danish Infants, Environ. Health Perspect. (2016). doi:10.1289/ehp.1409637.
[26] C. Philippat, J. Botton, A.M. Calafat, X. Ye, M.-A. Charles, R. Slama, EDEN Study Group, Prenatal exposure to phenols and growth in boys, Epidemiol. Camb. Mass. 25 (2014) 625–635. doi:10.1097/EDE.0000000000000132.
[27] R. Tang, M.-J. Chen, G.-D. Ding, X.-J. Chen, X.-M. Han, K. Zhou, L.-M. Chen, Y.-K. Xia, Y. Tian, X.-R. Wang, Associations of prenatal exposure to phenols with birth outcomes, Environ. Pollut. Barking Essex 1987. 178 (2013) 115–120. doi:10.1016/j.envpol.2013.03.023.
[28] M.S. Wolff, S.M. Engel, G.S. Berkowitz, X. Ye, M.J. Silva, C. Zhu, J. Wetmur, A.M. Calafat, Prenatal phenol and phthalate exposures and birth outcomes, Environ. Health Perspect. 116 (2008) 1092–1097. doi:10.1289/ehp.11007.

[29] J.R. Rochester, A.L. Bolden, Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes, Environ. Health Perspect. 123 (2015) 643–650. doi:10.1289/ehp.1408989.
[30] 2017 Premature Birth Report Card: Puerto Rico, March of Dimes, 2017. https://www.marchofdimes.org/peristats/tools/reportcard.aspx?frmodrc=1&reg=72 (accessed July 24, 2018).
[31] D.E. Cantonwine, J.F. Cordero, L.O. Rivera-González, L.V. Anzalota Del Toro, K.K. Ferguson, B. Mukherjee, A.M. Calafat, N. Crespo, B. Jiménez-Vélez, I.Y. Padilla, A.N. Alshawabkeh, J.D. Meeker, Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: Distribution, temporal variability, and predictors, Environ. Int. 62 (2014) 1–11. doi:10.1016/j.envint.2013.09.014.
[32] J.D. Meeker, D.E. Cantonwine, L.O. Rivera-González, K.K. Ferguson, B. Mukherjee, A.M. Calafat, X. Ye, L.V. Anzalota Del Toro, N. Crespo-Hernández, B. Jiménez-Vélez, A.N. Alshawabkeh, J.F. Cordero, Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico, Environ. Sci. Technol. 47 (2013) 3439–3447. doi:10.1021/es400510g.
[33] P.A. Harris, R. Taylor, R. Thielke, J. Payne, N. Gonzalez, J.G. Conde, Research Electronic Data Capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support, J. Biomed. Inform. 42 (2009) 377–381. doi:10.1016/j.jbi.2008.08.010.
[34] X. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 383 (2005) 638–644. doi:10.1007/s00216-005-0019-4.
[35] X. Ye, A.M. Bishop, J.A. Reidy, L.L. Needham, A.M. Calafat, Parabens as Urinary Biomarkers of Exposure in Humans, Environ. Health Perspect. 114 (2006) 1843–1846. doi:10.1289/ehp.9413.
[36] R.W. Hornung, L.D. Reed, Estimation of Average Concentration in the Presence of Nondetectable Values, Appl. Occup. Environ. Hyg. 5 (1990) 46–51. doi:10.1080/1047322X.1990.10389587.
[37] Committee on Obstetric Practice, the American Institute of Ultrasound in Medicine, and the Society for Maternal-Fetal Medicine, Committee Opinion No 700: Methods for Estimating the Due Date, Obstet. Gynecol. 129 (2017) e150–e154. doi:10.1097/AOG.0000000000002046.
[38] J. Villar, L.C. Ismail, C.G. Victora, E.O. Ohuma, E. Bertino, D.G. Altman, A. Lambert, A.T. Papageorghiou, M. Carvalho, Y.A. Jaffer, M.G. Gravett, M. Purwar, I.O. Frederick, A.J. Noble, R. Pang, F.C. Barros, C. Chumlea, Z.A. Bhutta, S.H. Kennedy, International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project, The Lancet. 384 (2014) 857–868. doi:10.1016/S0140-6736(14)60932-6.
[39] Fact Sheet Puerto Rico: Fast Facts, The Henry J. Kaiser Family Foundation, Menlo Park, CA, 2017. http://files.kff.org/attachment/Fact-Sheet-Puerto-Rico-Fast-Facts (accessed July 24, 2018).
[40] C. Philippat, M. Mortamais, C. Chevrier, C. Petit, A.M. Calafat, X. Ye, M.J. Silva, C. Brambilla, I. Pin, M.-A. Charles, S. Cordier, R. Slama, Exposure to phthalates and

phenols during pregnancy and offspring size at birth, Environ. Health Perspect. 120 (2012) 464–470. doi:10.1289/ehp.1103634.
[41] M. Krause, A. Klit, M. Blomberg Jensen, T. Søeborg, H. Frederiksen, M. Schlumpf, W. Lichtensteiger, N.E. Skakkebaek, K.T. Drzewiecki, Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters, Int. J. Androl. 35 (2012) 424–436. doi:10.1111/j.1365-2605.2012.01280.x.
[42] M.T. Aung, K.K. Ferguson, D.E. Cantonwine, K.M. Bakulski, B. Mukherjee, R. Loch-Caruso, T.F. McElrath, J.D. Meeker, Associations between maternal plasma measurements of inflammatory markers and urinary levels of phenols and parabens during pregnancy: A repeated measures study, Sci. Total Environ. 650 (2019) 1131–1140. doi:10.1016/j.scitotenv.2018.08.356.
[43] L.A. Geer, B.F.G. Pycke, J. Waxenbaum, D.M. Sherer, O. Abulafia, R.U. Halden, Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York, J. Hazard. Mater. 323, Part A (2017) 177–183. doi:10.1016/j.jhazmat.2016.03.028.
[44] C. Wu, W. Huo, Y. Li, B. Zhang, Y. Wan, T. Zheng, A. Zhou, Z. Chen, M. Qian, Y. Zhu, Y. Jiang, H. Liu, J. Hu, X. Chen, B. Xu, W. Xia, S. Xu, Maternal urinary paraben levels and offspring size at birth from a Chinese birth cohort, Chemosphere. 172 (2017) 29–36. doi:10.1016/j.chemosphere.2016.12.131.
[45] T.T.B. Vo, E.-M. Jung, K.-C. Choi, F.H. Yu, E.-B. Jeung, Estrogen receptor α is involved in the induction of Calbindin-D(9k) and progesterone receptor by parabens in GH3 cells: a biomarker gene for screening xenoestrogens, Steroids. 76 (2011) 675–681. doi:10.1016/j.steroids.2011.03.006.
[46] Y. Watanabe, H. Kojima, S. Takeuchi, N. Uramaru, S. Ohta, S. Kitamura, Comparative study on transcriptional activity of 17 parabens mediated by estrogen receptor α and β and androgen receptor, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 57 (2013) 227–234. doi:10.1016/j.fct.2013.03.036.
[47] A.M. Wróbel, E.Ł. Gregoraszczuk, Actions of methyl-, propyl- and butylparaben on estrogen receptor-α and -β and the progesterone receptor in MCF-7 cancer cells and non-cancerous MCF-10A cells, Toxicol. Lett. 230 (2014) 375–381. doi:10.1016/j.toxlet.2014.08.012.
[48] C. Taxvig, K. Dreisig, J. Boberg, C. Nellemann, A.B. Schelde, D. Pedersen, M. Boergesen, S. Mandrup, A.M. Vinggaard, Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARγ activation, Mol. Cell. Endocrinol. 361 (2012) 106–115. doi:10.1016/j.mce.2012.03.021.
[49] A. Veiga-Lopez, K. Kannan, C. Liao, W. Ye, S.E. Domino, V. Padmanabhan, Gender-Specific Effects on Gestational Length and Birth Weight by Early Pregnancy BPA Exposure, J. Clin. Endocrinol. Metab. 100 (2015) E1394-1403. doi:10.1210/jc.2015-1724.
[50] D.E. Cantonwine, K.K. Ferguson, B. Mukherjee, T.F. McElrath, J.D. Meeker, Urinary Bisphenol A Levels during Pregnancy and Risk of Preterm Birth, Environ. Health Perspect. 123 (2015) 895–901. doi:10.1289/ehp.1408126.
[51] F. Behnia, M. Peltier, D. Getahun, C. Watson, G. Saade, R. Menon, High bisphenol A (BPA) concentration in the maternal, but not fetal, compartment increases the risk of spontaneous preterm delivery, J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat.

Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. (2016) 1–7. doi:10.3109/14767058.2016.1139570.
[52] B. Weinberger, A.M. Vetrano, F.E. Archer, S.W. Marcella, B. Buckley, D. Wartenberg, M.G. Robson, J. Klim, S. Azhar, S. Cavin, L. Wang, D.Q. Rich, Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age, J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. 27 (2014) 323–327. doi:10.3109/14767058.2013.815718.
[53] G. Ding, C. Wang, A. Vinturache, S. Zhao, R. Pan, W. Han, L. Chen, W. Wang, T. Yuan, Y. Gao, Y. Tian, Prenatal low-level phenol exposures and birth outcomes in China, Sci. Total Environ. 607–608 (2017) 1400–1407. doi:10.1016/j.scitotenv.2017.07.084.
[54] S.H. Cho, Y. Choi, S.H. Kim, S.J. Kim, J. Chang, Urinary bisphenol A versus serum bisphenol A concentration and ovarian reproductive outcomes among IVF patients: Which is a better biomarker of BPA exposure?, Mol. Cell. Toxicol. 13 (2017) 351–359. doi:10.1007/s13273-017-0039-0.
[55] Y. Wan, W. Huo, S. Xu, T. Zheng, B. Zhang, Y. Li, A. Zhou, Y. Zhang, J. Hu, Y. Zhu, Z. Chen, S. Lu, C. Wu, M. Jiang, Y. Jiang, H. Liu, X. Yang, W. Xia, Relationship between maternal exposure to bisphenol S and pregnancy duration, Environ. Pollut. Barking Essex 1987. 238 (2018) 717–724. doi:10.1016/j.envpol.2018.03.057.
[56] T.M. Etzel, A.M. Calafat, X. Ye, A. Chen, B.P. Lanphear, D.A. Savitz, K. Yolton, J.M. Braun, Urinary triclosan concentrations during pregnancy and birth outcomes, Environ. Res. 156 (2017) 505–511. doi:10.1016/j.envres.2017.04.015.
[57] W. Huo, W. Xia, C. Wu, Y. Zhu, B. Zhang, Y. Wan, A. Zhou, Z. Qian, Z. Chen, Y. Jiang, H. Liu, J. Hu, B. Xu, S. Xu, Y. Li, Urinary level of triclosan in a population of Chinese pregnant women and its association with birth outcomes, Environ. Pollut. Barking Essex 1987. 233 (2018) 872–879. doi:10.1016/j.envpol.2017.08.073.
[58] F. Ouyang, N. Tang, H.-J. Zhang, X. Wang, S. Zhao, W. Wang, J. Zhang, W. Cheng, Maternal urinary triclosan level, gestational diabetes mellitus and birth weight in Chinese women, Sci. Total Environ. 626 (2018) 451–457. doi:10.1016/j.scitotenv.2018.01.102.
[59] P. Ashrap, D.J. Watkins, A.M. Calafat, X. Ye, Z. Rosario, P. Brown, C.M. Vélez-Vega, A. Alshawabkeh, J.F. Cordero, J.D. Meeker, Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends, Environ. Int. (2018). doi:10.1016/j.envint.2018.08.020.
[60] X. Ye, L.-Y. Wong, P. Dwivedi, X. Zhou, T. Jia, A.M. Calafat, Urinary Concentrations of the Antibacterial Agent Triclocarban in United States Residents: 2013–2014 National Health and Nutrition Examination Survey, Environ. Sci. Technol. 50 (2016) 13548–13554. doi:10.1021/acs.est.6b04668.
[61] J.R. Rochester, A.L. Bolden, K.E. Pelch, C.F. Kwiatkowski, Potential Developmental and Reproductive Impacts of Triclocarban: A Scoping Review, J. Toxicol. 2017 (2017). doi:10.1155/2017/9679738.

CHAPTER V. Interactions between Chemicals and Non-Chemical Stressors: The Modifying Effect of Life Events on the Association between Triclocarban, Phenols and Parabens with Gestational Length

Abstract

Background: Phenols, parabens and triclocarban are common additives to consumer products. There is evidence of adverse birth outcomes in association with prenatal exposure to phenols, parabens and triclocarban, as well as with psychosocial factors. Therefore, we examined the modifying effect of psychosocial stress on the association between chemicals and gestational length in 922 women among a cohort of pregnant women in Puerto Rico. Methods: Urinary triclocarban, phenols and parabens were measured at up to three time points in pregnancy (visit 1: 16-20 weeks, visit 2: 20-24 weeks and visit 3: 24-28 weeks gestation). Four measures of psychosocial stress were collected to score depression, perceived stress, social support and life events. Only the life events score (LES) was a significant modifier. Multiple linear regression (MLR) models were conducted to investigate the association between gestational length and the interaction between each woman's centered log average concentrations of exposure biomarkers and LES scores. MLR models regressing the exposure biomarkers in relation to gestational length were also stratified by Life Experiences Survey (LES), Negative LES and Positive LES scores, as well as by fetal sex. Results were transformed into the change in gestational length for an inter-quartile-range difference in the exposure. Results: Bisphenol-A, methylparaben and propyl paraben were associated with increase in gestational length, and triclocarban was associated with a decrease in gestational age. Associations between triclocarban, bisphenol S, methyl- and propyl-paraben in relation to gestational length were stronger among women with

negative Total LES scores. Among women with negative Total LES scores, bisphenol S and triclocarban were associated with a 3-5 day decrease in gestational length [(-3.15; 95% CI:-6.06, -0.24); (-4.68; 95% CI: -8.47, -0.89)], whereas methylparaben and propylparaben were associated with a 2-3 day increase in gestational length [(2.21; 95% CI: 0.02, 4.40); (2.92; 95% CI: 0.58, 5.26)]. Most interactions were driven by negative life events, but the association with triclocarban was driven by a lack of positive life events. Conclusions: Associations between exposure biomarkers and gestational length were stronger in the presence of negative life events. This provides evidence to the theory that stress makes the body more vulnerable to chemical exposure. Further research is required to substantiate these results.

Introduction

Phenols and parabens are a group of chemicals commonly found in personal care products and household items, and exposure to these chemicals has been associated with a number of growth parameters at birth, including birth weight, birth length and gestational length [1–5]. We previously reported on associations between bisphenols, parabens and triclocarban with various birth outcomes in this cohort of pregnant women in Puerto Rico [6]. We found an increase in gestational length in association with benzophenone-3, bisphenol-A (BPA), and methyl- and propyl-paraben, and a decrease in gestational length in association with triclocarban. Bisphenol-S (BPS) was associated with changes in birth size, depending on the timing of exposure.

Maternal stress has also been associated with adverse birth outcomes. A population-based case control study reported a 60% increased odds of a very low birth weight infant among women who reported always feeling stressed as compared to women reporting no stress [7]. Likewise, other studies have reported a shortened gestational length with maternal stress or a larger number of negative life events [8–11]. The effects of maternal stress on the fetus also extend beyond pregnancy, and potentially cause long-term adverse effects on the cognitive, behavioral and psychomotor development of the child [12,13].

There is a growing interest in looking at the combined effect of stressors in the environment on human health. In addition to exploring the effect of chemical mixtures on health, interactions between chemical and non-chemical stressors are important to consider in deepening our understanding of how the environment impacts humans. Although this area of research is

relatively new and limited, evidence supports the notion of an increased risk of adverse effects with exposure to high concentrations of environmental chemicals and the modifying effect of high psychosocial stress levels. For example, a systematic review found the effect of chemical exposures, such as smoking and traffic pollution, in combination with high stress or socio-economic stressors was associated with worse fetal growth parameters than with any of the chemical or socio-economic stressors on their own [14].

As a follow-up to our previous study, we examined the potential modifying effect of maternal stress on our associations between biomarkers of several common chemical exposures and gestational age. We tested this question using the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) project, a multi-disciplinary research center in Northern Puerto Rico that established a pregnancy cohort to investigate the role of environmental contamination in adverse birth outcomes. The analysis explored relationships between gestational length and the interaction between urinary concentrations of phenols, parabens and triclocarban, and psychosocial factors (depression, perceived stress, social support and life events). This Chapter focused on the exposure metabolites of interest based on our results from our previous analysis (benzophenone-3, BPA and BPS, triclocarban, methyl- and propyl-paraben).

Methods

Study Population

The details of the study population were previously described. Briefly, participants for this study were from the PROTECT cohort, an ongoing prospective cohort of pregnant women recruited from two hospitals and five affiliated health clinics in Puerto Rico. The present analysis includes 922 study participants recruited from 2011-2017 at 14 ± 2 weeks gestation. There were up to three study visits performed on each of the participants: visit 1 at 16-20 weeks gestation; visit 2 at 20-24 weeks gestation; and, visit at 24-28 weeks gestation. These study visits coincided with routine clinical visits and rapid fetal growth. All demographic data was collected at visit 1, and questionnaires on psychosocial status were administered at study visits 2 and 3 (further details found below). Spot urine samples for urinary exposure biomarker measurement were collected at each of the three study visits. Women were excluded from the study if they: lived outside the region, had multiple gestations, used oral contraceptives within three months of getting pregnant,

became pregnant through in vitro fertilization, or had any known medical health complications at time of recruitment (including diabetes, hypertension, etc.). This study was approved by the research and ethics committees of the University of Michigan School of Public Health, University of Puerto Rico, Northeastern University, and participating hospitals and clinics. All study participants provided full informed consent prior to participation.

Urinary Biomarker Measurement

Spot urine samples collected during the three study visits were divided into aliquots and frozen at -80^{o}C. They were shipped overnight in dry ice to the Centers for Disease C for analysis. Samples were analyzed for seven phenols (2,4-dichlorophenol, and 2,5-dichlorophenol, BPA, BPS, BPF, benzophenone-3, triclosan), four parabens (ethyl-paraben, methyl-paraben, butyl-paraben and propyl-paraben) and triclocarban using online solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry [15–17]. The sample size for BPS and triclocarban was smaller than for the other urinary biomarkers because they were added to the analytical panel mid-way through the study, and thus, only available on a subset of the cohort. Urinary specific gravity (SG) was measured using a digital handheld refractometer (AtagoCo., Ltd., Tokyo, Japan), and was used to account for urinary dilution. Samples below the limit of detection (LOD) were assigned a value of the LOD divided by $\sqrt{2}$ [18]. Urinary biomarkers were corrected for SG in preliminary analyses. For the purpose of clarity, this manuscript focuses on the exposure biomarkers associated with changes in birth outcomes from our previous analysis (Aker et al., 2018), which are benzophenone-3, BPA, BPS, triclocarban, methyl- and propyl-paraben.

Psychosocial Scores

The psychosocial status of study participants was determined via questionnaires. The questionnaire included questions from four instruments: 1) Center for Epidemiological Studies-Depression (CES-D) [19]; 2) Perceived Stress Scale (PSS) [20]; 3) ENRICHD Social Support Instrument (ESSI) [21]; and, 4) Life Experiences Survey (LES) [22]. The CES-D, PSS and ESSI instruments were administered at 28 weeks gestation in the clinic, whereas the LES questionnaire was administered during the in-home study visit (20-24 weeks gestation). All questions were translated and administered in Spanish. As described below, new categorical psychosocial scores were created for statistical analyses.

The CES-D is a 20-item score that asks participants how they felt in the past week to determine their depression status. A score of ≥16 for CES-D is typically used to determine depression [23]; therefore CES-D was categorized into scores greater than or equal to 16 or below 16.

The PSS is a 10-item score that aims to determine the participants' perceived stress levels during the previous month. There is no established cut-off for PSS. Therefore, we set the cut-off for PSS at the 75% percentile (score of 28, maximum=49). A cut-off of 28 was also used previously used in the literature [24].

ESSI is a 7-item score that asks respondents how they currently feel with regards to the social support that they have. There is no established cut-off for ESSI; however, two recent studies used a cut-off of 18 for the ESSI score to define low social support [25,26]; therefore we used the same cut-off for our analyses. This population reported very high scores of social support, so a cut off of 18 also allowed us to establish a mathematically meaningful lower social support group.

LES is a 38-item score that asks participants whether they experienced a list of events since becoming pregnant, and allows participants to rate how positive or negative they perceived the event to be on a scale of -3 to 3. The LES was categorized into three groups to take into account the negative and positive scores. The cut-off points were below -1, between -1 and 1, and above 1 (labelled "negative", "neutral" and "positive"). Two more scores were calculated from the LES score. A Positive LES score was calculated to include only life events scored > 0, and a Negative LES score was calculated to include only life events scored < 0. The categories for Positive and Negative LES scores were created to have a sufficient number of participants per group. The cut-offs were scores below 2, between 2 and 5, and above 5 (labelled "low", "medium", and "high").

Gestational Age Calculation

The American College of Gynecologists (ACOG) recommendations were used for the gestational length calculation of complete pregnancies [27]. In brief, ACOG recommends determining gestational length using either information from the first ultrasound or the last menstrual cycle date (LMP), depending on two cutoffs: 1) how far along the pregnancy the ultrasound was conducted, and 2) the difference in the estimated date of delivery (EDD) calculated from the LMP and from the ultrasound. Further details on this calculation can be found in the ACOG Committee Opinion document [27].

Statistical Analyses

A detailed description of the preliminary analyses conducted for exposure biomarkers, gestational length, and important covariates can be found in CHAPTER IV. Briefly, after distributions of key demographic characteristics were calculated, multiple linear regressions (MLR) were conducted to examine associations between gestational length and each average exposure biomarker. We re-ran the bivariate analyses on the potential covariates and urinary exposure biomarkers, stratified by Total LES scores. To compare the differences in frequencies of categorical variables across the LES categories, we used the Chi square test. The continuous variables (age and urinary exposure biomarkers) were not normally distributed; therefore, to compare the continuous variables in the groups, we calculated the Mann–Whitney U test. Following this, we conducted univariate analysis on the four psychosocial scores we had available. The mean, median and select percentiles were calculated for each of the scores. In addition, the frequency of each life event was calculated in the case of LES.

We constructed multiple linear regression (MLR) models to investigate associations between each categorical psychosocial score and gestational length. There was no evidence of non-linearity after the addition of penalized and smoothing splines, so we constructed MLR models to regress the interaction between each mean-centered log average urinary exposure biomarker concentration and each categorical psychosocial score in relation to gestational length. The average urinary exposure biomarker concentration was calculated using the urinary exposure biomarker concentrations across the three study visits. In the case of a missing value at one of the study visits, the average was taken of the two remaining concentrations. In the case of two missing concentrations, the "average" phenol or paraben concentration was equal to the available concentration. Crude analyses included only the average specific gravity as a covariate in addition to the urinary biomarker and psychosocial score. We maintained a covariate if it changed the main effect estimate by >10%. Final models were controlled for the following confounders: specific gravity, maternal age, insurance type, alcohol use, and self-reported exposure to second-hand smoking.

An interaction between a biomarker and a psychosocial parameter was considered of interest if the p value was less than 0.10. Only interactions with LES variables resulted in p values below 0.10; therefore, the remainder of the analyses focused on interactions between biomarkers of

exposure and the Total LES, Positive LES and Negative LES scores. To better interpret the interaction results, the MLR models were stratified by LES categories. To study the effect of infant sex, we further stratified the MLR models by infant sex. All effect estimates from MLR models were transformed to the change in gestational length in relation to the interquartile range (IQR) increase in urinary biomarker concentrations. The alpha level was set at 0.05 for stratified analysis. All statistical analyses were conducted in R Version 3.4.2.

As a sensitivity analysis, we included Positive LES scores as a covariate in models for Negative LES scores, and included Negative LES scores as a covariate in models for Positive LES scores. There were no differences in the results with the addition of these covariates. Second, we restricted our analysis to women with data from only one study visit, only two study visits, and all three study visits. The sample sizes in each group were too small to detect significant interactions, but the directions of the associations remained the same. Based on this, and the high intra-class correlation values, we proceeded with our calculation of the average exposure biomarker as described above.

Results

Our study population included a total of 908 pregnant women with gestational length data. Table V.1 presents demographic characteristics of the study population stratified by Total LES scores. The study population was on average 26 years of age; over half had BMI levels <25; 3% were current smokers; 11% were exposed to second-hand smoking for at least 1 hour a day; and, approximately 20% were single. Women with overall positive Total LES scores were generally younger (25 years versus 27 years) and less likely to have more than one more child, whereas women with overall negative Total LES scores were more likely to be current smokers (4.9% versus 2%).

Of the 38 life events included in the LES questionnaire, 14 life events were experienced by at least 10% of the study participants (Table V.2). Half of the participants experienced changes in eating and sleeping habits. Approximately 25% experienced mean negative changes in social and recreational activities, a mean negative change in financial status, and a mean positive change in personal achievements. Ten percent experienced an illness and/or death of a close family member. No psychosocial variables were associated with gestational length, and all p-values exceeded 0.50 (Table V.6). None of the interaction terms between any of the urinary biomarkers

and CES-D, PSS and ESSI were statistically significant (Table V.7). Therefore, the remainder of this manuscript focuses on the results of the interaction between phenol, paraben and triclocarban concentrations with Total LES, Positive LES and Negative LES on gestational length.

In a previous study, we observed 1-2 day increases in gestational length in relation to urinary benzophenone-3, BPA, methyl-paraben and propyl-paraben, as well as a 2 day decrease in gestational length in relation to urinary triclocarban (Table V.3). **Error! Reference source not found.** shows the results of the MLR models regressing these urinary exposure biomarkers against gestational length when stratified by Total LES categories. There was a general linear trend across the Total LES categories such that the strongest associations between the urinary biomarker and gestational length were among women with negative Total LES scores, which the exception of BPA where no trends emerged. Among women with overall positive Total LES scores, associations between the urinary biomarkers and gestational length were largely not statistically significant (Table V.8). When treated as an ordinal variable, the interaction between Total LES and BPS, triclocarban and propylparaben was statistically significant (Table V.7). BPS and triclocarban were associated with a 3-5 day decrease in gestational length among women with negative Total LES scores [(-3.15 change in gestational length days per IQR increase in phenol or paraben (Δ); 95% CI:-6.06, -0.24); (Δ -4.68; 95% CI: -8.47, -0.89), respectively], whereas methylparaben and propylparaben were associated with a 2-3 day increase in gestational length among women with negative Total LES scores [(Δ 2.21; 95% CI: 0.02, 4.40); (Δ 2.92; 95% CI: 0.58, 5.26), respectively]. As compared to the neutral Total LES scores, the association between BPS and gestational length was suggestively different among women with negative LES scores (p value = 0.06). Although BPS was not independently associated with gestational length, the strength of the interaction between LES and BPS, as well as the linear trend observed across the Total LES categories lend confidence to these results. Nevertheless, these associations should be interpreted with caution. Associations between benzophenone-3 and gestational length remained unchanged across any of the LES categories (not shown).

Figure V.2 shows the MLR results stratified by Negative LES scores. No clear patterns emerged, although associations between BPA, methylparaben and propylparaben and gestational length were strongest among women with Negative LES scores in the medium category. The association between BPS and gestational length was not statistically significant in any of the

Negative LES categories; however, the 2 day decrease in gestational length among women with medium Negative LES was statistically different compared to the change in gestational length among women in the low Negative LES category (p value = 0.08).

Figure V.3 shows the MLR results stratified by Positive LES scores. There was a strong association between triclocarban and reduced gestational length among women with low Positive LES scores (Δ -4.58; 95% CI: -7.70, -1.46). Given the lack of significant associations between triclocarban and gestational length when the results were stratified by Negative LES scores, this association could indicate that the relationship between triclocarban and gestational length among women with negative Total LES scores is driven by the lack of positive life events, rather than the presence of negative life events. Methylparaben and propylparaben (Spearman correlation = 0.78; Aker et al, 2018) were significantly associated with gestational length among women with low Positive LES scores; however, these associations were also observed among women with high Positive LES scores.

After stratification by fetal sex, the associations with parabens discussed above were only observed in girls, and had larger magnitudes (Table V.9). For example, propylparaben was associated with a 4.7 day increase in gestational length among women with negative Total LES scores and female pregnancies (Δ 4.66; 95% CI: 1.53, 7.80). On the other hand, associations with BPA and BPS were only observed among males. For example, BPS was associated with a 5-day decrease in gestational length among women with negative Total LES scores and male pregnancies (Δ-5.06; 95% CI: -9.4, -0.72), and BPA was associated with a 3 day increase in gestational length among women with medium Negative LES scores carrying males (Δ 3.43; 95% CI: 0.29, 6.57). However, the sample sizes for each group in this analysis were relatively small (60-90 women), so caution must be taken when interpreting these results.

Discussion

Women with negative Total LES scores had strong associations between some exposure biomarkers and gestational length, especially in the case of BPS, triclocarban, methylparaben and propylparaben. The inverse association between triclocarban and gestational length was strongest among women with low Positive LES scores, suggesting a protective effect of positive life events against the impact of triclocarban on pregnancy duration. Fetal sex may also add

another layer of complexity, given that associations with parabens were stronger among female pregnancies, and associations with bisphenols were stronger in male pregnancies.

Life events, a proxy for measuring maternal stress, have been linked to adverse birth outcomes [8–10,28–30]. However, in our population, LES scores were not associated with gestational length in adjusted models. This indicates that experiencing negative life events, and thus experiencing stress, may not be sufficient to illicit an adverse effect. It was only the combination of overall negative LES scores and higher concentrations of exposure biomarkers that we observe changes in gestational length of up to five days.

An allostatic load framework could help describe the potential biological mechanisms at play. The allostatic load refers to the wear and tear of stress on the body, wherein the allostatic load increases as the body copes with stressors [31,32]. Olson et al. [33] use this framework to predict preterm birth risk. In their study, they prescribe to a "two-hit" theory whereby each "hit" or stressor cumulatively leads to changes in the complex mechanisms involved in parturition, potentially leading to a tertiary outcome with an increased number of "hits". Rat dams were exposed to both stress and the pro-inflammatory cytokine, IL-1β. Even though neither stressor elicited a response on their own, the combined effect led to preterm birth in the rats. Thus, our results could be described under this framework, where the first "hit" may be maternal stress, and the second "hit" is the chemical exposure. When only one of our stressors was present in the model (LES or exposure biomarker), a small or no effect was observed on gestational length, but the interaction of the two led to changes in parturition and gestational length, consistent with a "two-hit" mechanism.

Allostatic load assessments use a variety of physiological biomarkers, including inflammation and hormonal markers. The prevailing theory on the mechanism between maternal stress and gestational length is hypothesized to occur via the maternal-placental-fetal hypothalamic-pituitary-adrenal (HPA) axis or inflammatory responses [11,12,34,35]. Maternal stress' influence on the maternal HPA axis stimulates cortisol production via corticotropin-releasing hormone (CRH) [34]. Cortisol concentration is an important physiological marker because higher levels of maternal cortisol are linked to increased intrauterine constriction and preterm birth [36–38].

CRH also stimulates the release of prostaglandins and inflammatory responses in the maternal-placental-fetal systems, thereby preparing women for labor and stimulating myometrial

contractions [39–41]. In fact, women in preterm labor have higher levels of CRH compared to gestational age-matched women, as well as accelerated rates of CRH over the course of their gestation [41,42]. So it is possible that through this mechanism, an increase in CRH levels from maternal stress could lead to an earlier parturition. Elevated levels of circulating CRH in the early third trimester were reported among women with high perceived stress levels [43,44]. In addition, our team previously reported an increase in CRH in association with methyparaben and propylparaben, and a decrease in CRH in association with BPS in the PROTECT cohort (Aker et al., in press). However, the direction of these associations are in the opposite direction of what one would expect to cause an increase or decrease in gestational length, respectively. While our previous analyses on exposure-hormone associations provide valuable insight on the potential mechanisms leading to adverse outcomes, our models did not adjust for diurnal hormone changes within women. Hormone levels vary greatly throughout the day, and we not controlling for these changes in our models may describe some of the unexpected directions of associations observed in this study.

In addition to the effects CRH has on prostaglandins and inflammatory mediators, chronic stress leads to inhibition of the hypothalamic-pituitary-adrenal axis. During chronic stress, prolonged periods of elevated levels of circulating cortisol inhibit hypothalamic production of thyroid stimulating hormone (TSH), leading to a decreased release of TSH from the pituitary gland, and subsequent decreased release of thyroxine (T4) from the thyroid. Furthermore, CRH inhibits conversion of thyroxine (T4) to the bioactive triiodothyronine (T3) [45,46]. However, these effects depend on the duration and intensity of the stressor [45]. In one study, decreased levels of FT4 was associated with higher rates of perinatal syndromal depression [47], which may be a result of decreased release of T4. There is evidence that circulating levels of thyroid hormones, particularly decreased FT4 and increased T3, are associated with preterm birth [48]. Our previous analysis found associations between urinary concentrations of BPA and triclocarban and an increase in T3 (Aker et al., in press). Thus, decreased FT4 levels from depression or high stress may be the first hit, and the increase in T3 from TCC could be the second hit, leading to a shortened gestational length. BPA was also associated with an increase in FT4, which may explain why we do not observe a shortened gestational length in this case.

Besides the neuroendocrine pathway, a potential mechanism of action could be via inflammatory responses. Some stressful life events during pregnancy were suggestively associated with elevated levels of 8-iso-$PGF_{2\alpha}$ (an oxidative stress marker) measured at a median of 32 weeks [49]. Additionally, chronic stress is related to immunosuppression and changes in the response to antigens [50], which is related to a decreased gestational age at birth. Exposure to some phenol and parabens have also been associated with oxidative stress and inflammatory biomarkers, including BPA and propylparaben [17,51]. In summary, we suggest several pathways that could lead to an increase in allostatic load with the combined effect of chemical exposure and maternal stress, including: 1) a direct effect on cortisol; 2) a direct effect on CRH; 3) an indirect effect on thyroid hormones via CRH and cortisol; and 4) a direct effect on inflammatory responses.

Our results also showed a difference in the associations by fetal sex. Although our sample sizes were too small to interpret these findings confidently, a difference in the effects by sex is plausible. Because both estrogens and androgens play a role in the regulation of the HPA axis via modulation of the adrenal, anterior pituitary and hypothalamic functions [52], it is possible that fetal reproductive hormones could impact the levels of placental CRH, thereby leading to adverse effects on gestational length that differ by fetal sex. Furthermore, placental CRH production can be suppressed by estrogen [53]. This further demonstrates the complexity of the effects of stress and chemicals on gestational length, given the additional importance of fetal sex.

The association between triclocarban and gestational length appeared to be influenced by a lack of positive life events more so than an excess of negative life events. This is consistent with the literature that suggests positive life events and perceived social support may act as a buffer to stress and negative events [54]. For example, positive life events were correlated with lower salivary cortisol levels among a group of 60 pregnant women, while negative life events were not [55].

We did not observe any interactions between exposure biomarkers and other psychosocial scores. One reason may be due to the different timing the scores attempt to capture. The ESSI score asks participants how they currently feel; the CES-D score asks participants how they felt in the past week, the PSS score focuses on the previous month, and LES pertains to events since becoming pregnant. While the scores are dependent on each other given they include similar questions, it is interesting to note that the only significant interactions we observed included the

psychosocial score that has the widest time frame and would presumably include the least subjective questions, LES.

We did not identify other studies that have investigated the same associations as this study. We did, however, identify a study that looked at the combined effect of phthalate exposure and stressful life events on the anogenital distance (AGD) among over 700 women in a well-educated cohort based in four medical centers across the U.S. [56]. The study found that among boys, prenatal exposure to stressful life events modified the association between first trimester di(2-ethylhexyl) phthalate (DEHP) metabolite concentrations and altered genital measurements, such that the association between DEHP and altered genital measurements was only significant among mothers with low stress (and not high stress). While our results are not directly comparable, both studies support further exploration of the effect of chemical and non-chemical stressors on perinatal health.

While our study is among the first to explore the effects of endocrine disruptors in addition to psychosocial variables, there were a few limitations. The timing of maternal stress could influence the effect on the fetus [44]; however, we do not have precise data on when the life event took place apart from happening sometime between the beginning of pregnancy and the second study visit (20-24 weeks gestation). Similarly, we have no data on the participants' psychosocial profiles past the second study visit. Evidence also suggests that the type of life event is an important factor to consider (i.e. a family death versus changes in diet), rather than combining all life events as equal in magnitude and/or importance [30,49]. However, because our LES scores allowed the participant to rate how positive or negative the event was, this limitation is unlikely to have greatly affected our results. While our analysis used urinary exposure biomarker data from three time points, our analyses still resembles a cross-sectional study in this context. In addition, we constructed multiple models in this exploratory study, which increases the risk of Type 1 error.

Our study also had many strengths. Our large sample size allowed us to explore the modifying effects of both psychosocial variables and fetal sex. We also had data on a total of four psychosocial scores, which allowed us to capture different methods of exploring stress during pregnancy. Most studies that examine the effect of life events focus solely on negative life events. However, our study shows the importance of both negative and positive life events, and

our most consistent results were based on the Total LES scores rather than the Negative and Positive LES scores separately. Furthermore, our urinary exposure biomarker panel included BPS and triclocarban which have not been studied in detail to date. Our cohort also has higher levels of triclocarban as compared to other populations [57], allowing us to study the association between this biomarker, maternal stress, and gestational length in a vulnerable population.

Conclusion

This study is one of the first to examine the effect of chemical exposure in the presence of maternal stress. Associations were stronger in the presence of negative life events, and positive events had some protective effect on the association between exposure biomarkers and gestational length. This indicates that stress makes the body more vulnerable to the effects of chemical exposure. Pregnancy is a stressful period in a woman's life, and the increased vulnerability to chemical exposure could have adverse effects on her and the fetus. More research is required to verify these associations.

Table V.1: Summary demographics of the 908 pregnant women in the study population stratified by Total LES Scores

	Total	**Negative Total LES Score**	**Neutral Total LES Score**	**Positive Total LES Score**	**p**
N	908	330	272	306	
Maternal Age (median [IQR])	26 [22.00, 31.00]	27 [23.00, 31.00]	27 [22.00, 31.00]	25 [21.00, 29.00]	<0.001
BMI (%)					0.16
0-25	419 (56.5)	151 (54.9)	118 (53.6)	150 (61)	
25-29.9	200 (27.0)	84 (30.5)	57 (25.9)	59 (24)	
>29.9	122 (16.5)	40 (14.5)	45 (20.5)	37 (15)	
Insurance type (%)					0.71
Mi Salud	586 (63.3)	216 (65.5)	180 (66.2)	190 (62.1)	
Private	322 (36.7)	114 (35.5)	92 (36.1)	116 (38.5)	
Household Income (%)					0.91
<10,000	229 (29.5)	89 (31)	69 (29.4)	71 (28)	
10,000-30,000	244 (31.4)	87 (30.3)	70 (29.8)	87 (34.3)	

30,000-50,000	182 (23.5)	68 (23.7)	55 (23.4)	59 (23.2)	
>50,000	121 (15.6)	43 (15)	41 (17.4)	37 (14.6)	
Maternal Education (%)					0.78
<High School/GED	185 (20.6)	68 (20.7)	57 (21.4)	60 (19.9)	
Some College	324 (36.2)	124 (37.8)	88 (33.1)	112 (37.1)	
College graduate	387 (43.2)	136 (41.5)	121 (45.5)	130 (43)	
Smoking (%)					0.02
Never	752 (83.7)	269 (82)	237 (88.4)	246 (81.5)	
Ever	118 (13.1)	43 (13.1)	25 (9.3)	50 (16.6)	
Current	28 (3.1)	16 (4.9)	6 (2.2)	6 (2)	
Exposure to second hand smoking (%)					0.86
None	769 (88.4)	278 (87.1)	232 (89.9)	259 (88.4)	
Up to 1 hour	44 (5.1)	19 (6)	11 (4.3)	14 (4.8)	
More than 1 hour	57 (6.6)	22 (6.9)	15 (5.8)	20 (6.8)	
Alcohol Consumption (%)					0.15
None	454 (50.8)	165 (50.6)	148 (55.4)	141 (46.8)	
Before pregnancy	380 (42.5)	136 (41.7)	108 (40.4)	136 (45.2)	
Yes within the last few months	60 (6.7)	25 (7.7)	11 (4.1)	24 (8)	
Marital Status (%)					0.09
Single	178 (19.8)	63 (19.2)	48 (17.9)	67 (22.1)	
Married	510 (56.7)	186 (56.7)	168 (62.7)	156 (51.5)	
Divorced	11 (1.2)	7 (2.1)	1 (0.4)	3 (1)	
Living together	200 (22.2)	72 (22)	51 (19)	77 (25.4)	
Number of previous children (%)					0.002
0	430 (47.9)	142 (43.3)	118 (44)	170 (56.5)	
1	358 (39.9)	143 (43.6)	108 (40.3)	107 (35.5)	
>1	109 (12.2)	43 (13.1)	42 (15.7)	24 (8)	

P values calculated from Chi squared test for categorical variables and Kruskal test for continuous variables.
Totals may not add up to 100% due to missing values.

Table V.2: Life events that occurred in at least 10% of the study population and their mean scores

Life Event	**N (%)**	**Mean score**
Drastic change in eating habits	463 (51.9)	0.21
Drastic change in sleeping habits	462 (51.7)	-0.52
Major change in social activities	246 (27.6)	-0.29
Change in amount/type recreation	231 (25.9)	-0.35
Change in financial status	215 (24.1)	-0.39

Successful personal achievement	211 (23.7)	1.16
Change in number of discussions with spouse	192 (21.5)	-0.70
Change in church activities	150 (16.8)	0.37
Change in proximity to family member	149 (16.7)	0.65
Moved to a new location	163 (15.6)	1.55
Changes in the workplace	127 (14.2)	-0.14
Changes in spouse's workplace	127 (14.2)	0.22
Death of a close family member	95 (10.7)	-1.70
Illness in a close family member	91 (10.2)	-1.56

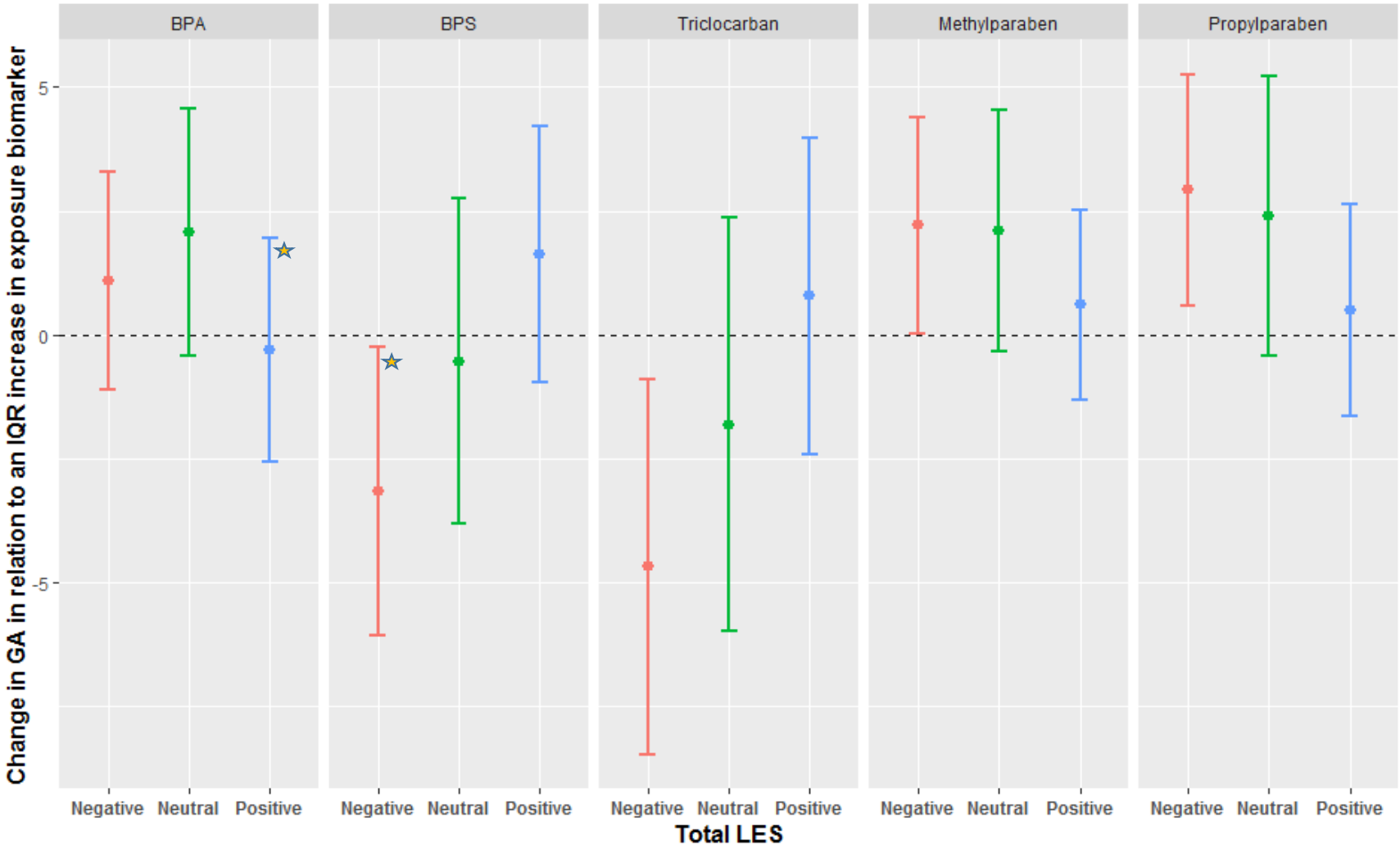


Figure V.1: Adjusted change in gestational length in relation to average mean-centered exposure biomarker concentration across three time points during pregnancy, stratified by Total LES. Beta coefficients were transformed to change in gestational days for an IQR increase in exposure biomarker concentration. The star represents a statistically significant interaction as compared to the Neutral category.

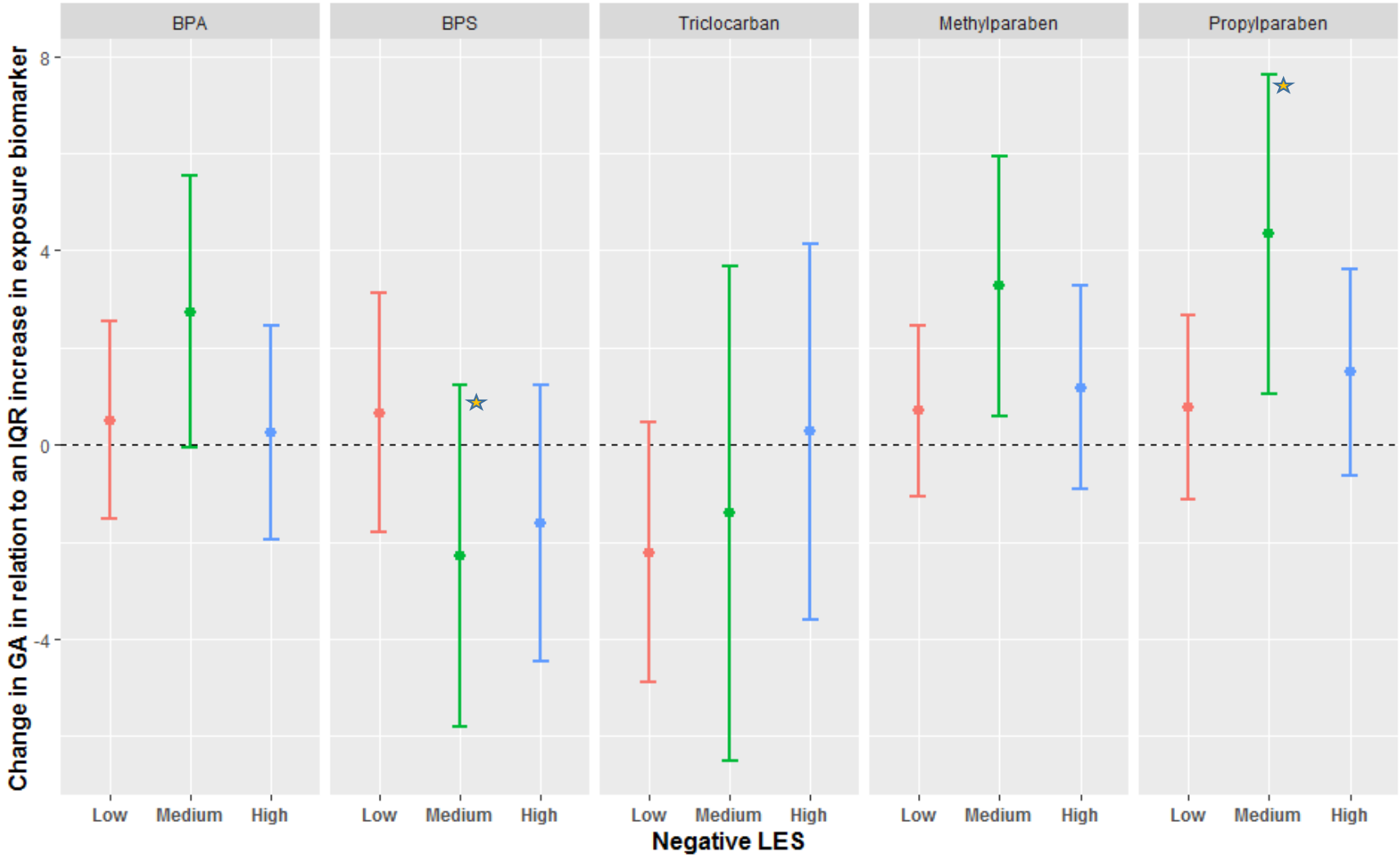


Figure V.2: Adjusted change in gestational length in relation to average mean-centered exposure biomarker concentration across three time points during pregnancy, stratified by Negative LES. Beta coefficients were transformed to change in gestational days for an IQR increase in exposure biomarker concentration. The star represents a statistically significant interaction as compared to the Low category.

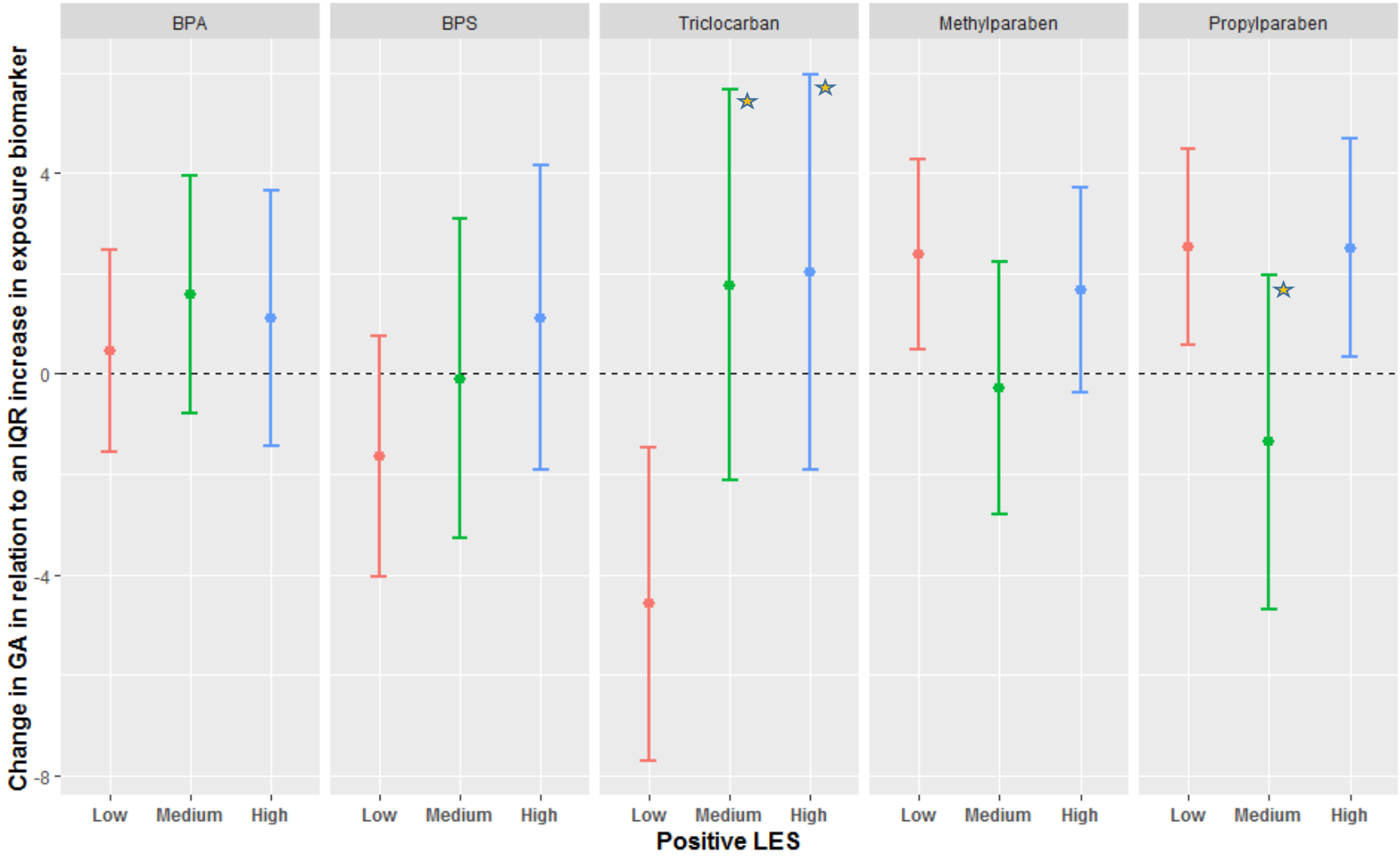


Figure V.3: Adjusted change in gestational length in relation to average mean-centered exposure biomarker concentration across three time points during pregnancy, stratified by Positive LES. Beta coefficients were transformed to change in gestational days for an IQR increase in exposure biomarker concentration. The star represents a statistically significant interaction as compared to the Low category.

Table V.3: Adjusted[a] change in gestational age in relation to average exposure biomarker concentration across three time points during pregnancy. Beta coefficients were transformed to change in gestational days for an IQR increase in exposure biomarker concentration.

	N	Change in days/IQR (95% CI)	p
BPA	748	0.98 (-0.41, 2.37)	0.17
BPS	540	-0.52 (-2.14, 1.09)	0.53
Benzophenone-3	749	1.90 (0.54, 3.26)	0.006**
Triclocarban	544	-1.96 (-4.11, 0.19)	0.08*
Methyl-paraben	751	1.63 (0.37, 2.89)	0.01**
Propyl-paraben	752	2.06 (0.63, 3.48)	0.005**

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking.
* 0.05 < p value < 0.10; ** p value < 0.05

Table V.4: SG-corrected urinary bisphenol, paraben and triclocarban biomarker concentrations (µg/mL) from all three study visits stratified by Total LES Scores

	Negative Total LES Score	Neutral Total LES Score	Positive Total LES Score	p
N	330	272	306	
	Median [IQR]	**Median [IQR]**	**Median [IQR]**	
BP-3	40.92 [14.64, 148.37]	30.05 [11.97, 159.75]	30.81 [12.25, 125.58]	0.25
BPA	2.39 [1.42, 4.05]	2.23 [1.49, 3.52]	2.02 [1.31, 3.65]	0.33
BPS	0.55 [0.30, 1.10]	0.45 [0.27, 0.99]	0.54 [0.25, 0.94]	0.43
TCC	5.75 [0.60, 41.01]	3.27 [0.60, 31.31]	3.38 [0.63, 27.33]	0.62
MPB	119.18 [38.93, 261.22]	108.46 [35.33, 257.22]	119.26 [43.58, 287.11]	0.63
PPB	30.64 [5.95, 76.28]	24.15 [5.13, 81.62]	27.38 [7.89, 81.07]	0.74

BP-3: Benzophenone; TCC: Triclocarban; MPB: Methylparaben; PPB: Propylparaben

Table V.5: Specific gravity corrected ICC values across the three study visits for PROTECT phenol, paraben and triclocarban concentrations

	SG-corrected ICC	95% CI
BPA	0.26	(0.20, 0.32)
BPS	0.17	(0.10, 0.24)
Benzophenone-3	0.65	(0.61, 0.69)
Triclocarban	0.74	(0.70, 0.77)
Methyl-paraben	0.51	(0.46, 0.56)
Propyl-paraben	0.48	(0.44, 0.53)

Table V.6: Adjusted change in gestational length in relation to categorical psychosocial variables. Beta coefficients are untransformed.

	N	β 95% CI ()	p
LES	835	-0.09 (-0.41, 0.23)	0.58
Negative LES	835	-0.028 (-0.34, 0.29)	0.86
Positive LES	835	-0.098 (-0.48, 0.28)	0.62
CES-D	835	0.19 (-0.25, 0.63)	0.39
SS	753	-0.103 (-0.83, 0.62)	0.78
PSS	758	0.043 (-0.24, 0.33)	0.77

Models were adjusted for maternal age, insurance type, smoking status and alcohol consumption.

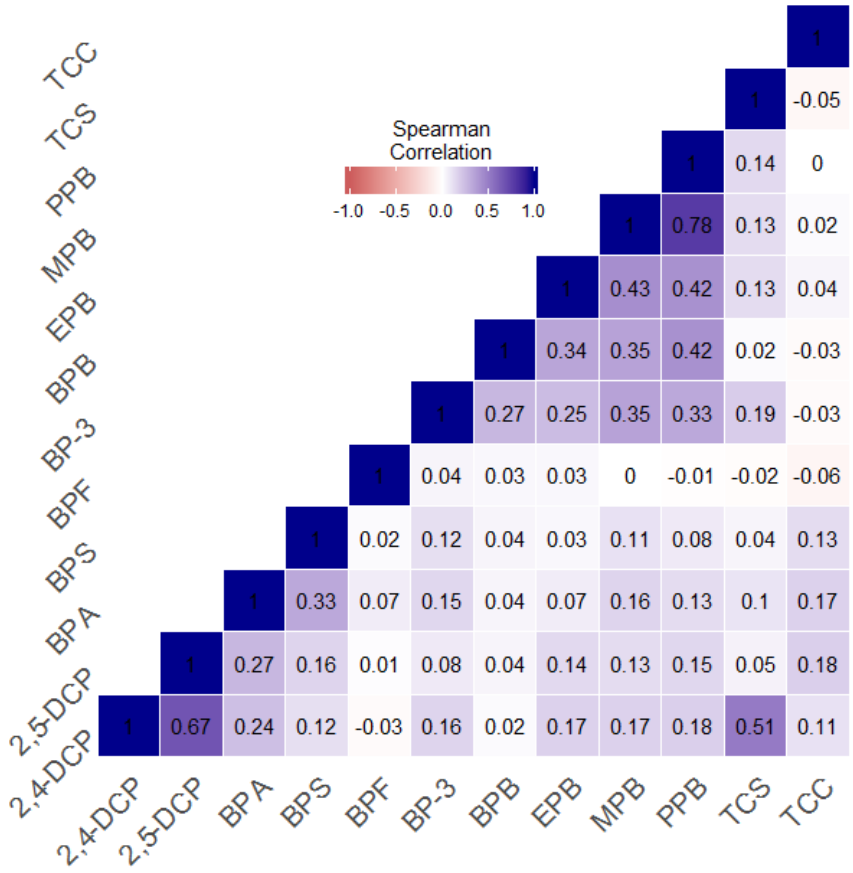


Figure V.4: Heat map of Spearman correlation coefficients of specific gravity corrected average triclocarban, phenol and paraben urinary biomarkers

Table V.7: Adjusted[a] change in gestational length in relation to mean-centered average exposure biomarker concentration across three time points during pregnancy, and the interaction between exposure biomarkers and categorical psychosocial scores.

Biomarker	N	Exp β	p	Int β	p	N	Exp β	p	Int β	p
			CES-D					**PSS**		
BP-3	670	0.10	0.04	-0.01	0.93	693	0.08	0.08	0.06	0.46
BPA	668	0.11	0.33	0.06	0.75	692	0.06	0.56	0.30	0.14
BPS	484	-0.12	0.27	0.33	0.08*	503	0.01	0.95	-0.06	0.77
TCC	487	-0.07	0.11	0.00	0.97	506	-0.04	0.31	-0.09	0.30
MPB	671	0.06	0.30	0.07	0.48	694	0.05	0.35	0.15	0.14
PPB	672	0.05	0.27	0.05	0.54	695	0.06	0.21	0.09	0.28
			SS					**LES**		
BP-3	689	0.00	0.98	0.11	0.64	749	0.09	0.35	0.01	0.84
BPA	687	-0.17	0.76	0.31	0.57	748	0.31	0.17	-0.09	0.41
BPS	498	-0.43	0.53	0.42	0.54	540	-0.54	0.02	0.23	0.03**
TCC	502	-0.20	0.49	0.15	0.61	544	-0.25	0.01	0.09	0.03**
MPB	689	-0.05	0.85	0.14	0.60	751	0.27	0.03	-0.07	0.19
PPB	690	-0.08	0.73	0.16	0.47	752	0.30	0.00	-0.09	0.05**
			Negative LES					**Positive LES**		
BP-3	749	0.11	0.35	0.00	0.96	749	0.10	0.26	0.01	0.88
BPA	748	0.20	0.42	-0.03	0.79	748	0.07	0.74	0.04	0.69
BPS	540	-0.42	0.13	0.16	0.17	540	-0.30	0.14	0.15	0.18
TCC	544	-0.02	0.85	-0.02	0.65	544	-0.28	0.00	0.14	0.003**
MPB	751	0.20	0.14	-0.03	0.54	751	0.21	0.05	-0.05	0.37
PPB	752	0.18	0.09	-0.03	0.46	752	0.16	0.07	-0.03	0.57

Exp β: effect estimate of the urinary biomarker; Int β: effect estimate of the interaction term between the urinary biomarker and psychosocial variable

BP-3: Benzophenone; TCC: Triclocarban; MPB: Methylparaben; PPB: Propylparaben

Cutoff points for psychosocial scores are as follows: 16 for CES-D; 28 for PSS; 25 for SS; -1 & 1 for LES; -5,-2 for Negative LES; 2,5 for Positive LES.

Interaction β and p values assume linearity between psychosocial categories.

** p value <0.05; * 0.05<p value<0.10.

Table V.8: Adjusted[a] change in gestational length in relation to average exposure biomarker concentration across three time points during pregnancy, stratified by LES variables. Beta coefficients were transformed to the change in the number of gestational days per IQR change in exposure biomarker concentration.

	LES								
	Score: < -1			Score: (-1, 1]			Score: > 1		
	N	GA Change 95% CI ()	p	N	GA Change 95% CI ()	p	N	GA Change 95% CI ()	p
2,4-DCP	277	0.45 (-1.23, 2.13)	0.60	219	0.19 (-1.96, 2.34)	0.86	252	-0.97 (-3.31, 1.36)	0.41
			0.99			-			*0.23*
2,5-DCP	276	-0.68 (-2.61, 1.25)	0.49	220	0.02 (-2.31, 2.34)	0.99	253	-0.74 (-2.88, 1.39)	0.50
			0.52			-			*0.40*
BP-3	279	0.85 (-1.29, 2.99)	0.44	219	2.50 (-0.24, 5.24)	0.08	251	1.92 (-0.36, 4.20)	0.10
			0.39			-			*0.51*
BPA	279	1.09 (-1.11, 3.29)	0.33	220	2.08 (-0.41, 4.57)	0.10	249	-0.30 (-2.55, 1.96)	0.80
			0.25			-			*0.05**
BPS	194	-3.15 (-6.06, -0.24)	0.04	161	-0.54 (-3.82, 2.74)	0.75	185	1.62 (-0.96, 4.20)	0.22
			*0.06**			-			*0.86*
BPF	178	-0.58 (-1.19, 0.03)	0.06	144	0.19 (-0.48, 0.85)	0.58	178	0.36 (-0.20, 0.92)	0.21
			0.17			-			*0.56*
TCS	277	1.47 (-1.47, 4.40)	0.33	221	-0.46 (-3.88, 2.95)	0.79	251	-1.32 (-4.23, 1.58)	0.37
			0.33			-			*0.66*
TCC	194	-4.68 (-8.47, -0.89)	0.02	161	-1.81 (-5.99, 2.37)	0.40	189	0.78 (-2.40, 43.96)	0.63
			0.18			-			*0.48*
EPB	198	-0.55 (-1.12, 0.02)	0.06	161	0.19 (-0.46, 0.84)	0.56	191	0.21 (-0.33, 0.76)	0.44
			0.25			-			*0.44*
MPB	280	2.21 (0.02, 4.40)	0.05	220	2.09 (-0.35, 4.53)	0.09	251	0.61 (-1.31, 2.53)	0.53
			0.99			-			*0.19*
BPB	277	0.88 (-1.86, 3.62)	0.53	219	1.61 (-2.19, 5.41)	0.41	250	-0.60 (-3.85, 2.65)	0.72
			0.70			-			*0.31*
PPB	280	2.92 (0.58, 5.26)	0.01	219	2.40 (-0.43, 5.23)	0.01	253	0.51 (-1.63, 2.65)	0.64
			0.73			-			*0.10**

	LES Positive								
	Low Score: [0, 2]			Medium Score: (2, 5]			High Score: (5, 27]		
	N	GA Change 95% CI ()	p	N	GA Change 95% CI ()	p	N	GA Change 95% CI ()	p
2,4-DCP	407	0.27 (-1.21, 1.75)	0.72	157	0.16 (-2.02, 2.35)	0.88	184	-0.63 (-3.15, 1.90)	0.63
			-			*0.80*			*0.52*
2,5-DCP	407	-0.63 (-2.38, 1.12)	0.48	158	0.12 (-2.06, 2.29)	0.92	184	-0.75 (-3.13, 1.63)	0.54
			-			*0.62*			*0.95*
BP-3	409	1.67 (-0.17, 3.52)	0.08	156	2.07 (-0.59, 4.74)	0.13	184	2.48 (-0.34, 5.29	0.09
			-			*0.46*			*0.68*
BPA	408	0.46 (-1.56, 2.48)	0.66	159	1.58 (-0.78, 3.94)	0.19	181	1.11 (-1.44, 3.66)	0.39
			-			*0.49*			*0.77*
BPS	313	-1.65 (-4.05, 0.74)	0.18	105	-0.09 (-3.28, 3.10)	0.95	122	1.12 (-1.92, 4.12)	0.47
			-			*0.19*			*0.25*
BPF	288	-0.23 (-0.76, 0.29)	0.38	97	0.58 (-0.06, 1.22)	0.08	115	0.34 (-0.30, 0.98)	0.30
			-			*0.23*			*0.19*
TCS	409	0.67 (-1.93, 3.26)	0.61	156	-0.86 (-4.29, 2.58)	0.63	184	-0.79 (-3.95, 2.37)	0.63
			-			*0.42*			*0.46*
TCC	314	-4.58 (-7.70, -1.46)	0.004	106	1.77 (-2.12, 5.67)	0.38	124	2.03 (-1.91, 5.97)	0.31
			-			*0.01***			*0.01***
EPB	319	-0.19 (-0.67, 0.28)	0.43	107	0.35 (-0.31, 1.01)	0.30	124	-0.14 (-0.76, 0.48)	0.66
			-			*0.26*			*0.64*
MPB	409	2.39 (0.50, 4.28)	0.01	157	-0.29 (-2.79, 2.22)	0.82	185	1.68 (-0.36, 3.72)	0.11
			-			*0.05**			*0.56*
BPB	406	1.42 (-1.19, 4.03)	0.29	156	-0.41 (-3.76, 2.93)	0.81	184	-0.53 (-4.10, 3.05)	0.77
			-			*0.22*			*0.38*
PPB	409	2.54 (0.58, 4.49)	0.01	158	-1.35 (-4.68, 1.98)	0.43	185	2.51 (0.33, 4.96)	0.03
			-			*0.02***			*0.85*

	LES Negative								
	Low Score: [0, -2)			Medium Score: [-2, -5)			High Score: [-5, -30]		
	N	GA Change 95% CI ()	p	N	GA Change 95% CI ()	p	N	GA Change 95% CI ()	p
2,4-DCP	359	-0.46 (-2.58, 1.67)	0.67	186	-0.20 (-2.91, 2.51)	0.88	203	1.07 (-0.44, 2.58)	0.17
			-			*0.52*			*0.21*
2,5-DCP	360	-1.10 (-3.08, 0.89)	0.28	186	-0.42 (-3.14, 2.30)	0.76	203	0.57 (-1.20, 2.34)	0.53
			-			*0.70*			*0.21*
BP-3	360	1.92 (-0.01, 3.85)	0.05	184	0.72 (-2.39, 3.84)	0.65	205	2.20 (-0.06, 4.45)	0.06
			-			*0.94*			*1.00*
BPA	359	0.50 (-1.53, 2.53)	0.63	184	2.74 (-0.06, 5.53)	0.06	205	0.24 (-1.97, 2.44)	0.83
			-			*0.86*			*0.85*
BPS	274	0.65 (-1.81, 3.11)	0.61	136	-2.29 (-5.80, 1.23)	0.21	130	-1.61 (-4.45, 1.23)	0.27
			-			*0.08**			*0.23*
BPF	258	0.34 (-0.15, 0.84)	0.18	124	-0.61 (-1.42, 0.20)	0.14	118	-0.04 (-0.61, 0.54)	0.90
			-			*0.09**			*0.47*
TCS	361	0.37 (-2.19, 2.94)	0.78	184	-1.35 (-5.18, 2.48)	0.49	204	1.67 (-1.27, 4.60)	0.27
			-			*0.40*			*0.65*
TCC	276	-2.21 (-4.88, 0.47)	0.11	139	-1.41 (-6.50, 3.68)	0.59	129	0.27 (-3.59, 4.13)	0.89
			-			*0.60*			*0.53*
EPB	279	0.18 (-0.31, 0.66)	0.47	140	-1.02 (-1.76, -0.28)	0.008	131	0.22 (-0.32, 0.77)	0.42
			-			*0.01***			*0.85*
MPB	361	0.70 (-1.06, 2.46)	0.44	184	3.27 (0.60, 5.94)	0.02	206	1.18 (-0.90, 3.27)	0.27
			-			*0.20*			*0.70*
BPB	357	0.42 (-2.77, 3.62)	0.80	184	2.14 (-1.86, 6.14)	0.30	205	-0.43 (-3.02, 2.15)	0.74
			-			*0.39*			*0.68*
PPB	362	0.77 (-1.13, 2.67)	0.43	184	4.34 (1.04, 7.63)	0.01	206	1.49 (-0.65, 3.62)	0.17
			-			*0.07**			*0.63*

2,4-DCP: 2,4-dichlorophenol; 2,5-DCP: 2,5-dichlorophenol; BP-3: Benzophenone; TCS: Triclosan; TCC: Triclocarban; EPB: Ethyl-paraben; MPB: Methylparaben; BPB: Butylparaben; PPB: Propylparaben

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking. ** interaction p value <0.05; * 0.05< interaction p value<0.10.

Table V.9: Adjusted[a] change in gestational length in relation to average exposure biomarker concentration across three time points during pregnancy, stratified by LES variables and infant sex. Beta coefficients were transformed to the change in the number of gestational days per IQR change in exposure biomarker concentration.

Total LES

	Females						Males					
	Negative		Neutral		Positive		Negative		Neutral		Positive	
Urinary Biomarker	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p
BPA	2.93 (-0.6, 6.46)	0.11	0.71 (-2.42, 3.85)	0.66	1.48 (-2.75, 5.7)	0.50	1.7 (-1.78, 5.19)	0.34	2.97 (-1.02, 6.97)	0.15	-0.51 (-3.5, 2.45)	0.74
BPS	-0.79 (-6.14, 4.55)	0.77	-1.47 (-6.2, 3.28)	0.55	1.38 (-2.98, 5.74)	0.54	-5.06 (-9.4, -0.72)	0.03	-0.77 (-5.66, 4.1)	0.76	1.15 (-2.06, 4.36)	0.48
TCS	0.74 (-3.88, 5.35)	0.76	-2.36 (-6.4, 1.69)	0.26	-4.50 (-9.04, 0.04)	0.06	2.40 (-1.54, 6.34)	0.24	3.36 (-2.49, 9.2)	0.26	2.00 (-1.83, 5.82)	0.31
TCC	-6.41 (-13, 0.19)	0.06	1.29 (-4.3, 6.88)	0.65	3.59 (-1.84, 9.02)	0.20	-4.08 (-9.02, 0.85)	0.11	-6.95 (-13.2, -0.7)	0.03	1.43 (-2.69, 5.56)	0.50
MPB	2.83 (0.17, 5.48)	0.04	1.22 (-1.69, 4.12)	0.42	1.19 (-2.33, 4.7)	0.51	-0.93 (-4.47, 2.62)	0.61	3.31 (-0.75, 7.37)	0.11	0.56 (-1.67, 2.79)	0.62
PPB	4.66 (1.53, 7.8)	0.00	0.23 (-2.74, 3.21)	0.88	1.56 (-2.08, 5.21)	0.40	0.75 (-2.75, 4.25)	0.68	4.05 (-0.67, 8.78)	0.10	0.37 (-2.32, 3.05)	0.79

Negative LES

	Females						Males					
	Low		Medium		High		Low		Medium		High	
Urinary Biomarker	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p	GA Change 95% CI ()	p
BPA	0.97 (-1.74, 3.69)	0.48	3.15 (-2.99, 9.29)	0.32	2.52 (-0.53, 5.57)	0.11	-0.18 (-3.37, 3.02)	0.91	3.43 (0.29, 6.57)	0.04	0.25 (-2.92, 3.43)	0.88
BPS	0.83 (-2.54, 4.19)	0.63	0.94 (-5.17, 7.06)	0.76	1.25 (-4.71, 7.22)	0.68	-0.53 (-4.51, 3.45)	0.79	-3.44 (-7.35, 0.5)	0.09	-1.26 (-4.8, 2.27)	0.49
TCC	0.23 (-4.01, 4.47)	0.92	-5.90 (-15.8, 4.0)	0.25	2.25 (-4.33, 8.83)	0.51	-4.09 (-8.65, 0.47)	0.08	0.47 (-3.89, 4.83)	0.83	-1.96 (-6.01, 2.1)	0.35
MPB	-0.35 (-2.71, 2)	0.77	2.16 (-1.21, 5.53)	0.21	4.52 (1.11, 7.93)	0.01	1.53 (-1.29, 4.35)	0.29	1.98 (-0.79, 4.75)	0.16	-1.29 (-4.8, 2.22)	0.47

PPB	-0.51 (-2.9, 1.88)	0.67	4.66 (0.34, 8.98)	0.04	2.88 (-0.35, 6.12)	0.08	1.05 (-1.69, 3.79)	0.45	2.11 (-1.99, 6.22)	0.32	2.21 (-1.35, 5.76)	0.23

Positive LES

	Females						**Males**					
	Low		**Medium**		**High**		**Low**		**Medium**		**High**	
Urinary Biomarker	**GA Change 95% CI ()**	**p**	**GA Change 95% CI ()**	**p**	**GA Change 95% CI ()**	**p**	**GA Change 95% CI ()**	**p**	**GA Change 95% CI ()**	**p**	**GA Change 95% CI ()**	**p**
BPA	0.89 (-1.85, 3.62)	0.53	2.27 (-1.61, 6.15)	0.26	4.39 (-0.46, 9.24)	0.08	0.77 (-2.58, 4.11)	0.65	1.74 (-1.84, 5.32)	0.35	0.82 (-1.99, 3.63)	0.57
BPS	0 (-3.53, 3.53)	1.00	0.43 (-4.3, 5.17)	0.86	1.37 (-4.58, 7.32)	0.66	-2.15 (-6.35, 2.04)	0.32	0.83 (-4.06, 5.73)	0.74	0.39 (-2.54, 3.32)	0.80
TCC	-3.28 (-8.05, 1.48)	0.18	-0.1 (-6.24, 6.04)	0.98	7.94 (-1.86, 17.75)	0.12	-6.53 (-11.3, -1.8)	0.01	5.65 (-0.25, 11.5)	0.07	1.86 (-1.85, 5.58)	0.33
MPB	1.77 (-0.44, 3.97)	0.12	-0.71 (-4.36, 2.9)	0.70	4.07 (0.31, 7.83)	0.04	2.08 (-1.03, 5.19)	0.19	0.25 (-2.57, 3.08)	0.86	0.55 (-2.42, 3.53)	0.72
PPB	2.16 (-0.23, 4.55)	0.08	-0.53 (-3.55, 2.5)	0.73	5.18 (1.08, 9.28)	0.02	2.42 (-1.08, 5.93)	0.18	-0.95 (-4.14, 2.2)	0.56	1.76 (-1.45, 4.97)	0.29

TCC: Triclocarban; MPB: Methylparaben; PPB: Propylparaben

[a] Models adjusted for specific gravity, maternal age, insurance type, alcohol use, and exposure to second-hand smoking.

References

[1] K.K. Ferguson, J.D. Meeker, D.E. Cantonwine, B. Mukherjee, G.G. Pace, D. Weller, T.F. McElrath, Environmental phenol associations with ultrasound and delivery measures of fetal growth, Environ. Int. 112 (2018) 243–250. doi:10.1016/j.envint.2017.12.011.

[2] T.H. Lassen, H. Frederiksen, H.B. Kyhl, S.H. Swan, K.M. Main, A.-M. Andersson, D.V. Lind, S. Husby, C. Wohlfahrt-Veje, N.E. Skakkebæk, T.K. Jensen, Prenatal Triclosan Exposure and Anthropometric Measures including Anogenital Distance in Danish Infants, Environ. Health Perspect. (2016). doi:10.1289/ehp.1409637.

[3] C. Philippat, J. Botton, A.M. Calafat, X. Ye, M.-A. Charles, R. Slama, EDEN Study Group, Prenatal exposure to phenols and growth in boys, Epidemiol. Camb. Mass. 25 (2014) 625–635. doi:10.1097/EDE.0000000000000132.

[4] R. Tang, M.-J. Chen, G.-D. Ding, X.-J. Chen, X.-M. Han, K. Zhou, L.-M. Chen, Y.-K. Xia, Y. Tian, X.-R. Wang, Associations of prenatal exposure to phenols with birth outcomes, Environ. Pollut. Barking Essex 1987. 178 (2013) 115–120. doi:10.1016/j.envpol.2013.03.023.

[5] M.S. Wolff, S.M. Engel, G.S. Berkowitz, X. Ye, M.J. Silva, C. Zhu, J. Wetmur, A.M. Calafat, Prenatal phenol and phthalate exposures and birth outcomes, Environ. Health Perspect. 116 (2008) 1092–1097. doi:10.1289/ehp.11007.

[6] A.M. Aker, K.K. Ferguson, Z.Y. Rosario, B. Mukherjee, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico, Environ. Res. 169 (2018) 41–51. doi:10.1016/j.envres.2018.10.030.

[7] M.R. Sable, D.S. Wilkinson, Impact of perceived stress, major life events and pregnancy attitudes on low birth weight, Fam. Plann. Perspect. 32 (2000) 288–294.

[8] G.A. Barbosa, The association of life events to gestational age at delivery among low-income, urban, African American women, J. Perinatol. Off. J. Calif. Perinat. Assoc. 20 (2000) 438–442.

[9] N. Dole, D.A. Savitz, I. Hertz-Picciotto, A.M. Siega-Riz, M.J. McMahon, P. Buekens, Maternal stress and preterm birth, Am. J. Epidemiol. 157 (2003) 14–24.

[10] T.P. Dominguez, C.D. Schetter, R. Mancuso, C.M. Rini, C. Hobel, Stress in African American pregnancies: testing the roles of various stress concepts in prediction of birth outcomes, Ann. Behav. Med. Publ. Soc. Behav. Med. 29 (2005) 12–21. doi:10.1207/s15324796abm2901_3.

[11] J.W. Rich-Edwards, T.A. Grizzard, Psychosocial stress and neuroendocrine mechanisms in preterm delivery, Am. J. Obstet. Gynecol. 192 (2005) S30-35. doi:10.1016/j.ajog.2005.01.072.

[12] D. Kingston, S. Tough, H. Whitfield, Prenatal and postpartum maternal psychological distress and infant development: a systematic review, Child Psychiatry Hum. Dev. 43 (2012) 683–714. doi:10.1007/s10578-012-0291-4.

[13] M. Weinstock, The long-term behavioural consequences of prenatal stress, Neurosci. Biobehav. Rev. 32 (2008) 1073–1086. doi:10.1016/j.neubiorev.2008.03.002.

[14] H.M. Vesterinen, R. Morello-Frosch, S. Sen, L. Zeise, T.J. Woodruff, Cumulative effects of prenatal-exposure to exogenous chemicals and psychosocial stress on fetal growth: Systematic-review of the human and animal evidence, PLoS ONE. 12 (2017). doi:10.1371/journal.pone.0176331.

[15] X. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry, Anal. Bioanal. Chem. 383 (2005) 638–644. doi:10.1007/s00216-005-0019-4.
[16] X. Ye, A.M. Bishop, J.A. Reidy, L.L. Needham, A.M. Calafat, Parabens as Urinary Biomarkers of Exposure in Humans, Environ. Health Perspect. 114 (2006) 1843–1846. doi:10.1289/ehp.9413.
[17] D.J. Watkins, K.K. Ferguson, L.V. Anzalota Del Toro, A.N. Alshawabkeh, J.F. Cordero, J.D. Meeker, Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico, Int. J. Hyg. Environ. Health. 218 (2015) 212–219. doi:10.1016/j.ijheh.2014.11.001.
[18] R.W. Hornung, L.D. Reed, Estimation of Average Concentration in the Presence of Nondetectable Values, Appl. Occup. Environ. Hyg. 5 (1990) 46–51. doi:10.1080/1047322X.1990.10389587.
[19] L.S. Radloff, The CES-D Scale: A Self-Report Depression Scale for Research in the General Population, Appl. Psychol. Meas. 1 (1977) 385–401. doi:10.1177/014662167700100306.
[20] S. Cohen, T. Kamarck, R. Mermelstein, A global measure of perceived stress, J. Health Soc. Behav. 24 (1983) 385–396.
[21] ENRICHD Investigators, Enhancing Recovery in Coronary Heart Disease (ENRICHD) study intervention: rationale and design, Psychosom. Med. 63 (2001) 747–755.
[22] I.G. Sarason, J.H. Johnson, J.M. Siegel, Assessing the impact of life changes: development of the Life Experiences Survey, J. Consult. Clin. Psychol. 46 (1978) 932–946.
[23] P.M. Lewinsohn, J.R. Seeley, R.E. Roberts, N.B. Allen, Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults, Psychol. Aging. 12 (1997) 277–287.
[24] M. Shah, S. Hasan, S. Malik, C.T. Sreeramareddy, Perceived Stress, Sources and Severity of Stress among medical undergraduates in a Pakistani Medical School, BMC Med. Educ. 10 (2010) 2. doi:10.1186/1472-6920-10-2.
[25] B. Aggarwal, M. Liao, J.P. Allegrante, L. Mosca, Low Social Support Level is Associated with Non-Adherence to Diet at 1-Year in the Family Intervention Trial for Heart Health (FIT Heart), J. Nutr. Educ. Behav. 42 (2010) 380–388. doi:10.1016/j.jneb.2009.08.006.
[26] E.M. Bucholz, K.M. Strait, R.P. Dreyer, M. Geda, E.S. Spatz, H. Bueno, J.H. Lichtman, G. D'Onofrio, J.A. Spertus, H.M. Krumholz, Effect of Low Perceived Social Support on Health Outcomes in Young Patients With Acute Myocardial Infarction: Results From the VIRGO (Variation in Recovery: Role of Gender on Outcomes of Young AMI Patients) Study, J. Am. Heart Assoc. Cardiovasc. Cerebrovasc. Dis. 3 (2014). doi:10.1161/JAHA.114.001252.
[27] Committee on Obstetric Practice, the American Institute of Ultrasound in Medicine, and the Society for Maternal-Fetal Medicine, Committee Opinion No 700: Methods for Estimating the Due Date, Obstet. Gynecol. 129 (2017) e150–e154. doi:10.1097/AOG.0000000000002046.

[28] P.H.C. Rondó, R.F. Ferreira, F. Nogueira, M.C.N. Ribeiro, H. Lobert, R. Artes, Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation, Eur. J. Clin. Nutr. 57 (2003) 266–272. doi:10.1038/sj.ejcn.1601526.
[29] R.J. Ruiz, J. Fullerton, C.E.L. Brown, D.J. Dudley, Predicting Risk of Preterm Birth: The Roles of Stress, Clinical Risk Factors, and Corticotropin-Releasing Hormone, Biol. Res. Nurs. 4 (2002) 54–64. doi:10.1177/1099800402004001007.
[30] N.M. Talge, C. Neal, V. Glover, Early Stress, Translational Research and Prevention Science Network: Fetal and Neonatal Experience on Child and Adolescent Mental Health, Antenatal maternal stress and long-term effects on child neurodevelopment: how and why?, J. Child Psychol. Psychiatry. 48 (2007) 245–261. doi:10.1111/j.1469-7610.2006.01714.x.
[31] M. Shannon, T.L. King, H.P. Kennedy, Allostasis: a theoretical framework for understanding and evaluating perinatal health outcomes, J. Obstet. Gynecol. Neonatal Nurs. JOGNN. 36 (2007) 125–134. doi:10.1111/j.1552-6909.2007.00126.x.
[32] P. Sterling, J. Eyer, Allostasis: A new paradigm to explain arousal pathology, in: Handb. Life Stress Cogn. Health, John Wiley & Sons, Oxford, England, 1988: pp. 629–649.
[33] D.M. Olson, E.M. Severson, B.S.E. Verstraeten, J.W.Y. Ng, J.K. McCreary, G.A.S. Metz, Allostatic Load and Preterm Birth, Int. J. Mol. Sci. 16 (2015) 29856–29874. doi:10.3390/ijms161226209.
[34] C. de Weerth, J.K. Buitelaar, Physiological stress reactivity in human pregnancy--a review, Neurosci. Biobehav. Rev. 29 (2005) 295–312. doi:10.1016/j.neubiorev.2004.10.005.
[35] M.R. Kramer, C.R. Hogue, What Causes Racial Disparities in Very Preterm Birth? A Biosocial Perspective, Epidemiol. Rev. 31 (2009) 84–98. doi:10.1093/ajerev/mxp003.
[36] T. Carpenter, S.M. Grecian, R.M. Reynolds, Sex differences in early-life programming of the hypothalamic-pituitary-adrenal axis in humans suggest increased vulnerability in females: a systematic review, J. Dev. Orig. Health Dis. 8 (2017) 244–255. doi:10.1017/S204017441600074X.
[37] S.J. Cherak, G.F. Giesbrecht, A. Metcalfe, P.E. Ronksley, M.E. Malebranche, The effect of gestational period on the association between maternal prenatal salivary cortisol and birth weight: A systematic review and meta-analysis, Psychoneuroendocrinology. 94 (2018) 49–62. doi:10.1016/j.psyneuen.2018.04.023.
[38] R. Smith, Parturition, N. Engl. J. Med. 356 (2007) 271–283. doi:10.1056/NEJMra061360.
[39] R. Smith, R.C. Nicholson, Corticotrophin releasing hormone and the timing of birth, Front. Biosci. J. Virtual Libr. 12 (2007) 912–918.
[40] M. Thomson, The physiological roles of placental corticotropin releasing hormone in pregnancy and childbirth, J. Physiol. Biochem. 69 (2013) 559–573. doi:10.1007/s13105-012-0227-2.
[41] P.D. Wadhwa, C.A. Sandman, T.J. Garite, Chapter 9 The neurobiology of stress in human pregnancy: implications for prematurity and development of the fetal central nervous system, in: Prog. Brain Res., Elsevier, 2001: pp. 131–142. doi:10.1016/S0079-6123(01)33010-8.
[42] P.D. Wadhwa, T.J. Garite, M. Porto, L. Glynn, A. Chicz-DeMet, C. Dunkel-Schetter, C.A. Sandman, Placental corticotropin-releasing hormone (CRH), spontaneous preterm

birth, and fetal growth restriction: a prospective investigation, Am. J. Obstet. Gynecol. 191 (2004) 1063–1069. doi:10.1016/j.ajog.2004.06.070.
[43] P.D. Wadhwa, C. Dunkel-Schetter, A. Chicz-Demet, M. Porto, C.A. Sandman, Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy, Psychosom. Med. 58 (1996) 432–446. doi:10.1097/00006842-199609000-00006.
[44] M. Weinstock, The potential influence of maternal stress hormones on development and mental health of the offspring, Brain. Behav. Immun. 19 (2005) 296–308. doi:10.1016/j.bbi.2004.09.006.
[45] D.L. Helmreich, D.B. Parfitt, X.-Y. Lu, H. Akil, S.J. Watson, Relation between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis during repeated stress, Neuroendocrinology. 81 (2005) 183–192. doi:10.1159/000087001.
[46] P. Heyma, R.G. Larkins, Glucocorticoids decrease in conversion of thyroxine into 3, 5, 3'-tri-iodothyronine by isolated rat renal tubules, Clin. Sci. Lond. Engl. 1979. 62 (1982) 215–220.
[47] C. Pedersen, J. Leserman, N. Garcia, M. Stansbury, S. Meltzer-Brody, J. Johnson, Late pregnancy thyroid-binding globulin predicts perinatal depression, Psychoneuroendocrinology. 65 (2016) 84–93. doi:10.1016/j.psyneuen.2015.12.010.
[48] L.E. Johns, K.K. Ferguson, T.F. McElrath, B. Mukherjee, E.W. Seely, J.D. Meeker, Longitudinal Profiles of Thyroid Hormone Parameters in Pregnancy and Associations with Preterm Birth, PLOS ONE. 12 (2017) e0169542. doi:10.1371/journal.pone.0169542.
[49] S.M. Eick, E.S. Barrett, T.J. van 't Erve, R.H.N. Nguyen, N.R. Bush, G. Milne, S.H. Swan, K.K. Ferguson, Association between prenatal psychological stress and oxidative stress during pregnancy, Paediatr. Perinat. Epidemiol. 32 (2018) 318–326. doi:10.1111/ppe.12465.
[50] null Elenkov, null Chrousos, Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease, Trends Endocrinol. Metab. TEM. 10 (1999) 359–368.
[51] K. Neier, E.H. Marchlewicz, D.C. Dolinoy, V. Padmanabhan, Assessing Human Health Risk to Endocrine Disrupting Chemicals: a Focus on Prenatal Exposures and Oxidative Stress, Endocr. Disruptors Austin Tex. 3 (2015). doi:10.1080/23273747.2015.1069916.
[52] X. Ni, Y. Hou, B.R. King, X. Tang, M.A. Read, R. Smith, R.C. Nicholson, Estrogen receptor-mediated down-regulation of corticotropin-releasing hormone gene expression is dependent on a cyclic adenosine 3',5'-monophosphate regulatory element in human placental syncytiotrophoblast cells, J. Clin. Endocrinol. Metab. 89 (2004) 2312–2318. doi:10.1210/jc.2003-030948.
[53] F. Petraglia, A. Imperatore, J.R.G. Challis, Neuroendocrine Mechanisms in Pregnancy and Parturition, Endocr. Rev. 31 (2010) 783–816. doi:10.1210/er.2009-0019.
[54] L.H. Cohen, J. McGowan, S. Fooskas, S. Rose, Positive life events and social support and the relationship between life stress and psychological disorder, Am. J. Community Psychol. 12 (1984) 567–587.
[55] M. Pluess, H. Wurmser, A. Buske-Kirschbaum, M. Papousek, K.-M. Pirke, D. Hellhammer, M. Bolten, Positive life events predict salivary cortisol in pregnant women, Psychoneuroendocrinology. 37 (2012) 1336–1340. doi:10.1016/j.psyneuen.2012.01.006.
[56] E.S. Barrett, L.E. Parlett, S. Sathyanarayana, J.B. Redmon, R.H.N. Nguyen, S.H. Swan, Prenatal stress as a modifier of associations between phthalate exposure and reproductive

development: results from a multicenter pregnancy cohort study, Paediatr. Perinat. Epidemiol. 30 (2016) 105–114. doi:10.1111/ppe.12264.
[57] P. Ashrap, D.J. Watkins, A.M. Calafat, X. Ye, Z. Rosario, P. Brown, C.M. Vélez-Vega, A. Alshawabkeh, J.F. Cordero, J.D. Meeker, Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends, Environ. Int. (2018). doi:10.1016/j.envint.2018.08.020.

CHAPTER VI. Conclusions and Integration of Findings

This dissertation presents findings from four studies on the relationships between phenol, paraben and triclocarban exposure during pregnancy, maternal hormone levels, and birth outcomes. These results provide strong evidence for a role these chemicals have in impacting gestational length and birth size, suggesting a possible mediating effect of maternal hormones, as well as a possible modifying effect of maternal stress. Previous studies in this area have been limited, were cross-sectional in nature, and did not include the same breadth of exposure biomarkers and hormones. This is also the first study to report interactions between maternal stress and these exposure biomarkers on birth outcomes. Figure VI.1 summarizes the interaction between the hormone pathways explored in this dissertation that could be disrupted via chemical exposure and maternal stress, leading to changes in gestational length. While additional investigations are required, this dissertation provides implications for future health policies aimed to reduce adverse birth outcomes in the population.

Exposure biomarkers and hormones. Aim 1 of this dissertation examined the effect of phenols, parabens and triclocarban on maternal thyroid and reproductive hormones. The first study was conducted on the LifeCodes cohort of pregnant women in Boston, MA, and only included thyroid hormones. The associations differed by exposure biomarker, but the majority of the significant associations were with T3 and TSH, and few exposure biomarkers were associated with changes in FT4 and T4. BP-3, triclosan, triclocarban, and BPB were associated with a decrease in T3, while MPB was associated with an increase in T3. BPS and TCS were associated with an increase in TSH, whereas triclocarban was associated with a decrease in TSH. There appeared to be windows of vulnerability: BPS, MPB and PPB were associated with thyroid hormone at earlier time points in pregnancy, whereas BPB was associated with thyroid hormone later in pregnancy.

A second study was conducted on a cohort of pregnant women in Northern Puerto Rico. This study included reproductive hormones as well thyroid hormones, and included more exposure biomarkers than the first study. However, the women in this cohort had serum hormone analyses conducted at two time points during pregnancy, rather than the four time points the LifeCodes cohort included. There were differences in the associations observed. Triclosan was also associated with a decrease in T3; however, triclocarban was associated with an increase in T3 in this study. BPS was associated with a decrease in TSH at study visit 1, in contrast to the increase in TSH at study visit 1 in LifeCodes. There were no strong associations between MPB and thyroid hormones in the PROTECT cohort, as opposed to the increase in T4 and T3 with MPB in LifeCodes. However, MPB was associated with a decrease in TSH at the third study visit, similar to the LifeCodes cohort. Associations with BPS and MPB were stronger at the earlier time point in pregnancy, whereas associations with triclosan and triclocarban were stronger at the later time point.

Differences in the results may be explained by differences in the study populations. The largest differences observed between the two cohorts included associations with triclocarban and BPS. Concentrations of triclocarban in the PROTECT cohort were much higher than the levels detected in LifeCodes, partly due to the differences in the LODs across both studies. The geometric mean of triclocarban was 4.3 μg/L in PROTECT with less than 6% below the LOD (0.1 μg/L), and geometric mean in LifeCodes was below the LOD (2 μg/L). Similarly, the geometric mean of BPS in PROTECT was 0.5 μg/L with less than 10% of samples below the LOD (0.1 μg/L), whereas the geometric mean of BPS concentrations in LifeCodes was below the LOD (0.4 μg/L). The differences in the LODs and higher concentrations observed in PROTECT may, at least partly, explain the differences observed between the two cohorts. Other exposure biomarkers in the PROTECT cohort also had lower LODs than the corresponding biomarkers in LifeCodes (including 2,4-DCP, 2,5-DCP, BPB and PPB) providing an advantage in exploring the dose-response curves of the exposure biomarkers at lower levels of exposure. No evidence of non-linearity was observed in the PROTECT cohort in exposure-hormone models.

In addition, the two study populations differed in their timing and characteristics. LifeCodes includes mostly White women of high income, whereas the PROTECT cohort is predominantly

Hispanic and dependent on public health insurance. Thus, there may be other residual confounders unaccounted for that explain the differences observed.

With regards to reproductive hormones, parabens were associated with a decreased level of SHBG, particularly at the earlier time point. In conference with the decrease in SHBG, MPB was also associated with a decrease in testosterone, estriol, and progesterone at the earlier time point. The testosterone, estriol and progesterone concentrations measured are all total hormone concentrations; therefore, these concentrations include the SHBG-bound hormones. This could indicate a direct effect of MPB on the levels of SHBG, which subsequently decreased the total concentrations of SHBG-bound hormones. An in vitro study showed that parabens effectively bind to SHBG [1], which supports the hypothesis that parabens can directly impact the binding affinity of SHBG. This is further evidenced by the strength of associations observed in our study. SHBG binds strongly to testosterone, followed by estriol, and weakly to progesterone [2]. My analysis showed the strongest association at the earlier time point was between MPB and testosterone, followed by estriol, and finally progesterone, mimicking SHBG's order of affinity. A decrease in SHBG may allow the mother to be more vulnerable to fetal sex hormones that pass through the placenta [3]. A decrease in SHBG has also been associated with hypothyroidism [4]; however, MPB in this study was also associated with a decrease in TSH. A decrease in TSH is evidence of hyperthyroidism, rather than hypothyroidism. Alternatively, this decrease in SHBG could indicate liver toxicity given that SHBG is produced in the liver.

Other associations between exposure biomarkers and hormones varied. BPS was strongly associated with a decrease in CRH, whereas triclosan was strongly associated with an increase in CRH. Triclosan was also associated with an increase in testosterone and progesterone. BPA, on the other hand, was associated with a decrease in testosterone.

There were limitations across both studies. While both studies improved upon the cross-sectional design, the included time points may not be sufficient in understanding the complexities of these associations due to the changing hormone levels, and the high variation in exposure biomarkers throughout the day and throughout pregnancy. Furthermore, we did not take into account the iodine status, nor did we measure thyroperoxidase antibodies and human chorionic gonadotropin (hCG), any of which could impact thyroid function and confound our results. There were many models run, and there may be associations observed simply due to chance. However, I did not

apply any adjustments to correct for the multiple comparisons (such as the Bonferroni adjustment) because the methods are too conservative, risking Type II error, and potentially missing important relationships in this exploratory analysis. Despite the limitations, there were many strengths to the studies. The collection of exposure biomarkers and hormone data at multiple time points during pregnancy helps account for the biomarkers' short lifespan in the body, and the varying levels of hormones throughout pregnancy. Our robust sample size also allowed us to be able to explore potential windows of susceptibility for these associations.

In conclusion, my findings from Aim 1 provide evidence of endocrine disruption properties of phenols, parabens and triclocarban. This endocrine disruption could potentially lead to adverse health outcomes, particularly in the context of pregnancy, where there are many developmental changes occurring and even slight changes to hormone levels could impact the growing fetus. Further studies are needed to understand these associations better to protect the mother and her fetus.

Exposure biomarkers and birth outcomes. Aim 2 of this dissertation examined the effect of phenols, parabens and triclocarban on birth outcomes. I first looked at the average exposure of each exposure biomarker across three study visits, and then examined windows of susceptibility by stratifying the models by study visit. We found that BP-3, MPB and PPB were associated with an increase in gestational length. The associations with MPB, PPB and BPA were strongest at the first study visit indicating earlier time points are more vulnerable to changes in gestational length. BP-3 associations did not differ by study visit. Triclocarban was suggestively associated with a decrease in gestational length, and the association was also stronger at earlier time points in pregnancy. Associations with triclosan, on the other hand, differed by fetal sex. Triclosan was associated with an increase in gestational length among male fetuses, and a decrease in gestational length among female fetuses. We wanted to look into differences in preterm birth (spontaneous versus placental), but were limited by the sample size. Furthermore, most of our significant associations indicated an increase in gestational length, rather than a decrease.

None of the exposure biomarkers were associated with birthweight; there were however associations with birth size. Parabens were protective of SGA, but were not associated with LGA. Triclosan was associated with a higher odds of SGA, and BP-3 was associated with a higher odds of LGA, and these associations were strongest at 20-24 weeks gestation. The

relationship between BPS and birth size differed by study visit. At 16-20 weeks gestation, BPS was associated with LGA; however, at 24-28 weeks gestation, BPS was associated with SGA. To date, no other studies have looked into the association between BPS and birth outcomes in humans, and this finding of opposite directions of associations by time point needs to be verified by future studies as our significant results could be due to chance. Nevertheless, this finding highlights the importance of investigating effects of endocrine disruptors at different time points during pregnancy. It can also help explain why results in this area of research can vary from one study to another.

Few studies have looked into the relationships between these exposure biomarkers and birth outcomes. One study examined the association between BP-3 and gestational length, and reported a decrease in gestational length, in conflict with our results [5]. However, as previously mentioned, it appears that the timing of exposure is important in determining the outcome. In the mentioned study, BP-3 was measured at delivery, whereas our measurements were conducted at up to 28 weeks, i.e. the beginning of the third trimester. Similarly, studies that looked at the association between BPA and gestational length also measured BPA concentrations at delivery, all of which reported a decrease in gestational length with higher concentrations of BPA [5–7], in contrast to our finding of an increase in gestational length at 16-20 weeks gestation. These studies that look at BPA concentrations at time of delivery also risk reverse causation. Higher risk pregnancies involve more invasive medical care, leading to higher BPA exposure through medical equipment. Thus, the shortened gestational length could result in higher BPA concentrations rather than BPA leading to that adverse change. There was one study that looked at BPA levels at a similar time point to our study, and also reported an increase in gestational length with higher maternal plasma concentrations of BPA [8].

Previous reports on parabens were more in line with our results. Geer et al. also reported a protective effect on preterm birth [9], similar to our findings. We detected a decrease in SHBG and an insignificant decrease in estriol in association with paraben biomarkers in Aim 1. Estrogens increase steadily during pregnancy, and play a role in gestational length by stimulating uterine prostaglandins and contractions [10,11]. Therefore, it is possible that a decrease in these hormones by parabens could influence this important rise that triggers parturition, leading to a longer gestational length. Parabens have also been reported to increase birthweight and birth

length, which could indicate an increase in birth size [12,13]. While we did not report an increase in birth size with paraben biomarkers, we did observe a protective effect against SGA at the 20-24 weeks gestation time point.

There were no previous studies that looked into triclocarban's impact on gestational length. However, triclocarban was associated with an increase in T3 in Aim 1. A previous report found that high levels of T3 at similar time points to the study visits reported in PROTECT was associated with an increased odds of preterm birth [14]. Therefore, our finding could indicate a potential mediating effect of T3 in the association between triclocarban and decreased gestational length.

With regards to our association between gestational length and triclosan, four other studies also reported on significant associations between triclosan and birth outcomes among boys only [15–18]. Fetal sex can impact the gestational environment, and cause different birth outcomes. For example, preterm births due to pre-eclampsia are more common in female infants versus male infants [19], and there are differences in placentation by fetal sex that could lead to adverse birth outcomes [20]. Thus, it is plausible that the interaction between triclosan and fetal sex could lead to different birth outcomes, possibly mediated by an androgenic effect from triclosan [34]. In fact, as reported in CHAPTER III, triclosan was associated with an increase in maternal testosterone levels in the PROTECT cohort.

The analyses included in Aim 2 is the largest human study to date to look at the relationship between phenols, parabens and triclocarban in relation to birth outcomes that includes several time points. While there were many strengths to this study, there were some limitations. Similar to the limitations pertaining to Aim 1, the variation of concentrations of these exposure biomarkers over time may introduce potential bias. We also had a smaller sample size for some of the emerging phenols of concern, such as BPA replacement chemicals, and triclocarban as they were added to the analytical panel part-way through the study. Unlike most other studies in this area, however, we were able to explore windows of susceptibility over a number of birth outcomes. Additionally, even though our sample sizes were smaller for some analytes, we are among the first to explore associations with some emerging chemicals of interest. Our population had an exceptionally high concentration of triclocarban as compared to other populations, allowing us to study this association in a vulnerable population. While further studies are

required to substantiate our results, the work presented in Aim 2 provides evidence to caution parents about the kind of products used during pregnancy.

Interaction between exposure biomarkers and psychosocial stress. Aim 3 expanded the analysis of Aim 2 by exploring the interaction between exposure biomarkers and psychosocial stress indices on gestational length. Interactions between chemical and non-chemical stressors are important to consider in deepening our understanding on how the environment impacts humans. Stress can impact the human body via similar mechanisms of action to those proposed for phenols, parabens and triclocarban, leading to multiple "hits" on a system, eventually increasing the allostatic load sufficiently to cause an adverse effect.

Four psychosocial indices were available from the PROTECT cohort, including perceived stress, depression, social support and a life events survey (LES). Only interactions with the LES score were statistically significant, and the rest of Aim 3 focused on the results of these interactions. The LES score was further divided into only the positive life events and the negative life events.

Associations with BPS, triclocarban, MPB and PPB were strongest when the total LES scores were negative, indicating that gestational length was most affected in the presence of higher concentrations of the exposure biomarkers, as well as overall negative life events. In Aim 2, associations between the average BPS and triclocarban concentrations were not significantly associated with gestational length; however, Aim 3 reports significant associations in the presence of negative life events. Associations between BP-3 and gestational length were not modified by LES. In the examination of negative life events alone, women with medium and high scores of Negative LES had stronger associations between the exposure biomarkers and gestational length. The association between triclocarban and gestational length was strongest when the Positive LES score was low, indicating the lack of positive life events modified the association more so than the presence of negative life events.

None of the psychosocial indices were independently associated with gestational length in our dataset. Therefore, this indicates that the presence of psychosocial stress was not sufficient to illicit a response. It was only in the presence of both higher concentrations of exposure biomarkers and negative scores of Total LES that we observe up to five-day changes in gestational length. These results support the multiple hit theory described in **Error! Reference**

source not found.. Aim 3 further highlights the importance of examining the impact of multiple stressors on health, rather than looking at individual chemicals or other stressors in silo.

Given the relationships observed between the exposure biomarkers and hormones in Aim 1, it is possible that the associations observed in Aims 2 and 3 were at least partly mediated through endocrine disruption. Life events is used as a proxy measure for maternal stress, and maternal stress has been implicated to negatively impact the maternal-placental-fetal hypothalamic-pituitary-adrenal axis [21,22]. A pertinent hormone in stress-caused responses is cortisol, which is mediated by levels of CRH. While cortisol and CRH are part of the HPA axis, they also influence thyroid hormones part of the HPT axis [23], and are influenced themselves by reproductive hormones such estrogens and progesterone [24]. This complex interplay of hormones make it difficult to disentangle how the combined effect of exposure biomarkers and stress could in unison impact gestational length, but it does speak to the potential vulnerability in the system in the case of multiple hits. For example, excess stress increases levels of cortisol, which is implicated in stimulating preterm birth [25]. Cortisol, however, also inhibits T4 to T3 deodination; T3 is another hormone implicated in stimulating preterm birth [14]. As previously mentioned, none of the stress indices were associated with gestational length in this cohort. As shown in Aim 1, triclocarban was associated with an increase in T3. Thus, it may be possible that the combined effect of an increase in cortisol via negative life events, as well as an increase in T3 via triclocarban caused a sufficient allostatic overload, leading to a significant decrease in gestational length.

The associations with parabens were especially difficult to interpret. Parabens were associated with an increase in gestational length; however, maternal stress is associated with a decrease in gestational length. Parabens were associated with a decrease in estriol, while stress is associated with an increase in CRH [26] and subsequent increase in estriol [11]. Additionally, parabens have antimicrobial properties which could decrease inflammation, while stress is associated with an increase in inflammation [27]. Because of these opposite biological effects, I would have expected to see no effect on gestational length with high levels of stress and paraben exposure. This conflicting finding could indicate that parabens affect gestational length through a different mechanism than the one proposed, or it could indicate that interaction between stressors act differently than in the presence of each stressor alone. This was also observed in an animal study

that exposed rats to various stressors across two generations, and also observed different effects on birth outcomes with different combination of stressors [28].

Aim 3 was the first study to look at the interaction between this set of exposure biomarkers and psychosocial stressors. A unique strength of this study was its exploration of positive, as well as negative life events. Most studies examine the effect of negative events and/or maternal stress without exploring the buffering effects of positive life events. This study the importance of looking at both in understanding the modifying effect of stress on chemical exposures and birth outcomes. While the robust sample size allowed us to look for interactions effectively, there were some limitations to the study. The timing of maternal stress could influence the effect on the fetus [26]; however, we did not have data on when the life event took place apart from happening sometime between the beginning of pregnancy and the mid-way through the second trimester. Similarly, we had no data on the participants' psychosocial profiles past this time point. Given this, we had to rely on average biomarker concentrations as our exposure proxies. The three time points used to calculate the average concentration is more robust than using a single time point, but the models resemble a cross-sectional design.

Future Research Recommendations

These results add to the growing evidence on the risk of phenol, paraben and triclocarban exposure on adverse birth outcomes. While this dissertation examines these associations in a large cohort, future studies are required to validate our findings. Of note, we found differences across the associations by time point in pregnancy, so future studies should include more time points to look for potential windows of vulnerability, and confirm our findings.

I originally proposed to estimate the extent to which hormones mediate the associations between the exposure biomarkers and gestational length. However, the effect estimates of the associations between the biomarkers and gestational length were relatively small, and the mediation analysis would have further diluted the estimates, making it difficult to observe meaningful results. There were also no associations between any of the hormones and gestational length in our dataset, possibly due to the smaller sample size for which hormone levels have been measured. Thus, future studies should look to estimate this mediation in a larger sample size. Furthermore, evidence suggests hormone trajectories provide better prediction of pregnancy health as compared to point estimates due to the large variation in hormone levels between women. High

variation also exists within women, and our models did not adjust for these diurnal hormone changes. Future studies should try to incorporate more time points that are further apart to better estimate hormone trajectories and their subsequent mediating effects on pregnancy health. Other pathways should also be considered. Associations between phenols and parabens in relation to inflammation and oxidative stress have been observed, and could also explain the adverse effects observed in this cohort. Other pathways could also include epigenetic changes. Further studies are required to demonstrate the pathways involved.

There has been a push to examine chemical mixtures, rather than studying the effects one chemical at a time. The chemicals included in this dissertation (as well as many others not included) are ubiquitous in our anthropogenic environment. Therefore, it is imperative that future work looks at the combined effect of these chemicals on health to understand the implications of any possible interactions.

In addition to looking at chemical mixtures, interactions between chemical and non-chemical stressors is another largely untapped area of research in the area of endocrine disruptors. This dissertation examines the interaction with psychosocial stressors, but there are other equally important stressors than could modify associations between chemical exposure and health. In the context of Puerto Rico specifically, the data collected for this dissertation occurred prior to Hurricane Maria. Thus, stress will contribute to health even more so now, and studying the effects of this stress will be important in implementing effective strategies to improve overall health and well-being of the Puerto Rican population.

Finally, the majority of research on endocrine disruptors has focused on the biology and statistics of the issue. However, more studies on practically reducing exposure to such chemicals are required. Some researchers have attempted to study the effect of switching personal care products and making dietary changes, but results on the efficacy of such changes have varied [29–31].

Public Health Impact

Exposure to phenols, parabens and triclocarban may contribute to changes in gestational length, including the high preterm birth rate in Puerto Rico. There has been a push to educate and communicate these risks to the general public in order to reduce exposure. There is some

evidence supporting lower concentrations of exposure biomarkers after switching certain products [29–31], and this dissertation supports the need for such programs.

However, there are several issues with the approach of relying on public education alone. First, education does not necessarily lead to behavior change. In spite of high risk perception, change in cosmetic use among pregnant women did not occur [32]. Second, products that avoid use of endocrine disrupting chemicals tend to be cost-prohibitive and can exclude those of lower socioeconomic status. In fact, there is evidence of higher concentrations of phthalates in less educated pregnant women with lower incomes [33]. Therefore, there is concern regarding burdening those with lower socioeconomic status with guilt in the case of an inability to change the products they use.

A better approach would be to make the safety of all products a priority and requirement. Currently, it has been difficult to apply policies to limit the use of these endocrine disruptors given industry's push to raise doubt on their health impacts. Instead, a more comprehensive approach to chemical safety may be a better route. Green chemistry principles have been introduced to consider the health and environmental safety of chemicals during the initial chemical engineering design and process. Such initiatives may be difficult to implement, but would be highly worthwhile in the future, given the burden of impact hazardous chemicals have on population health. Thus, this research grows our understanding of the impacts and potential pathways involved in phenols, parabens and triclocarban exposure on pregnancy, thereby, helping to create overall safer chemicals.

The results pertaining to the modifying effect of maternal stress on this population in Puerto Rico is especially relevant today. Hurricane Maria, a Category 5 Hurricane, hit Puerto Rico in late 2017, leading to catastrophic damage. Many were left without electricity, running water and/or homeless, and remain in need today. The data collected as part of this dissertation were collected prior to the event, and do not reflect the effects post-hurricane, but can serve as a baseline for future comparisons. Based on this analysis, in addition to the direct effects of Hurricane Maria on the health of the Puerto Rican population, the elevated levels of stress as a result of Hurricane Maria created a biological state more vulnerable to various stressors, including chemical exposures. Chemical exposures were likely to be especially elevated due to the destruction of buildings, increased consumption of canned foods, increased cooking over open fires, and other

coping strategies. Thus, maternal and fetal health will be especially affected from this event. In addition to the continued emergency assistance the Puerto Rican population requires, interventions should be put in place to alleviate the effects of these multiple stressors on pregnant women.

Overall Conclusions

In conclusion, this dissertation provides evidence for the endocrine disrupting properties of phenols, parabens and triclocarban during pregnancy, which could potentially mediate the relationship between the exposure biomarkers and birth outcomes. Additionally, this dissertation provides evidence for a modifying effect of psychosocial stress, where negative life events can strengthen associations between exposure biomarkers and gestational length. This body of work significantly improves upon the body of knowledge in this area of research by utilizing a large prospective cohort study, and by exploring the modifying effect of psychosocial factors during pregnancy. This is also the first study to include this breadth of exposures and birth outcomes in a cohort of pregnant women. Thus, these results provide a strong argument for the role of phenols, parabens and triclocarban in the risk of adverse birth outcomes, and can be used to support stronger policies in the future.

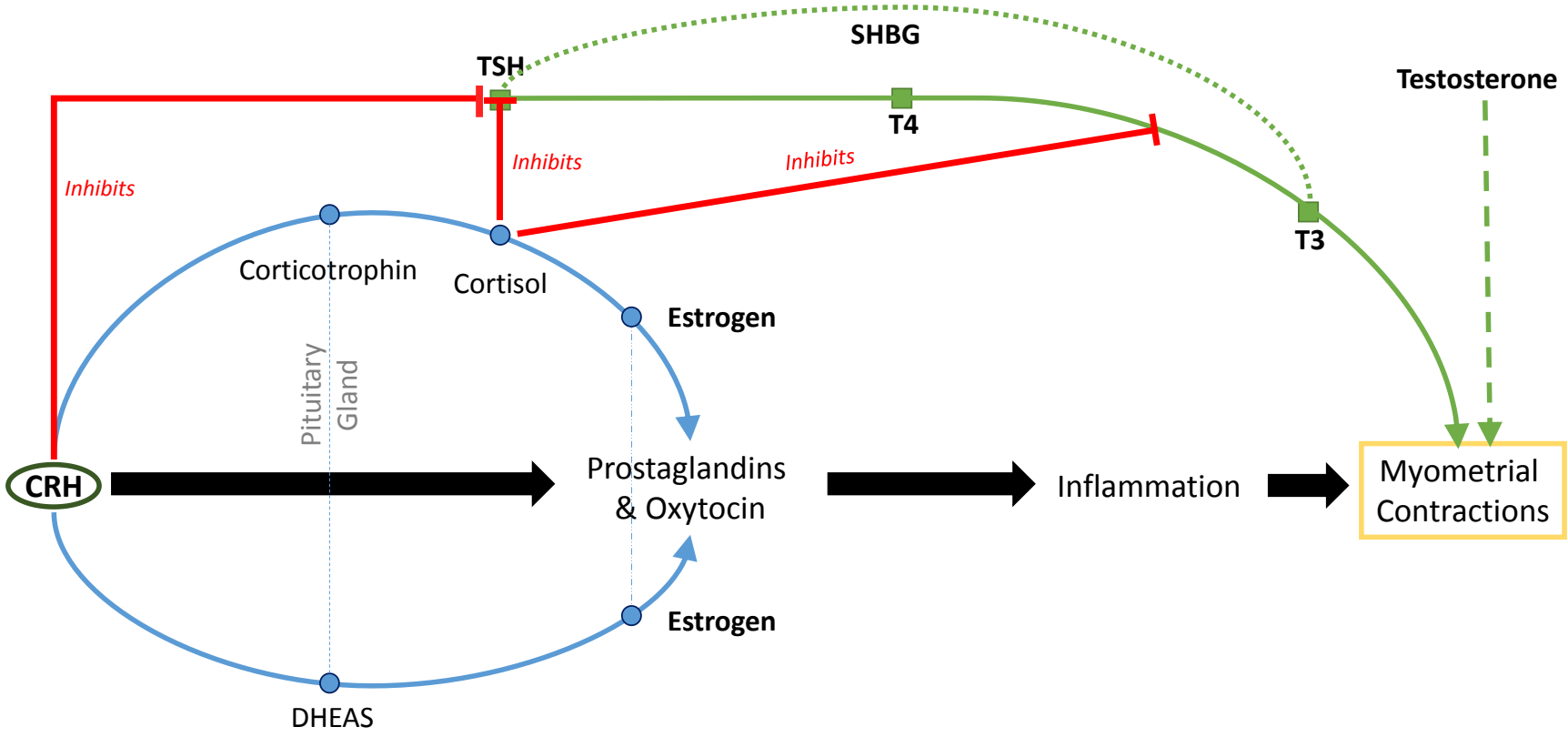


Figure VI.1: Depiction of hormone pathways during pregnancy. Bolded hormones are hormones studied in this dissertation. SHBG rises and falls with thyroid hormones. Some evidence supports testosterone's role in initiating parturition, but it is unclear how. CRH is produced by hypothalamus and placenta, and has an important role in setting biological clock of pregnancy duration. CRH is also produced by the hypothalamus with maternal stress to stimulate cortisol production. Phenols, parabens and triclocarban are associated with changes in these hormones during pregnancy, which could affect gestational length.

References

[1] H. Hong, W.S. Branham, H.W. Ng, C.L. Moland, S.L. Dial, H. Fang, R. Perkins, D. Sheehan, W. Tong, Human Sex Hormone-Binding Globulin Binding Affinities of 125 Structurally Diverse Chemicals and Comparison with Their Binding to Androgen Receptor, Estrogen Receptor, and α-Fetoprotein, Toxicol. Sci. 143 (2015) 333–348. doi:10.1093/toxsci/kfu231.

[2] C. Selby, Sex hormone binding globulin: origin, function and clinical significance, Ann. Clin. Biochem. 27 (Pt 6) (1990) 532–541. doi:10.1177/000456329002700603.

[3] G.L. Hammond, Diverse Roles for Sex Hormone-Binding Globulin in Reproduction, Biol. Reprod. 85 (2011) 431–441. doi:10.1095/biolreprod.111.092593.

[4] S.C. Dumoulin, B.P. Perret, A.P. Bennet, P.J. Caron, Opposite effects of thyroid hormones on binding proteins for steroid hormones (sex hormone-binding globulin and corticosteroid-binding globulin) in humans, Eur. J. Endocrinol. 132 (1995) 594–598.

[5] R. Tang, M.-J. Chen, G.-D. Ding, X.-J. Chen, X.-M. Han, K. Zhou, L.-M. Chen, Y.-K. Xia, Y. Tian, X.-R. Wang, Associations of prenatal exposure to phenols with birth outcomes, Environ. Pollut. Barking Essex 1987. 178 (2013) 115–120. doi:10.1016/j.envpol.2013.03.023.

[6] F. Behnia, M. Peltier, D. Getahun, C. Watson, G. Saade, R. Menon, High bisphenol A (BPA) concentration in the maternal, but not fetal, compartment increases the risk of spontaneous preterm delivery, J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. (2016) 1–7. doi:10.3109/14767058.2016.1139570.

[7] B. Weinberger, A.M. Vetrano, F.E. Archer, S.W. Marcella, B. Buckley, D. Wartenberg, M.G. Robson, J. Klim, S. Azhar, S. Cavin, L. Wang, D.Q. Rich, Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age, J. Matern.-Fetal Neonatal Med. Off. J. Eur. Assoc. Perinat. Med. Fed. Asia Ocean. Perinat. Soc. Int. Soc. Perinat. Obstet. 27 (2014) 323–327. doi:10.3109/14767058.2013.815718.

[8] A. Veiga-Lopez, K. Kannan, C. Liao, W. Ye, S.E. Domino, V. Padmanabhan, Gender-Specific Effects on Gestational Length and Birth Weight by Early Pregnancy BPA Exposure, J. Clin. Endocrinol. Metab. 100 (2015) E1394-1403. doi:10.1210/jc.2015-1724.

[9] L.A. Geer, B.F.G. Pycke, J. Waxenbaum, D.M. Sherer, O. Abulafia, R.U. Halden, Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York, J. Hazard. Mater. 323, Part A (2017) 177–183. doi:10.1016/j.jhazmat.2016.03.028.

[10] V.D. Castracane, Endocrinology of preterm labor, Clin. Obstet. Gynecol. 43 (2000) 717–726.

[11] R. Smith, J. Paul, K. Maiti, J. Tolosa, G. Madsen, Recent advances in understanding the endocrinology of human birth, Trends Endocrinol. Metab. 23 (2012) 516–523. doi:10.1016/j.tem.2012.05.002.

[12] C. Philippat, M. Mortamais, C. Chevrier, C. Petit, A.M. Calafat, X. Ye, M.J. Silva, C. Brambilla, I. Pin, M.-A. Charles, S. Cordier, R. Slama, Exposure to phthalates and phenols during pregnancy and offspring size at birth, Environ. Health Perspect. 120 (2012) 464–470. doi:10.1289/ehp.1103634.

[13] C. Wu, W. Huo, Y. Li, B. Zhang, Y. Wan, T. Zheng, A. Zhou, Z. Chen, M. Qian, Y. Zhu, Y. Jiang, H. Liu, J. Hu, X. Chen, B. Xu, W. Xia, S. Xu, Maternal urinary paraben levels and offspring size at birth from a Chinese birth cohort, Chemosphere. 172 (2017) 29–36. doi:10.1016/j.chemosphere.2016.12.131.

[14] L.E. Johns, K.K. Ferguson, T.F. McElrath, B. Mukherjee, E.W. Seely, J.D. Meeker, Longitudinal Profiles of Thyroid Hormone Parameters in Pregnancy and Associations with Preterm Birth, PLOS ONE. 12 (2017) e0169542. doi:10.1371/journal.pone.0169542.

[15] K.K. Ferguson, J.D. Meeker, D.E. Cantonwine, B. Mukherjee, G.G. Pace, D. Weller, T.F. McElrath, Environmental phenol associations with ultrasound and delivery measures of fetal growth, Environ. Int. 112 (2018) 243–250. doi:10.1016/j.envint.2017.12.011.

[16] T.H. Lassen, H. Frederiksen, H.B. Kyhl, S.H. Swan, K.M. Main, A.-M. Andersson, D.V. Lind, S. Husby, C. Wohlfahrt-Veje, N.E. Skakkebæk, T.K. Jensen, Prenatal Triclosan Exposure and Anthropometric Measures including Anogenital Distance in Danish Infants, Environ. Health Perspect. (2016). doi:10.1289/ehp.1409637.

[17] C. Philippat, J. Botton, A.M. Calafat, X. Ye, M.-A. Charles, R. Slama, EDEN Study Group, Prenatal exposure to phenols and growth in boys, Epidemiol. Camb. Mass. 25 (2014) 625–635. doi:10.1097/EDE.0000000000000132.

[18] M.S. Wolff, S.M. Engel, G.S. Berkowitz, X. Ye, M.J. Silva, C. Zhu, J. Wetmur, A.M. Calafat, Prenatal phenol and phthalate exposures and birth outcomes, Environ. Health Perspect. 116 (2008) 1092–1097. doi:10.1289/ehp.11007.

[19] Global Pregnancy Collaboration:, S. Schalekamp-Timmermans, L.R. Arends, E. Alsaker, L. Chappell, S. Hansson, N.K. Harsem, M. Jälmby, A. Jeyabalan, H. Laivuori, D.A. Lawlor, C. Macdonald-Wallis, P. Magnus, J. Myers, J. Olsen, L. Poston, C.W. Redman, A.C. Staff, P. Villa, J.M. Roberts, E.A. Steegers, Fetal sex-specific differences in gestational age at delivery in pre-eclampsia: a meta-analysis, Int. J. Epidemiol. 46 (2017) 632–642. doi:10.1093/ije/dyw178.

[20] Z.A. Brown, S. Schalekamp-Timmermans, H.W. Tiemeier, A. Hofman, V.W.V. Jaddoe, E.A.P. Steegers, Fetal sex specific differences in human placentation: A prospective cohort study, Placenta. 35 (2014) 359–364. doi:10.1016/j.placenta.2014.03.014.

[21] C. de Weerth, J.K. Buitelaar, Physiological stress reactivity in human pregnancy--a review, Neurosci. Biobehav. Rev. 29 (2005) 295–312. doi:10.1016/j.neubiorev.2004.10.005.

[22] D. Kingston, S. Tough, H. Whitfield, Prenatal and postpartum maternal psychological distress and infant development: a systematic review, Child Psychiatry Hum. Dev. 43 (2012) 683–714. doi:10.1007/s10578-012-0291-4.

[23] D.L. Helmreich, D.B. Parfitt, X.-Y. Lu, H. Akil, S.J. Watson, Relation between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenal (HPA) axis during repeated stress, Neuroendocrinology. 81 (2005) 183–192. doi:10.1159/000087001.

[24] R.J. Handa, M.J. Weiser, Gonadal steroid hormones and the hypothalamo-pituitary-adrenal axis, Front. Neuroendocrinol. 35 (2014) 197–220. doi:10.1016/j.yfrne.2013.11.001.

[25] M.A. Diego, T. Field, M. Hernandez-Reif, S. Schanberg, C. Kuhn, V.H. Gonzalez-Quintero, Prenatal Depression Restricts Fetal Growth, Early Hum. Dev. 85 (2009) 65–70. doi:10.1016/j.earlhumdev.2008.07.002.

[26] M. Weinstock, The potential influence of maternal stress hormones on development and mental health of the offspring, Brain. Behav. Immun. 19 (2005) 296–308. doi:10.1016/j.bbi.2004.09.006.
[27] S.L. Bronson, T.L. Bale, Prenatal Stress-Induced Increases in Placental Inflammation and Offspring Hyperactivity Are Male-Specific and Ameliorated by Maternal Antiinflammatory Treatment, Endocrinology. 155 (2014) 2635–2646. doi:10.1210/en.2014-1040.
[28] B.S.E. Verstraeten, J.K. McCreary, S. Weyers, G.A.S. Metz, D.M. Olson, Prenatal two-hit stress affects maternal and offspring pregnancy outcomes and uterine gene expression in rats: match or mismatch?, Biol. Reprod. (n.d.). doi:10.1093/biolre/ioy166.
[29] E.S. Barrett, M. Velez, X. Qiu, S.-R. Chen, Reducing Prenatal Phthalate Exposure Through Maternal Dietary Changes: Results from a Pilot Study, Matern. Child Health J. 19 (2015) 1936–1942. doi:10.1007/s10995-015-1707-0.
[30] C.-Y. Chen, Y.-Y. Chou, S.-J. Lin, C.-C. Lee, Developing an intervention strategy to reduce phthalate exposure in Taiwanese girls, Sci. Total Environ. 517 (2015) 125–131. doi:10.1016/j.scitotenv.2015.02.021.
[31] Harley Kim G., Kogut Katherine, Madrigal Daniel S., Cardenas Maritza, Vera Irene A., Meza-Alfaro Gonzalo, She Jianwen, Gavin Qi, Zahedi Rana, Bradman Asa, Eskenazi Brenda, Parra Kimberly L., Reducing Phthalate, Paraben, and Phenol Exposure from Personal Care Products in Adolescent Girls: Findings from the HERMOSA Intervention Study, Environ. Health Perspect. 124 (2016) 1600–1607. doi:10.1289/ehp.1510514.
[32] C. Marie, S. Cabut, F. Vendittelli, M.-P. Sauvant-Rochat, Changes in Cosmetics Use during Pregnancy and Risk Perception by Women, Int. J. Environ. Res. Public. Health. 13 (2016) 383. doi:10.3390/ijerph13040383.
[33] A.G. Wenzel, J.W. Brock, L. Cruze, R.B. Newman, E.R. Unal, B.J. Wolf, S.E. Somerville, J.R. Kucklick, Prevalence and predictors of phthalate exposure in pregnant women in Charleston, SC, Chemosphere. 193 (2018) 394–402. doi:10.1016/j.chemosphere.2017.11.019.
[34] M. Yueh, R.H. Tukey, Triclosan: A widespread environmental toxicant with many biological effects, Annu Rev Pharmacol Toxicol. 56 (2016) 251–272.