ENDOCRINE DISRUPTING CHEMICALS AND GESTATIONAL HORMONES: ELUCIDATING THE ROLES OF EXOGENOUS AND ENDOGENOUS FACTORS UNDERLYING PERSISTENT NAUSEA IN PREGNANCY

By

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ABSTRACT

Nausea is the most common symptom pregnant women experience. Although nausea symptoms typically subside by the first trimester, many women continue to experience symptoms later into pregnancy, with possible impacts on women's quality of life and future health. While the exact mechanisms are unknown, hormonal changes during pregnancy have been implicated as plausible causes of symptoms. Nearly all pregnant women are exposed to known hormone (endocrine) disrupting chemicals (EDCs), including phthalates, phenols, and their novel replacements from common consumer products, including food packaging/processing materials, personal care products, and various cleaning products. These chemicals are linked to other pregnancy complications, but no studies have assessed whether exposure to these EDCs is related to nausea symptoms. Furthermore, most prior studies focusing on maternal and perinatal health have investigated single chemicals, but pregnant women are exposed to a myriad of chemicals simultaneously. Thus, to better understand the relationship of EDC exposure with health outcomes, chemicals need to be considered jointly. Therefore, our research was designed to understand the relationships between EDCs, gestational hormones, and nausea during pregnancy by utilizing information collected in the Illinois Kids Development Study. Specifically, we evaluated associations of EDC mixtures with persistent nausea during pregnancy (**Chapter Two**), assessed relationships of EDC mixtures with mid-pregnancy sex-steroid and thyroid hormones (**Chapter Three**), and identified hormonal predictors of persistent nausea (**Chapter Four**). To address limitations in prior studies, we assessed persistent nausea rather than typical nausea and used various statistical mixture methods to consider exposures to many EDCs jointly, including several newer replacements. Findings from this dissertation have the potential to identify modifiable contributors to persistent nausea during pregnancy that could be targeted through targeted lifestyle interventions, including reducing the use of consumer products that contain phthalates and phenols.

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Foremost, I thank my advisor, Dr. Rita Strakovsky. While I cannot prove a causal relationship between her mentorship and my PhD success, the Bradford-Hill criteria strengthen my claim. First, as her mentorship started a long six years prior to the completion of my research, we have a clear temporal relationship. Second, as my exposure to her mentorship increased, my productivity increased, demonstrating a strong dose-response relationship. Third, as good mentoring is critical for successful PhD completion and poor mentoring often results in failure, her mentoring leading to my success is coherent with this principle. Fourth, a plausible biological mechanism is her perfectionism and hands-on mentoring style increasing my circulating stress hormones, which fueled my motivation and persistence and led to me accomplishing my research goals. Fifth, her past successful mentoring of Dr. Diana Pacyga provides an excellent analogy to my own experience. Unfortunately, unless Dr. Strakovsky has unknown murine mentorship experiments ongoing, I cannot rely on experimental evidence; however, if she did have mice under her tutelage, I am sure they would be submitting numerous manuscripts for publication and grants for review! Lastly, regarding strength of association, I am certain that my odds of success were significantly increased, and the risk of failure significantly reduced, under her mentorship. I definitively state that Dr. Strakovsky's encouragement and guidance were invaluable in my success and her mentorship will make me a better scientist and physician.

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LIST OF ABBREVIATIONS

Σ	Sum of
<	Less than
>	Greater than
2,4-DCP	2,4-dichlorophenol
2,5-DCP	2,5-dichlorophenol
AAFP	American Academy of Family Physicians
ACOG	American College of Obstetricians and Gynecologists
AHEI-2010	Alternative Healthy Eating Index 2010
BP-3	Benzophenone-3
BPA	Bisphenol A
BPS	Bisphenol S
BPF	Bisphenol F
BKMR	Bayesian kernel machine regression
BMI	Body mass index
°C	Degrees Celsius
CDC	Centers for Disease Control and Prevention
CI	Confidence/credible interval
DAG	Directed acylic graph
DBP	Di-n-butyl phthalate
DEHP	Di-2-ethylhexyl phthalate
DEHTP	Di-2-ethylhexyl terephthalate
DiBP	Di-isobutyl phthalate
DiNCH	Di(isononyl) cyclohexane-1,2-dicarboxylate
DiNP	Di-isononyl phthalate

EDC	Endocrine disrupting chemical
FFQ	Food frequency questionnaire
FT4	Free thyroxine
GDF-15	Placental hormone growth/differentiation factor 15
hCG	Human chorionic gonadotropin
HG	Hyperemesis gravidarum
I-KIDS	Illinois Kids Development Study
ln	Natural log
LOD	Limit of detection
MBP	Mono-n-butyl phthalate
MBzP	Monobenzyl phthalate
MCNP	Monocarboxynonyl phthalate
MCOCH	Cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester
MCOP	Monocarboxyoctyl phthalate
MCPP	Mono(3-carboxypropyl) phthalate
MDRC	University of Michigan Diabetes Research Center Laboratory
MECPTP	Mono(2-ethyl-5-carboxypentyl) terephthalate
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHHTP	Mono(2-ethyl-5-hydroxyhexyl) terephthalate
MEHP	Mono(2-ethylhexyl) phthalate
MEOHP	Mono(2-ethyl-5-oxohexyl) phthalate
MECPP	Mono(2-ethyl-5-carboxypentyl) phthalate
MEP	Mono-ethyl phthalate
MHBP	Mono-hydroxybutyl phthalate
MHiBP	Mono-hydroxyisobutyl phthalate

MiBP	Mono-isobutyl phthalate
MiNP	Mono-isononyl phthalate
MHiNCH	Cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester
MONP	Mono-oxononyl phthalate
NHANES	National Health and Nutrition Examination Survey
NVP	Nausea and vomiting during pregnancy
OR	Odds ratio
PIP	Posterior inclusion probability
PSS	Perceived stress scale
PUQE	Pregnancy-Unique Quantification of Emesis and Nausea
QGComp	Quantile-based g-computation
RR	Risk ratio
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TCS	Triclosan
TPOAb	Thyroid peroxidase antibody
TSH	Thyroid stimulating hormone
TT4	Total thyroxine
WQSR	Weighted quantile sum regression

CHAPTER ONE:

INTRODUCTION

Nausea and vomiting during pregnancy (NVP), commonly known as morning sickness, is the most common symptom women experience during pregnancy, with prevalence estimates between 50 and 90% (Bustos et al., 2017; Herrell, 2014; Lee and Saha, 2011; Niebyl, 2010). Various factors have been identified that predict whether or not a woman will experience NVP, and include older maternal age, family or past history of NVP, twin pregnancies, and fetal sex (Niebyl, 2010). NVP has been linked to many adverse maternal health outcomes, including high blood pressure, preeclampsia, and depression, with potential impacts on employment and family life (Attard et al., 2002; Chortatos et al., 2015; Mazzotta et al., 2000; Niebyl, 2010; Smith et al., 2000). In addition, NVP is costly, with the economic impact of NVP in the United States estimated at $1.7 billion in 2012, with healthcare costs of treating one symptomatic woman estimated at nearly $2,000 (Piwko et al., 2013). Despite negative maternal health impacts, some researchers have theorized an evolutionary basis for NVP where women with nausea restrict their diets and avoid potential teratogens (Flaxman and Sherman, 2000), and some studies have reported that some NVP characteristics are associated with better birth outcomes (Koren et al., 2014; Schrager et al., 2023). However, the identified protective effect may be confounded by hormonal changes that are related to both NVP and better birth outcomes. One major limitation of prior research investigating NVP is focusing on early pregnancy symptoms that are the most prevalent. While NVP does occur more frequently in the first trimester (Herrell, 2014), many women's symptoms continue later in pregnancy, with prevalence estimates around 40%, as late as the third trimester (Einarson et al., 2013; Kramer et al., 2013). It is uncertain whether these seemingly positive associations between NVP and pregnancy would also be observed with NVP symptoms that persist beyond the first trimester. As NVP impacts women's quality of life and it is not limited to the first trimester, research is needed to better understand the underlying mechanisms of symptoms that persist later into pregnancy.

Decades of, almost exclusively, epidemiologic research has explored the biological underpinnings of NVP. While there are experimental models for nausea and vomiting in non-pregnant animals (Horn, 2014; King, 1990), NVP is uniquely human, and there are no animal models capable of elucidating hormonal mechanisms of pregnancy-related nausea. Prior epidemiologic research identified a relationship of higher levels of early-pregnancy human chorionic gonadotropin (hCG) with NVP (Masson et al., 1985); however, as hCG rapidly declines near the end of the first trimester, it is unlikely to explain persistent nausea

symptoms. Sex-steroid hormones (progesterone, estradiol, and testosterone) and thyroid hormones (free thyroxine (FT4), total thyroxine (TT4), and thyroid stimulating hormone (TSH)) increase across pregnancy (Dukic and Ehlert, 2023; Soldin et al., 2004) and have key roles in maintaining pregnancy, preventing uterine contractions, and increasing uterine blood supply (Hacker et al., 2010), with disruptions to these hormones linked to preterm delivery, preeclampsia, fetal growth restriction, and developmental disabilities (Silva et al., 2018). Some studies have considered the roles of sex-steroid and thyroid hormones in NVP symptomology, although findings have been mixed (Carlsen et al., 2003; Dekkers et al., 2020; Lagiou et al., 2003). For example, one study of nausea and vomiting in 129 Scandinavian women with uncomplicated pregnancies reported a positive association of serum testosterone (at mean 17 and 33 weeks gestation) with nausea and vomiting at 33 weeks gestation (Carlsen et al., 2003), whereas another study of 262 White women from Boston reported negative associations of prolactin (but not other hormones) with nausea at 16 and 27 weeks gestation and positive associations of estradiol (but not other hormones) with nausea at 16 and 27 weeks gestation (Lagiou et al., 2003). Additionally, a recent study of 1,682 pregnant women from the Holistic Approach to Pregnancy and the first Postpartum Year (HAPPY) study in the Netherlands reported that hCG was positively associated with first trimester nausea and vomiting, but thyroid hormones were not associated with nausea during pregnancy (Dekkers et al., 2020). Recently, researchers have reported associations of the placental hormone growth/differentiation factor 15 (GDF15) with hyperemesis gravidarum (HG), the most severe form of nausea and vomiting during pregnancy (Fejzo et al., 2023; Fejzo et al., 2019a; Fejzo et al., 2018; Fejzo et al., 2019b). However, it is unclear how much of pregnancy nausea is explained exclusively by GDF15 or whether this hormone interacts with other key pregnancy hormones (e.g., sex-steroid and thyroid hormones).

One limitation of these prior studies is they did not consider the hormonal milieu in pregnancy, which leads to considerable correlation between hormones, making it difficult to discern important independent drivers of nausea symptoms. Exploring the hormonal milieu could be accomplished by evaluating each hormone's relationship with a health outcome while simultaneously adjusting for other hormones in regression models; however, given that gestational hormones share common pathways of synthesis and regulation, this method could result in multicollinearity and poor model fit (Kim, 2019). Epidemiologic research should explore the hormonal milieu rather than attempting to identify the roles of single hormones in

NVP, as it is unlikely NVP etiology can be explained by one hormone alone. Because the pathological mechanisms behind NVP are unknown, current clinical treatments are mostly untargeted.

Currently used pharmacological and non-pharmacological treatments for NVP have uncertain efficacy. Cochrane Database Systematic Reviews surveyed the evidence of various treatments studied in randomized controlled trials, including acupressure, acupuncture, ginger, chamomile, lemon oil, vitamin B6 (pyridoxine), and antiemetic drugs, and concluded that, of the many possible treatments, only vitamin B6 and anti-emetic drugs had support, although with limited evidence from trials (Matthews et al., 2014). They also concluded there was limited information on the maternal and fetal impacts of these treatments. Based on this research, the American Academy of Family Physicians (AAFP) and the American College of Obstetricians and Gynecologists (ACOG) recommend dietary modifications, despite limited evidence, and vitamin B6 with or without doxylamine (Committee on Practice, 2018; Herrell, 2014). Clinicians' reticence to treat NVP pharmacologically is rooted in the thalidomide scandal where thalidomide was introduced in Europe as an anti-emetic for NVP. After many women were treated, they gave birth to children with birth defects, predominately severe limb malformations (Leck and Millar, 1962; Lenz, 1988). Because of the limited evidence for current treatments and the understandable hesitance for pharmacological treatment of pregnant women, it is imperative to understand potential modifiable factors involved in NVP.

Environmental exposures could be one possible modifiable determinant of NVP, but to our knowledge, this relationship has only been assessed in one study. A prospective cohort in Bangladesh assessed arsenic in drinking water and reported increased odds of self-reported NVP with higher arsenic exposure (Kile et al., 2014). However, as levels of arsenic are much lower in the U.S. compared to Bangladesh, and have been decreasing over time, arsenic exposure may be less relevant to most U.S. populations (Welch et al., 2018). Others studies in U.S. pregnant populations have assessed cannabis use during pregnancy and reported increased odds of NVP with use of cannabis (Metz et al., 2022; Vanderziel et al., 2023); however, the vast majority of pregnant women do not consume cannabis, and the identified relationship may actually be due to reverse causation. To date, no studies have investigated common environmental exposures found in daily-use consumer products to which pregnant women are ubiquitously exposed. Additionally, as the most plausible hypothesis for the cause

of NVP is that symptoms are related to altered hormones during pregnancy, as described above, these same hormonal shifts could be influenced by environmental chemicals that are known to disrupt hormones.

Pregnant women are ubiquitously exposed to non-persistent endocrine disrupting chemicals (EDCs), with virtually all women having detectable concentrations of EDC biomarkers in their blood and urine, despite rapid metabolism and excretion from the body (CDC, 2019; Woodruff et al., 2011). As opposed to persistent EDCs, such as per- and polyfluorinated substances (PFAS), which can have half-lives of days to years, non-persistent EDCs have short half-lives of around 8-24 hours, depending on the chemical (CDC, 2019). Non-persistent EDCs are used as functional ingredients in many common consumer products (CDC, 2019; Haggerty et al., 2021). For example, di-2-ethylhexyl phthalate (DEHP) is a plasticizer used in food processing and packaging materials, whereas diethyl phthalate (DEP) is a scent stabilizer used in personal care products and cosmetics (2008; Guo and Kannan, 2013; Hauser and Calafat, 2005). Phenols are a broad group of chemicals used for many purposes. For example, parabens, such as propylparaben, are predominately used as antimicrobials in personal care products and cosmetics but also used as food additives (Guo and Kannan, 2013; Wei et al., 2021). Other phenols, such bisphenol A (BPA), are plasticizers, whereas benzophenone-3 (BP-3) is a UV blocker, and dichlorophenols, such as 2,4-dichlorophenol (2,4-DCP), are found in pesticides (Chen et al., 2016; Chen et al., 2023; Dodson et al., 2007; Mao et al., 2022; Sun et al., 2023; Vandenberg et al., 2007). These and similar chemicals have been shown to disrupt hormones in *in vitro* and *in vivo* experimental models (Vandenberg et al., 2012). Interestingly, these chemicals can exhibit low dose non-monotonic dose-response relationships with the hormones they disrupt (Gore et al., 2015; Vandenberg, 2014; Vandenberg et al., 2007). Importantly, epidemiologic studies have reported that these EDCs are associated with many hormonally-mediated pregnancy-related disorders, such as gestational diabetes, pre-eclampsia, and gestational weight gain (Cantonwine et al., 2016; James-Todd et al., 2016; Pacyga et al., 2023). Because of potential reproductive and developmental hazards of several EDCs, such as DEHP, replacements like di-2-ethylhexyl terephthalate (DEHTP) and di(isononyl) cyclohexane-1,2-dicarboxylate (DiNCH) were introduced in the U.S. in the early 2000s (Silva et al., 2013; Silva et al., 2015; Silva et al., 2017; Zota et al., 2014). Likewise, bisphenol S (BPS) and F (BPF) were introduced as replacements for BPA (Ye et al., 2015). Unfortunately, consistent with the concept of

regrettable substitution, recent studies have demonstrated that some phthalate and bisphenol replacements may have similar reproductive (Lee et al., 2020; Yland et al., 2022), cardiovascular (Abrantes-Soares et al., 2022), and oncological (Edaes and de Souza, 2022) impacts as the chemicals they have replaced, likely due to their endocrine disrupting properties. Because women are increasingly being exposed to newer chemicals introduced to replace well-characterized EDCs, future research must consider if and how replacement chemicals are associated with health outcomes of interest.

Although most prior research has assessed one chemical at a time, in reality, pregnant women are exposed to complex mixtures of non-persistent EDCs. However, as these chemicals share exposure sources and exhibit similar toxicokinetic properties, they are highly correlated, and as such, studies investigating health risks associated with these chemicals may underestimate their cumulative effects. Because of limitations of traditional methods, environmental epidemiologists have developed statistical methods to model chemicals as a single mixture (Braun et al., 2016). Among these methods, supervised machine-learning statistical mixture methods, such as weighted quantile sum regression (WQSR), quantile-based g-computation (QGComp), and Bayesian kernel machine regression (BKMR), are capable of identifying a joint association of multiple chemical co-exposures with a health outcome of interest, while also handling moderately and highly correlated environmental exposures (Bobb et al., 2018; Carrico et al., 2015; Hamra and Buckley, 2018; Keil et al., 2020). Additionally, these methods can evaluate the relative importance of each co-exposure within the context of the mixture, which can be helpful for identifying particular chemicals to target for intervention. Unlike WQSR and QGComp, BKMR also has the ability to identify non-linear relationships and chemical-chemical interactions within the EDC mixture. Going forward, studies assessing non-persistent EDCs and health outcomes should model the relationship using multiple complex mixture methods in order to better understand the actual exposure profile.

Because of these gaps in knowledge, the overall objective of this dissertation is to understand the individual and joint relationships between non-persistent EDCs (phthalates/replacements and phenols), mid-pregnancy gestational hormones (sex-steroid and thyroid), and nausea during pregnancy (never, typical, persistent, and intermittent nausea). As these chemicals are known to disrupt hormones, are associated with various pregnancy complications, and

NVP likely has hormonal pathophysiology, we hypothesized that non-persistent EDCs are associated with increased risk of nausea during pregnancy, as well as changes in mid-pregnancy hormone levels, and that those same hormones are associated with increased nausea during pregnancy. To accomplish this objective, we utilized information collected from the Illinois Kids Development Study (I-KIDS), an ongoing pregnancy and birth cohort from the University of Illinois, Champaign-Urbana, Illinois that has followed 535 pregnant women and their children from pregnancy through childhood. In **Chapter 2**, we explored overall and fetal sex-specific associations of non-persistent EDCs with nausea during pregnancy using traditional regression approaches, QGComp, and BKMR. Major strengths of our approach include assessing EDCs as a mixture and evaluating nausea persistence in pregnancy rather than focusing on first trimester symptoms. In **Chapter 3**, we investigated overall and fetal sex-specific associations of non-persistent EDC biomarkers with maternal sex-steroid and thyroid hormones using traditional regression methods, WQSR, and BKMR. These mixture methods allowed us to better assess the complex exposure profile in I-KIDS women compared to assessing biomarkers one at a time. In **Chapter 4**, we evaluated hormonal determinants of persistent nausea during pregnancy in all women and by fetal sex. Importantly, to better model the hormonal milieu (sex-steroid and thyroid hormones), we modeled a statistical hormone mixture using WQSR. Finally, **Chapter 5** summarizes our findings and provides some commentary on pitfalls and potential future directions.

Keywords: Endocrine disrupting chemical, phthalate, phenol, paraben, persistent nausea, pregnancy, fetal sex

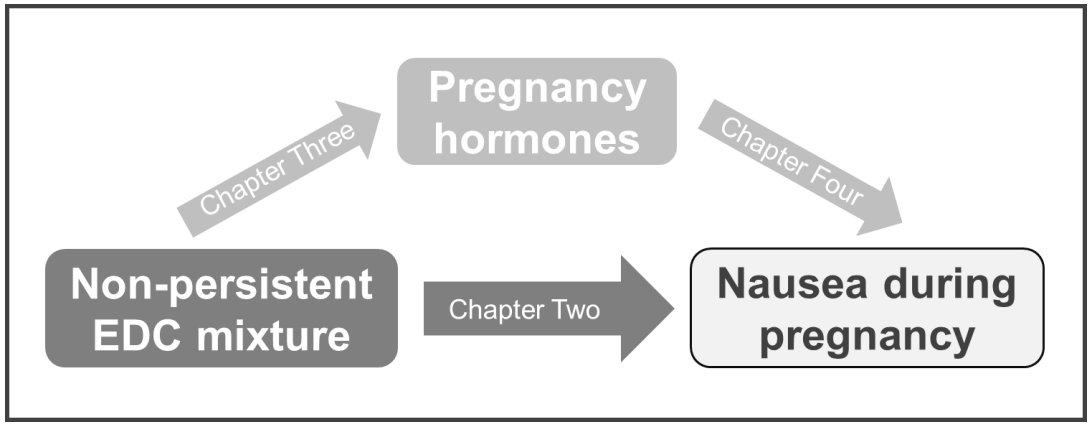

Figure 1. Summary of dissertation objectives.

CHAPTER TWO:
A MIXTURE OF ENDOCRINE DISRUPTING CHEMICALS IS ASSOCIATED WITH INCREASED RISK OF PERSISTENT NAUSEA DURING PREGNANCY

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2.1. ABSTRACT

Pregnant women are exposed to numerous endocrine disrupting chemicals (EDCs). Pregnancy-related nausea is common, persists beyond the first trimester, and likely has hormonal etiology. We aimed to determine the relationship between EDC biomarkers and pregnancy nausea characteristics. Illinois Kids Development Study (I-KIDS) pregnant women (n = 467) reported nausea symptoms monthly from conception to delivery. We categorized women as never having nausea (9%), or as having typical (ends by 17 weeks gestation; 42%), persistent (ends after 17 weeks gestation; 25%), or irregular (24%) nausea. Women provided five urine samples across pregnancy, which we pooled and analyzed for phthalate/replacement, phenol, and triclocarban biomarkers. Using covariate-adjusted logistic regression, we evaluated relationships of EDCs with nausea and used quantile-based g-computation (QGComp) and Bayesian kernel machine regression (BKMR) to evaluate joint associations of EDCs with nausea symptoms. We also considered differences in associations by fetal sex. Only the sum of urinary biomarkers of di(isononyl) cyclohexane-1,2-dicarboxylate (ΣDiNCH) was associated with persistent nausea in all women. However, using QGComp, each 10% increase in the EDC mixture was associated with 14% higher risk of persistent nausea (RR: 1.14; 95% CI: 1.01, 1.30), due to ΣDiNCH, ethylparaben, and the sum of di-2-ethylhexyl phthalate (ΣDEHP) metabolites. Similarly, using BMKR, we identified a marginally positive relationship of the mixture with persistent nausea in all women. In women carrying males, ethylparaben was associated with persistent nausea, and each 10% increase in the QGComp mixture was associated with 26% higher risk of persistent nausea (RR: 1.26; 95% CI: 1.13, 1.41), driven by ethylparaben and ΣDiNCH. Consistently, using BKMR, EDCs were positively associated with persistent nausea in women carrying males. We did not identify meaningful relationships of EDCs in women carrying females or with other nausea patterns. Non-persistent EDCs are associated with persistent nausea in pregnancy, primarily in women carrying males. Future work should explore possible mechanisms, clinical implications, and interventions to reduce exposures and symptoms.

2.2. Introduction

Nausea is the most common symptom women experience in pregnancy (Bustos et al., 2017; Herrell, 2014; Lee and Saha, 2011; Niebyl, 2010). In 2012, the cost of medically managing one symptomatic woman was estimated at nearly $2,000 (Piwko et al., 2013). Additionally, nausea during pregnancy has been linked to adverse maternal health outcomes, including high blood pressure, preeclampsia, and depression, with potential impacts on work capacity and family life (Attard et al., 2002; Chortatos et al., 2015; Mazzotta et al., 2000; Niebyl, 2010; Smith et al., 2000). Symptoms can also result in hundreds of hours of lost work, which may have other long-term impacts (Mazzotta et al., 2000). In many women, nausea occurs in the first trimester, with peak symptoms at nine weeks and symptomatic improvement by 16 to 18 weeks (Herrell, 2014; Judith A Smith, 2023); however, up to 40% of women can have nausea symptoms that continue later in pregnancy (Einarson et al., 2013; Kramer et al., 2013). Although the exact mechanisms are not fully understood, the leading hypothesis for the cause of nausea in pregnancy is hormonal changes that occur (especially) in early pregnancy (Lee and Saha, 2011). Because of the substantial impacts of nausea during pregnancy and the understandable hesitance for pharmacological treatment of pregnant women, it is imperative to identify possible modifiable factors individuals and clinicians can target to decrease persistent nausea in pregnancy.

Exposure to environmental contaminants is one possible modifiable cause of nausea in pregnancy, but to our knowledge, only one study has considered this relationship. In this study of women from Bangladesh, higher arsenic exposure from drinking water was associated with increased odds of self-reported nausea and vomiting in pregnancy (Kile et al., 2014). To date, no studies have investigated contaminant exposures from daily-use consumer products, including non-persistent endocrine disrupting chemicals (EDCs), such as phthalates and phenols, found in food packaging materials, medications, and cosmetics (CDC, 2019; Chen et al., 2016; Wei et al., 2021). Virtually all women in the U.S. have detectable concentrations of urinary biomarkers of these EDCs, despite rapid metabolism and excretion from the body (CDC, 2019; Woodruff et al., 2011). Importantly, certain phthalates and phenols exhibit endocrine disrupting properties, based on experimental and epidemiologic studies (Pacyga et al., 2021; Ryva et al., 2024; Vandenberg et al., 2012). In addition, epidemiologic studies have reported that EDCs are associated with other hormonally-mediated pregnancy-related conditions, including hypertension, preeclampsia,

gestational diabetes, and inappropriate gestational weight gain (Cantonwine et al., 2016; James-Todd et al., 2016; Pacyga et al., 2023). Therefore, further research is needed to understand whether EDCs also play a role in nausea etiology in pregnancy, especially in relation to symptoms that persist beyond the first trimester.

Because nausea in pregnancy is so prevalent and likely has a hormonal basis, and because pregnant women are frequently exposed to EDCs, our objective was to evaluate the relationship of ubiquitous non-persistent EDC biomarkers (phthalates and phenols) with nausea during pregnancy. Additionally, because little is currently understood about persistent sub-clinical nausea that continues throughout pregnancy, our hypothesis was that higher EDC exposures are related to increased risk of persistent nausea. As a secondary analysis, we also considered differences in these associations by fetal sex, as nausea characteristics are known to differ between women carrying females compared to those carrying males.

2.3. Materials and Methods

Illinois Kids Development Study (I-KIDS) study design and analytic sample

Pregnant women were recruited into I-KIDS, a prospective pregnancy and birth cohort, from two local obstetric clinics in Champaign-Urbana, Illinois to evaluate associations of prenatal environmental chemical exposures with neurodevelopment. Recruitment, enrollment, and eligibility criteria have been previously detailed (Pacyga et al., 2021; Pacyga et al., 2022a; Pacyga et al., 2023). Our current study includes 467 women who enrolled in I-KIDS between 2013 and 2019, remained in the study through their child's birth, had nausea symptom information at all timepoints across pregnancy, and had measurable levels of at least one maternal urinary EDC biomarker. All women provided written informed consent, and the study was approved by the University of Illinois' Institutional Review Board.

Collection of maternal sociodemographic, lifestyle, and health information

I-KIDS staff conducted home visits to interview enrolled women about various sociodemographic and lifestyle factors. Pre-pregnancy body mass index (kg/m^2) was calculated from self-reported pre-pregnancy weight and height. To measure early pregnancy stress levels, women completed the Perceived Stress Scale (PSS), a ten-item questionnaire asking about thoughts and feelings during the last month scored from 0 to 40 (Cohen et al., 1983; Cohen and Williamson, 1988). Scores 0 to 13 indicate low stress, whereas scores 14

or greater signify moderate or high stress. At their first visit, women completed a semi-quantitative food frequency questionnaire (FFQ) adapted from the full-length Block-98 FFQ (NutritionQuest, Berkely, CA) and validated in pregnant populations (Bodnar and Siega-Riz, 2002; Boucher et al., 2006; Laraia et al., 2007). Dietary intakes representing diet patterns from the previous three months were used to calculate an early pregnancy Alternative Healthy Eating Index 2010 (AHEI-2010) average, which is an 11-component diet quality index (totaling 110 total points) based on foods and nutrients known to be predictive of chronic disease risk and mortality, with higher scores indicating better overall diet quality (Chiuve et al., 2012; McCullough et al., 2002). Since AHEI-2010 considers moderate alcohol consumption as beneficial to health, but clinical guidelines recommend pregnant women abstain from alcohol (Bertrand et al., 2005; CDC, 2023; Cook et al., 2016), we removed the alcohol component from the index to create a ten-component diet quality index (maximum: 100 points). Fragrant personal care and cleaning product use was determined at baseline when women answered, “never or almost never”, “sometimes”, or “always” to: 1) “How often do you use personal care products that are fragrance-free?” and 2) “How often do you use fragrance-free cleaning, laundry, and other household products?” From this, we created a composite variable of women who never or almost never used any fragrance-free products and women who used fragrance-free products sometimes or always.

Assessment of urinary phthalate/replacement, phenol, and triclocarban biomarker concentrations

Because non-persistent EDCs have relatively short biological half-lives (6–24 hours depending on the chemical) and high within-person variability (Shin et al., 2019a; Shin et al., 2023; Shin et al., 2019b), we measured EDC biomarkers in five across-pregnancy urine samples pooled physically before chemical biomarker measurement. At study clinic/home visits or routine prenatal care clinic visits, women provided at least three and up to five first-morning urine samples at median 13, 17, 23, 28, 34 weeks gestation. Details about urine collection, processing, and storage have been previously detailed (Pacyga et al., 2023). Briefly, women collected first-morning urine into polypropylene urine cups and refrigerated them for up to 24 hours until we aliquoted samples for long-term storage. To create the pooled sample, we added 900 µL of urine from the first urine sample to a 5 mL cryovial tube. At each visit, we layered fresh urine onto the previous frozen sample and immediately stored the sample at −80 °C. At the end of pregnancy, we thawed, vortexed, and measured the specific

gravity of pooled samples.

Frozen pooled urines were shipped to the CDC's Division of Laboratory Sciences in four batches (batch 1: enrolled December 2013 – February 2015; batch 2: enrolled February 2015 - July 2016; batch 3: enrolled July 2016 – August 2018; batch 4: enrolled September 2018 – November 2019). Using previously published isotope-dilution mass spectrometry methods with rigorous quality assurance/quality control protocols and high long-term reproducibility (Calafat et al., 2006; Calafat et al., 2010; Schantz et al., 2015; Silva et al., 2013; Silva et al., 2007; Silva et al., 2019; Ye et al., 2014), CDC laboratory staff quantified biomarkers for 19 phthalate/replacement metabolites, 11 phenols, and triclocarban (Ryva et al., 2024). Many women (n = 156) did not have measured levels of mono(2-ethyl-5-hydroxyhexyl) terephthalate (MEHHTP), mono(2-ethyl-5-carboxypentyl) terephthalate (MECPTP), and monooxoononyl phthalate (MONP), as these biomarkers were not assessed in the first batch.

Self-reporting of nausea during pregnancy

Women reported nausea symptoms approximately monthly across pregnancy (13, 17, 23, 28, and 34 median weeks gestation, and at a hospital research visit within 24 hours after birth). Research home visits were conducted at median 13 and 34 weeks, phone interviews were conducted at median 23 and 28 weeks, and a separate clinic visit for blood collection and interview was conducted at median 17 weeks. At the first prenatal visit (median 13 weeks gestation), women were asked if they had experienced nausea since conception (answer: "yes", "no"). At the next visit, women were asked if they still have nausea (answer: "yes", "no") and when it ended if "no". They were also asked if they started experiencing any new nausea since the last visit (answers: "yes", "no") and when it started if "yes". We categorized women as "never having nausea" if they did not report nausea at any point in pregnancy.

Statistical Analysis

Derivation of analytic sample

The derivation of our analytic sample is detailed in Figure S1. Briefly, of the 688 enrolled I-KIDS women, 531 remained until the birth of their child. Some women (n = 64) were excluded from this analysis as they did not have sufficient information to create the nausea persistence variable. Our final analytic sample included 467 women who have at least one measured EDC biomarker and nausea persistence information. We summarized information on

sociodemographic, health, and lifestyle factors in the reference population and our analytic sample as frequency (percent) or median (25^{th}, 75^{th} percentile) (**Table 1**).

Modeling of urinary chemical concentrations

For non-zero biomarker concentrations below the limit of detection (LOD), we used instrument-read values to avoid bias associated with imputing concentrations < LOD (Succop et al., 2004). In our statistical analyses, regardless of the number of women with values > LOD, we only included chemical biomarkers with concentrations greater than 0 ng/mL in at least 90% of women (**Table 2**). This resulted in butylparaben, BPF, and triclocarban being excluded from single-pollutant and mixture analyses. To avoid undefined estimates for ln-transformed zero concentrations (ethylparaben n = 4; BPA n = 3; and ΣDiNCH, BPS, BP-3, and 2,5-DCP n = 1), we used the formula [ln(chemical concentration + 0.0001)] for each chemical value in linear regression and mixture models.

We evaluated specific gravity adjusted phthalate/replacement, phenol, and triclocarban biomarkers as molar sums for parent compounds for which more than one metabolite was measured or individual biomarkers (ng/mL) adjusted for specific gravity (Meeker et al., 2009). For phthalates/replacements, we approximated women's exposure to phthalate/replacement parent compounds using their urinary metabolite concentrations. Specifically, we calculated parent molar sums (nmol/mL) by summing metabolites from common precursors: MEHP, MEHHP, MEOHP, and MECPP for the sum of DEHP metabolites (ΣDEHP); MCOP, MiNP, and MONP for the sum of metabolites of di-isononyl phthalate (ΣDiNP); MBP and MHBP for the sum of di-n-butyl phthalate metabolites (ΣDBP); MiBP and MHiBP for the sum of di-isobutyl phthalate metabolites (ΣDiBP); MHiNCH and MCOCH for the sum of DiNCH metabolites (ΣDiNCH); and MEHHTP and MECPTP for the sum of DEHTP metabolites (ΣDEHTP). Specific formulas were previously published (Pacyga et al., 2021) and are reported in table footers. Molar concentrations were back-converted to ng/mL by multiplying ΣDEHP, ΣDiNP, ΣDBP, ΣDiBP, ΣDiNCH, and ΣDEHTP by the molecular weights of MECPP, MCOP, MBP, MiBP, MHiNCH, and MECPTP, respectively (Pacyga et al., 2022a; Rodriguez-Carmona et al., 2020; Zhang et al., 2020a). We estimated exposure to di-isodecyl phthalate (DIDP), di-n-octyl phthalate (DnOP), benzylbutyl phthalate (BBzP), and di-ethyl phthalate (DEP) using ng/mL concentrations of their urinary metabolites MCNP, MCPP, MBzP, and MEP, respectively. Biomarker concentrations and LODs are reported in **Table 2**.

Modeling persistent nausea during pregnancy

Using self-reported nausea symptoms across pregnancy, we categorized women as having "typical nausea" if they reported having nausea since conception and their symptoms ended by median 17 weeks gestation. We categorized women as having "persistent nausea" if they reported having nausea since conception and their symptoms persisted past 17 weeks gestation. Lastly, we categorized women as having "irregular symptoms" if they reported nausea symptoms that started and stopped more than once during pregnancy. We selected the 17-week gestation cut-off to delineate persistent from typical nausea as nausea that most women experience commonly resolves between 16 and 18 weeks gestation (Judith A Smith, 2023). As most women experience some nausea during pregnancy, we used typical nausea as our reference group in all models.

Covariate selection

Based on prior literature and our data, we generated a directed acylic graph (DAG) to identify a minimum sufficient adjustment set of covariates (Herrell, 2014; Niebyl, 2010). We assessed correlations between covariates to test for potential multicollinearity; however, all covariates were only weakly or moderately correlated ($r < 0.4$; **data not shown**). All models accounted for maternal age, race/ethnicity, educational attainment, pre-pregnancy BMI, early pregnancy diet quality (AHEI-2010) (as a potential source of some EDCs), fragrant product use (as a potential source of some EDCs), early pregnancy stress (PSS 10), alcohol use since conception, parity, and fetal sex. Age, pre-pregnancy BMI, diet quality, stress, and gestational age were modeled as continuous variables, whereas all others were categorized with the reference group indicated in **Table 1**.

Evaluating associations of EDC biomarkers with persistent nausea during pregnancy

To address our primary objective, we evaluated whether EDC biomarkers are associated with persistent nausea compared to typical nausea using covariate-adjusted logistic regression models, with the covariates detailed above. To improve model fit, we natural log (ln)-transformed all EDC biomarkers. In secondary analyses, we also considered the relationships of EDC biomarkers with atypical nausea patterns (never having nausea or having irregular nausea).

Evaluating associations of an EDC biomarker mixture with nausea persistence during

pregnancy

We utilized two methods to evaluate covariate-adjusted, joint associations of phthalates/replacements and phenol biomarkers (excluding butylparaben, BPF, and triclocarban biomarkers, as described above) with persistent nausea. First, we used quantile-based g-computation (QGComp), which was designed to address possible limitations in the weighted quantile sum regression (WQSR) mixture method by relaxing the assumption that all co-exposures are associated with outcomes in the same direction (Keil et al., 2020). We used QGComp to estimate the association of the EDC mixture with persistent nausea using logistic regression. We generated results without bootstrapping to obtain partial negative and partial positive associations and weights, which indicate relative importance and direction of each co-exposure to the joint association. Then, because persistent nausea is not a rare outcome, we fit models with 500 bootstraps to estimate risk ratios with more precise confidence intervals, avoiding potentially overestimating our effect estimates. As we transformed all biomarker concentrations into deciles, the resulting RR and 95% CIs are interpreted as the risk of nausea persistence if the EDC biomarker mixture increased by 10%.

Second, we used Bayesian kernel machine regression (BKMR), which estimates a non-parametric, high-dimensional exposure–response function to identify a relationship between a mixture of co-exposures and a health outcome of interest (Bobb et al., 2018). Additionally, BKMR can identify non-linear dose–response relationships and chemical-chemical interactions within a mixture. We ln-transformed, centered, and scaled all co-exposures and continuous covariates. We fit hierarchical BKMR models, using the binominal family and 200,000 iterations to determine the joint relationship of EDC biomarker mixture with probit odds of persistent nausea. Hierarchical BKMR allowed us to group the phthalates/replacements and phenols that we included in the mixture as two separate groups. To assess a joint association, we created dose–response curves where we modeled the relationship of the EDC mixture at various quantiles across its distribution relative to the median with persistent nausea. We calculated group and individual posterior inclusion probabilities (PIPs) to identify important EDC classes and biomarkers. Lastly, we interpreted univariable dose-response curves to identify non-linear relationships and bivariable plots to identify chemical-chemical interactions (i.e., the relationship of one EDC biomarker with persistent nausea differs by another biomarker's level of exposure).

Evaluating differences in associations by fetal sex

As nausea prevalence in pregnancy may differ by fetal sex (Mitsuda et al., 2019; Young et al., 2021), we investigated if associations of EDCs (individual and joint) with persistent nausea differ between women carrying females and those carrying males. In logistic regression models, we included a multiplicative interaction term to identify differences and reported interaction *p*-values. To simplify the interpretation of results from interaction models in QGComp and BKMR analyses, we stratified our sample by fetal sex and fit separate models.

Sensitivity analyses

We performed various sensitivity analyses to better understand the relationship between EDC exposure and persistent nausea in pregnancy. To determine the influence of individual dietary components on the relationship of EDCs with nausea during pregnancy, we considered each individual dietary component of the AHEI-2010 rather than the total index; however, as this approach minimally changed either the direction or precision of our estimates (**data not shown**), we accounted for total dietary index score in final models. As previously discussed, a large number of participants did not have data on DEHTP metabolites (MEHHTP and MECPTP) and a third metabolite of DiNP (MiNP) because the CDC began measuring these after study onset. Thus, we conducted a sensitivity analysis in a smaller subset of women to understand the potential role of these chemicals within the joint EDC mixture.

Reporting of findings and interpreting meaningful results

For single pollutant logistic regression results, ORs and 95% CIs represent the odds of nausea (never, persistent, or irregular) for a two-fold increase in each EDC biomarker concentration compared to typical nausea. Our main QGComp results are interpreted as the risk ratio (RR) associated with a 10% increase in all EDC biomarker concentrations combined. For BKMR, we assessed trends visually and reported meaningful PIPs based on the largest PIPs selected in each group. To ensure model assumptions were met, we performed regression diagnostics for single-pollutant analyses and checked for convergence with the Markov Chain Monte Carlo procedure in BKMR. Rather than focusing on statistical significance thresholds, we identified potentially meaningful findings by assessing the direction, strength, and precision of the associations, as recommended by the American Statistical Association (Wasserstein and Lazar, 2016). As such, we did not adjust for multiple comparisons. We followed the Strengthening the Reporting of Observational Studies in

Epidemiology (STROBE) reporting guidelines (Table S14). We performed logistic regression analyses in SAS version 9.4 (SAS Institute Inc. Cary, NC) using PROC LOGISTIC. We conducted QGComp and BKMR in R Statistical Software using R packages: "qgcomp: Quantile G-Computation" (Keil, 2023) and "bkmr: Bayesian Kernel Machine Regression" (Bobb, 2022).

2.4. Results

Participant characteristics and nausea prevalence

Most of the 467 women included in this study were non-Hispanic White (81%), college-educated (82%), with a total family income greater than $60,000 (72%) and did not consume alcohol since conception (58%) (**Table 1**). Only 9% of women never had nausea during pregnancy, with 42% of women experiencing typical nausea, followed by 25% with persistent nausea, 24% with irregular nausea. Some characteristics (alcohol use since conception, pre-pregnancy diet quality index, pre-pregnancy stress scores, pre-pregnancy BMI, and fragrance-free product use) differed by the nausea characteristics (Table S1).

Concentrations and correlations of maternal urinary chemical biomarkers

Urinary biomarker concentrations are presented in **Table 2**. Most chemicals had concentrations ≥ LOD in the vast majority of women, except MiNP, MCOCH, butyl paraben, ethyl paraben, bisphenol F, and triclocarban, which were only detectable (≥ LOD) in 42.5%, 50.6%, 42.9%, 54%, 63.6%, 29.9% of participants, respectively. Some EDC biomarkers were moderately-to-strongly correlated with each other (Figure S2), including ethylparaben with propylparaben (r=0.7); 2,4-DCP with 2,5-DCP (r=0.7); and MCPP with ΣDiNP (two metabolite and three metabolite sums) (r=0.8). Additionally, ethylparaben was weakly correlated with methylparaben (r=0.4), and MCNP was weakly correlated with MCPP (r=0.3) (Figure S2).

Associations of individual EDC biomarkers with persistent nausea during pregnancy

In all women, only ΣDiNCH was associated with persistent nausea during pregnancy (**Table 3**). Specifically, each two-fold increase in ΣDiNCH was associated with 18% higher odds of persistent nausea compared to typical nausea (OR: 1.18; 95% CI: 1.01, 1.37). We observed differences by fetal sex, such that in women carrying males, each two-fold increase in ethylparaben was associated with 12% increased odds of persistent nausea (OR: 1.12; 95% CI: 0.99, 1.26) (**Table 3**). Individual EDCs were not associated with persistent nausea in

women carrying females.

Associations of an EDC biomarker mixture with persistent nausea during pregnancy

When we evaluated associations jointly as a mixture using QGComp, in all women, each 10% increase in the EDC biomarker mixture was associated with 14% increased risk of persistent nausea compared to typical nausea (RR: 1.14; 95% CI: 1.01, 1.30), with ΣDiNCH (17%), with ethylparaben (17%), ΣDEHP (16%), and MEP (13%) contributing most to the positive direction (**Figure 2;** Table S4). This overall association appeared to be driven by women carrying males, in whom each 10% increase in the mixture was associated with 26% increased risk of persistent nausea compared to typical nausea (RR: 1.26; 95% CI: 1.13, 1.41), with ethylparaben (18%), ΣDiNCH (17%), MEP (14%), MBzP (13%), BPA (12%), and DiNP (2 metabolites; 12%) contributing the most to the positive direction (**Figure 1;** Table S4). We did not observe a relationship between the EDC biomarker mixture and persistent nausea in women carrying females (RR: 1.01; 95% CI: 0.80, 1.26) (**Figure 2;** Table S4). In sensitivity analysis that included ΣDEHTP and ΣDiNP (three metabolites versus two metabolites in main analysis) in the mixture and thus decreased the sample size, our effect estimates were smaller, with less precision, and there were some changes to meaningful weights (Table S5). Specifically, in all women, a 10% increase in the biomarker mixture was associated with a 10% increased risk of persistent nausea (RR: 1.10; 95% CI: 0.93, 1.29), due to ethylparaben (27%), ΣDiNP (3 metabolites; 15%), and BPA (12%). Furthermore, in women carrying males, a 10% increase in the biomarker mixture was associated with a 23% increased risk of persistent nausea (RR: 1.23; 95% CI: 1.00, 1.51), due to ethylparaben (26%), ΣDiNCH (15%), and ΣDiNP (3 metabolites; 14%) (Table S5)**.** Consistent with our main analyses, the EDC biomarker mixture was not associated with persistent nausea in women carrying females.

Using hierarchical BKMR, the relationship in all women trended in the positive direction, due to ΣDiNCH (PIP: 0.35), TCS (PIP: 0.20), BPA (PIP: 0.19), MCPP (PIP: 0.18), and ethylparaben (PIP: 0.16) (**Figure 2;** Table S6). Similar to QGComp, we observed differences by fetal sex. In women carrying males, we identified a potential increasing probability of persistent nausea with increasing EDC biomarker mixture concentration (**Figure 1**). Phthalates/replacements (PIP: 0.73) and phenols (PIP: 0.67) were strongly selected in the model, with MEP (PIP: 0.37), ΣDiNCH (PIP: 0.17), BPA (0.20), and methylparaben (PIP: 0.15)

being of particular importance (Table S6). In women carrying females, there was no relationship between the EDC biomarker mixture and persistent nausea. We did not identify any non-linearities or chemical-chemical interactions in all women, women carrying males, or women carrying females (Figures S4-S7). In the sensitivity analysis that included additional phthalate biomarkers (in a smaller sample of women), the relationship in all women remained null, whereas the relationship in women carrying males had similar trending positive estimates as the main analysis but with considerably less precision (Figure S8). Additionally, the PIPs in women carrying males differed from the main analysis, with ethylparaben (PIP: 0.82), ΣDiBP (PIP: 0.40), and MEP (PIP: 0.29) being the most important (Table S7). Consistent with our main analysis, the EDC mixture was not associated with persistent nausea in women carrying females.

Secondary analysis: associations of EDC biomarkers and mixture with atypical nausea patterns in pregnancy

In all women, EDCs were not associated with never having nausea compared to typical nausea, except potentially at higher levels of exposure. But, in women carrying females, some phthalates (ΣDEHP, ΣDiBP) were associated with higher odds of never having nausea, some phenols (methylparaben, 2,5-DCP) were associated with lower odds of never having nausea, and the EDC mixture was associated with lower odds of never having nausea. In women carrying males, while methylparaben and propylparaben were associated with higher odds of never having nausea, joint associations were inconsistent, with possible higher odds of never developing nausea at higher exposure levels (Tables S3, S8-S10; Figure S9). Furthermore, despite some individual phthalate biomarkers being associated with irregular nausea compared to typical nausea (MEP, ΣDBP in all women; ΣDiBP in women carrying females), there were no observed joint associations, except in women carrying females, where ΣDiBP may be responsible for a weak and imprecise joint association only at higher levels of exposure (Tables S3, S11-S13; Figure S10).

2.5. Discussion

Summary of major findings

Ours is the first study to investigate the relationship between EDCs and nausea during pregnancy, an understudied condition that has potential short- and long-term implications for women's health, including mental health during pregnancy and cardiovascular disease post-

partum (Attard et al., 2002; Cecile et al., 2023; Fossum et al., 2018; Smith et al., 2000). Our most salient findings were in women carrying males, showing that some EDCs, individually and jointly, were associated with persistent nausea compared to typical nausea. The primary EDC biomarkers of importance were the phthalate replacement plasticizer DiNCH and ethylparaben - commonly used as an antibacterial in personal care and cleaning products. In contrast, findings related to atypical nausea patterns were less compelling. Our results suggest that EDC exposure in pregnancy could be related to having nausea that persists across pregnancy; however, additional studies are needed to elucidate potential underlying biological pathways, including likely hormone-mediated relationships, as well as to understand the long-term implications for both mother and child.

EDCs were associated with increased risk of persistent nausea during pregnancy, primarily in women carrying males

We reported positive relationships of the phthalate replacement, ΣDiNCH, with persistent nausea in all women, and of ethylparaben with persistent nausea in women carrying males. Furthermore, ethylparaben, ΣDiNCH, and MEP were primary drivers of the joint association with persistent nausea in all women and in women carrying males. There is some evidence from epidemiologic studies that both DiNCH and ethylparaben are associated with adverse pregnancy and birth outcomes (Kek et al., 2024; Pacyga et al., 2023; Zhang et al., 2020b), as well as changes in women's hormonal, inflammatory, and metabolic biomarker levels (Derakhshan et al., 2021b; Minguez-Alarcon et al., 2016; Pacyga et al., 2022b; Ryva et al., 2024; Weng et al., 2023). Specifically, our prior work in I-KIDS showed that ethylparaben, alone and as part of a mixture, was associated with lower TSH concentrations in all women and in women carrying males (Ryva et al., 2024). However, exact biological mechanisms from experimental studies are unclear. One *in vitro* study reported that DiNCH disrupted steroidogenesis at supraphysiological doses, but was not estrogenic or anti-androgenic (Moche et al., 2021), whereas another *in vitro* study reported that DiNCH did not impact steroidogenesis, but that its metabolites activated estrogen, androgen, and peroxisome proliferator-activated receptor gamma (PPARγ) receptors at high concentrations (Engel et al., 2018). Additionally, experimental studies have shown that parabens only weakly bind to estrogen receptors (Golden et al., 2005). Thus, it may be that DiNCH and ethylparaben's mechanisms of action are not through sex-steroid pathways, which needs to be more extensively investigated.

Our fetal sex-specific findings are not surprising, as many pregnancy complications, such as early pregnancy loss, stillbirth, and preeclampsia, differ by fetal sex (Inkster et al., 2021). Furthermore, previous research has reported sexually-dimorphic responses to EDCs in relation to pregnancy sex-steroid and thyroid hormones (Pacyga et al., 2021; Ryva et al., 2024), as well as pregnancy outcomes, such as preeclampsia (Cantonwine et al., 2016) and gestational weight gain (Pacyga et al., 2023). Additionally, some studies suggest that NVP is a sexually dimorphic condition (Mitsuda et al., 2019; Young et al., 2021). These findings could be explained by placental differences between male and female fetuses as placentae are sexed organs with differences in both function and morphology (Gabory et al., 2013; Graves, 2010; Meakin et al., 2021; Rich-Edwards et al., 2001). In addition, X chromosome inactivation in female fetuses, Y chromosome presence in male fetuses, and sex-steroid hormone (e.g. testosterone) differences in male and female fetuses could explain our findings (Inkster et al., 2021; Meulenberg and Hofman, 1991). Our sex-specific findings may strengthen the biological plausibility of the relationship between EDCs and persistent nausea, as we would be unlikely to observe starkly sexually-dimorphic findings by chance alone.

Research on environmental exposures and NVP is sparse and has not focused on hormonally-mediated exposure/outcome relationships

EDCs have been linked to other pregnancy-related adverse health outcomes, including gestational diabetes, gestational hypertension, and inappropriate gestational weight gain (James-Todd et al., 2016; Liu et al., 2024; Pacyga et al., 2023). However, there are no studies considering the role of EDCs in nausea symptomology during pregnancy, and prior research related to the roles of other environmental exposures in NVP is sparse. Specifically, while one study of 1,458 pregnant Bangladeshi women reported higher drinking water arsenic concentrations were associated with increased odds of self-reported NVP (Kile et al., 2014), acute arsenic toxicity is associated with nausea and vomiting in non-pregnant individuals, so this relationships may not be pregnancy-specific but is rather due to arsenic's known toxic properties (Ratnaike, 2003). Some other studies have reported increased odds of NVP with marijuana use (Vanderziel et al., 2023; Young-Wolff et al., 2018). It is possible these findings are related to Cannabinoid Hyperemesis Syndrome, where heavy users of cannabis experience intense episodes of nausea and vomiting (Galli et al., 2011). However, reverse causality is also a likely explanation, as women with NVP may use marijuana to alleviate their symptoms. As nausea that is unique to pregnancy likely has hormonal underpinnings, the

relationship between EDCs and nausea in pregnancy is biologically plausible. Specifically, studies have identified relationships of sex-steroid and thyroid hormones in nausea symptomology, although findings have been mixed (Carlsen et al., 2003; Dekkers et al., 2020; Lagiou et al., 2003). Recently, the placental hormone growth/differentiation factor 15 (GDF15) was implicated in hyperemesis gravidarum (HG), the most severe form of NVP, but its role in less severe nausea is unknown (Fejzo et al., 2023; Fejzo et al., 2019a; Fejzo et al., 2018; Fejzo et al., 2019b). Based on the paucity of prior literature related to this work, substantially more research is needed on environmental drivers of nausea during pregnancy. Future epidemiologic studies should also continue to explore hormonal predictors of NVP to determine if risk of persistent nausea is due to changes in hormones. While no experimental models of NVP currently exist, future experimental studies could continue to explore EDC mechanisms of toxicity beyond those that act via hormone receptors.

Possible behavioral and lifestyle modifications to reduce EDC exposures

Our results indicate EDCs of concern may have both dietary and personal care product (PCP) exposure sources. Specifically, as DiNCH is used as a plasticizer in various food contact materials (Silva et al., 2013), pregnant women are exposed to DiNCH through their diets, and both we and National Health and Nutrition Examination Survey (NHANES) have reported that urinary biomarker concentrations of DiNCH have been increasing across time (CDC, 2019; Pacyga et al., 2022a). Furthermore, pregnant women who use more PCPs have higher measurable levels of parabens and monoethyl phthalate (MEP) (Ashrap et al., 2018; Braun et al., 2014; Fisher et al., 2017; Guo and Kannan, 2013; Rosen et al., 2024). Recent review articles have summarized interventions (e.g., changes to diet or PCP usage) to reduce EDC exposure (Martin et al., 2022; Park et al., 2022; Sieck et al., 2024; Yang et al., 2023). Few studies have focused on pregnant women, with mixed results. For example, one study of ten low-income pregnant women provided women with organic foods for three days prepared using stainless steel and reported no changes in phthalate metabolites at the end of the study (Barrett et al., 2015). However, a more recent study of 35 pregnant women provided education on reducing exposure through diet and PCP use as the intervention and reported reductions in phthalate metabolite biomarkers (Wu et al., 2021). One limitation of these studies is that they focused primarily on DEHP, not its replacements, as well as on BPA but no other phenols. Recently, one randomized controlled trial of 230 pregnant women (152 in intervention and 78 in control) provided workshops on reducing EDC exposures and did not

identify changes in paraben levels following the intervention (El Ouazzani et al., 2021). However, a different study in only eight non-pregnant women reported lower urinary paraben and triclosan levels when women were provided with replacement products that did not contain parabens, benzophenones, triclocarban, triclosan, or BPA (Koch et al., 2014). Paradoxically, a study of 100 adolescent females reported higher ethylparaben levels after an intervention to alter PCP use; however, this may have been due to mislabeled replacement products and may not be as relevant for pregnant populations (Harley et al., 2016). Interventions to reduce chemical exposures through modifying diet or PCP use may be necessary to better target interventions to women to potentially decrease nausea symptomology or persistence; however, care will need to be taken not to inadvertently increase exposures to other chemicals.

Strengths and Limitations

This study has some limitations, but also many strengths. First, I-KIDS did not assess nausea using the Pregnancy-Unique Quantification of Emesis and Nausea (PUQE), which assesses symptoms in the last 24-hour period. However, our survey addressed many of the same questions as PUQE that allowed us to query nausea at multiple timepoints across pregnancy to model persistent nausea during pregnancy. Second, our pooled EDC biomarker assessment strategy resulted in some exposure occurring after our outcome of interest (persistent nausea). However, we measured EDC biomarkers in a pool of up to five first-morning urine samples collected throughout pregnancy, providing a more stable estimate of gestational exposure than a single urine sample that reflects exposure at any one point during pregnancy (Rosen et al., 2023; Shin et al., 2019a; Vernet et al., 2019). We also investigated a panel of many non-persistent EDCs (and replacement chemicals) from multiple chemical classes, and we have reported previously that I-KIDS women have concentrations of these chemicals comparable to reproductive-aged women in NHANES (Pacyga et al., 2022a). Third, as with any observational study, we cannot rule out unmeasured confounding or make causal conclusions. However, I-KIDS collected pertinent baseline information that allowed us to account for important covariates, such as diet quality and fragrant product use, and we selected covariates using a directed acyclic graph. Additionally, based on what we know about NVP and EDC's mechanisms of action and the strength of our reported associations, our hypothesis is biologically plausible. Fourth, we cannot rule out reverse causation; however, if symptoms resulted in women reducing fragrant product use, that would

presumably lead to a reduction in EDC exposure. It is unclear whether NVP alters habits due to aversive smells, and there are conflicting reports about which type of smells are associated with NVP. For example, one study reported that “cleaning solvents, perfumes, and soaps” were at fault (O'Brien et al., 1997), whereas a different study reported aversive smells are primarily fatty foods with minimal impact of scented personal care or cleaning products (Swallow et al., 2005). Future longitudinal exposure assessment research is needed to help resolve this issue. Fifth, the I-KIDS cohort is a relatively homogeneous sample of non-Hispanic White, well-educated, married women, which may limit generalizability; however, as we are investigating biological hypotheses, a homogeneous sample could reduce residual confounding. Sixth, some of our analyses are likely underpowered, such as those with women never experiencing nausea, and should be replicated in a larger cohort; but, in many analyses, even with small sample sizes, we were able to identify meaningful relationships. Finally, our mixture did not contain all chemicals of potential concern and BKMR results can be difficult to interpret within the context of human health (Hoskovec et al., 2021). But, we used QGComp and BKMR, which are robust machine-learning methods, to calculate joint associations, identify meaningful drivers of the mixture, and assess non-linearities.

2.6. Conclusion

In this study, we confirmed our hypothesis that non-persistent EDCs from both food and personal care product sources are associated with nausea during pregnancy, an understudied pregnancy condition that affects the majority of women during pregnancy and impacts quality of life and long-term health. Specifically, higher levels of ethylparaben, DiNCH, MEP, and MBzP exposure were associated with increased risk of persistent nausea in women carrying males. Our research may identify a potentially modifiable contributor to nausea that could be targeted with various interventions. Future research is needed to understand the clinical implications of our findings, such as determining whether behavioral and lifestyle modifications that reduce EDC exposure (e.g., DiNCH from diet, parabens from scented products and cosmetics) can ameliorate some nausea symptoms. Of utmost clinical importance, future studies are needed to determine whether persistent nausea is associated with adverse birth outcomes, such as pre-term birth and low birthweight, and pregnancy disorders, such as pre-eclampsia and gestational diabetes.

Tables

Table 1. Demographics of I-KIDS women in analytic sample (n=467).

Characteristic	n (%)
[1]**Race/Ethnicity**	
Non-Hispanic White (*ref*)	376 (80.7)
Other[a]	90 (19.3)
[1]**Education**	
Some college or less (*ref*)	85 (18.2)
College graduate or higher	382 (81.8)
Income	
<$60,000	130 (28.1)
$60,000-$99,999	177 (38.2)
>$100,000	156 (33.7)
[1]**Alcohol since conception**	
None (*ref*)	271 (58.2)
Any alcohol consumed	195 (41.8)
[1]**Fragrance-free product use**	
Sometimes/Always (*ref*)	291 (62.3)
Never	176 (37.7)
[1]**Parity**	
No children (*ref*)	242 (51.8)
At least 1 child	225 (48.2)
[1]**Fetal Sex**	
Male (*ref*)	224 (48.0)
Female	243 (52.0)
Nausea during pregnancy	
Typical nausea (*ref*)	198 (42.4)
Never nausea	43 (9.2)
Persistent nausea	115 (24.6)
Irregular nausea	111 (23.8)
	Median (25^{th}, 75^{th} percentile)
[1]**Maternal age (years)**	29.9 (27.3, 32.7)
[1]**Pre-pregnancy body mass index (kg/m^2)**	24.5 (21.9, 29.2)
[1]**Early pregnancy Alternative Healthy Eating Index 2010***	51.6 (44.2, 59.8)
[1]**Early pregnancy perceived stress**	10.8 (6.8, 16.1)

[1]Variables included in adjusted models. *Alcohol intake was removed from the index (total score out of 100). [a]Includes non-Hispanic Black, Asian, Native Hawaiian or other Pacific Islander, American Indian or Alaska Native, Multiracial, and Others. Some women are missing covariates (race/ethnicity: n=1; diet quality index: n=19; perceived stress score: n=8; alcohol since conception: n=1).

Table 2. Distributions of pooled urinary EDC biomarkers in I-KIDS women.

EDC Metabolite Biomarker	n	LOD (ng/mL)	% ≥ LOD	% > 0	Median (25th, 75th percentile)
Phthalate/replacement					
Mono(3-carboxypropyl) phthalate (MCPP)	467	0.4	97.1	100.0	1.41 (0.93, 2.45)
Monobenzyl phthalate (MBzP)	467	0.3	99.6	100.0	5.42 (2.78, 11.3)
Monoethyl phthalate (MEP)	467	1.2	100.0	100.0	26.19 (14.19, 48.57)
Monocarboxynonyl phthalate (MCNP)	467	0.2	100.0	100.0	2.07 (1.48, 3.09)
Mono(2-ethylhexyl) phthalate (MEHP)	467	0.8	73.2	96.8	1.29 (0.80, 2.10)
Mono(2-ethyl5-carboxypentyl) phthalate (MECPP)	467	0.4	100.0	100.0	8.92 (6.65, 13.70)
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	467	0.4	100.0	100.0	5.68 (3.99, 8.86)
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	467	0.2	100.0	100.0	4.54 (3.20, 6.71)
Di(2-ethylhexyl) phthalate metabolites (ΣDEHP) sum*	467	--	--	100.0	21.2 (15.6, 32.3)
Mono-n-butyl phthalate (MBP)	467	0.4	100.0	100.0	13.05 (9.15, 18.36)
Mono-hydroxybutyl phthalate (MHBP)	467	0.4	91.0	98.9	1.30 (0.80, 1.91)
Di-n-butyl phthalate metabolites (ΣDBP) sum*	467	--	--	100.0	14.37 (10.24, 19.87)
Mono-isobutyl phthalate (MiBP)	467	0.8	99.8	100.0	8.76 (5.93, 13.34)
Mono-hydroxy-isobutyl phthalate (MHiBP)	467	0.4	99.8	100.0	3.20 (2.24, 4.99)
Di-iso-butyl phthalate metabolites (ΣDiBP) sum*	467	--	--	100.0	11.89 (8.28, 17.83)
Monocarboxyoctyl phthalate (MCOP)	467	0.3	100.0	100.0	11.20 (5.59, 23.69)
Mono-isononyl phthalate (MiNP)	453	0.9	42.5	99.1	0.73 (<LOD, 1.48)
[a]Monooxononyl phthalate (MONP)	311	0.4	66.7	99.7	2.61 (1.71, 4.63)
Di(isononyl) phthalate metabolites (ΣDiNP) sum*	453	--	--	100.0	12.41 (6.34, 26.25)
Cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester (MHiNCH)	467	0.4	78.9	99.4	0.85 (0.50, 1.72)
Cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester (MCOCH)	467	0.5	50.6	91.4	0.53 (<LOD, 0.98)
Di(isononyl) cyclohexane-1,2-dicarboxylate metabolites (ΣDiNCH) sum*	467	--	--	99.8	1.41 (0.83, 2.57)
Mono-2-ethyl-5-hydroxyhexyl terephthalate (MEHHTP)	311	0.4	66.7	100.0	8.65 (3.85, 20.55)
Mono-2-ethyl5-carboxypentyl terephthalate (MECPTP)	311	0.2	66.7	100.0	61.52 (25.85, 143.41)
Di(2-ethylhexyl) terephthalate metabolites (ΣDEHTP) sum	311	--	--	100.0	70.75 (30.56, 166.42)
Phenol					
Butyl paraben	467	0.1	42.9	62.2	<LOD (<LOD, 0.32)
Ethyl paraben	467	1.0	54.0	99.1	1.25 (<LOD, 6.13)
Methyl paraben	467	1.0	100.0	100.0	49.49 (18.74, 138.59)
Propyl paraben	467	0.1	99.8	100.0	9.44 (2.27, 29.07)
Bisphenol A (BPA)	467	0.2	97.3	99.4	0.94 (0.56, 1.53)
Bisphenol S (BPS)	467	0.1	99.2	99.8	0.47 (0.30, 0.80)
Bisphenol F (BPF)	467	0.2	63.6	82.7	0.38 (<LOD, 1.11)

Table 2 (cont'd).

Benzophenone 3 (BP-3)	467	0.4	99.8	99.8	105.84 (32.45, 268.46)
Triclosan (TCS)	467	1.7	93.3	100.0	11.52 (4.08, 58.96)
2,4-Dichlorophenol (2,4-DCP)	467	0.1	100.0	99.8	0.59 (0.38, 0.96)
2,5-Dichlorophenol (2,5-DCP)	467	0.1	99.8	99.8	1.42 (0.85, 2.96)
Other					
Triclocarban	467	0.1	29.9	45.2	<LOD (<LOD, 0.15)

Specific gravity-adjusted EDC biomarker concentrations in ng/mL. *Indicates phthalate sum included in primary statistical models. [a]Metabolite included in ΣDiNP (3 metabolites). ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308). Molar concentrations of sums were back-converted to ng/mL by multiplying ΣDEHP, ΣDiNP, ΣDBP, ΣDiBP, ΣDiNCH, and ΣDEHTP by the molecular weights of MECPP, MCOP, MBP, MiBP, MHiNCH, and MECPTP, respectively. Abbreviations: EDCs, endocrine disrupting chemicals; LOD, limit of detection.

Table 3. Associations of EDCs with persistent nausea during pregnancy by fetal sex.

	Persistent nausea compared to typical nausea (n=313)			
Biomarker	**All women**	**Female (n=151)**	**Male (n=144)**	P_{int}
Phthalates/replacements	**Odds Ratio (95% Confidence Interval)**			
ΣDEHP	1.17 (0.91, 1.52)	1.37 (0.92, 2.03)	1.08 (0.77, 1.50)	0.36
MCPP	1.19 (0.96, 1.48)	1.17 (0.86, 1.59)	1.23 (0.91, 1.68)	0.80
MCNP	1.00 (0.78, 1.27)	0.99 (0.71, 1.38)	0.99 (0.69, 1.44)	0.98
MBzP	1.02 (0.86, 1.21)	0.94 (0.75, 1.18)	1.14 (0.89, 1.46)	0.26
MEP	1.12 (0.92, 1.35)	1.08 (0.83, 1.41)	1.15 (0.88, 1.50)	0.74
ΣDiNP (2 metabolites)	1.06 (0.89, 1.26)	1.04 (0.81, 1.32)	1.10 (0.86, 1.41)	0.73
ΣDiNP (3 metabolites)	1.16 (0.92, 1.45)	1.22 (0.90, 1.64)	1.10 (0.75, 1.61)	0.67
ΣDBP	1.22 (0.93, 1.61)	1.31 (0.91, 1.87)	1.12 (0.74, 1.69)	0.57
ΣDiBP	1.03 (0.82, 1.30)	1.25 (0.91, 1.71)	0.81 (0.56, 1.17)	**0.08**
ΣDiNCH	**1.18 (1.01, 1.37)**	1.17 (0.95, 1.45)	1.18 (0.94, 1.49)	0.96
ΣDEHTP	0.93 (0.77, 1.12)	0.82 (0.63, 1.07)	1.05 (0.81, 1.36)	0.19
Phenols				
BPA	1.13 (0.94, 1.36)	1.08 (0.88, 1.33)	1.28 (0.89, 1.82)	0.43
BPS	1.06 (0.88, 1.28)	1.09 (0.82, 1.44)	1.08 (0.81, 1.45)	0.98
Methylparaben	1.00 (0.88, 1.14)	0.90 (0.75, 1.08)	1.12 (0.93, 1.36)	0.09
Ethylparaben	1.07 (0.98, 1.16)	1.01 (0.89, 1.14)	**1.12 (0.99, 1.26)**	0.23
Propylparaben	1.01 (0.91, 1.11)	0.94 (0.82, 1.09)	1.07 (0.93, 1.23)	0.21
BP-3	0.95 (0.86, 1.06)	0.93 (0.80, 1.07)	0.98 (0.82, 1.17)	0.62
TCS	0.93 (0.84, 1.03)	0.89 (0.76, 1.03)	0.98 (0.84, 1.13)	0.36
2,4-DCP	0.92 (0.74, 1.15)	0.87 (0.64, 1.19)	0.98 (0.72, 1.32)	0.60
2,5-DCP	0.97 (0.85, 1.12)	0.96 (0.78, 1.19)	0.98 (0.81, 1.18)	0.92

Odds ratio and 95% confidence intervals are interpreted as odds of persistent nausea for each two-fold increase in biomarker compared to reference group (typical nausea; n=187). Models accounted for age, race/ethnicity, education, diet quality, fragrant product use, pre-pregnancy BMI, early pregnancy perceived stress, alcohol since conception, parity, and fetal sex. Models assessing fetal sex included a multiplicative interaction term. Some women are missing covariates (n=25; race/ethnicity: n=1; diet quality index: n=19; perceived stress score: n=8; alcohol since conception: n=1).

Abbreviations: BP-3, benozophenone-3; BPA, bisphenol A; BPS, bisphenol S; EDCs, endocrine disrupting chemicals; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate, MCPP, mono(3-carboxypropyl)phthalate; MEP, monoethyl phthalate; P_{int}, $P_{interaction}$; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol.

Figures

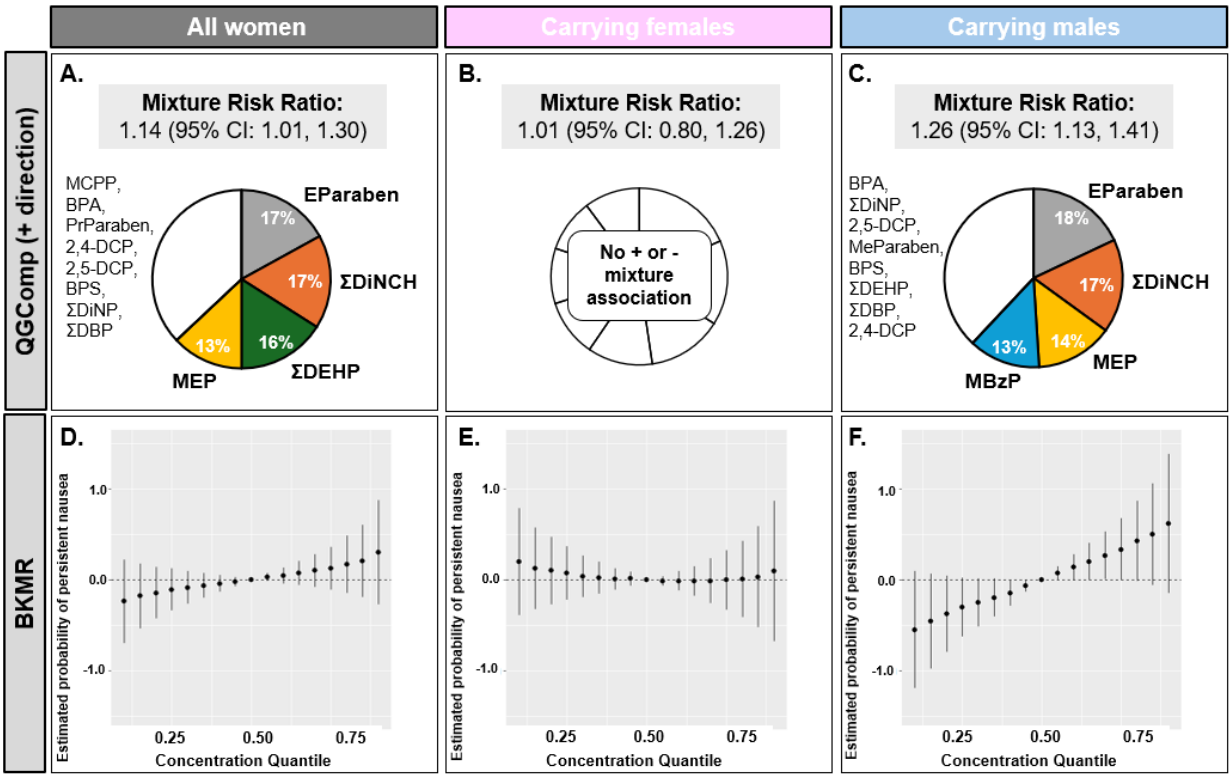

Figure 2. Associations of an EDC mixture with persistent nausea during pregnancy: QGComp in A) all women, B) women carrying females, C) women carrying males; BKMR in D) all women, E) women carrying females, and F) women carrying males. Risk ratios and 95% confidence intervals were generated from QGComp models fit with 500 bootstraps. Pie charts display percentages of EDC biomarker weights generated from non-bootstrapped QGComp models. Only positive associations are displayed as the overall risk ratios were in the positive directions. Probit BKMR models were fit using 200,000 iterations to generate plots (estimates and 95% credible intervals at various quantiles of exposure relative to the median), which are interpreted as the estimated probability of persistent nausea as the EDC biomarker mixture concentration increases. All models accounted for maternal age, race/ethnicity, educational attainment, diet quality, fragrant product use, pre-pregnancy BMI, early pregnancy stress, alcohol since conception, parity, and fetal sex. Models were stratified by fetal sex to estimate association in women carrying females and males. Sample sizes (all women: 295; women carrying females: 151; women carrying males: 144). Reference group was typical nausea in all models. Abbreviations: 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; BKMR, Bayesian kernel machine regression; BPA, bisphenol A, BPS, bisphenol S, CI, confidence interval; DBP, di-n-butyl phthalate; DEHP, di-2-ethylhexyl phthalate; DiNCH, di(isononyl) cyclohexane-1,2-dicarboxylate; DiNP, di-isononyl phthalate; EDC, endocrine disrupting chemical; EParaben, ethylparaben; MBzP, monobenzyl phthalate; MCCP, mono(3-carboxypropyl) phthalate; MEP, monoethyl phthalate; MeParaben, methylparaben; PrParaben: propylparaben; QGComp, quantile-based g-computation; Σ, sum; +, positive; -, negative.

CHAPTER THREE:
ASSOCIATIONS OF URINARY NON-PERSISTENT ENDOCRINE DISRUPTING CHEMICAL BIOMARKERS WITH EARLY-TO-MID PREGNANCY PLASMA SEX-STEROID AND THYROID HORMONES

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3.1. ABSTRACT

Pregnant women are exposed to numerous endocrine disrupting chemicals (EDCs) that can affect hormonal pathways regulating pregnancy outcomes and fetal development. Thus, we evaluated overall and fetal sex-specific associations of phthalate/replacement, paraben, and phenol biomarkers with sex-steroid and thyroid hormones. Illinois women (n = 302) provided plasma for progesterone, estradiol, testosterone, free T4 (FT4), total T4 (TT4), and thyroid stimulating hormone (TSH) at median 17 weeks gestation. Women also provided up-to-five first-morning urine samples monthly across pregnancy (8–40 weeks), which we pooled to measure 19 phthalate/replacement metabolites (reflecting ten parent compounds), three parabens, and six phenols. We used linear regression to evaluate overall and fetal sex-specific associations of biomarkers with hormones, as well as weighted quantile sum and Bayesian kernel machine regression (BKMR) to assess cumulative associations, non-linearities, and chemical interactions. In women of relatively high socioeconomic status, several EDC biomarkers were associated with select hormones, without cumulative or non-linear associations with progesterone, FT4, or TT4. The biomarker mixture was negatively associated with estradiol (only at higher biomarker concentrations using BKMR), testosterone, and TSH, where each 10% mixture increase was associated with −5.65% (95% CI: −9.79, −1.28) lower testosterone and −0.09 μIU/mL (95% CI: −0.20, 0.00) lower TSH. Associations with progesterone, testosterone, and FT4 did not differ by fetal sex. However, in women carrying females, we identified an inverted u-shaped relationship of the mixture with estradiol. Additionally, in women carrying females, each 10% increase in the mixture was associated with 1.50% (95% CI: −0.15, 3.18) higher TT4, whereas in women carrying males, the mixture was associated with −1.77% (95% CI: −4.08, 0.58) lower TT4 and −0.18 μIU/mL (95% CI: −0.33, −0.03) lower TSH. We also identified select chemical interactions. Some biomarkers were associated with early-to-mid pregnancy hormones. There were some sex-specific and non-linear associations. Future studies could consider how these findings relate to pregnancy/birth outcomes.

3.2. INTRODUCTION

Successful, healthy pregnancies require a multitude of coordinated physiological changes, including shifts in many hormones. Sex-steroid hormones, such as progesterone, estradiol, and testosterone, are derived from cholesterol, synthesized along the same biosynthetic pathways, and play various roles in healthy pregnancy, including maintaining pregnancy, preventing uterine contractions, and increasing uterine blood supply (Hacker et al., 2010). Thyroid hormones, such as free T4 (FT4), total T4 (TT4), and thyroid stimulating hormone (TSH), also have roles in pregnancy and fetal development, with both hypothyroidism and hyperthyroidism linked to preterm delivery, preeclampsia, intrauterine growth restriction, and developmental disabilities (Silva et al., 2018). There is also sex-steroid and thyroid hormone crosstalk, with thyroid hormones influencing sex-steroid hormone synthesis, transport, and elimination and sex-steroid hormones influencing thyroid hormones through feedback loops (Duarte-Guterman et al., 2014; Ren and Zhu, 2022). As normal hormonal processes need to be intricately maintained during pregnancy, any factors that perturb gestational hormones could pose health concerns and should be identified.

Pregnant women are ubiquitously exposed to non-persistent endocrine disrupting chemicals (EDCs), with virtually all women having detectable concentrations of EDC biomarkers in their urine, despite rapid metabolism and excretion from the body (CDC, 2019; Woodruff et al., 2011). EDCs are found in many consumer products (CDC, 2019; Haggerty et al., 2021). For example, di-2-ethylhexyl phthalate (DEHP) is a plasticizer used in food processing and diethyl phthalate (DEP) is a scent stabilizer used in personal care products and cosmetics (Council, 2008; Guo and Kannan, 2013; Hauser and Calafat, 2005). Parabens, such as propylparaben, are predominately used as antimicrobials in personal care products and cosmetics (Guo and Kannan, 2013; Wei et al., 2021). Phenols are a broad chemical group used for many purposes. For example, bisphenols, such bisphenol A (BPA), are used in plastics, benzophenone-3 (BP-3) is used in UV blockers, and dichlorophenols, such as 2,4-dichlorophenol (2,4-DCP), are found in pesticides (Chen et al., 2016; Chen et al., 2023; Dodson et al., 2007; Mao et al., 2022; Sun et al., 2023; Vandenberg et al., 2007). Because of potential reproductive and developmental hazards of several EDCs, such as DEHP, replacements like di-2-ethylhexyl terephthalate (DEHTP) and di(isononyl) cyclohexane-1,2-dicarboxylate (DiNCH) were introduced (Silva et al., 2013; Silva et al., 2015; Silva et al., 2017; Zota et al., 2014). Likewise, bisphenol S (BPS) and F (BPF) were introduced as replacements

for BPA (Ye et al., 2015). Unfortunately, consistent with the concept of regrettable substitution, recent studies have demonstrated that some phthalate and bisphenol replacements may have similar reproductive (Lee et al., 2020; Yang et al., 2022; Yland et al., 2022), cardiovascular (Abrantes-Soares et al., 2022), and oncological (Edaes and de Souza, 2022) impacts as the chemicals they were meant to replace, likely due to their endocrine disrupting properties.

Certain phthalates, parabens, and phenols have been characterized as EDCs based on decades of experimental evidence (Vandenberg et al., 2012). Unsurprisingly, many epidemiologic studies have investigated relationships of single non-persistent EDCs with maternal sex-steroid (Aker et al., 2019; Banker et al., 2021; Cathey et al., 2019; Johns et al., 2015; Kolatorova et al., 2018; Pacyga et al., 2021; Sathyanarayana et al., 2014; Sathyanarayana et al., 2017) and thyroid (Aker et al., 2019; Aker et al., 2016; Aung et al., 2017; Berger et al., 2018; Derakhshan et al., 2021a; Derakhshan et al., 2019; Huang et al., 2022; Nakiwala et al., 2022; Romano et al., 2018; Sarzo et al., 2022; Souter et al., 2020; Yang et al., 2022; Yland et al., 2022) hormones. Generally, most studies investigating associations of phthalates, parabens, and phenols with sex-steroid hormones have been mixed. For example, a study from Michigan Mother-Infant Pairs (MMIP) studied six phenols and four parabens and reported negative associations of BPS with estradiol and propylparaben and methylparaben with progesterone but reported no associations with testosterone. In contrast, the PROTECT study from Puerto Rico investigated the same EDC biomarkers and only reported that triclosan was positively associated with 2nd trimester testosterone (Aker et al., 2019). Similarly, studies assessing associations of phthalates with sex-steroid hormones have reported null findings (Banker et al., 2021), select positive associations (Cathey et al., 2021; Pacyga et al., 2021), and select negative associations (Cathey et al., 2019; Johns et al., 2015; Sathyanarayana et al., 2014; Sathyanarayana et al., 2017). Studies assessing EDCs and thyroid hormones are similarly mixed, though, in general, higher exposure has been associated with disrupted T4 and TSH concentrations (Berger et al., 2018; Huang et al., 2018; Romano et al., 2018; Wang et al., 2017; Yao et al., 2016).

Although pregnant women are not exposed to single chemicals, few studies have considered potential mixture effects and non-linear interactions. To the best of our knowledge, no studies have assessed associations of EDC mixtures with gestational sex-steroid hormones. In

contrast, recent studies have utilized various mixtures approaches to assess cumulative associations of EDCs and thyroid hormones, but with mixed results, likely due to use of potentially problematic methods, such as including co-exposures as covariates or only assessing single classes of EDCs (Berger et al., 2018; Derakhshan et al., 2021a; Huang et al., 2022; Nakiwala et al., 2022; Romano et al., 2018; Sarzo et al., 2022; Souter et al., 2020; Yang et al., 2022; Yland et al., 2022). While many studies reported null findings, some identified negative associations of various non-persistent EDC mixtures with maternal serum free T3 (FT3), total T3 (TT3), total T4 (TT4), thyroid peroxidase antibody (TPOAB), and T3/T4 ratio. Despite the fact that maternal hormone levels differ by fetal sex (Meulenberg and Hofman, 1991; Sitoris et al., 2022; Toriola et al., 2011), only a few studies assessed and identified differences in the relationship between single EDCs and hormones by fetal sex (Banker et al., 2021; Pacyga et al., 2021; Sathyanarayana et al., 2014). Of the studies assessing EDCs as a mixture, only one explored differences by fetal sex, reporting a negative association of a bisphenol mixture with FT3 in women carrying males and a positive association in women carrying females (Huang et al., 2022; Yang et al., 2022).

Because women are exposed to multiple non-persistent EDCs during pregnancy and proper hormone balance is critical for pregnancy health, our primary objective was to evaluate individual and cumulative associations of multiple classes of non-persistent EDC biomarkers with early-to-mid pregnancy sex-steroid and thyroid hormones. One difficulty in studying EDCs is the potential presence of non-monotonic dose response curves, such as low dose responses, which have been identified in *in vitro* and *in vivo* experiments for many EDCs, including phthalates and phenols (Vandenberg et al., 2012; Vandenberg et al., 2007). Therefore, to better understand how individual biomarkers within the mixture interact to affect gestational hormones, we additionally explored potential non-linear dose–response relationships and chemical-chemical interactions. As maternal sex-steroid and thyroid hormone levels may differ in women carrying females and males (Klinga et al., 1978; Sitoris et al., 2022; Toriola et al., 2011), we also evaluated whether associations differed by fetal sex.

3.3. MATERIALS AND METHODS

Illinois KIDS development study (I-KIDS) study design and population

Pregnant women were recruited into I-KIDS, a prospective pregnancy and birth cohort, from

two local obstetric clinics in Champaign-Urbana, Illinois to evaluate associations of prenatal environmental chemical exposures with neurodevelopment. Recruitment and enrollment have been previously detailed (Pacyga et al., 2021; Pacyga et al., 2022a; Pacyga et al., 2023)). To be eligible to participate, women had to be ≤ 15 weeks pregnant at enrollment, 18–40 years old, fluent in English, in a low-risk pregnancy (determined by a medical provider), not carrying multiples, residing within 30 min of the study site, and not planning on moving before their child's first birthday. The current study includes 302 women enrolled between 2015 and 2018, who remained in the study through the birth of their child, had urinary EDC biomarkers measured, and had available measurements of at least one sex-steroid or thyroid hormone in maternal plasma (Figure S11). All women provided written informed consent, and the study was approved by the University of Illinois' Institutional Review Board. The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute human subjects' research.

Collection of maternal sociodemographic, lifestyle, and health information

After enrollment (median 13 weeks gestation), I-KIDS staff conducted home visits to interview women about various sociodemographic and lifestyle factors. Pre-pregnancy body mass index (BMI) (kg/m^2) was calculated from self-reported pre-pregnancy weight and height. To measure early pregnancy stress levels, women completed the Perceived Stress Scale (PSS), a ten-item questionnaire asking about thoughts and feelings during the last month (Cohen et al., 1983; Cohen and Williamson, 1988). At median 13 weeks gestation, women also completed a semi-quantitative food-frequency questionnaire (FFQ) adapted from the full-length Block-98 FFQ (NutritionQuest, Berkely, CA) and validated in pregnant populations (Bodnar and Siega-Riz, 2002; Boucher et al., 2006; Laraia et al., 2007). Dietary intakes representing diet patterns from the previous three months were used to calculate early pregnancy Alternative Healthy Eating Index (AHEI-2010), an 11-component diet quality index (out of 110 total points) based on foods and nutrients shown to be predictive of chronic disease risk and mortality, where a higher score indicates better overall diet quality (Chiuve et al., 2012; McCullough et al., 2002). As the AHEI-2010 considers moderate alcohol consumption beneficial but guidelines recommend avoiding alcohol in pregnancy, we removed the alcohol component to create a ten-component diet quality index (maximum: 100 points).

Assessment of urinary phthalate/replacement, paraben, triclocarban, and phenol biomarker concentrations

Because non-persistent EDCs have relatively short biological half-lives (6–24 hours depending on the chemical) and high within-person variability (Shin et al., 2019a; Shin et al., 2023; Shin et al., 2019b), we measured EDC biomarkers in five across-pregnancy urine samples that were physically pooled prior to chemical biomarker measurement. At study clinic/home visits or routine prenatal care clinic visits, women provided at least three and up to five first-morning urine samples at median 13, 17, 23, 28, 34 weeks gestation. Most women contributed all five samples (97%) and the rest provided three or four samples (Pacyga et al., 2023). Details about urine collection, processing, and storage have been previously published (Pacyga et al., 2023). Briefly, women collected urine into polypropylene urine cups and refrigerated them for up to 24 h until we aliquoted samples for long-term storage. To create the pool, we added 900 µL of urine from the first collection to a five mL cryovial tube. At each subsequent visit, we layered fresh urine onto the previous frozen sample and immediately stored the sample at −80 °C. At the end of pregnancy, we thawed and vortexed the sample to measure specific gravity.

Frozen pooled urines were shipped to the CDC's Division of Laboratory Sciences in four batches (batch 1: enrolled December 2013 – February 2015; batch 2: enrolled February 2015 - July 2016; batch 3: enrolled July 2016 – August 2018; batch 4: enrolled September 2018 – November 2019). Only women with chemicals measured in batches 2 and 3 were included in the current study because they had all chemical data and at least some hormone data. Using previously published isotope-dilution mass spectrometry methods with rigorous quality assurance/quality control protocols and high long-term reproducibility (Calafat et al., 2006; Calafat et al., 2010; Schantz et al., 2015; Silva et al., 2013; Silva et al., 2007; Silva et al., 2019; Ye et al., 2014), CDC laboratory staff quantified 19 phthalate/replacement metabolites: monocarboxynonyl phthalate (MCNP), monocarboxyoctyl phthalate (MCOP), monooxononyl phthalate (MONP), mono-isononyl phthalate (MiNP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono(3-carboxypropyl) phthalate (MCPP), monobenzyl phthalate (MBzP), mono-n-butyl phthalate (MBP), monohydroxybutyl phthalate (MHBP), mono-isobutyl phthalate (MiBP), monohydroxy-isobutyl phthalate (MHiBP), monoethyl phthalate (MEP), cyclohexane-1,2-dicarboxylic acid-

mono(carboxyoctyl) ester (MCOCH), cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester (MHiNCH), mono(2-ethyl-5-hydroxyhexyl) terephthalate (MEHHTP), and mono(2-ethyl-5-carboxypentyl) terephthalate (MECPTP). In addition, the CDC measured concentrations of triclocarban, four parabens (butylparaben, ethylparaben, methylparaben, propylparaben), and seven phenols (bisphenol A (BPA), bisphenol F (BPF), bisphenol S (BPS), triclosan (TCS), benzophenone-3 (BP-3), 2,4-dichlorphenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP)). The limits of detection (LOD) were 0.1–1.7 ng/mL, depending on the biomarker (**Table 5**).

Collection and quantification of plasma maternal hormone concentrations

We collected maternal plasma at median 17 weeks gestation in glass heparin-containing vacutainer tubes, centrifuged them at room temperature for 20 min, and aliquoted them for storage at −80 °C. We sent the samples to the University of Michigan Diabetes Research Center (MDRC) Clinical Core Chemistry Laboratory for quantification of progesterone, estradiol, total testosterone, FT4, TT4, and TSH using solid-phase, enzyme-labeled chemiluminescent competitive immunoassay (IMMULITE 1000, Siemens). Briefly, 10–75 µL of plasma was incubated for 30 min with a polyclonal rabbit antibody coated bead (solid-phase) and bovine calf intestine alkaline phosphate conjugated to hormone (liquid-phase). After repeated washes and centrifugation to remove unbound hormone, the chemiluminescent substrate was added to measure the signal indicating the proportion of hormone bound. Progesterone had a reportable range of 0.20 – 40.0 ng/dL, an LOD of 0.20 ng/dL, and a functional sensitivity of 0.46 ng/mL. Estradiol had a reportable range of 20.0 – 2000.0 pg/mL and an analytic sensitivity of 15.0 pg/mL. Testosterone had a calibration range of 20.0 – 1600.0 ng/dL and an analytic sensitivity of 15.0 ng/dL. FT4 had a reportable range of 0.3 – 6.0 ng/dL, an analytic sensitivity of 0.13 ng/dL, an LOD of 0.28 ng/dL, and a functional sensitivity of 0.3 ng/dL. TT4 had a calibration range of 1.0 – 24.0 µg/dL and an analytic sensitivity of 0.4 µg/dL. Finally, TSH had a calibration range of up to 75.0 µIU/mL and an analytic sensitivity of 0.01 µIU/mL. Based on protocol recommendations for when estradiol concentrations are above the assay's reportable range, estradiol samples were diluted 10x before analysis and machine-read values were multiplied by ten to obtain final concentrations. For total testosterone, we considered values below the calibration range as missing (n = 37). We used the following formula for calculating one missing TSH value: minimum detected TSH concentration /√2.

3.4. STATISTICAL ANALYSIS

Derivation of analytic sample

The derivation of our analytic sample is detailed in Figure S11**.** Briefly, of 10,178 women who completed a reply card, 688 enrolled in the study and 531 remained active through the birth of their child. Of these women, 302 had chemical biomarkers > 0, at least some hormone information, and all covariate information (all women were in chemical analysis batches 2 and 3). Of our analytic sample, 295 women had complete data on progesterone and estradiol, 250 women had complete testosterone data, and 294 women had complete FT4, TT4, and TSH data (Figure S11). We summarized sociodemographic, health, and lifestyle factors in the reference population and our analytic sample as frequency (percent) or median (25th, 75th percentile) (**Table 4**).

Modeling of urinary chemical concentrations

For non-zero biomarker concentrations below the LOD, we used instrument-read values to avoid bias associated with imputing concentrations < LOD (73). In our statistical analyses, we only included chemical biomarkers with concentrations > 0 in at least 60% of women (**data not shown**). This resulted in butylparaben, BPF, and triclocarban being excluded from further analyses. To avoid undefined estimates for ln-transformed zero concentrations (ethylparaben n = 3; BPA n = 2; and BPS, BP-3, and 2,4-DCP n = 1), we used the formula [ln(chemical concentration + 0.0001)] in linear regression and weighted quantile sums regression (WQSR). In Bayesian kernel machine regression (BKMR) models, we used the following formula in place of zero concentrations as it improved model fit and convergence: minimum measured concentration/$\sqrt{2}$. Including BPF in WQSR models had minimal impact on estimates and weights (**data not shown**).

We evaluated specific gravity adjusted phthalate/replacement, paraben, and phenol biomarkers as molar sums or individual biomarkers using the previously reported formula for specific gravity adjustment (Meeker et al., 2009). For phthalates/replacements, we used the urinary metabolite concentrations to approximate pregnant women's exposure to phthalate/replacement parent compounds. We calculated parent molar sums (nmol/mL) by summing metabolites from common precursors: MEHP, MEHHP, MEOHP, and MECPP for the sum of DEHP metabolites (ΣDEHP); MCOP, MiNP, and MONP for the sum of metabolites of di-isononyl phthalate (ΣDiNP); MBP and MHBP for the sum of di-n-butyl phthalate

metabolites (ΣDBP); MiBP and MHiBP for the sum of di-isobutyl phthalate metabolites (ΣDiBP); MHiNCH and MCOCH for the sum of DiNCH metabolites (ΣDiNCH); and MEHHTP and MECPTP for the sum of DEHTP metabolites (ΣDEHTP). Specific formulas have been published elsewhere (Pacyga et al., 2021) and are reported in table footers. Molar concentrations were back-converted to ng/mL by multiplying ΣDEHP, ΣDiNP, ΣDBP, ΣDiBP, ΣDiNCH, and ΣDEHTP by the molecular weights of MECPP, MCOP, MBP, MiBP, MHiNCH, and MECPTP, respectively (Pacyga et al., 2022a; Rodriguez-Carmona et al., 2020; Zhang et al., 2020a). We estimated exposure to di-isodecyl phthalate, di-n-octyl phthalate, benzylbutyl phthalate (BBzP), and DEP using ng/mL concentrations of their urinary metabolites MCNP, MCPP, MBzP, and MEP, respectively.

Covariate selection

Based on previous literature and our data (Huang et al., 2022; Nakiwala et al., 2022; Romano et al., 2018; Souter et al., 2020; Yang et al., 2022; Yland et al., 2022), we generated a directed acyclic graph (DAG) to identify a minimum sufficient adjustment set of covariates (Supp. Fig. 2). We assessed correlations between covariates to test for potential multicollinearity; however, all covariates were only weakly or moderately correlated ($r < 0.4$; **data not shown**). Our final linear regression, WQSR, and BKMR models accounted for maternal age, race/ethnicity, educational attainment, pre-pregnancy BMI, early pregnancy diet quality (AHEI-2010), stress (PSS 10), ever smoking, parity, gestational age at hormone assessment, and fetal sex. These variables may represent latent constructs, such as reproductive health (maternal age and parity), socioeconomic status (race/ethnicity and education), and health/lifestyle (pre-pregnancy BMI, stress, ever smoking, and diet quality). Age, pre-pregnancy BMI, diet quality, stress, and gestational age were continuous variables, whereas all other variables were categorized with the reference group as indicated in **Table 4**.

Evaluating associations of non-persistent EDC biomarkers with maternal hormones

To address our main objective, we evaluated associations between EDC biomarkers and gestational hormones using unadjusted and multivariable linear regression and WQSR. When modeled individually, we ln-transformed all phthalate/replacement, paraben, and phenol biomarkers due to their right-skewed distributions. In single-pollutant and WQSR models, because of non-normal distributions, we ln-transformed progesterone, estradiol, testosterone, FT4, and TT4. TSH was normally distributed and thus not transformed.

WQSR models cumulative associations and identifies individual biomarkers responsible for most of the mixture effect, while handling moderately and highly correlated co-exposures. Our mixture included 19 non-persistent EDC biomarkers, including ten phthalate/replacement metabolites or sums, three parabens, and six phenols (as listed in **Tables 7-9**). WQSR is a supervised mixture method that creates a weighted index by transforming exposure biomarkers into quantiles (deciles in this study) and evaluates the cumulative association of the index with the outcome using multiple linear regression (Carrico et al., 2015). We generated a distribution of results using 100 iterations (repeated holdouts), each with 100 bootstrap replications. Within each iteration, data were randomly split 40/60% into training and validation datasets, respectively (Tanner et al., 2019). To determine the relative importance (weight) of single co-exposures within the mixture, we used the standard cut-off (1/# of co-exposures; 1/19 = 0.05) to identify meaningful contributors (Carrico et al., 2015).

Identifying non-linear relationships and chemical-chemical interactions with the EDC biomarker mixture

BKMR uses kernel machine regression to estimate a non-parametric, high-dimensional exposure–response function to identify non-linear chemical and hormone dose–response relationships and chemical-chemical interactions within a mixture (Bobb et al., 2018; Bobb et al., 2015). After ln-transforming, centering, and scaling our co-exposures, outcomes, and continuous covariates, we fit BKMR models with 200,000 iterations and 50 knots for the same mixture of EDC biomarkers described above. To assess a cumulative non-linear mixture association, we created dose–response curves where the full mixture increases by various quantiles. We also calculated posterior inclusion probabilities (PIPs) to identify important chemical biomarkers contributing to associations between the mixture and hormones (Table S19). By interpreting univariable dose–response relationships where all other exposure biomarkers are fixed at their median, we can identify non-linear relationships, within the range of the cohort's exposures, between single EDC biomarkers and gestational hormones. To identify interactions between biomarkers, we interpreted bivariate exposure–response plots where we visualized one biomarker's dose–response relationship with hormones while a second chemical biomarker was held at 10th, 25th, 50th, 75th, and 90th percentiles. We only explored chemical-chemical interactions when there was evidence of potential mixture associations from either WQSR or BKMR, and we determined interactions by identifying non-parallel or non-overlapping dose–response curves.

Evaluating differences in association of EDC biomarkers with maternal hormones by fetal sex

Because pregnancy hormones differ by fetal sex, our second objective was to identify fetal sex-specific associations between EDC biomarker mixtures and early-to-mid pregnancy hormones. In linear regression, WQSR, and BKMR models, we assessed fetal-sex specific associations of EDC biomarkers individually and as a mixture with maternal sex-steroid and thyroid hormones. Specifically, in linear regression models, we included a multiplicative interaction ($P_{interaction}$) between chemical biomarkers and fetal sex. We examined general trends and reported potentially meaningful results based on the associations' direction, strength, and precision, regardless of interaction *P*-value. For WQSR and BKMR, we stratified our sample by fetal sex and identified potentially meaningful results by comparing direction and strength of association.

Reporting of findings and interpreting meaningful associations

For single-chemical biomarker linear regression results, except TSH, our β-estimates and 95% confidence intervals (CIs) represent the percentage change (%Δ) in gestational hormone concentration associated with a 10% increase in chemical biomarker concentration as we performed the back-transformation $[(1.10^{\beta} - 1)*100]$ for progesterone, estradiol, testosterone, FT4, and TT4 and $[\beta^{\ln(1.10)}]$ for TSH. For WQSR results, except TSH, the β-estimates and 95% CIs represent the percentage change (%Δ) in hormone concentration associated with a 10% increase in the mixture index as our mixture exposure biomarkers were divided into deciles, all hormones were ln-transformed, and we back-transformed our mixtures results using $[(e^{\beta} - 1)*100]$. For TSH, the β-estimates and 95% CIs represent the μIU/mL change in TSH for each 10% increase in chemical biomarker concentrations or the EDC mixture. We identified potentially meaningful findings from single pollutant and WQSR models by assessing the direction, strength, and precision of the associations. To ensure we met model assumptions, we performed regression diagnostics based on residuals for single pollutant models, assessed scatterplots in WQSR, and checked for convergence with the Markov Chain Monte Carlo procedure in BKMR. We performed linear regression analyses in SAS version 9.4 (SAS Institute Inc. Cary, NC) using PROC GLM and completed WQSR and BKMR analyses in R Statistical Software using R packages "gWQS: Generalized Weighted Quantile Sum Regression" (Stefano Renzetti) and "bkmr: Bayesian Kernel Machine Regression" (Bobb, 2022).

3.5. RESULTS

Illinois KIDS development study (I-KIDS) characteristics

Most women were non-Hispanic White (82%), college-educated (83%), with an annual household income >$60,000 (73%) (**Table 4**), and characteristics did not differ greatly from the full I-KIDS sample of 531 women (those with at least one chemical biomarker measurement, reflecting women who stayed in the study through the birth of their infant). The median age was 30.4 years. The median pre-pregnancy BMI was 24.7 kg/m^2 with 47% of women having overweight or obesity. The median (25th, 75th percentile) diet quality (AHEI-2010) was 51.7 (44.6, 59.3) out of 100 points. Most women never smoked cigarettes (83%). Most women reported having low early-pregnancy stress (62%) and more than half of women were nulliparous (54%). Fetal sex was approximately evenly distributed between females (51%) and males (49%).

Concentrations of maternal urinary chemical biomarkers

Most chemicals had concentrations ≥ LOD in the vast majority of women, except MiNP, MCOCH, butyl paraben, ethyl paraben, bisphenol F, and triclocarban, which were only detectable (≥ LOD) in 31.5%, 66.9%, 33.4%, 57.0%, 57.0%, and 29.8% of women, respectively (**Table 5**). Only a few chemical biomarkers were strongly correlated with each other, including ΣDiNP with MCPP (r = 0.8), 2,4-DCP with 2,5-DCP (r = 0.7), and methylparaben with propylparaben (r = 0.7; Figure S13).

Maternal plasma hormone concentrations

All women had measurable concentrations of progesterone, estradiol, FT4, and TT4; however, 87% and 99% of women had concentrations at or above the lower limit of the reportable ranges for testosterone and TSH, respectively (**Table 6**). The median (25th, 75th percentile) concentration of hormones are reported in **Table 6**.

Linear, non-linear, and interactive relationships of EDC biomarkers with early-to-mid pregnancy sex-steroid hormones

Associations with progesterone

In general, despite a few single pollutant associations, in all women or women carrying females or males, there were no cumulative or non-linear associations of EDC biomarkers with progesterone or chemical-chemical interactions (**Table 7; Figure 3; Figure 4;** Figures

S14, S20-S22).

Associations with estradiol

There was a potentially non-linear relationship between the mixture and estradiol, with negative associations at higher EDC biomarker mixture concentrations (**Figure 4**). Additionally, BPA exhibited an s-shaped relationship with estradiol when all co-exposures were held at their median. This relationship was attenuated at lower concentrations of MEP and higher concentrations of BP-3, ΣDiNP, methylparaben, and propylparaben (Figures S15, S23)**.** When modeled individually, BPS was associated with higher estradiol in women carrying females and BPA was associated with lower estradiol in women carrying males (**Table 8**), but sex-specific WQSR mixture associations were only marginally meaningful (Tables S16-S17). However, using BKMR, the mixture was negatively associated with estradiol at both lower and higher concentrations in women carrying females, driven by BPA (PIP: 0.95) and propylparaben (PIP: 0.75) (**Figure 4;** Table S19). Importantly, in women carrying females, BPA had an inverted u-shape relationship with estradiol when all EDC biomarker concentrations were fixed at their median, which was attenuated at the highest concentrations of propylparaben (Figures S15b, S24). In women carrying males, the relationship of BPA with estradiol was attenuated at higher concentrations of MEP and lower concentrations of MCPP (Figures S15c, S25).

Associations with testosterone

Propylparaben, triclosan, 2,4-DCP, and 2,5-DCP were negatively associated with testosterone (**Table 9**), which, along with BPS and ΣDEHTP, drove the WQSR mixture association, such that a 10% increase in the mixture was associated with a −5.65% (95% CI: −9.79, −1.28) lower testosterone (**Figure 3;** Tables S16-S17). Using BKMR, there were no cumulative or non-linear associations (**Figure 4**); however, there was a non-linear relationship between BPS and testosterone when all co-exposures were fixed at their median (Figure S16a). We also identified some chemical-chemical interactions, such that negative relationships of propylparaben, TCS, and 2,5-DCP with testosterone were stronger at lower concentrations of MEP, whereas associations of TCS and 2,4-DCP with testosterone were attenuated at lower concentrations of BP-3 (Figure S26). There was no evidence of meaningful sex-specific or non-linear relationships (**Table 9**; **Figure 3, Figure 4**; Figures S16b,c). However, in women carrying females, propylparaben interacted with BPA, and in

women carrying males, propylparaben interacted with ΣDEHTP, 2,5-DCP, ΣDiNCH, and methylparaben, whereas 2,5-DCP interacted with ΣDEHTP and methylparaben (Figures S27–S28).

Linear, non-linear, and interactive relationships of EDC biomarkers with early-to-mid pregnancy sex-steroid hormones

Associations with FT4

Overall, neither individual EDC biomarkers nor the mixture were associated with FT4 (**Table 10**; **Figure 3; Figure 5;** Table S17). Only in women carrying females, 2,4-DCP was associated with higher FT4, whereas only in women carrying males, ΣDiNP was associated with lower FT4 (**Table 10**). There was no evidence of meaningful sex-specific associations, or cumulative/non-linear associations (**Figure 3; Figure 5;** Figures S17; S29–S31).

Associations with TT4

Despite a few individual chemical associations, we found no evidence of cumulative or non-linear associations of the EDC biomarkers mixture with TT4 or chemical-chemical interactions (**Table 11**; **Figure 3; Figure 5**; Figures S18). Only in women carrying males, higher ΣDiBP was associated with lower TT4, whereas associations of MBzP and 2,5-DCP with TT4 were stronger in women carrying females (**Table 11**). There was also a sex-specific association of the WQSR mixture with TT4 (**Figure 3**), such that each 10% mixture increase in women carrying females was associated with 1.50% (95% CI: −0.15, 3.18) higher TT4 (driven by MBzP and 2,5-DCP) and each 10% mixture increase in women carrying males was associated with −1.77% (95% CI: −4.08, 0.58) lower TT4 (driven by DiBP, BPS, and ΣDiNCH) (Tables S16, S18). Using BKMR, there were no cumulative non-linear associations of EDC biomarkers with TT4 (**Figure 5**). However, in women carrying males, there were non-linear relationship of BPS and TCS with TT4 when other biomarkers were held at their medians, and associations of TCS and BPS with TT4 were attenuated at higher concentrations of ΣDEHP (Figures S18c, S34).

Associations with TSH

In all women, methyl-, ethyl-, and propylparaben were inversely associated with TSH, whereas MBzP was positively associated with TSH (**Table 12**). Parabens, along with BPA, BPS, 2,5-DCP, ΣDiNCH, ΣDiBP, and TCS, drove the WQSR mixture association, such that

each 10% mixture increase was associated with −0.09 μIU/mL (95% CI: −0.19, 0.00) lower TSH (**Figure 3;** Tables S16, S18). Using BKMR, there were no cumulative or non-linear associations of EDC biomarkers with TSH (**Figure 5**); however, there was a non-linear relationship of BPA with TSH that was attenuated at higher concentrations of BP-3 (Figures S19a, 35). In women carrying females, higher ΣDiBP was associated with lower TSH, whereas in women carrying males, higher MBzP and ΣDBP were associated with higher TSH and higher ethylparaben was associated with lower TSH (**Table 12**). In women carrying males, ethylparaben, along with 2,5-DCP, DiNP, MEP, BPA, MCNP, TCS, and methylparaben, drove the WQSR mixture association, such that each 10% mixture increase was associated with −0.18 μIU/mL (95% CI: −0.33, −0.03) lower TSH (**Figure 3;** Tables S16, S18). Using BKMR, there was no evidence of sex-specific non-linear associations or chemical-chemical interactions (**Figure 5;** Figures S19b,c, S36–S37).

3.6. DISCUSSION

Summary of major findings

In a relatively homogenous, higher socioeconomic status sample of midwestern U.S. pregnant women, a mixture of phthalate/replacement, paraben, and phenol metabolites was associated with lower early-to-mid pregnancy testosterone in all women (driven by propylparaben and triclosan), TT4 in women carrying females (driven by MBzP, 2,5-DCP, and propylparaben), and TSH in women carrying males (driven by 2,5-DCP and propylparaben). We also identified potential non-linear associations between the EDC biomarker mixture and estradiol in all women (at high concentrations) and in women carrying females (at low and high concentrations). In general, the mixture was not associated with progesterone or FT4.

A mixture of non-persistent EDC biomarkers was not associated with early-to-mid pregnancy progesterone

Despite identifying a negative association between propylparaben and progesterone, we did not observe any meaningful mixture associations, consistent with recent studies evaluating single chemicals (Aker et al., 2019; Banker et al., 2021; Cathey et al., 2019; Johns et al., 2015; Kolatorova et al., 2018; Sathyanarayana et al., 2014; Sathyanarayana et al., 2017). Similar to our results, the Michigan Mother-Infant Pairs (MMIP) study reported negative associations of methylparaben and propylparaben with first trimester progesterone (Banker

et al., 2021). However, a study of Puerto Rican women (PROTECT), who had higher paraben and phthalate biomarker concentrations and lower biomarkers of phthalate replacement concentrations compared to I-KIDS, reported no associations of four parabens and seven phenols with 2nd and 3rd trimester progesterone (Aker et al., 2019) and negative associations between some phthalates biomarkers and progesterone (Cathey et al., 2019; Johns et al., 2015). Our findings, using two robust mixture methods, suggest that a mixture of non-persistent EDC biomarkers does not affect early-to-mid pregnancy progesterone; however, single chemical results suggest a relationship of parabens with progesterone that warrants further investigation, especially because methylparaben and propylparaben share common sources of exposure and thus could have cumulative effects.

An EDC biomarker mixture was associated with estradiol, with evidence of sex-specific and non-linear relationships

Our single-pollutant null findings add to already-mixed literature with regards to EDCs and estradiol. For example, PROTECT reported no associations of EDC biomarkers with estriol or estradiol (Aker et al., 2019; Cathey et al., 2019; Johns et al., 2015). However, the Infant Development and Environment Study (TIDES), comprised of women with slightly higher phthalate biomarker concentrations compared to I-KIDS, identified positive associations of phthalate biomarkers with estradiol (Sathyanarayana et al., 2017), whereas the MMIP study reported negative associations of BPS with estradiol and of methylparaben with estradiol and estrone (Banker et al., 2021). Previously, we identified many positive associations between phthalate/replacement biomarkers and urinary estrogens across pregnancy (Pacyga et al., 2021). Those prior results could differ from our current results in part due to hormone assessment timing (median 13, 28, and 34 weeks in our prior study versus 17 weeks in the current study) and hormone assessment medium (urine in the prior study versus blood in the current study). While reported sex-specific associations of EDC biomarkers with estrogens are mixed (Banker et al., 2021; Pacyga et al., 2021; Sathyanarayana et al., 2014), our results support sex-specific associations of BPS and BPA with estradiol, which should be further explored.

We identified a non-linear relationship of BPA and propylparaben with estradiol in women carrying females, which is consistent with experimental and epidemiologic studies showing various non-linear dose–response curves when evaluating EDCs and estradiol (u-shaped

curves, inverted u-shape curves, s-shape curves, etc.) (reviewed by (Vandenberg et al., 2012)), likely due to dose-dependent disruption of genes, proteins, and receptors. However, as the range of urinary biomarker concentrations in I-KIDS is modest, associations at our highest concentrations may represent average concentrations in higher exposed populations, such as women in the PROTECT cohort. A recent study of pregnant Chinese women, with much higher urinary concentrations of BPA, reported non-linear negative low-dose relationships of BPA with estriol, estradiol, and estrone, with no fetal-sex differences (Li et al., 2020). However, this study also differs from ours in study design (spot urine samples for BPA and estrogen assessment) and method (single-pollutant models). Interestingly, in our study, BPA interacted with other EDC biomarkers, such as BP-3, methylparaben, and propylparaben, and the negative relationship between BPA and estradiol in women carrying females was stronger when propylparaben concentrations were relatively low. Parabens and bisphenols both disrupt estrogen (Liang et al., 2023), so it is plausible they both act at similar cellular targets. To this end, one recent experimental study reported mixtures of bisphenols and benzophenone derivatives showed synergistic or additive effects at human-relevant concentration (Kudlak et al., 2022).

Non-persistent EDC biomarkers, individually and as a mixture, were associated with lower testosterone

Several phthalates and phenols have been characterized as anti-androgenic based on animal and human epidemiologic studies (Gray et al., 2006; Parks et al., 2000); however, many studies were conducted within the context of male reproductive health. Our results suggest that select non-persistent EDC biomarkers are negatively associated with early-to-mid pregnancy testosterone, primarily driven by phenols and propylparaben, which differs somewhat from prior studies. In a previous study investigating urinary rather than plasma hormones, we identified positive associations of MBzP and MEP with urinary testosterone at 28 weeks gestation (Pacyga et al., 2021), and the Study for Future Families (SFF) reported positive associations of MEP with serum second and third trimester testosterone (Sathyanarayana et al., 2014). Despite no association between MEP and testosterone in this study, we identified that MEP attenuates some negative relationships. Associations of TCS, propylparaben, 2,4-DCP, and 2,5-DCP with lower testosterone in our study differed from one PROTECT study that reported non-significant positive associations of TCS, 2,4-DCP, and 2,5-DCP with second trimester serum testosterone (Aker et al., 2019). However, this study

relied on urinary biomarker concentrations from spot urine samples collected at 16–20 weeks gestation, whereas we utilized concentrations from a pooled sample composed of up to five first-morning urine samples collected throughout gestation. Importantly, unlike prior studies that assessed single EDC biomarkers, our study used two different mixture methods that identified TCS as being a meaningful predictor of testosterone. The roles of androgens in pregnancy are not entirely clear, but testosterone at normal levels regulates key processes of pregnancy and birth, such as cervical remodeling (Makieva et al., 2014), and at higher levels is associated with pregnancy conditions like gestational diabetes, preeclampsia, and pre-term birth (Cathey et al., 2021; Morisset et al., 2013; Salamalekis et al., 2006). Future studies should investigate if lower testosterone levels are linked to adverse pregnancy health or birth outcomes.

Non-persistent EDC biomarkers were associated with total T4 but not with free T4

We did not identify any cumulative or non-linear associations between EDC biomarkers and FT4 overall or by fetal sex, which is consistent with prior studies (Huang et al., 2018; Nakiwala et al., 2022; Romano et al., 2018; Sarzo et al., 2022; Souter et al., 2020; Yang et al., 2022; Yland et al., 2022). However, our and others' results identified relationships between EDC biomarkers and TT4. We identified positive associations of MBzP and 2,5-DCP with TT4; however, we did not identify a cumulative association of the EDC mixture with TT4. In contrast, the Health Outcomes and Measures of the Environment (HOME) study reported an inverse association of a nine-phthalate biomarkers mixture using WQSR with maternal serum TT4 (at 16 weeks gestation) that was primarily driven by MEP and MCPP, neither of which met the WQSR threshold in our study (Romano et al., 2018). One major difference between this study and ours is that HOME measured phthalate biomarkers in two spot urines at 16 and 26 weeks gestation (compared to our pooled sample of phthalates/replacements, parabens, and phenols), which could affect both temporality and precision of the exposure assessment. One recent study assessed biomarkers of phthalates, parabens, and phenols as an 11-chemical mixture using BKMR and reported a negative association with TT3/TT4 ratio (nine weeks gestation), which may indicate EDCs are associated with higher TT4 (Nakiwala et al., 2022). This study utilized a dimension reduction method by limiting chemical biomarkers included in their mixture to those exhibiting biological activity in a toxicological database, whereas we included any phthalate/replacement, paraben, and phenol biomarkers analyzed by the CDC with measurable concentrations. While no other studies identified sex-

specific associations of EDC biomarkers mixtures with TT4, our results suggest a positive relationship between EDCs and TT4 in women carrying females and a negative relationship in women carrying males. Overall, our findings, and those from prior studies, suggest non-persistent EDC biomarkers may affect early-to-mid pregnancy maternal TT4 but not FT4. FT4 and TT4 are both biomarkers of maternal thyroid function, but it is unclear what implications altered TT4 would have (compared to FT4), as TT4 exhibits higher variability during early pregnancy, is poorly related to TSH levels, and is not associated with adverse pregnancy outcomes, such as pre-eclampsia, premature delivery, and abnormal birthweight (Korevaar et al., 2016).

Non-persistent EDC biomarkers were associated with lower TSH, primarily in women carrying males

We observed negative associations of parabens with TSH and identified a negative mixture association with TSH, driven by BPA, BPS, ethylparaben, and 2,5-DCP. However, no prior studies assessing non-persistent EDC biomarker mixtures with TSH have reported meaningful associations (Berger et al., 2018; Derakhshan et al., 2021a; Huang et al., 2022; Nakiwala et al., 2022; Romano et al., 2018; Sarzo et al., 2022; Souter et al., 2020; Yang et al., 2022; Yland et al., 2022). Key differences between our study and prior studies include urine measurement timing, single spot urine versus pooled urine sampling, and the mixture composition. The only other study that modeled biomarkers of phthalates, parabens, and phenols (using an *a priori*-driven method for chemical inclusion described above) did not identify any associations with TSH (Nakiwala et al., 2022). While low TSH levels can occur in normal pregnancy (Laurberg et al., 2016), hyperthyroidism, characterized by high thyroid hormones and low TSH, are linked to increased risk of pre-eclampsia, miscarriage, and low birthweight (Marx et al., 2008). TSH is secreted by the pituitary gland, acts on the thyroid gland, and is regulated by TT4 and TT3. Because of complex hormonal feedback loops, EDCs could act at the pituitary or thyroid gland. The exact mechanism of action is hard to elucidate; however, animal studies have reported that bisphenols can act as thyroid hormone receptor antagonists (Kim and Park, 2019). As thyroid hormones play significant roles in pregnancy, fetal growth, and neurodevelopment (Marx et al., 2008), future studies should investigate the potential effects of other EDCs on TSH.

Unlike prior studies, we also identified a negative association of the mixture with TSH in

women carrying males, which could be due to various factors. The placenta, which is responsible for thyroid hormone regulation and transport during pregnancy, is a sexed organ (XX and XY), as it develops from the zygote. Numerous studies have demonstrated that there are differences between male and female placentas in terms of gene expression, function, and morphology (Gabory et al., 2013; Graves, 2010; Meakin et al., 2021; Rich-Edwards et al., 2001). Additionally, other studies have shown that male placentas are more responsive to stressors than female placentas (Bale, 2016; Bronson and Bale, 2016; Eriksson et al., 2010). Potentially due to placental differences, the concentrations of maternal TSH have been shown to differ between women carrying male or female fetuses, which could indicate differences in thyroid homeostasis (Sitoris et al., 2022; Wang et al., 2019). Additionally, maternal sex-steroid hormones, which we have shown to also to be sexually dimorphic, play a role in regulating thyroid hormones that could explain some of the differences in TSH by fetal sex. There are likely other mechanisms that could be further investigated using experimental models.

Strengths and limitations

This current study has some limitations and many strengths. First, I-KIDS quantified EDC biomarkers in a pool of up to five first-morning urine samples collected throughout pregnancy, and some exposures occurred after our outcomes of interest. The pooled sample reduces exposure assessment error, provides a more stable estimate of gestational exposure, and can be considered a reflection of exposure during pregnancy (Shin et al., 2019a; Vernet et al., 2019). Additionally, the urinary concentrations for some biomarkers were much lower compared to other cohorts; however, this study investigated a large panel of non-persistent EDCs from multiple chemical classes and I-KIDS women have comparable EDC biomarker concentrations to reproductive-aged women in the nationally-representative National Health and Nutrition Examination Survey (Pacyga et al., 2022a). Second, because we were limited to a single early second-trimester measurement of select hormones, our findings might not be generalizable to other timepoints and hormones (such as pregnancy triiodothyronine; TT3). However, we assessed six early-to-mid pregnancy plasma hormones that reflect sex-steroid and thyroid hormones known to be critical for pregnancy health and fetal development. Third, we cannot rule out unmeasured confounding, such as by poor sleep quality during pregnancy which may impact hormone levels and indirectly impact chemical exposure through alterations of behaviors and habits; however, I-KIDS collected pertinent

sociodemographic, lifestyle, and health information that allowed us to account for many other important covariates, and we utilized *a priori* consideration and previous literature to inform decisions about covariate selection. Fourth, we may have been underpowered for analyses stratified by fetal sex, but we had adequate sample sizes overall. Fifth, the I-KIDS cohort is a relatively homogenous sample of non-Hispanic White, well-educated, married women, which limits generalizability. However, as we are investigating biological hypotheses, a homogenous sample may reduce unmeasured confounding. Lastly, BKMR results can be difficult to interpret within the context of human health (Hoskovec et al., 2021) and WQSR assumes homogeneity in direction of association (Carrico et al., 2015; Czarnota et al., 2015); however, these two robust and reliable methods allowed us to estimate cumulative effects, identify meaningful drivers of associations, and assess non-linearities and chemical-chemical interactions.

3.7. CONCLUSION

To our knowledge, this is the first study to investigate the relationship between a broad mixture of EDC urinary biomarkers and plasma pregnancy sex-steroid hormones. Additionally, we have contributed to a growing body of literature investigating EDC biomarker mixtures and pregnancy thyroid hormones. Our results suggest a mixture of non-persistent EDC biomarkers is associated with testosterone, estradiol, and TSH in this population of women. We also identified important sex-specific results, non-linear relationships, and chemical-chemical interactions. Future studies should explore similar relationships in more diverse cohorts, including those with women in high-risk pregnancies, such as pregnancies complicated by gestational diabetes, hypertension, or pre-eclampsia, to increase the understanding of associations of EDCs with pregnancy and birth outcomes. Additionally, more expansive mixtures will need to be considered that include other classes of EDCs, such as pesticides, herbicides, and per- and polyfluoroalkyl substances. Furthermore, mixture approaches could consider *a priori* classifying chemical biomarkers using unsupervised statistical methods, such as principal component analysis, or grouping individual biomarkers by class or proposed mechanism of action to better understand the relationship of EDCs as classes with hormonal outcomes.

Tables

Table 4. Characteristics of I-KIDS women in analytic sample

	Full sample[a] (n = 531)	Analytic sample[b] (n = 302)	Women carrying females (n = 155)	Women carrying males (n = 147)
Characteristic	**n (%)**			
Race/ethnicity				
Non-Hispanic White (ref)	424 (80.0)	246 (81.5)	124 (80.0)	122 (83.0)
Others	106 (20.0)	56 (18.5)	31 (20.0)	25 (17.0)
Education				
Some college or less (ref)	103 (19.4)	51 (16.9)	21 (13.5)	30 (20.4)
College graduate or higher	428 (80.6)	251 (83.1)	134 (86.5)	117 (79.6)
Ever smoker				
No (ref)	435 (82.2)	250 (82.8)	123 (79.4)	127 (86.4)
Yes	94 (17.8)	52 (17.2)	32 (20.6)	20 (13.6)
Parity				
No children (ref)	272 (51.3)	163 (54.0)	83 (53.5)	80 (54.4)
1 + children	258 (48.7)	139 (46.0)	72 (46.5)	67 (45.6)
Fetal sex				
Male (ref)	271 (51.1)	147 (48.7)	–	–
Female	259 (48.9)	155 (51.3)	–	–
	Median (25th, 75th percentile)			
Maternal age (years)	30.0 (27.1, 32.7)	30.4 (27.5, 32.8)	30.5 (27.9, 32.7)	30.3 (27.8, 32.9)
Pre-pregnancy BMI (kg/m^2)	24.6 (21.9, 29.4)	24.7 (21.9, 28.9)	24.4 (21.6, 28.2)	25.0 (22.5, 30.3)
Early pregnancy Alternative Healthy Eating Index 2010*‡	51.5 (43.9, 59.7)	51.7 (44.6, 59.3)	53.2 (45.8, 61.8)	50.1 (43.4, 57.8)
Early pregnancy perceived stress	10.9 (6.9, 16.1)	11.1 (7.1, 16.5)	11.5 (7.2, 16.9)	10.5 (6.9, 16.1)
Gestational age at blood collection	16.9 (16.3, 17.7)	16.9 (16.3, 17.6)	16.8 (16.3, 17.7)	17.0 (16.3, 17.6)

Percentages may not add up to 100% due to missing (n missing): perceived stress score (8 missing in reference population; 2 missing in analytic sample), gestational age (1 missing in reference population). *Alcohol intake was removed from the index (total score out of 100). ‡Median (25th, 75th percentile) Alternative Healthy Eating Index 2010 excludes women whose diet data have not yet been analyzed (n = 49). [a]Women with at least one chemical biomarker. [b]Women with all chemical biomarkers and at least one hormone measurement. BMI, body mass index; I-KIDS, Illinois Kids Development Study.

Table 5. Distribution of pooled urinary EDC biomarkers (n=302) (2015-2018).

Biomarker	LOD (ng/mL)	% ≥ LOD	Median (25^{th}, 75^{th} percentile)
Phthalate/replacement			
Mono(2-ethylhexyl) phthalate (MEHP), ng/mL	0.8	76.2	1.31 (0.85, 2.13)
Mono(2-ethyl5-carboxypentyl) phthalate (MECPP), ng/mL	0.4	100.0	8.32 (6.07, 12.70)
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), ng/mL	0.4	100.0	5.36 (3.61, 8.17)
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), ng/mL	0.2	100.0	3.99 (2.86, 6.32)
Mono(3-carboxypropyl) phthalate (MCPP), ng/mL	0.4	96.4	1.28 (0.87, 1.91)
Monobenzyl phthalate (MBzP), ng/mL	0.3	99.3	5.17 (2.55, 10.31)
Monoethyl phthalate (MEP), ng/mL	1.2	100.0	26.72 (13.68, 47.38)
Monocarboxynonyl phthalate (MCNP), ng/mL	0.2	100.0	1.83 (1.35, 2.62)
Mono-n-butyl phthalate (MBP), ng/mL	0.4	100.0	12.67 (8.50, 17.17)
Mono-hydroxybutyl phthalate (MHBP), ng/mL	0.4	90.1	1.20 (0.75, 1.82)
Mono-isobutyl phthalate (MiBP), ng/mL	0.8	99.7	8.66 (5.75, 13.99)
Mono-hydroxy-isobutyl phthalate (MHiBP), ng/mL	0.4	99.7	3.11 (2.13, 5.43)
Monocarboxyoctyl phthalate (MCOP), ng/mL	0.3	100.0	7.11 (4.59, 13.65)
Mono-isononyl phthalate (MiNP), ng/mL	0.9	31.5	0.61 (<LOD, 1.08)
Monooxononyl phthalate (MONP), ng/mL	0.4	100.0	2.63 (1.72, 4.63)
Cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester (MHiNCH), ng/mL	0.4	91.1	1.13 (0.69, 2.21)
Cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester (MCOCH), ng/mL	0.5	66.9	0.70 (<LOD, 1.20)
Mono(2-ethyl-5-hydroxyhexyl terephthalate (MEHHTP), ng/mL	0.4	100.0	8.21 (3.83, 20.26)
Mono(2-ethyl5-carboxypentyl terephthalate (MECPTP), ng/mL	0.2	100.0	59.56 (25.32, 138.29)
Paraben			
Butyl paraben, ng/mL	0.1	33.4	<LOD (< LOD, 0.15)
Ethyl paraben, ng/mL	1.0	57.0	1.37 (<LOD, 6.64)
Methyl paraben, ng/mL	1.0	100.0	49.01 (19.30, 138.03)
Propyl paraben, ng/mL	0.1	99.7	8.75 (2.31, 28.34)
Phenol			
Bisphenol A (BPA), ng/mL	0.2	96.7	0.85 (0.52, 1.46)
Bisphenol S (BPS), ng/mL	0.1	99.0	0.48 (0.29, 0.80)
Bisphenol F (BPF), ng/mL	0.2	57.0	0.29 (<LOD, 1.05)
Benzophenone 3 (BP-3), ng/mL	0.4	99.7	117.44 (37.47, 293.71)
Triclosan (TCS), ng/mL	1.7	92.1	8.67 (3.51, 56.28)
2,4-Dichlorophenol (2,4-DCP), ng/mL	0.1	100.0	0.58 (0.36, 0.99)
2,5-Dichlorophenol (2,5-DCP), ng/mL	0.1	99.7	1.40 (0.84, 3.28)
Other			
Triclocarban, ng/mL	0.1	29.8	< LOD (< LOD, 0.14)

Specific gravity-adjusted EDC biomarker concentrations. EDCs, endocrine disrupting chemicals; LOD, limit of detection.

Table 6. Distribution of early-to-mid pregnancy (median 17 weeks gestation) plasma hormones (n=302) (2015-2018).

	Reportable range	% ≥ lower limit of reportable range	Median (25^{th}, 75^{th} percentile)
Hormone			
Progesterone, ng/mL	0.20 – 40.00	100.0	28.30 (23.60, 32.90)
Estradiol, pg/mL	20.00 – 2000.00	100.0	2745.00 (1983.30, 3830.00)*
Testosterone, ng/dL	20.00 – 1600.00	87.29	46.50 (34.90, 66.70)
Free thyroxine (FT4), ng/dL	0.30 - 0.60	100.0	0.93 (0.86, 0.99)
Total thyroxine (TT4), µg/dL	1.00 – 24.00	100.0	8.94 (7.97, 9.89)
Thyroid stimulating hormone (TSH), µIU/mL	Up to 75.00	99.7	1.77 (1.16, 2.46)
*Values are outside of manufacturer's reportable range because estradiol was diluted 10x prior to analysis and machine-read values were multiplied by 10.			

Table 7. Associations between urinary EDC biomarkers and mid-pregnancy progesterone in I-KIDS women.

Biomarkers	All women (n=295)	Carrying females (n=151)	Carrying males (n=144)	P_{int}
	%Δ (95% CI)			
Phthalates & Replacements[1]				
ΣDEHP	-0.03 (-0.44, 0.39)	-0.15 (-0.74, 0.45)	0.09 (-0.48, 0.66)	0.57
MCPP	0.02 (-0.36, 0.39)	0.09 (-0.40, 0.59)	-0.09 (-0.67, 0.49)	0.63
MCNP	0.05 (-0.42, 0.52)	-0.21 (-0.83, 0.41)	0.40 (-0.31, 1.11)	0.20
MBzP	-0.04 (-0.31, 0.23)	-0.17 (-0.54, 0.20)	0.10 (-0.29, 0.48)	0.32
MEP	0.02 (-0.29, 0.33)	-0.24 (-0.67, 0.18)	0.28 (-0.15, 0.71)	**0.08**
ΣDiNP	0.08 (-0.24, 0.40)	0.15 (-0.25, 0.56)	-0.04 (-0.57, 0.49)	0.57
ΣDBP	0.03 (-0.41, 0.47)	-0.21 (-0.77, 0.34)	0.38 (-0.28, 1.05)	0.16
ΣDiBP	-0.01 (-0.36, 0.34)	-0.14 (-0.58, 0.29)	0.23 (-0.35, 0.83)	0.31
ΣDiNCH	0.00 (-0.29, 0.30)	-0.19 (-0.59, 0.20)	0.24 (-0.19, 0.68)	**0.14**
ΣDEHTP	-0.16 (-0.41, 0.09)	**-0.38 (-0.73, -0.02)***	0.04 (-0.30, 0.38)	**0.09**
Parabens				
Ethylparaben	-0.02 (-0.16, 0.12)	-0.08 (-0.28, 0.12)	0.03 (-0.15, 0.21)	0.44
Methylparaben	-0.13 (-0.35, 0.08)	**-0.26 (-0.56, 0.04)**	0.00 (-0.31, 0.31)	0.23
Propylparaben	**-0.13 (-0.29, 0.02)**	-0.17 (-0.40, 0.05)	-0.10 (-0.32, 0.12)	0.64
Phenols				
BPA	-0.11 (-0.38, 0.15)	-0.13 (-0.43, 0.16)	-0.01 (-0.62, 0.61)	0.72
BPS	-0.02 (-0.29, 0.25)	0.11 (-0.3, 0.52)	-0.12 (-0.49, 0.25)	0.41
BP-3	-0.01 (-0.18, 0.16)	-0.11 (-0.32, 0.10)	0.17 (-0.11, 0.44)	**0.12**
TCS	0.04 (-0.12, 0.20)	0.07 (-0.15, 0.29)	0.01 (-0.22, 0.23)	0.70
2,4-DCP	0.17 (-0.17, 0.50)	0.11 (-0.37, 0.58)	0.22 (-0.24, 0.69)	0.73
2,5-DCP	0.08 (-0.14, 0.30)	0.02 (-0.32, 0.37)	0.11 (-0.16, 0.39)	0.68

Data are presented as the percent change (%Δ) and 95% CI in plasma hormone concentrations with every 10% increase in chemical biomarker. All models account for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, and fetal sex. Linear regression models evaluated associations of individual chemical biomarkers with plasma hormones. Bold signifies potentially meaningful findings with asterisk (*) denoting statistically significant findings at $P < 0.05$.
1ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322) + (MONP/306); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308).
Abbreviations: CI, confidence interval; EDCs, endocrine disrupting chemicals; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol. Women missing covariates in analyses (n=5). Diet (n=3), stress (n=2).

Table 8. Associations between urinary EDC biomarkers and mid-pregnancy estradiol in I-KIDS women.

Biomarkers	All women (n=295)	Carrying females (n=151)	Carrying males (n=144)	P_{int}
	%Δ (95% CI)			
Phthalates & Replacements[1]				
ΣDEHP	-0.11 (-0.83, 0.61)	-0.35 (-1.38, 0.70)	0.10 (-0.89, 1.10)	0.54
MCPP	-0.42 (-1.08, 0.24)	-0.49 (-1.36, 0.39)	-0.33 (-1.34, 0.69)	0.82
MCNP	-0.52 (-1.33, 0.29)	-0.20 (-1.28, 0.88)	-0.94 (-2.15, 0.30)	0.38
MBzP	-0.17 (-0.64, 0.30)	-0.38 (-1.03, 0.27)	0.05 (-0.62, 0.72)	0.36
MEP	-0.35 (-0.88, 0.19)	-0.33 (-1.07, 0.42)	-0.36 (-1.10, 0.39)	0.96
ΣDiNP	-0.17 (-0.74, 0.39)	-0.36 (-1.07, 0.35)	0.14 (-0.78, 1.07)	0.40
ΣDBP	-0.63 (-1.38, 0.13)	-0.74 (-1.70, 0.23)	-0.47 (-1.62, 0.68)	0.72
ΣDiBP	-0.49 (-1.08, 0.11)	-0.38 (-1.13, 0.38)	-0.67 (-1.63, 0.30)	0.64
ΣDiNCH	-0.07 (-0.58, 0.44)	-0.26 (-0.95, 0.44)	0.16 (-0.59, 0.91)	0.42
ΣDEHTP	-0.17 (-0.61, 0.27)	-0.06 (-0.69, 0.57)	-0.27 (-0.87, 0.33)	0.63
Parabens				
Ethylparaben	-0.16 (-0.40, 0.09)	-0.06 (-0.41, 0.30)	-0.24 (-0.56, 0.08)	0.44
Methylparaben	-0.03 (-0.41, 0.35)	0.12 (-0.40, 0.64)	-0.19 (-0.74, 0.35)	0.41
Propylparaben	0.05 (-0.23, 0.33)	0.20 (-0.19, 0.60)	-0.11 (-0.49, 0.28)	0.27
Phenols				
BPA	-0.12 (-0.58, 0.35)	0.11 (-0.40, 0.63)	**-1.11 (-2.16, -0.05)***	**0.04**
BPS	0.07 (-0.40, 0.55)	**0.73 (0.02, 1.44)***	-0.46 (-1.09, 0.18)	**0.01**
BP-3	-0.10 (-0.39, 0.19)	-0.08 (-0.45, 0.28)	-0.14 (-0.62, 0.35)	0.86
TCS	0.03 (-0.25, 0.31)	-0.03 (-0.41, 0.36)	0.09 (-0.31, 0.49)	0.67
2,4-DCP	-0.18 (-0.76, 0.41)	-0.09 (-0.92, 0.75)	-0.26 (-1.07, 0.55)	0.77
2,5-DCP	-0.22 (-0.61, 0.16)	-0.06 (-0.66, 0.54)	-0.32 (-0.80, 0.16)	0.5

Data are presented as the percent change (%Δ) and 95% CI in plasma hormone concentrations with every 10% increase in chemical biomarker. All models account for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, and fetal sex. Linear regression models evaluated associations of individual chemical biomarkers with plasma hormones. Bold signifies potentially meaningful findings with asterisk (*) denoting statistically significant findings at P < 0.05. 1ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322) + (MONP/306); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308). Abbreviations: CI, confidence interval; EDCs, endocrine disrupting chemicals; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol. Women missing covariates in analyses (n=5). Diet (n=3), stress (n=2).

Table 9. Associations between urinary EDC biomarkers and mid-pregnancy testosterone in I-KIDS women.

Biomarkers	All women (n=295)	Carrying females (n=151)	Carrying males (n=144)	P_{int}
	%Δ (95% CI)			
Phthalates & Replacements[1]				
ΣDEHP	-0.65 (-1.49, 0.20)	-0.49 (-1.72, 0.76)	-0.79 (-1.93, 0.36)	0.72
MCPP	-0.64 (-1.42, 0.14)	-0.58 (-1.65, 0.51)	-0.71 (-1.84, 0.43)	0.86
MCNP	-0.11 (-1.12, 0.91)	0.22 (-1.06, 1.52)	-0.65 (-2.26, 1.00)	0.41
MBzP	0.01 (-0.54, 0.57)	-0.06 (-0.83, 0.73)	0.08 (-0.70, 0.87)	0.81
MEP	0.52 (-0.13, 1.18)	0.71 (-0.16, 1.58)	0.29 (-0.67, 1.25)	0.51
ΣDiNP	-0.23 (-0.91, 0.46)	-0.09 (-0.98, 0.80)	-0.41 (-1.46, 0.64)	0.64
ΣDBP	0.11 (-0.78, 1.02)	0.19 (-0.95, 1.35)	-0.01 (-1.39, 1.40)	0.83
ΣDiBP	0.37 (-0.33, 1.08)	0.32 (-0.55, 1.20)	0.46 (-0.73, 1.67)	0.85
ΣDiNCH	0.15 (-0.47, 0.77)	0.06 (-0.81, 0.93)	0.24 (-0.65, 1.14)	0.77
ΣDEHTP	-0.14 (-0.67, 0.39)	0.20 (-0.56, 0.97)	-0.45 (-1.16, 0.27)	0.22
Parabens				
Ethylparaben	-0.09 (-0.37, 0.20)	-0.19 (-0.59, 0.22)	0.00 (-0.38, 0.38)	0.49
Methylparaben	-0.30 (-0.75, 0.16)	-0.23 (-0.85, 0.39)	-0.38 (-1.03, 0.28)	0.75
Propylparaben	**-0.45 (-0.78, -0.11)***	**-0.49 (-0.96, -0.02)***	**-0.40 (-0.87, 0.07)**	0.79
Phenols				
BPA	0.24 (-0.29, 0.77)	0.48 (-0.10, 1.06)	-0.98 (-2.26, 0.33)	**0.05**
BPS	-0.35 (-0.94, 0.25)	-0.49 (-1.39, 0.41)	-0.23 (-1.01, 0.55)	0.67
BP-3	0.13 (-0.21, 0.47)	0.15 (-0.27, 0.58)	0.08 (-0.49, 0.65)	0.84
TCS	**-0.36 (-0.69, -0.02)***	-0.34 (-0.80, 0.11)	-0.37 (-0.86, 0.12)	0.93
2,4-DCP	**-0.73 (-1.40, -0.05)***	-0.32 (-1.28, 0.64)	**-1.11 (-2.03, -0.17)***	0.25
2,5-DCP	**-0.52 (-0.97, -0.07)***	-0.36 (-1.06, 0.35)	**-0.62 (-1.18, -0.06)***	0.55

Data are presented as the percent change (%Δ) and 95% CI in plasma hormone concentrations with every 10% increase in chemical biomarker. All models account for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, and fetal sex. Linear regression models evaluated associations of individual chemical biomarkers with plasma hormones. Bold signifies potentially meaningful findings with asterisk (*) denoting statistically significant findings at $P < 0.05$.
1ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322) + (MONP/306); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308).
Abbreviations: CI, confidence interval; EDCs, endocrine disrupting chemicals; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol.

Table 10. Associations between urinary EDC biomarkers and free T4 in I-KIDS women.

Biomarkers	All women (n=295)	Carrying females (n=151)	Carrying males (n=144)	P_{int}
	%Δ (95% CI)			
Phthalates & Replacements[1]				
ΣDEHP	0.02 (-0.16, 0.19)	-0.02 (-0.28, 0.24)	0.05 (-0.20, 0.29)	0.71
MCPP	0.05 (-0.12, 0.21)	0.15 (-0.06, 0.37)	-0.10 (-0.35, 0.15)	**0.13**
MCNP	0.02 (-0.18, 0.23)	0.09 (-0.18, 0.35)	-0.06 (-0.36, 0.25)	0.48
MBzP	0.02 (-0.10, 0.14)	-0.01 (-0.17, 0.16)	0.05 (-0.12, 0.21)	0.66
MEP	0.04 (-0.10, 0.17)	-0.02 (-0.20, 0.16)	0.10 (-0.09, 0.28)	0.37
ΣDiNP	-0.05 (-0.19, 0.09)	0.09 (-0.08, 0.26)	**-0.28 (-0.50, -0.06)***	**0.01**
ΣDBP	0.01 (-0.17, 0.20)	-0.01 (-0.25, 0.23)	0.04 (-0.24, 0.33)	0.80
ΣDiBP	0.03 (-0.12, 0.18)	0.06 (-0.13, 0.25)	-0.01 (-0.26, 0.24)	0.66
ΣDiNCH	0.06 (-0.07, 0.18)	0.08 (-0.09, 0.25)	0.03 (-0.15, 0.22)	0.73
ΣDEHTP	0.05 (-0.06, 0.16)	0.04 (-0.12, 0.19)	0.05 (-0.09, 0.20)	0.89
Parabens				
Ethylparaben	-0.01 (-0.07, 0.05)	-0.01 (-0.10, 0.08)	-0.01 (-0.09, 0.07)	0.97
Methylparaben	0.04 (-0.05, 0.13)	0.05 (-0.08, 0.18)	0.03 (-0.11, 0.16)	0.78
Propylparaben	0.02 (-0.05, 0.09)	0.05 (-0.05, 0.15)	-0.01 (-0.11, 0.08)	0.36
Phenols				
BPA	0.01 (-0.10, 0.13)	0.06 (-0.07, 0.19)	-0.19 (-0.45, 0.08)	**0.10**
BPS	-0.03 (-0.15, 0.08)	0.02 (-0.15, 0.20)	-0.08 (-0.24, 0.08)	0.39
BP-3	-0.03 (-0.10, 0.04)	-0.02 (-0.11, 0.07)	-0.05 (-0.17, 0.07)	0.71
TCS	-0.03 (-0.10, 0.03)	0.00 (-0.09, 0.10)	-0.07 (-0.17, 0.02)	0.26
2,4-DCP	0.10 (-0.04, 0.24)	**0.19 (-0.01, 0.40)**	0.01 (-0.18, 0.21)	0.22
2,5-DCP	0.05 (-0.05, 0.14)	-0.02 (-0.17, 0.13)	0.09 (-0.02, 0.21)	0.22

Data are presented as the percent change (%Δ) and 95% CI in plasma hormone concentrations with every 10% increase in chemical biomarker. All models account for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, and fetal sex. Linear regression models evaluated associations of individual chemical biomarkers with plasma hormones. Bold signifies potentially meaningful findings with asterisk (*) denoting statistically significant findings at $P < 0.05$. [1]ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322) + (MONP/306); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308). Abbreviations: CI, confidence interval; EDCs, endocrine disrupting chemicals; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; T4, thyroxine. Women missing covariates in thyroid hormones analyses (n=5). Diet (n=3), stress (n=2).

Table 11. Associations between urinary EDC biomarkers and total T4 in I-KIDS women.

Biomarkers	All women (n=295)	Carrying females (n=151)	Carrying males (n=144)	P_{int}
	%Δ (95% CI)			
Phthalates & Replacements[1]				
ΣDEHP	0.03 (-0.23, 0.30)	0.28 (-0.11, 0.66)	-0.19 (-0.55, 0.18)	**0.09**
MCPP	0.06 (-0.18, 0.31)	0.18 (-0.14, 0.51)	-0.09 (-0.46, 0.29)	0.28
MCNP	0.20 (-0.10, 0.51)	0.20 (-0.20, 0.60)	0.21 (-0.25, 0.67)	0.98
MBzP	**0.23 (0.05, 0.40)***	**0.36 (0.12, 0.60)***	0.09 (-0.16, 0.33)	**0.12**
MEP	-0.04 (-0.24, 0.16)	-0.07 (-0.35, 0.21)	0.00 (-0.28, 0.28)	0.74
ΣDiNP	0.01 (-0.20, 0.22)	0.10 (-0.16, 0.37)	-0.14 (-0.48, 0.20)	0.26
ΣDBP	0.00 (-0.28, 0.28)	0.12 (-0.24, 0.48)	-0.17 (-0.60, 0.26)	0.29
ΣDiBP	-0.08 (-0.31, 0.15)	0.07 (-0.21, 0.35)	**-0.36 (-0.73, 0.02)**	**0.08**
ΣDiNCH	-0.05 (-0.24, 0.14)	0.07 (-0.19, 0.32)	-0.18 (-0.46, 0.10)	0.21
ΣDEHTP	0.02 (-0.14, 0.18)	0.07 (-0.16, 0.31)	-0.03 (-0.26, 0.19)	0.51
Parabens				
Ethylparaben	-0.02 (-0.11, 0.07)	-0.05 (-0.18, 0.08)	0.00 (-0.12, 0.12)	0.53
Methylparaben	-0.02 (-0.16, 0.13)	0.11 (-0.09, 0.30)	-0.15 (-0.35, 0.05)	**0.07**
Propylparaben	0.02 (-0.09, 0.12)	0.11 (-0.03, 0.26)	-0.07 (-0.22, 0.07)	**0.07**
Phenols				
BPA	0.07 (-0.10, 0.24)	0.11 (-0.09, 0.30)	-0.07 (-0.47, 0.32)	0.43
BPS	-0.05 (-0.23, 0.12)	0.08 (-0.18, 0.34)	-0.16 (-0.39, 0.08)	**0.19**
BP-3	0.02 (-0.09, 0.12)	-0.02 (-0.16, 0.11)	0.08 (-0.10, 0.26)	0.36
TCS	-0.04 (-0.15, 0.06)	-0.02 (-0.17, 0.12)	-0.07 (-0.21, 0.08)	0.68
2,4-DCP	0.15 (-0.07, 0.36)	0.11 (-0.20, 0.42)	0.18 (-0.12, 0.48)	0.74
2,5-DCP	**0.17 (0.03, 0.32)***	**0.27 (0.04, 0.49)***	0.12 (-0.06, 0.30)	0.30

Data are presented as the percent change (%Δ) and 95% CI in plasma hormone concentrations with every 10% increase in chemical biomarker. All models account for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, and fetal sex. Linear regression models evaluated associations of individual chemical biomarkers with plasma hormones. Bold signifies potentially meaningful findings with asterisk (*) denoting statistically significant findings at $P < 0.05$. [1]ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322) + (MONP/306); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308). Abbreviations: CI, confidence interval; EDCs, endocrine disrupting chemicals; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; T4, thyroxine. Women missing covariates in thyroid hormones analyses (n=5). Diet (n=3), stress (n=2).

Table 12. Associations between urinary EDC biomarkers and TSH in I-KIDS women.

Biomarkers	All women (n=295)	Carrying females (n=151)	Carrying males (n=144)	P_{int}
	%Δ (95% CI)			
Phthalates & Replacements[1]				
ΣDEHP	0.01 (-0.01, 0.02)	0.01 (-0.02, 0.04)	0.00 (-0.02, 0.03)	0.62
MCPP	0.00 (-0.02, 0.01)	0.00 (-0.02, 0.02)	-0.01 (-0.03, 0.02)	0.74
MCNP	0.00 (-0.02, 0.02)	0.00 (-0.03, 0.03)	0.00 (-0.03, 0.03)	0.85
MBzP	**0.01 (0.00, 0.02)***	0.00 (-0.01, 0.02)	**0.02 (0.00, 0.04)***	**0.11**
MEP	0.00 (-0.02, 0.01)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.01)	0.66
ΣDiNP	0.00 (-0.02, 0.01)	0.00 (-0.01, 0.02)	-0.01 (-0.04, 0.01)	0.22
ΣDBP	0.01 (-0.01, 0.03)	-0.01 (-0.03, 0.02)	**0.04 (0.01, 0.07)***	**0.01**
ΣDiBP	-0.01 (-0.02, 0.01)	**-0.02 (-0.04, 0.00)***	0.01 (-0.02, 0.04)	**0.08**
ΣDiNCH	0.00 (-0.02, 0.01)	-0.01 (-0.02, 0.01)	0.00 (-0.02, 0.02)	0.68
ΣDEHTP	0.00 (-0.01, 0.01)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.01)	0.79
Parabens				
Ethylparaben	**-0.01 (-0.01, 0.00)***	0.00 (-0.01, 0.01)	**-0.01 (-0.02, 0.00)***	**0.20**
Methylparaben	**-0.01 (-0.02, 0.00)***	-0.01 (-0.02, 0.00)	-0.01 (-0.02, 0.00)	0.96
Propylparaben	**-0.01 (-0.01, 0.00)***	-0.01 (-0.02, 0.00)	-0.01 (-0.02, 0.00)	0.82
Phenols				
BPA	-0.01 (-0.02, 0.01)	0.00 (-0.02, 0.01)	-0.02 (-0.05, 0.01)	0.31
BPS	-0.01 (-0.02, 0.01)	-0.01 (-0.03, 0.00)	0.00 (-0.01, 0.02)	**0.17**
BP-3	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.00)	0.01 (0.00, 0.02)	**0.09**
TCS	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)	0.86
2,4-DCP	0.00 (-0.01, 0.02)	-0.01 (-0.03, 0.02)	0.01 (-0.02, 0.03)	0.49
2,5-DCP	0.00 (-0.01, 0.01)	0.00 (-0.02, 0.01)	-0.01 (-0.02, 0.01)	0.54

Data are presented as μIU/mL change and 95% CI in TSH with every 10% increase in chemical biomarker. All models account for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, and fetal sex. Linear regression models evaluated associations of individual chemical biomarkers with plasma hormones. Bold signifies potentially meaningful findings with asterisk (*) denoting statistically significant findings at $P < 0.05$. [1]ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣDiNP = (MiNP/292) + (MCOP/322) + (MONP/306); ΣDBP = (MBP/222) + (MHBP/238); ΣDiBP = (MiBP/222) + (MHiBP/238); ΣDiNCH = (MHiNCH/ 314) + (MCOCH/328); and ΣDEHTP = (MEHHTP/294) + (MECPTP/308). Abbreviations: CI, confidence interval; EDCs, endocrine disrupting chemicals; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; ΣDEHTP, sum of di-2-ethylhexyl terephthalate metabolites; ΣDiNP, sum of di-isononyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; BPA, bisphenol A; BPS, bisphenol S; BPF, bisphenol F; TCS, triclosan; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; TSH, thyroid stimulating hormone. Women missing covariates in thyroid hormones analyses (n=5). Diet (n=3), stress (n=2).

Figures

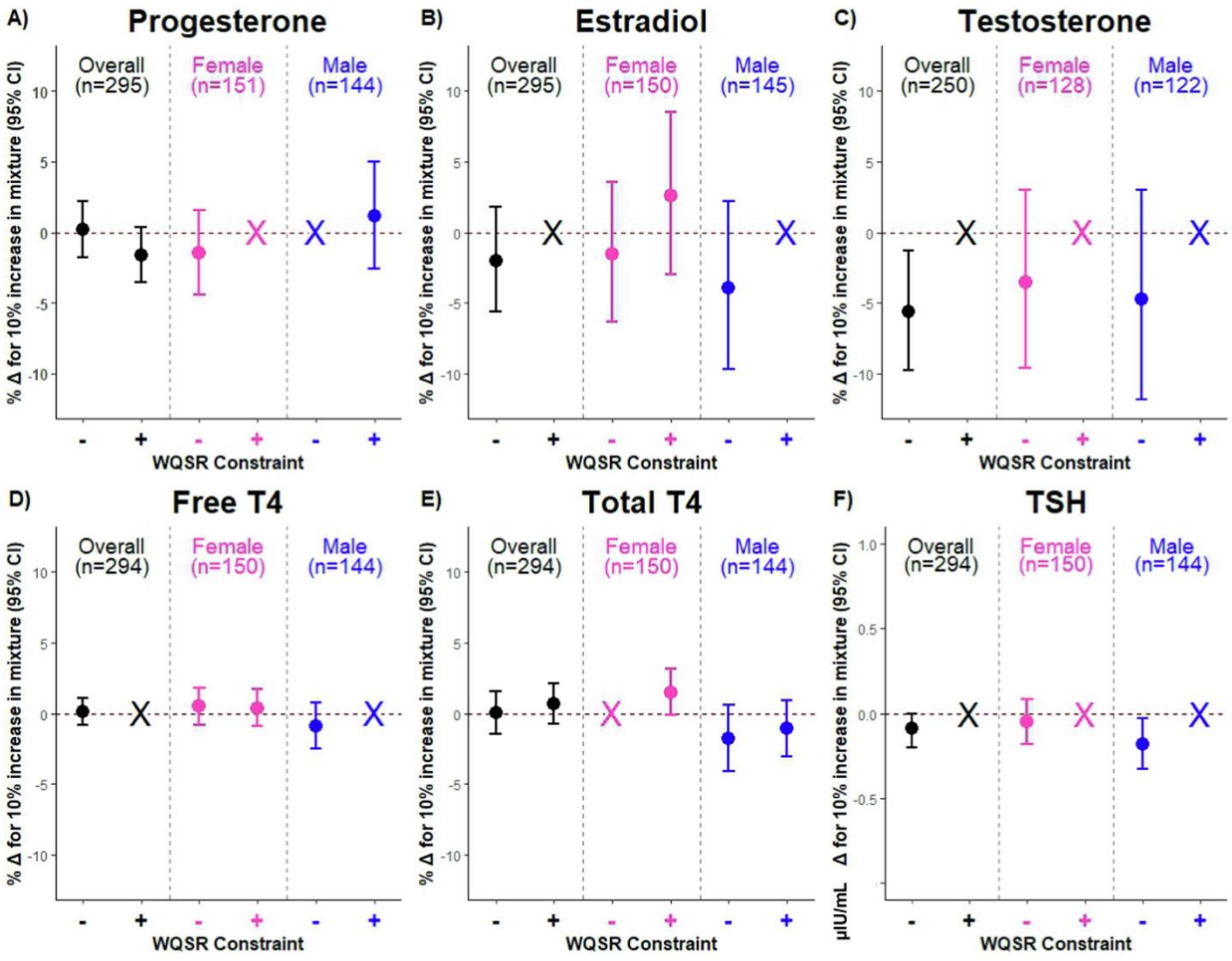

Figure 3. Cumulative associations of WQSR mixture with A) progesterone, B) estradiol, C) testosterone, D) free T4, E) total T4, F) TSH. Negatively (-) and positively (+) constrained WQSR models were fit with 100 bootstraps and 100 repeated holdouts accounting for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment, women carrying females, and women carrying males. Data are presented as the percent change (%Δ) and 95 % CI in plasma hormone concentrations with every 10 % increase in the WQSR mixture. For TSH, data are presented as μIU/mL change and 95 % CI in TSH for every 10 % increase in the WQSR mixture. Estimate and interval color signifies analytic sample (all women: black; women carrying females: pink; women carrying males: blue), and X indicates non-positive or non-negative β-estimates. For TSH, y-axis is on a different scale for data visualization. Abbreviations: CI, confidence interval; T4, thyroxine; TSH, thyroid stimulating hormone; WQSR, Weighted Quantile Sum Regression.

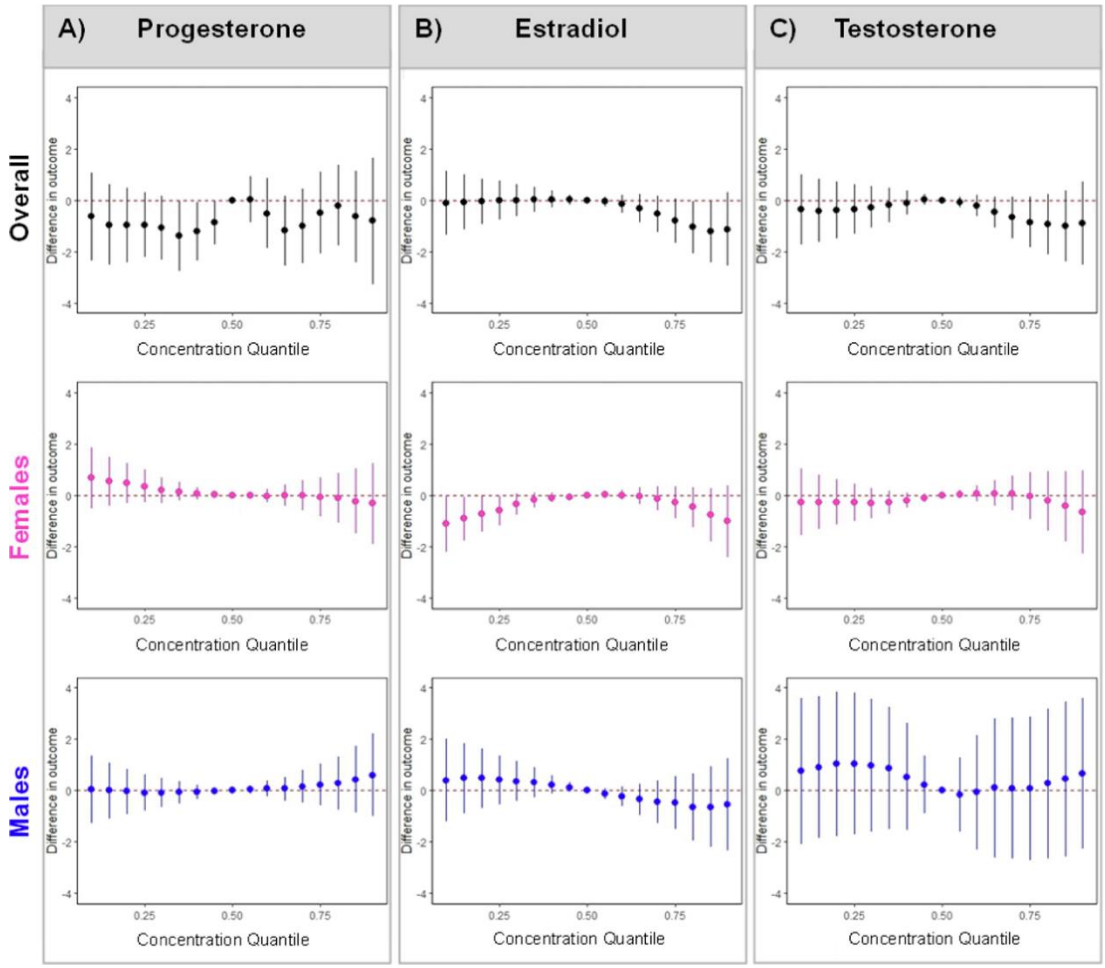

Figure 4. Associations of BKMR mixture with A) progesterone, B) estradiol, C) testosterone in all women, women carrying females, and women carrying males. BKMR models were fit with 200,000 iterations and 50 knots accounting for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment. Data are presented as effect estimates and 95% credible intervals which are interpreted as the association between the mixture at each quantile and gestational hormone compared to when all co-exposures are fixed at the median. Estimate and interval color signifies analytic sample (all women: black; women carrying females: pink; women carrying males: blue). Abbreviations: BKMR, Bayesian Kernel Machine Regression.

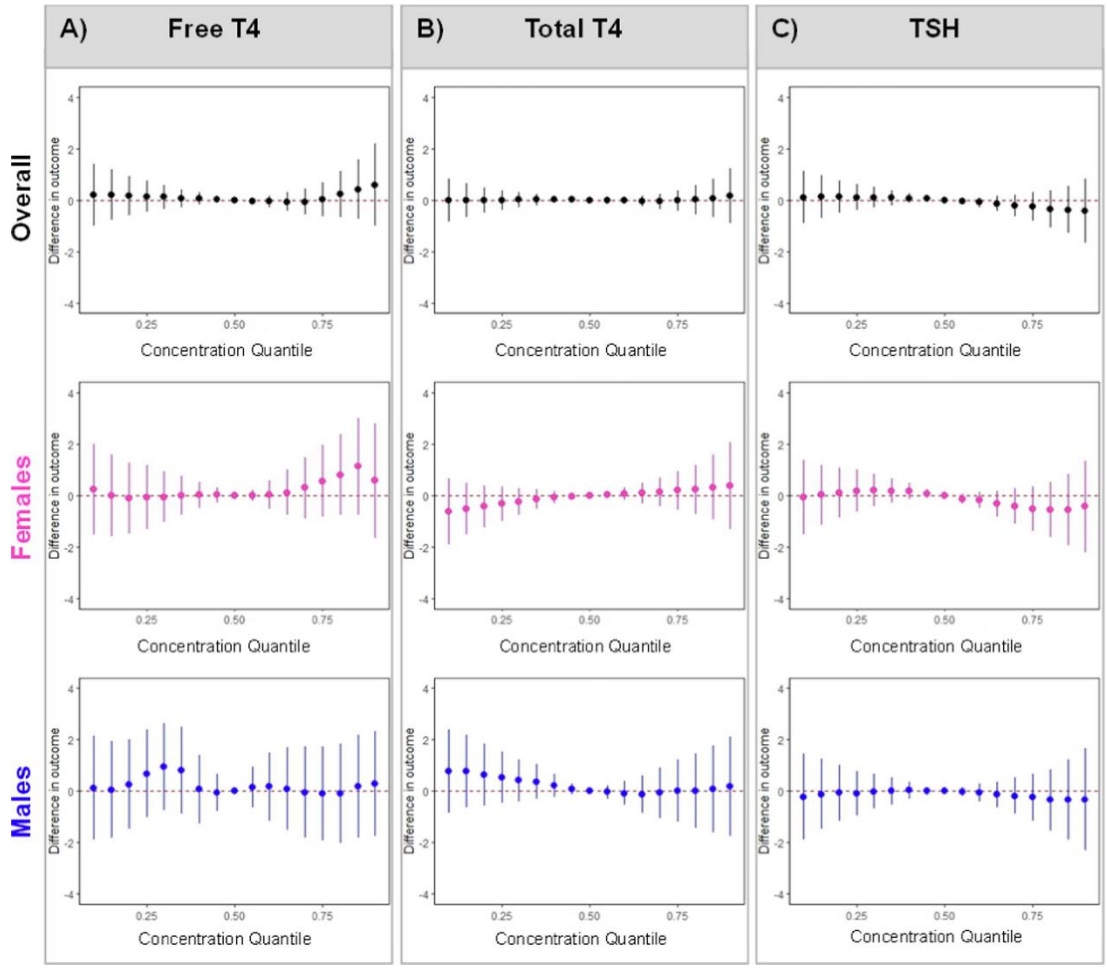

Figure 5. Associations of BKMR mixture with A) free T4, B) total T4, C) TSH in all women, women carrying females, and women carrying males. BKMR models were fit with 200,000 iterations and 50 knots accounting for educational attainment, age, diet quality, pre-pregnancy body mass index, perceived stress in early pregnancy, lifetime smoking status, parity, race/ethnicity, gestational age at plasma hormone assessment. Data are presented as effect estimates and 95% credible intervals which are interpreted as the association between the mixture at each quantile and gestational hormone compared to when all co-exposures are fixed at the median. Estimate and interval color signifies analytic sample (all women: black; women carrying females: pink; women carrying males: blue). Abbreviations: BKMR, Bayesian Kernel Machine Regression; T4, thyroxine; TSH, thyroid stimulating hormone.

CHAPTER FOUR:
PREVALENCE AND HORMONAL PREDICTORS OF PERSISTENT NAUSEA IN PREGNANT WOMEN FROM AN ILLINOIS PROSPECTIVE PREGNANCY COHORT

This chapter was submitted for publication in the *Journal of the Endocrine Society* on June 13, 2024; Ryva BA, Anderson KY, Nallabelli A, Wylie BJ, Schantz SL, Strakovsky RS. Supplemental tables and figures referenced in the chapter can be found in the dissertation supplemental file.

4.1. ABSTRACT

Persistent nausea in pregnancy is common with unclear etiology. We evaluated associations of early second-trimester maternal sex-steroid/thyroid hormones with pregnancy nausea persistence. We quantified second-trimester (median 17 weeks; n=410) concentrations of plasma progesterone, estradiol, testosterone, free thyroxine (FT4), total thyroxine (TT4), and thyroid stimulating hormone (TSH). We categorized women into four groups: those who never developed nausea, those with typical nausea (ending by 17 weeks gestation), those with persistent nausea (extending beyond 17 weeks gestation), and those with irregular nausea. We evaluated associations of hormones with nausea persistence categories using logistic regression and used weighted quantile sums regression to understand the contribution of each hormone to nausea persistence when accounting for the other hormones. We also considered differences by fetal sex. Compared to women with typical nausea, each 10% increase in testosterone was associated with 6% greater odds of persistent nausea (OR: 1.06; 95% CI: 1.00, 1.13), with associations observed mainly among women carrying male fetuses (OR: 1.09; 95% CI: 1.00, 1.19). Additionally, compared to women with typical nausea, each 10% increase in progesterone was associated with 8% (OR: 1.08; 95% CI: 0.98, 1.18) greater odds of persistent nausea. When all hormones were modeled jointly, testosterone, progesterone, and FT4 were the major drivers of persistent nausea. We identified some associations of hormones with never having nausea and having irregular nausea. Some early second-trimester sex-steroid and thyroid hormones are associated with elevated odds of having persistent nausea in pregnancy, with possible implications for maternal and fetal health.

4.2. Introduction

Nausea is the most common symptom women experience during pregnancy, with prevalence estimates of 50-90% (Bustos et al., 2017; Herrell, 2014; Lee and Saha, 2011; Niebyl, 2010). In most cases, nausea occurs in the first trimester and resolves on its own (Herrell, 2014); however, 20-40% of women continue to have nausea later in pregnancy (Einarson et al., 2013; Kramer et al., 2013). In 2012, the economic impact of nausea and vomiting during pregnancy (NVP) in the U.S. was estimated at $1.7 billion, with the health care costs of one symptomatic woman being nearly $2,000 (Piwko et al., 2013). Additionally, nausea during pregnancy has been linked to adverse maternal health outcomes, including high blood pressure, preeclampsia, and depression, with potential impacts on employment and family life (Attard et al., 2002; Chortatos et al., 2015; Mazzotta et al., 2000; Niebyl, 2010; Smith et al., 2000). Despite this burden, some studies have reported women with nausea symptoms have lower risk of miscarriage and birth defects (Koren et al., 2014; Schrager et al., 2023). However, it is unclear if these supposedly “protective effects” are limited to nausea experienced in the early months of pregnancy or if they are due to confounding by some other biological factor, such as gestational hormones. Therefore, there is still a critical need to better understand the fundamental mechanisms of nausea during pregnancy, especially that which persists later into the second and third trimesters, in order to clarify whether current lifestyle and pharmacological interventions for nausea can be modified or improved upon.

Prior epidemiologic research identified a relationship of higher levels of early-pregnancy human chorionic gonadotropin (hCG) with pregnancy nausea (Masson et al., 1985); however, as hCG rapidly declines near the end of the first trimester, it is unlikely to explain persistent nausea symptoms. Sex-steroid hormones (progesterone, estradiol, and testosterone) and thyroid hormones (free thyroxine (FT4), total thyroxine (TT4), and thyroid stimulating hormone (TSH)) increase across pregnancy (Dukic and Ehlert, 2023; Soldin et al., 2004) and have key roles in maintaining pregnancy, preventing uterine contractions, and increasing uterine blood supply (Hacker et al., 2010), with disruptions to these hormones linked to preterm delivery, preeclampsia, fetal growth restriction, and developmental disabilities (Silva et al., 2018). Some studies have considered the roles of sex-steroid and thyroid hormones in nausea symptomology, although findings have been mixed (Carlsen et al., 2003; Dekkers et al., 2020; Lagiou et al., 2003). For example, one study of nausea and vomiting in 129 Scandinavian women with uncomplicated pregnancies reported a positive association of serum

testosterone (average of 17- and 33 week-samples) with nausea and vomiting at 33 weeks gestation (Carlsen et al., 2003), whereas another study of 262 White women from Boston reported negative associations of prolactin (but not other hormones) with nausea at 16 and 27 weeks gestation and positive associations of estradiol (but not other hormones) with nausea at 16 and 27 weeks gestation (Lagiou et al., 2003). Additionally, a recent study of 1,682 pregnant women from the Holistic Approach to Pregnancy and the first Postpartum Year (HAPPY) study in the Netherlands reported that hCG was positively associated with first trimester nausea and vomiting, but thyroid hormones were not associated with nausea during pregnancy (Dekkers et al., 2020). Recently, researchers have reported associations of the placental hormone growth/differentiation factor 15 (GDF15) with hyperemesis gravidarum (HG), the most severe form of nausea and vomiting during pregnancy (Fejzo et al., 2023; Fejzo et al., 2019a; Fejzo et al., 2018; Fejzo et al., 2019b). However, it is unclear how much of less severe pregnancy nausea is explained exclusively by GDF15 and whether this hormone interacts with other key pregnancy hormones (e.g., sex-steroid and thyroid hormones).

One possible limitation of prior studies is they were unable to consider the hormonal milieu in pregnancy, which leads to considerable correlation between hormones, making it difficult to discern important independent drivers of nausea symptoms. One approach for identifying the role of each hormone, within the context of other hormones, is to evaluate each hormone's relationship with a health outcome while simultaneously adjusting for other hormones (Lagiou et al., 2003). Given that gestational hormones share common pathways of synthesis and regulation, this would result in poor model fit due to multicollinearity (Kim, 2019). In recent years, environmental epidemiologists have been working to rectify this issue by utilizing statistical mixture methods that can handle moderately and highly correlated environmental exposures. In general, these methods allow researchers to evaluate the relative importance of each co-exposure within the context of a mixture of several exposures and to identify a joint association of these chemical co-exposures with a health outcome of interest (Carrico et al., 2015; Hamra and Buckley, 2018; Keil et al., 2020). One commonly used mixture method is weighted quantile sums regression (WQSR), which is a supervised method that models a mixture of co-exposures dependent on the outcome (Carrico et al., 2015). Importantly, WQSR calculates effect estimates, such as odds ratios (OR) and confidence intervals (CIs), for reporting the overall relationship of all co-exposures (e.g., multiple pregnancy hormones) with

a health outcome (e.g., nausea in pregnancy), along with providing weights that are interpreted as the relative contribution of each co-exposure (e.g., individual hormone) towards the mixture association. To date, no study has utilized these methods to model the gestational hormonal milieu as an exposure for a health outcome.

Given the surprisingly high prevalence of persistent nausea in pregnancy and the lack of a clear understanding of the hormonal underpinnings, we evaluated associations of early second-trimester sex-steroid and thyroid-hormones with nausea persistence during pregnancy. We conceptualized nausea as being typical (early) nausea that may be normal or as persistent nausea that continues beyond what may be considered normal. We also considered women who never developed nausea and those with inconsistent nausea symptoms. We evaluated hormones individually and utilized WQSR to allow us to evaluate how each hormone within the milieu contributes to nausea persistence. Lastly, as prevalence of nausea during pregnancy has been reported to differ in women carrying male or female fetuses, we considered differences in associations by fetal sex (Mitsuda et al., 2019; Young et al., 2021).

4.3. Materials and Methods

Illinois Kids Development Study (I-KIDS) study design and population

Pregnant women in the current study participated in I-KIDS, a prospective pregnancy and birth cohort recruited from two local obstetric clinics in Champaign-Urbana, Illinois to evaluate associations of prenatal environmental chemical exposures with neurodevelopment. Recruitment and enrollment have been previously detailed (Pacyga et al., 2021; Pacyga et al., 2022a; Pacyga et al., 2023). To be eligible to participate, women had to be ≤15 weeks pregnant at enrollment, 18-40 years old, fluent in English, in a low risk and singleton pregnancy, residing within 30 minutes of the study site, and not planning on moving before their child's first birthday. Our current study includes 410 women who enrolled in I-KIDS between 2013 and 2019, remained in the study through their child's birth, had available nausea symptom information, had measurable maternal plasma levels of at least one sex-steroid or thyroid hormone measured at median 17 weeks gestation, and consented to participate in research outside of the original study aims related to chemical exposures (Figure S38). All women provided written informed consent, and the study was approved by the University of Illinois' Institutional Review Board.

Collection of maternal sociodemographic, lifestyle, and health information

After enrollment, I-KIDS staff conducted home visits to interview women about various sociodemographic and lifestyle factors. Pre-pregnancy body mass index (kg/m^2) was calculated from self-reported pre-pregnancy weight and height. To measure early pregnancy stress levels, women completed the Perceived Stress Scale (PSS), a ten-item questionnaire asking about thoughts and feelings during the last month that provides a score from 0 to 40 (Cohen et al., 1983; Cohen and Williamson, 1988). Scores of 0 to 13 indicate low stress and scores 14 or greater indicate moderate or high stress. At their first study visit at median 13 weeks gestation, women completed a semi-quantitative food-frequency questionnaire (FFQ) adapted from the full-length Block-98 FFQ (NutritionQuest, Berkely, CA) and validated in pregnant populations (Bodnar and Siega-Riz, 2002; Boucher et al., 2006; Laraia et al., 2007). Dietary intakes representing diet patterns from the previous three months were used to calculate the Alternative Healthy Eating Index (AHEI-2010), an 11-component diet quality index (out of 110 total points) based on foods and nutrients known to be predictive of chronic disease risk and mortality. For AHEI-2010, a higher score indicates better overall diet quality (Chiuve et al., 2012; McCullough et al., 2002). Since AHEI-2010 considers moderate alcohol consumption as beneficial to health, but clinical guidelines recommend pregnant women abstain from alcohol (Bertrand et al., 2005; CDC, 2023; Cook et al., 2016), we removed the alcohol component from the index to create a ten-component diet quality index (maximum: 100 points).

Collection and quantification of plasma maternal hormone concentrations

We assessed a one-time measurement of early second-trimester maternal plasma samples for sex-steroid (progesterone, estradiol, total testosterone) and thyroid (FT4, TT4, TSH) hormones. We collected samples in glass heparin-containing vacutainer tubes, centrifuged them at room temperature for 20 minutes, and aliquoted them for storage at -80°C. We sent the samples to the University of Michigan Diabetes Research Center (MDRC) Clinical Core Chemistry Laboratory for maternal hormone quantification using solid-phase, enzyme-labeled chemiluminescent competitive immunoassay (IMMULITE 1000, Siemens). The protocol has been described elsewhere (Ryva et al., 2024). Briefly, 10-75 µL of plasma (depending on hormone) was incubated for 30 minutes with a polyclonal rabbit antibody coated bead (solid-phase) and bovine calf intestine alkaline phosphate conjugated to hormone (liquid-phase). After repeated washes and centrifugation to remove unbound hormone, the

chemiluminescent substrate was added to measure the signal indicating the proportion of hormone bound. Each hormone's limit of detection and analytic sensitivity have been previously reported (Ryva et al., 2024) and their reportable ranges are listed in Table 2. Based on protocol requirements and pregnancy estradiol concentrations being greater than the assay's reportable range, estradiol samples were diluted 10x before analysis and machine-read values were multiplied by ten to obtain final concentrations. For total testosterone and TSH, we considered values below the calibration range as missing and excluded women with those values from analyses (n = 65 and n = 1, respectively).

Statistical Analysis

Derivation of analytic sample and covariate selection

The derivation of our analytic sample is presented in Figure S38. Of the 688 women who enrolled in I-KIDS, 531 remained active through the birth of their child between 2013 and 2019. Of those women, 466 had complete nausea information and data for at least one hormone of interest. We excluded women who did not consent to participate in research outside of studies related to chemical exposure (n = 56), resulting in our analytic sample of 410 women. We characterized sociodemographic, health, and lifestyle factors of these women as frequency (percent) or median (25^{th}, 75^{th} percentile) in **Table 13**.

Based on our data and prior literature (Carlsen et al., 2003; Chou et al., 2008; Lagiou et al., 2003; Masson et al., 1985), we considered many potential covariates and generated a directed acyclic graph (DAG) to identify a minimum sufficient adjustment set (Figure S39). We assessed correlations between covariates to test for potential multicollinearity; however, all included covariates were only weakly or moderately correlated (r<0.4; **data not shown**). All models accounted for maternal age, race/ethnicity, educational attainment, alcohol consumption since conception, diet quality (AHEI-2010), perceived stress (PSS10), pre-pregnancy BMI, conception season, and fetal sex. These covariates may represent latent constructs, such as reproductive health, general health and lifestyle, or socioeconomic status. Age, pre-pregnancy BMI, diet quality, and stress were modeled as continuous variables, whereas the remaining variables were categorized with the reference group indicated in **Table 13**.

Modeling nausea persistence during pregnancy

Women reported nausea symptoms approximately monthly across pregnancy (13, 17, 23, 28, and 34 median weeks gestation, and at a hospital research visit within 24 hours after birth). Research home visits were conducted at median 13 and 34 weeks, phone interviews were conducted at median 23 and 28 weeks, and a separate clinic visit for blood collection and interview was conducted at median 17 weeks. At the first prenatal visit, women were asked if they had experienced nausea since conception (answer: “yes”, “no”). At the next visit, women were asked if they still have nausea (answer: “yes”, “no”) and when it ended if “no”. They were also asked if they started experiencing any new nausea since the last visit (answers: “yes”, “no”) and when it started if “yes”. We categorized women as “never having nausea” if they did not report nausea at any point in pregnancy. We categorized women as having “typical nausea” if they reported having nausea since conception, but their symptoms ended by median 17 weeks gestation. We categorized women as having “persistent nausea” if they reported having nausea since conception and their symptoms persisted past 17 weeks gestation. Lastly, we categorized women as having “irregular symptoms” if they reported nausea symptoms that started and stopped more than once during pregnancy. In the current study, we used the cutoff of 17 weeks to delineate typical versus persistent nausea for two reasons. First, our study only collected maternal blood at median 17 weeks gestation, and selecting women with nausea that persisted past this point allowed us to prospectively evaluate the relationship of hormones with nausea and include the most women possible in our analyses. Second, the 17-week cutoff represents a timepoint that is well beyond the first trimester (or typical nausea experience previously attributed to hCG) when the placenta ramps up its production of hormones that regulate various gestational processes.

Evaluating associations of early second-trimester hormones with nausea during pregnancy

To address our primary objective, we evaluated whether early second-trimester hormone concentrations would be associated with persistent nausea compared to typical nausea using logistic regression. Our secondary objective was to evaluate whether hormone concentrations were associated with never developing nausea in pregnancy. Although some of the outcomes (nausea) will have occurred prior to the exposure (hormones) and the smaller sample size of women who never had nausea reduces statistical power, this additional analysis allowed us to understand potential hormonal characteristics of women with a non-typical pattern of pregnancy nausea. We also evaluated whether hormone concentrations

were associated with irregular nausea in pregnancy. In single-hormone analyses, because of non-normal distributions and to improve model fit, we natural log (ln)-transformed all hormones. In logistic regression models, we adjusted for the covariates described above.

Exploring the relationships of joint hormones with nausea during pregnancy

We used WQSR to explore the possible associations of a mixture of hormones with persistent nausea and identify individual hormones responsible for the mixture association (Carrico et al., 2015). Our hormone mixture included three sex-steroid (progesterone, estradiol, testosterone) and three thyroid (FT4, TT4, TSH) hormones. WQSR creates a weighted index by transforming exposure biomarkers into quantiles (deciles in this study) and evaluates the association of the index with the health outcomes of interest by fitting regression models with continuous or binary outcomes (Carrico et al., 2015). We fit our models using 100 iterations (repeated holdouts) and 100 bootstrap replications. Within each iteration, data were randomly split 40/60% into training and validation datasets, respectively (Tanner et al., 2019). WQSR fits two models for each mixture and outcome relationship: 1) where all co-exposures are fixed in a positive direction (positive constraint) and 2) where all co-exposures are fixed in the negative direction (negative constraint). We fit models constrained in both the positive and negative direction to obtain the OR, 95% CI, and weights (indicating hormones contributing the most to the overall association). We used the standard cut-off to determine if a hormone contributed meaningfully to the overall association (1/# of hormones; 1/6 = 0.17) (Carrico et al., 2015).

Investigating effect modification by fetal sex

As nausea during pregnancy has been reported to differ by fetal sex (Ben-Aroya et al., 2005; Mitsuda et al., 2019), we conducted a secondary analysis investigating if associations of hormones (individually and jointly) with nausea differ between women carrying females and women carrying males. For logistic regression, we included a multiplicative interaction term in our models to identify differences and reported interaction *p*-values. To simplify the interpretation of results from interaction models in WQSR, we stratified our sample by fetal sex and fit separate models. For women carrying females, positive and negative WQSR models did not converge for never having nausea compared to typical nausea, due to small sample sizes of conception season and educational attainment covariates; thus, we reported estimates and weights from models without these covariates.

Reporting of findings and interpreting meaningful associations

For single-hormone logistic regression results, our OR and 95% CIs represent the odds of nausea (never, persistent, or irregular) for a 10% increase in each second trimester hormone concentration compared to typical nausea. Our WQSR results are interpreted as the OR associated with a 10% increase in all hormones combined. We identified potentially meaningful findings from single and cumulative hormone models by assessing the direction, strength, and precision of the associations, rather than focusing on statistical significance thresholds, as recommended by the American Statistical Association (Wasserstein and Lazar, 2016). To ensure model assumptions were met, we performed regression diagnostics for single-hormone analyses and assessed WQSR scatterplots. We performed logistic regression analyses in SAS version 9.4 (SAS Institute Inc. Cary, NC) using PROC LOGISTIC and completed WQSR analyses in R Statistical Software using R package: "gWQS: Generalized Weighted Quantile Sum Regression" (Stefano Renzetti).

4.4. Results

Study demographics and distribution of nausea persistence during pregnancy

Most women in our study were non-Hispanic White (82%), college-educated (85%), and had an annual household income > $60,000 (73%) (**Table 13**). Most women never smoked cigarettes (84%) and did not consume alcohol since conception (58%). Approximately half of women had not yet given birth to a child (53%), and fetal sex in the current pregnancy was approximately evenly distributed between female (52%) and male (48%). The median age was 30 years old, and median pre-pregnancy BMI was 24.5 kg/m^2, with 47% of women classified as having overweight or obesity. The median (25th, 75th percentile) diet quality as measured by the AHEI-2010 was 51.8 (45.3, 60.0) out of 100 points. Most women reported having low early-pregnancy stress (65%), with a median PSS10 score of 10.7 (25th, 75th percentile: 6.8, 17.0) (**Table 13**).

Some maternal characteristics differed depending on maternal pregnancy nausea category (Table S20). For example, women with typical nausea were more likely to have graduated from college or received a higher degree compared to women with never, persistent, or irregular nausea (90% versus 82%, 80%, and 83%, respectively). Women with typical nausea were more likely to consume alcohol since conception compared to women with never, persistent, or irregular nausea (47% and 45% versus 31% and 35%). Women who never had

nausea, those who had persistent nausea, and those with irregular nausea had slightly higher pre-pregnancy BMI compared to those with typical nausea (25 kg/m^2 versus 24 kg/m^2). Furthermore, most women with typical nausea were underweight or normal weight (62%), whereas most women who never had nausea, those with persistent nausea, and those with irregular nausea had overweight/obesity (64%, 51%, 50%, respectively). In addition, women with persistent nausea had slightly lower diet quality scores (49 points) compared to those who never had nausea (52 points), those with typical nausea (53 points), and those with irregular nausea (52 points). Finally, women with persistent nausea had higher stress scores (13 points) compared to those who never had nausea (8 points), those with typical nausea (10 points), and those with irregular nausea (12 points), with 51% of women with persistent nausea classified as having moderate/high stress.

Distribution of nausea persistence during pregnancy

Nausea prevalence during pregnancy ranged from 22% to 90%, depending on the gestational timepoint (**Figure 6A**). Specifically, the vast majority of women reported having some nausea since conception when interviewed at median of 13 weeks gestation (90%), with the prevalence steadily decreasing across pregnancy. The largest group of women experienced typical nausea during pregnancy (n=166; 40%), followed by persistent nausea (n=104; 25%), and irregular nausea (n=101; 25%). Only 10% (n=39) reported never having nausea in pregnancy (n=39; 10%) (**Figure 6B;** Table S20).

Concentrations of maternal plasma hormones

Most women had measurable levels of all early second-trimester gestational hormones (**Table 14**). Specifically, 100% of women had progesterone, estradiol, FT4, and TT4 levels greater than or equal to the lower limit of the reportable range. For testosterone and TSH, 83% and 99% of women had levels greater than or equal to the lower limit of the reportable range. The median (25th, 75th percentile) concentration of progesterone, estradiol, and testosterone were 29.1 ng/mL (24.3, 33.9), 2727.5 pg/mL (1955.0, 3660.0), and 44.1 ng/dL (32.6, 64.4), respectively. The median (25th, 75th percentile) levels of FT4, TT4, and TSH were 0.9 ng/dL (0.8, 1.0), 8.9 µg/dL (8.0, 9.9), and 1.8 µIU/mL (1.2, 2.5), respectively. Progesterone concentrations differed significantly by fetal sex, with women carrying males having higher progesterone (31.0 ng/mL and 27.8 ng/mL, respectively) (Table S21). Testosterone levels were also slightly higher in women carrying males compared to females

(45.0 ng/dL and 41.7 ng/dL, respectively). Estradiol was weakly correlated with progesterone (r=0.25) and testosterone (r=0.20), whereas TT4 and FT4 were moderately correlated with each other (r=0.44) (Figure S40).

Associations of hormone concentrations individually and jointly with nausea persistence during pregnancy

Progesterone and testosterone were associated with persistent nausea during pregnancy (**Table 15**). Specifically, a 10% increase in progesterone concentration was associated with 8% higher odds of persistent nausea compared to typical nausea (OR: 1.08; 95% CI: 0.98, 1.19). Likewise, each 10% increase in testosterone was associated with 6% higher odds of persistent nausea compared to typical nausea (OR: 1.06; 95% CI: 1.00, 1.13). There were no meaningful associations of estradiol, FT4, TT4, and TSH with nausea during pregnancy. Only the relationship of testosterone with persistent nausea differed by fetal sex. Specifically, in women carrying males, a 10% increase in testosterone was associated with 9% higher odds of persistent nausea compared to typical nausea (OR: 1.09; 95% CI: 1.00, 1.19), whereas in women carrying females, there was no association of testosterone with nausea persistence (OR: 1.03; 95% CI: 0.94, 1.13) (Table S23).

When we modeled hormones cumulatively using WQSR, FT4 (27%), testosterone (21%), and progesterone (18%) contributed meaningfully to a positive association of increasing hormone concentrations with odds of having persistent nausea (OR: 1.23; 95% CI: 0.93, 1.64) (**Figure 7A,** Table S24). The WQSR model where the relationship of hormones with nausea persistence is constrained in the negative direction could not be fit (i.e., no negative β estimates), suggesting the hormone mixture was positively associated with minimal negative contribution. Similar to single-hormone analyses, in women carrying males, testosterone was the main contributor (27%) to a marginally meaningful positive association of all hormone levels with higher odds of persistent nausea compared to typical nausea (OR: 1.32; 95% CI: 0.82, 2.13) (Table S24). Interestingly, in women carrying males, FT4 (18%), and TT4 (19%) were also major contributors, despite not being associated with single hormone analyses. We did not identify any associations of hormones with nausea in women carrying females.

Associations of hormones individually and jointly with other atypical nausea patterns

Some hormones were associated with never developing nausea and with having irregular

nausea during pregnancy. Specifically, each 10% increase in progesterone and testosterone levels were associated with 10% and 9% higher odds of never developing nausea compared to having typical nausea, respectively (OR: 1.10; 95% CI: 0.96, 1.25 and OR: 1.09; 95% CI: 1.00, 1.18) (Table S23). In women carrying males, each 10% increase in progesterone concentration was associated with 20% higher odds of never developing nausea compared to typical nausea (OR 1.20; 95% CI: 1.02, 1.42) (Table S23). There were no meaningful associations of estradiol, FT4, TT4, or TSH with the odds of never developing nausea. When modeled as a mixture, testosterone (30%), progesterone (24%), and FT4 (19%) contributed meaningfully to a positive association of all hormone concentrations with higher odds of never experiencing nausea compared to typical nausea (OR: 1.26; 95% CI: 0.90, 1.76) (Table S24). As with persistent nausea, when hormones were modeled in the negative direction for models with never nausea using WQSR, the model could not be fit (i.e., no negative β estimates). When we stratified by fetal sex, we did not identify any meaningful sex-specific relationships of the hormone mixture with never having nausea (Table S24); however, in women carrying females, positively and negatively constrained WQSR models were unreliable due to sparse data in educational attainment and conception season. When we removed these covariates, the models produced reliable, but not meaningful, estimates and weights.

Progesterone was also associated with irregular nausea during pregnancy. Specifically, each 10% increase in progesterone concentration was associated with 8% higher odds of having irregular nausea compared to having typical nausea (OR: 1.08; 95% CI: 0.98, 1.19) (Table S23). This relationship was driven by women carrying males, in whom each 10% increase in progesterone level was associated with 17% higher odds of having irregular nausea compared to typical nausea (OR: 1.17; 95% CI: 1.02, 1.24) (Table S23). When modeled as a mixture of hormones, progesterone (37%) and testosterone (19%) contributed most towards a positive association of all hormone mixture levels with higher odds of experiencing irregular nausea compared to typical nausea (OR: 1.10; 95% CI: 0.86, 1.41) (Table S24). In models stratified by fetal sex, we did not identify any meaningful fetal sex-specific relationships of the hormone mixture with having irregular nausea (Table S24).

4.5. Discussion

Summary of major findings

To our knowledge, ours is the first study to utilize a mixtures approach to understand the

relationship of the pregnancy hormonal milieu with nausea persistence in pregnancy. In our sample of relatively high-SES women, jointly, second-trimester sex-steroid and thyroid hormones were associated with higher odds of having persistent nausea (due to FT4, testosterone, and progesterone), never having nausea (due to testosterone, progesterone, and FT4), and having irregular nausea (due to progesterone and testosterone). We did identify some meaningful differences in associations of hormones with nausea persistence by fetal sex. Specifically, the observed relationship of testosterone (and the hormone mixture) with nausea persistence may have been primarily due to women carrying males. Our results contribute to existing literature on the relationships of hormones with nausea during pregnancy and expand upon it by exploring the hormonal milieu rather than individual hormones and by considering nausea persistence.

Sex-steroid hormones are associated with increased odds of persistent nausea

Older and more recent epidemiologic studies have reported associations of hCG with nausea (Dekkers et al., 2020; Masson et al., 1985), suggesting it as a strong causal candidate; however, for many women, symptoms persist after hCG concentrations peak and hCG concentrations did not correlate well with symptom intensity (Einarson et al., 2013; Kramer et al., 2013). Thus, other potential mechanisms, such as those involving changes in sex-steroid or thyroid hormones, may contribute to persistent nausea. In this study, our single hormone and joint hormone findings suggest higher levels of progesterone and testosterone are associated with greater odds of having persistent nausea during pregnancy.

Some of our findings agree and some disagree with prior studies, although no prior studies have specifically focused on nausea persistence. For example, in one study of 129 Scandinavian women, higher androstenedione and dehydroepiandrosterone sulfate (DHEAS) were associated with nausea at 17- and 33-weeks gestation, and higher testosterone was associated with nausea at 33 weeks gestation (Carlsen et al., 2003). These findings are concordant with ours, suggesting androgens play a role in nausea during later pregnancy. However, this study differs from ours in that they assessed the relationship between two averaged hormone measurements (at 17 and 33 weeks) and reported nausea at individual timepoints. Thus, the women who reported nausea at these later timepoints may not have had symptoms since conception. Unlike our single and cumulative hormone findings, other studies did not identify any meaningful associations of progesterone with nausea during

pregnancy (Lagiou et al., 2003; Masson et al., 1985). One study of 262 white pregnant women from the Boston area investigated associations of serum estradiol, estriol, progesterone, prolactin, and serum hormone-binding globulin with nausea at 16- and 27-weeks gestation and reported that prolactin was associated with lower odds of nausea and estradiol was associated with higher odds of nausea (Lagiou et al., 2003). Interestingly, in this study, estradiol was only associated with nausea after accounting for all other measured hormones, implicating the other hormones as confounders in the relationship of estradiol with nausea. These results differ from ours, as we did not identify a relationship between estradiol and persistent nausea in single or joint hormone analyses. Importantly, rather than including all hormones in a single model (which could lead to multicollinearity of correlated hormones), we utilized a statistical method that model the hormonal milieu while handling moderately correlated co-exposures (hormones).

Another major difference between our study and prior studies is that rather than focusing on nausea symptoms at single gestational timepoints, we were interested in understanding the relationships between the mid-trimester hormonal milieu and the extent of nausea persistence. Interestingly, and possibly paradoxically, instead of identifying an exposure-response relationship where increasing testosterone is associated with increased nausea, we identified positive associations of testosterone with both never experiencing nausea and having nausea that persists well past the first trimester – compared to the more typical nausea symptomology that ends at the beginning of the second trimester. This may suggest that women with lower testosterone are more likely to have “typical” nausea symptoms, whereas women with dysregulated testosterone can either experience no nausea or experience persistent nausea. Understanding this relationship is critical as prior studies have identified a lower risk of pregnancy or birth complications in women who experience typical nausea (Koren et al., 2014; Schrager et al., 2023), suggesting dysregulated hormones that reduce nausea symptoms may have adverse consequences for pregnant women and their developing fetuses. Therefore, future studies should investigate the relationships of persistent nausea with maternal and fetal outcomes. It is possible that mechanisms underlying not experiencing nausea during pregnancy and having more persistent nausea during pregnancy differ even if they both involve testosterone; future research should explore this possibility.

FT4, but not TT4 or TSH, are positively associated with never having or having persistent nausea

Because of shared structural characteristics between TSH and hCG, researchers have hypothesized that thyroid hormones play a role in nausea during pregnancy. Interestingly, we identified that FT4 was the largest contributor to the relationship between hormones and persistent nausea, but only when accounting for all other hormones. In contrast, one recent study of 1,682 pregnant women in the HAPPY study, investigating both hormonal and psychological determinants of nausea and vomiting during pregnancy (NVP), reported no associations of TSH or FT4 with nausea (Dekkers et al., 2020). Our results highlight the strength of our mixture method in assessing the relationship of the hormonal milieu with nausea during pregnancy because potential relationships between hormones and nausea may be missed using traditional methods, as they would have been in our study. A different study investigated TSH and TT4 and reported only elevated TSH was associated with increased nausea and vomiting scores in early pregnancy (Nijsten et al., 2021). These findings differ from our single hormone results that suggest null or potentially very slight negative associations of TSH with both never nausea and persistent nausea during pregnancy. In models that accounted for all hormones simultaneously, TSH contributed the least to the positive association of all hormones with never nausea and persistent nausea; however, the cumulative model did not identify any meaningful associations in the negative direction. Our findings may have potential clinical implications for women with higher circulating testosterone and FT4 in early second trimester. Future studies should investigate if persistent nausea in pregnancy from disrupted thyroid hormones is related to pregnancy and birth outcomes.

The role of fetal sex in early second-trimester hormone levels and nausea status during pregnancy

Previous studies have reported women carrying females have higher odds of nausea during pregnancy compared to women carrying males, thus suggesting that fetal sex plays a role in this relationship (Mitsuda et al., 2019; Young et al., 2021). Despite observing no differences in the distribution of our nausea categories by fetal sex, we wanted to evaluate whether associations of hormones with nausea differ between women carrying males or females. To our knowledge, no other studies have evaluated the role of fetal sex in the relationship of hormones with nausea during pregnancy. In this study, we reported that the association of

testosterone with persistent nausea is potentially driven by women carrying male fetuses. Similarly, the relationship between all hormones and persistent nausea may also be driven by women carrying males, although with less precision.

Differences by fetal sex may be due to variation in the placenta which could result in different gestational hormone concentrations in women carrying males or females. As it develops from the zygote, the placenta is a sexed organ (XX and XY), and many studies have reported differences in function and morphology by fetal sex (Gabory et al., 2013; Graves, 2010; Meakin et al., 2021; Rich-Edwards et al., 2001). These differences may also impact hormone levels. In our study and the literature (Meulenberg and Hofman, 1991), women carrying males had slightly higher testosterone levels compared to women carrying females; however, as we only assessed hormones at one time point, we were unable to assess levels at later gestational timepoints, when these differences might be more pronounced. We also reported that women carrying males had higher median progesterone concentrations, but the relationship of progesterone with persistent nausea did not differ by fetal sex. In contrast to our results, one recent study reported no differences in sex-steroid hormones by fetal sex, but some differences by cytokine levels and angiogenic factors (Enninga et al., 2015). In our study, we did not identify any fetal sex differences in thyroid hormone levels, and thyroid hormones only seemed to play a role in nausea during pregnancy within the context of all hormones. In contrast, a recent study reported higher levels of TSH, but not FT4 levels, in women carrying males (Sitoris et al., 2022). Our results and others highlight the complexity of sexually dimorphic hormonal drivers of nausea. Future work is needed to investigate other potential hormonal and non-hormonal pathways, especially at different timepoints during pregnancy.

Strengths and Limitations

The current study has some limitations and many strengths. First, I-KIDS did not assess nausea using more validated questionnaires such as Pregnancy-Unique Quantification of Emesis and Nausea (PUQE); however, I-KIDS collected information in a similar manner as PUQE. One important distinction is that PUQE assesses symptoms over the last 24-hour period, whereas I-KIDS assessed nausea since the last visit at multiple timepoints across pregnancy which allowed us to model nausea persistence during pregnancy. Additionally, while we also collected data related to vomiting during pregnancy, we were underpowered to

consider vomiting persistence, which may be important to consider in future studies. Second, we were limited to a single early second-trimester measurement of select sex-steroid and thyroid hormones, so our findings may not be generalizable to other gestational timepoints and other hormones. We were unable to investigate GDF15, which has recently been implicated in the mechanism of nausea and vomiting during pregnancy, but we assessed six hormones that have been previously linked with nausea symptoms during pregnancy and reflect sex-steroid and thyroid hormones known to be critical for pregnancy health and fetal development. Third, while we cannot rule out unmeasured confounding, I-KIDS collected pertinent demographic, lifestyle, and health information that allowed us to account for many important covariates, and we utilized *a priori* consideration informed by previous literature review to inform decisions about covariate selection. Fourth, we may have been underpowered when investigating women who never reported nausea and, in some sex,-specific analyses; but, in general, our study had adequate power to identify meaningful associations. Fifth, the I-KIDS cohort is a relatively homogenous sample of non-Hispanic White, well-educated, married women, which limits generalizability; however, as we are investigating biological hypotheses, a homogenous sample may reduce unmeasured confounding. Lastly, we did not use a mixture method that can potentially identify hormone-hormone interactions and non-linearities in associations of hormones with nausea (e.g., Bayesian Machine Kernel Regression or others), but we used WQSR, a robust and reliable method to identify meaningful hormones related to nausea during pregnancy and to estimate a cumulative association.

4.6. Conclusion

In the current study, we reported that early second-trimester progesterone and testosterone concentrations were associated with higher odds of persistent nausea in a relatively homogenous sample of midwestern United States pregnant women. When all hormones were modeled together, progesterone and testosterone remained as determinants of persistent nausea, but we also identified FT4 as the major contributor to the elevated odds of having persistent nausea. Similarly, we also identified that testosterone, progesterone, and FT4 were associated with higher odds of never developing nausea, which suggests that not having any nausea could be another non-typical pregnancy nausea phenotype. Finally, we concluded that associations of our panel of hormones measured at median 17 weeks gestation with nausea persistence during pregnancy were likely due to women carrying males. Our

approach of investigating the hormonal milieu could also help clarify or possibly establish the independent relationship of GDP15 with nausea during pregnancy by accounting for sex-steroid and thyroid hormones. Our method of characterizing persistent nausea during pregnancy could be useful for clinicians, and future studies will need to consider hormones at additional pregnancy timepoints, and also to investigate whether persistent nausea leads to similar adverse pregnancy outcomes as hyperemesis gravidarum, including pre-term birth, abnormal birthweight, and placental abruption (Jansen et al., 2023).

Tables

Table 13. Characteristics of I-KIDS women included in the analytic sample (n=410).

Characteristic	n (%)
Race/Ethnicity	
Non-Hispanic White (*ref*)	336 (82.0)
Others	74 (18.0)
Education	
Some college or less (*ref*)	61 (14.9)
College graduate or higher	349 (85.1)
Income	
<$60,000	109 (26.7)
$60,000-$99,999	155 (38.0)
>$100,000	144 (35.3)
Alcohol since conception	
None (*ref*)	238 (58.2)
Any alcohol consumed	171 (41.8)
Parity	
No children (*ref*)	217 (52.9)
At least 1 child	193 (47.1)
Conception Season	
Winter (*ref*)	97 (23.6)
Spring	107 (26.1)
Summer	95 (23.2)
Fall	111 (27.1)
Fetal Sex	
Male (*ref*)	197 (48.0)
Female	213 (52.0)
	Median (25^{th}, 75^{th} percentile)
Maternal age (years)	30.0 (27.5, 32.7)
Pre-pregnancy body mass index (kg/m^2)	24.5 (21.9, 29.1)
Early pregnancy Alternative Healthy Eating Index 2010*	51.8 (45.3, 60.0)
Early pregnancy perceived stress	10.7 (6.8, 17.0)
Gestational age at hormone assessment	17.0 (16.4, 17.7)
*Alcohol intake was removed from the index (total score out of 100).	

Table 14. Distribution of early second-trimester gestational hormones (n=410).

	n	Reportable range	% ≥ lower limit of reportable range	Median (25th, 75th percentile)
Gestational hormone				
Progesterone, ng/mL	408	0.2 – 40.0	100	29.1 (24.3, 33.9)
Estradiol, pg/mL	408	20.0 – 2000.0	100	2727.5 (1955.0, 3660.0)
Testosterone, ng/dL	326	20.0 – 1600.0	83.0	44.1 (32.6, 64.4)
Free thyroxine (FT4), ng/dL	407	0.3 - 0.6	100	0.9 (0.8, 1.0)
Total thyroxine (TT4), µg/dL	407	1.0 – 24.0	100	8.9 (8.0, 9.9)
Thyroid Stimulating Hormone (TSH), µIU/mL	407	Up to 75.0	99.8	1.8 (1.2, 2.5)

*Values are outside of manufacturer's reportable range because estradiol was diluted 10x prior to analysis and machine-read values were multiplied by 10.
Progesterone limit of detection (LOD) of 0.20 ng/dL and functional sensitivity of 0.46 ng/mL. Estradiol analytic sensitivity of 15.0 pg/mL. Testosterone analytic sensitivity of 15.0 ng/dL. FT4 analytic sensitivity of 0.13 ng/dL, LOD of 0.28 ng/dL, and functional sensitivity of 0.3 ng/dL. TT4 analytic sensitivity of 0.4 µg/dL. TSH analytic sensitivity of 0.01 µIU/mL.

Table 15. Associations of early second-trimester hormones with nausea during pregnancy (n=410).

	Never nausea v typical nausea (n=205)	Persistent nausea v typical nausea (n=270)	Irregular nausea v typical nausea (n=267)
Gestational hormone	**Odds Ratio (95% Confidence Interval)**		
Progesterone	1.10 (0.96, 1.25)	1.08 (0.98, 1.19)	1.08 (0.98, 1.19)
Estradiol	1.04 (0.96, 1.12)	1.03 (0.97, 1.09)	1.00 (0.95, 1.06)
Testosterone	1.09 (1.00, 1.18)	1.06 (1.00, 1.13)	1.03 (0.96, 1.09)
FT4	1.19 (0.85, 1.66)	1.20 (0.93, 1.54)	1.07 (0.86, 1.34)
TT4	1.00 (0.81, 1.24)	1.02 (0.87, 1.20)	0.95 (0.81, 1.10)
TSH	0.96 (0.92, 1.01)	0.97 (0.93, 1.01)	0.99 (0.95, 1.03)

Data are presented as odds ratio and 95% confidence intervals of nausea outcome for a 10% increase in the hormone. Nausea type modeled with never nausea, persistent nausea, and irregular nausea compared to typical nausea (n=166). Fully adjusted models accounted for maternal age, race/ethnicity, education level, alcohol since conception, diet quality, perceived stress score, pre-pregnancy body mass index, conception season, and fetal sex.

Sample sizes: progesterone (n=385; f=197; m=188) and estradiol (n=385; F=196; M=189); testosterone (n=311; f=154; m=157); FT4, TT4, TSH (n=384; f=196; m=188). Some women are missing covariate information. Progesterone, estradiol, FT4, TT4, TSH (n=23; diet quality: n=18; stress score: n=4; alcohol: n=1); testosterone (n=15; diet quality: n=10, stress score: n=4; alcohol: n=1).

Abbreviations: FT4, free thyroxine; TT4, total thyroxine; TSH, thyroid stimulating hormone.

Figures

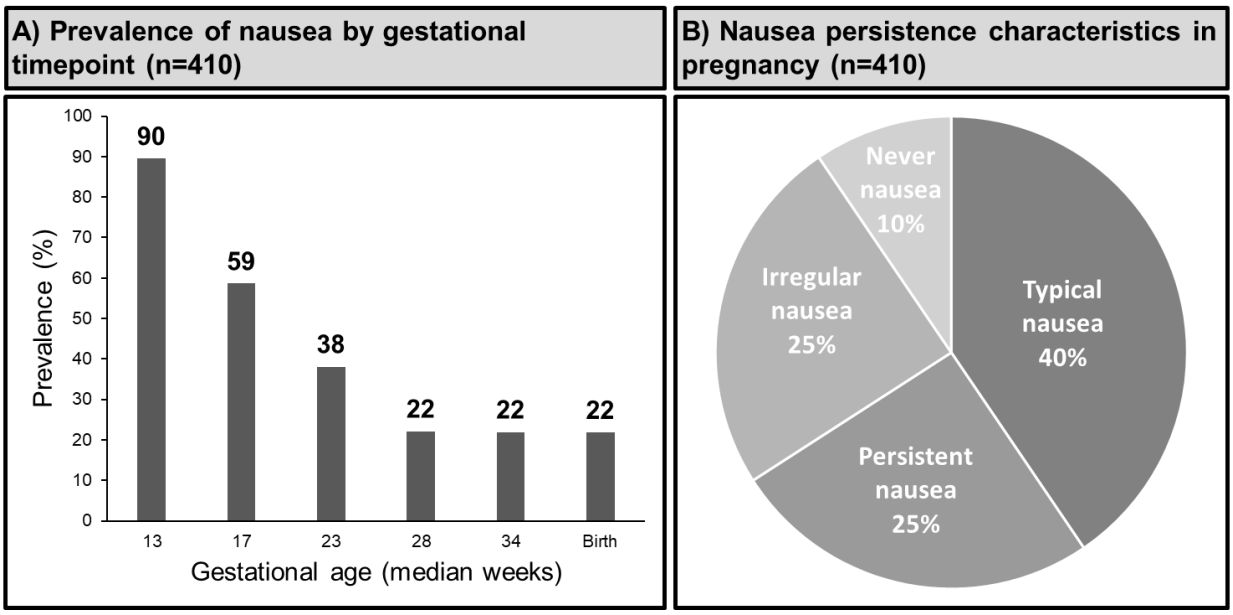

Figure 6. Prevalence of nausea across pregnancy (A) and nausea persistence characteristics during pregnancy (B). Women reported nausea symptoms (yes/no) approximately monthly across pregnancy (13, 17, 23, 28, and 34 median weeks gestation, and at a hospital research visit within 24 hours after birth). Women were categorized as "never having nausea" if they did not report nausea at any point in pregnancy; having "typical nausea" if they reported nausea since conception, but their symptoms ended by median 17 weeks gestation; having "persistent nausea" if they reported nausea since conception and their symptoms persisted past 17 weeks gestation; and having "irregular symptoms" if they reported nausea symptoms that started and stopped more than once during pregnancy. n=410.

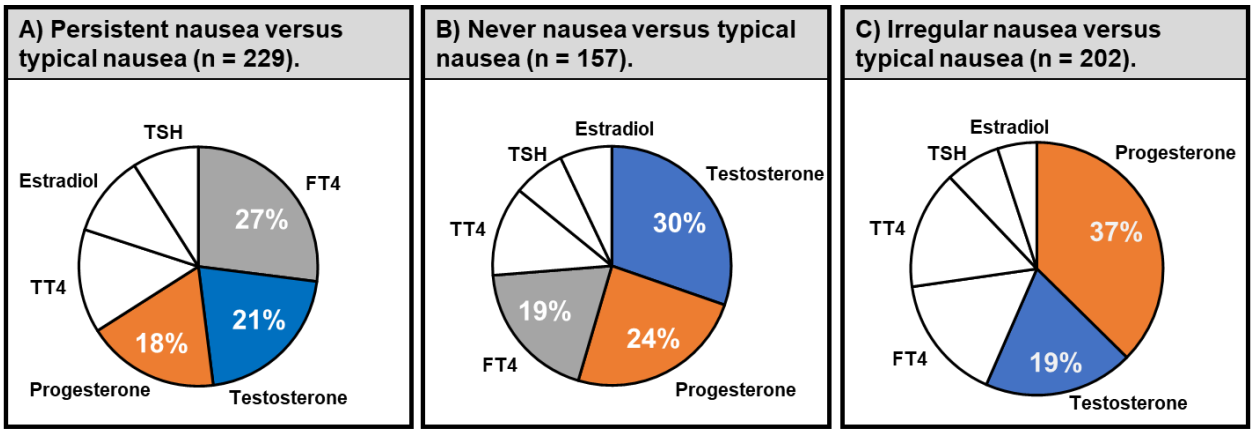

Figure 7. Hormonal contributors to having persistent nausea (A), never developing nausea (B), and having irregular nausea (C) using weighted quantile sums regression (WQSR). Pie charts display percentages of hormone weights generated from WQSR (positively constrained models). Models account for maternal age, race/ethnicity, education level, alcohol since conception, diet quality, conception season, perceived stress score, pre-pregnancy body mass index, and fetal sex. No negative beta estimates were generated in WQSR models. Values (%) in pie charts are reported for hormones that met the WQSR threshold (> 1/# hormones; 17%). Abbreviations: FT4: free thyroxine; TT4: total thyroxine; TSH: thyroid stimulating hormone. n=157-229.

CHAPTER FIVE:
DISCUSSION

Summary of major findings

Within the three central chapters of this dissertation, we explored our central hypothesis that higher concentrations of non-persistent endocrine disrupting chemicals (EDCs), such as phthalates and phenols from common consumer products, are associated with nausea during pregnancy due to relationships of EDCs with mid-pregnancy hormones and of mid-pregnancy hormones with nausea during pregnancy. To accomplish our objectives, we used information from the Illinois Kids Development Study (I-KIDS), an ongoing prospective pregnancy cohort at the University of Illinois, Urbana-Champaign. First, in Chapter Two, we explored overall and fetal sex-specific associations of EDCs with nausea during pregnancy using a traditional regression approach and two robust statistical mixture methods: quantile-based g-computation (QGComp) and Bayesian kernel machine regression (BKMR). Our most salient findings were related to persistent nausea rather than atypical nausea patterns, such as never having nausea or having irregular nausea. Although only the sum of urinary biomarkers of di(isononyl) cyclohexane-1,2-dicarboxylate (ΣDiNCH) was associated with persistent nausea in all women, when all chemicals were modeled jointly (using QGComp), an increase in the EDC mixture was associated with higher risk of persistent nausea, due to ΣDiNCH, ethylparaben, and the sum of di-2-ethylhexyl phthalate (ΣDEHP) metabolites; consistently, using BMKR, we identified a marginally positive relationship between all EDCs and persistent nausea. These associations appeared to differ by the sex of the fetus, such that in women carrying males, ethylparaben was associated with persistent nausea. Also, in women carrying males, the EDC mixture was associated with higher risk of persistent nausea modeled using QGComp (driven by ethylparaben and ΣDiNCH) and BKMR. We did not identify any associations in women carrying females or any non-linear relationships or chemical-chemical interactions in any group.

To better understand the pathways underlying this relationship, we explored associations of EDCs with hormones and of hormones with persistent nausea. In Chapter Three, we investigated overall and fetal sex-specific associations of non-persistent EDC biomarkers with maternal sex-steroid and thyroid hormones using traditional regression methods, as well as weighted quantile sums regression (WQSR) and BKMR to model an EDC mixture. This research was published in the journal *Environment International* (Ryva et al., 2024). We confirmed our hypothesis and identified associations of non-persistent EDCs (and their mixture) with sex-steroid and thyroid hormones. Specifically, we reported that a mixture of

phthalate/replacement and phenol metabolites was associated with lower early-to-mid pregnancy testosterone in all women (driven by propylparaben and triclosan), higher total T4 in women carrying females (driven by monobenzyl phthalate (MBzP), 2,5-dichlorophenol, and propylparaben), and lower thyroid stimulating hormone (TSH) in women carrying males (driven by 2,5-dichlorophenol and propylparaben). Using BKMR, we identified associations of the EDC mixture at higher levels of exposure with lower levels of estradiol in all women, and we reported a potential non-linear association of the EDC biomarker mixture with estradiol in women carrying females. In general, the mixture was not associated with progesterone or free T4. Lastly, in Chapter Four, we evaluated hormonal determinants of persistent nausea in all women and considered differences by fetal sex using linear regression for single hormone analyses and WQSR to model the hormone milieu. In models evaluating hormones individually, we observed that testosterone and progesterone were associated with increased odds of persistent nausea. When modeled jointly, hormones were associated with higher odds of having persistent nausea (due to free T4, testosterone, and progesterone), never having nausea (due to testosterone, progesterone, and free T4), and having irregular nausea (due to progesterone and testosterone). We also identified meaningful differences by fetal sex. Specifically, the observed relationship of testosterone (and the hormone mixture) with persistent nausea was primarily driven by women carrying males.

Plausible biological mechanisms of identified associations

Despite the observational nature of our studies, determining sensible, biological mechanisms underlying the association of EDCs with persistent nausea during pregnancy is critical. To accomplish this, we aimed to understand mechanisms underlying endocrine disruption and nausea during pregnancy. Obviously, as these chemicals are classified as endocrine disruptors, phthalates and phenols are known to alter hormones in cell culture, animals, and humans (Vandenberg et al., 2012). However, the literature focusing on relationships between EDCs and sex-steroid or thyroid hormones during pregnancy is inconsistent, with studies reporting positive, negative, and null associations (Aker et al., 2019; Aker et al., 2016; Aung et al., 2017; Berger et al., 2018; Cathey et al., 2019; Derakhshan et al., 2021a; Derakhshan et al., 2019; Huang et al., 2022; Johns et al., 2015; Kolatorova et al., 2018; Nakiwala et al., 2022; Pacyga et al., 2021; Romano et al., 2018; Sarzo et al., 2022; Souter et al., 2020; Yang et al., 2022). Our findings in Chapter Three, consistent with some other studies, suggest that

both sex-steroid and thyroid hormones were associated with mid-pregnancy hormone levels. Our results showing possible anti-androgenic action of EDCs are supported by prior *in vitro* and *in vivo* studies that demonstrated weak binding to androgen receptors, as well as anti-androgenic properties; however, most prior studies were within the context of male reproductive health (Gray et al., 2006; Howdeshell et al., 2017; Parks et al., 2000). In addition, we reported a non-linear relationship of an EDC mixture with estradiol in women carrying females, where both the lowest and highest levels of exposure were associated with lower estradiol. As with testosterone, various experimental studies have shown weak estrogenic activity of EDCs (Harris et al., 1997; Jobling et al., 1995; Liang et al., 2023). Our non-linear findings were not surprising but somewhat unexpected within the context of epidemiologic research, as previous experimental studies have reported non-linear relationships, such as u-shaped curves, inverted u-shape curves, and s-shape curves, due to dose-dependent disruption of genes, proteins, and receptors (reviewed by (Vandenberg et al., 2012)). Beyond sex-steroid hormones, we showed that EDCs are also associated with thyroid hormones, such as TT4 and TSH. Because of complex endocrine feedback loops, EDCs could be acting at the hypothalamus, pituitary gland, or thyroid gland. While the exact mechanism of action is hard to elucidate in epidemiologic studies, animal studies have reported that bisphenols can act as thyroid hormone receptor antagonists (Kim and Park, 2019). Overall, we identified associations of EDCs with pregnancy hormones in women that may be related to mechanisms of action identified in experimental studies.

As the exact mechanisms underlying nausea during pregnancy are not known, in this dissertation, our primary hypothesis focused on the most agreed-upon mechanism, where nausea symptoms are caused by perturbation to pregnancy hormones (Fejzo et al., 2019b; Liu et al., 2021). Thus, in Chapter Four, we evaluated if mid-pregnancy sex-steroid or thyroid hormones were associated with nausea during pregnancy. In our study, testosterone—individually and after taking into account other hormones—was associated with persistent nausea, which agrees with a prior epidemiologic study in 129 Scandinavian women that identified an association between testosterone and nausea at 33 weeks gestation (Carlsen et al., 2003). Interestingly, one older study in baboons identified androgen receptors in the gastrointestinal tract, primarily the tunica muscularis which is responsible for peristalsis (Winborn et al., 1987), which could indicate the mechanism of action is occurring at the gut. A more recent study demonstrated that female mice with irritable bowel syndrome,

characterized by having both diarrhea and constipation, had lower levels of circulating testosterone (Rastelli et al., 2022). As this mouse study also reported that lower androgen levels slow gastric transit time, it is conceivable that higher levels of androgens may increase gastric emptying time and contribute to nausea. However, as both slowed and faster gastric emptying time can contribute to nausea symptoms, it may be difficult to tease out whether this is the mechanism responsible for nausea in pregnant women. In contrast to testosterone, progesterone was not associated with nausea during pregnancy in prior epidemiologic studies (Lagiou et al., 2003; Masson et al., 1985). However, in our study, progesterone was associated (both individually and within the context of other hormones) with persistent nausea. In animal studies, progesterone has been shown to impact the gastrointestinal tract by altering gastric emptying in both dose and sex-dependent ways, specifically through slowing gut motility, altering gallbladder response, and reducing esophageal sphincter tone (Coquoz et al., 2022). Regarding sex-steroid hormones, an older study investigated the role of progesterone, estradiol, and testosterone in gastric transit and emptying and reported that testosterone did not play a role, progesterone increased gastric emptying, and a mixture of estradiol and progesterone inhibited gastric emptying (Chen et al., 1995), which highlights the importance of investigating hormone mixtures, as we did in this study. Interestingly, despite not being associated with nausea individually, FT4 was the major driver of the identified joint association between hormones and persistent nausea. Consistent with our individual-hormone findings, the Holistic Approach to Pregnancy and the first Postpartum Year (HAPPY) study, a large pregnancy cohort (n=1,682), also did not observe any relationships between thyroid hormones and nausea during pregnancy (Dekkers et al., 2020); however, they did not model hormones jointly. It is known clinically, that in non-pregnant individuals, lower levels of thyroid hormones, hypothyroidism (and to a lesser extent higher levels of thyroid hormones, hyperthyroidism), can cause nausea and vomiting (Rosenthal et al., 1976; Sweet et al., 2010). Furthermore, triiodothyronine (T3), the active metabolite of T4 induces expression of *RYR2*, which encodes ryanodine receptor 2, a calcium channel located in the brain's vomiting center (Fejzo et al., 2017). These results and potential mechanisms highlight the complexity of nausea during pregnancy, which likely involves multiple components of the GI tract, as well as the nausea and vomiting centers in the brain.

Within our central hypothesis, was an implicit proposal that EDCs are associated with persistent nausea through their disruption of gestational hormones; however, despite

identifying strong associations of EDCs with nausea, of EDCs with hormones, and of hormones with nausea in Chapters Two, Three, and Four, we were not able to elucidate strong hormonal candidates that could explain the relationships between EDCs and nausea. Specifically, although higher levels of ΣDiNCH, ethylparaben, an EDC mixture, progesterone, testosterone, and a hormone mixture were associated with persistent nausea, there was no specific EDC-to-hormone relationships that could explain this particular relationship of EDCs with persistent nausea. One possible causal pathway was the relationship between ΣDiNCH and persistent nausea, partially explained by FT4; however, ΣDiNCH and FT4 were not associated, and FT4 was only associated with persistent nausea as part of the hormone mixture. We did confirm a lack of mediation using a formal mediation analysis that was not included in this dissertation. Other potential candidates linking EDC exposure to persistent nausea are described in the Future Directions section below.

The role of fetal sex in EDC exposure and nausea during pregnancy

Throughout this dissertation, we identified that many important findings overall differed depending on whether a woman was carrying a female or male fetus. For example, both the relationship of the EDC mixture with persistent nausea and the association of testosterone with persistent nausea were strongest in women carrying males. Differences such as these are not surprising, as many pregnancy complications, like early pregnancy loss, stillbirth, and preeclampsia differ by fetal sex (Inkster et al., 2021). Furthermore, previous research has reported sexually-dimorphic responses to EDCs in relation to pregnancy sex-steroid hormones (Pacyga et al., 2021) and pregnancy outcomes, such as preeclampsia (Cantonwine et al., 2016) and gestational weight gain (Pacyga et al., 2023). In addition, other studies suggest that NVP is a sexually dimorphic condition (Mitsuda et al., 2019; Young et al., 2021), although persistent nausea did not differ meaningfully by fetal sex in our study. Our sex-specific findings could be explained by placental differences between male and female fetuses as placentae are sexed organs with differences in both function and morphology (Gabory et al., 2013; Graves, 2010; Meakin et al., 2021; Rich-Edwards et al., 2001). In addition, X chromosome inactivation in female fetuses, Y chromosome presence in male fetuses, and sex-steroid hormone differences in male and female fetuses could explain why relationship differ by sex (Inkster et al., 2021). For example, women carrying males have higher circulating testosterone concentrations relative to women carrying females (Inkster et al., 2021; Meulenberg and Hofman, 1991). Sex-specific findings may strengthen the

biological plausibility of our identified relationship in this dissertation, as we would be unlikely to observe starkly sexually dimorphic findings by chance alone. Our findings further support the need for all future research investigating environmental exposures within the context of pregnancy, especially nausea during pregnancy, to consider differences in all relationships by fetal sex.

Strengths, Limitations, and Future Directions:

The I-KIDS cohort and collection of covariate information

While these studies have many strengths, there were limitations that should be addressed in future scholarship. A critical strength of our work was leveraging the vast information collected from the I-KIDS pregnancy and birth cohort, which included copious information for use in modeling exposures, outcomes, and covariates. We were able to account for many potential confounders, such as factors related to socioeconomic status (race/ethnicity, education), reproductive health (maternal age, parity), and general health (body mass index, early pregnancy alcohol use, early pregnancy perceived stress score). Importantly, I-KIDS collected information on early pregnancy diet quality and fragrant-product usage, which are not commonly assessed in environmental epidemiologic studies, but may confound the relationship between EDCS and nausea during pregnancy. Additionally, we were able to assess whether relationships differed depending on whether a woman was carrying a male or female fetus in all analyses. However, because most women in I-KIDS are Non-Hispanic White, well-educated, and relatively healthy, as I-KIDS excluded women in a high-risk pregnancy, our findings may have limited generalizability. That being said, the cohort's homogeneity may reduce potential unmeasured confounding and isolate biological relationships. Future studies should evaluate the relationship of EDC biomarkers with nausea during pregnancy in more diverse cohorts that include higher-risk pregnancies.

Evaluation of nausea during pregnancy

A critical strength of the I-KIDS cohort was the evaluation of nausea symptoms five times across pregnancy that allowed us to assess a unique outcome—nausea persistence—in relation to typical nausea that subsides near the end of the first trimester. However, I-KIDS did not assess nausea during pregnancy using validated questionnaires such as Pregnancy Unique Quantification of Emesis (PUQE), which provides information on nausea and vomiting symptoms and severity within the last 24-hours, or Nausea and Vomiting during Pregnancy

Quality of Life (NVP QOL), which highlight impacts on women's quality of life (Lacasse and Berard, 2008; Nelson-Piercy et al., 2024). As these scales are used clinically, it may be necessary to replicate our findings in a cohort that assesses nausea during pregnancy using validated outcome measurements; however, our approach of repeatedly querying nausea symptomology allowed us to capture nausea that persists beyond early pregnancy, which was the primary goal of our study. Furthermore, while I-KIDS did collect information on vomiting during pregnancy, there were very few women who had persistent vomiting, so we were underpowered to assess associations of chemical biomarkers with pregnancy vomiting. As electronic medical records abstraction is ongoing in our study, we did not have formal diagnosis of hyperemesis gravidarum. However, early in our study, we were informed that our medical abstraction protocol did not identify any women with hyperemesis gravidarum; this informal report, together with the healthy nature of the women in our study make it unlikely that diagnoses of hyperemesis gravidarum drove our findings. Therefore, future studies may be needed to identify associations of EDCs with more severe forms of nausea and vomiting in pregnancy.

Measurement of endocrine disrupting chemical concentrations

Another major strength of our study was the rigorous assessment of chemical exposure in I-KIDS, which has data on 31 urinary EDC biomarkers from important chemical classes to approximate exposure to eight phthalates, two phthalate replacements, and 11 phenols, as well as triclocarban (a non-phthalate, non-phenol EDC). Importantly, relatively newer replacement chemicals, such as DEHTP, DiNCH, and bisphenol S, were measured. Key to the robustness of our findings was I-KIDS' use of multiple, across pregnancy urinary biomarkers assessed at the Center for Disease Control and Prevention (CDC), which is the gold standard for assessment of non-persistent EDC biomarkers with short half-lives and high inter-person variability (Silva et al., 2007; Vernet et al., 2019). Even though our pooled approach was performed to reduce the high variability in EDC measurement, it resulted in some of our exposure occurring after our outcomes of interest (hormones and persistent nausea). However, as the variability of phthalate and phenol concentrations across pregnancy is more likely due to random variation rather than actual concentration changes, our pooled assessment likely reflects a stable estimate of exposure at any one point during pregnancy (Rosen et al., 2023). Despite this likelihood, in order to rule out reverse causation, future studies should assess EDC biomarkers prior to pregnancy, at multiple timepoints

across pregnancy, and particularly before the health outcomes of interest (nausea). Although we assessed many important non-persistent EDCs as individual exposures and as a mixture of co-exposures using multiple statistical mixture methods, there are numerous environmental exposures that were not addressed in this dissertation, such as pesticides (e.g., glyphosate), persistent chemicals (e.g., per- and polyfluoroalkyl substances), and heavy metals (e.g., arsenic). These chemicals, along with any additional chemicals considered as potentially harmful to human health by the CDC National Biomonitoring Program in the future, should be studied within the context of nausea as both single exposures and as co-exposures, ideally alongside the non-persistent EDCs evaluated in our research.

Quantification of gestational hormone levels

Another strength was that we measured six critical mid-pregnancy sex-steroid and thyroid hormones in blood (plasma), which is considered the gold standard for hormone assessment. In prior studies, these hormones have been shown to be important for pregnancy health (Hacker et al., 2010; Silva et al., 2018) and have been implicated in nausea during pregnancy (Carlsen et al., 2003; Dekkers et al., 2020; Lagiou et al., 2003). To our knowledge, we were also the first group to use a statistical mixture method developed for environmental mixtures to more accurately model the complex hormonal milieu in pregnant women. However, as we already discussed, none of our measured hormones appeared to be responsible for the relationship of EDCs with persistent nausea, and, as with the measure chemical exposures, although we measured critical pregnancy hormones, there are many other hormones to be studied in the future. For example, serotonin, the neurotransmitter and hormone, plays a critical role in nausea and vomiting outside of pregnancy, and ondansetron, a serotonin agonist, has been used as an anti-emetic agent for decades. While prior research has demonstrated that serotonin is produced by the placenta and that pregnant women have higher levels of circulating serotonin at the second trimester and at birth (Adibi et al., 2024; Lonstein, 2019), ondansetron is not FDA-approved for nausea and vomiting during pregnancy and is currently only used off-label, despite potential adverse health effects (Ashour, 2023; Kaplan et al., 2019; Kennedy, 2016). There are several experimental studies investigating environmental exposures and serotonin; however, no studies have investigated the relationship of EDCs with serotonin in human pregnancies (Sarrouilhe et al., 2021). Thus, the relationship of EDCs with serotonin and of serotonin with persistent nausea should be explored. Another hormone to study is placental human growth/differentiation factor 15

(GDF15) which has been implicated in nausea and vomiting during pregnancy, as well as hyperemesis gravidarum, in recent research (Fejzo et al., 2023; Fejzo et al., 2019a; Fejzo et al., 2018; Fejzo et al., 2019b). While these studies implicate GDF15 in nausea and vomiting during pregnancy, no one has investigated whether this hormone interacts with other pregnancy hormones or whether endocrine disruptors alter GDF15 levels.

While phthalates and phenols are classically known as endocrine disruptors, they are also thought of as metabolic disruptors. However, because of the vast number of potential causal candidates proposed for nausea during pregnancy, including hormones, immune biomarkers, metabolic factors, etc., it will likely require untargeted metabolomic or proteomic methods. Beyond investigating other hormones (and hormone mixtures) or metabolic biomarkers, in order to identify sensitive time windows, future studies should assess possible biological pathways at multiple timepoints across pregnancy, which would require biospecimen collection at each trimester, at least. If one or several causal candidates were identified, then we could use causal mediation methods to determine what portion of the relationship of EDCs with persistent nausea is explained by these biological factors. However, one limitation of current methodology is that most mediation methods cannot model the relationship of a mixture of exposures with a mixture of mediators within the context of health outcomes of interest (Bellavia et al., 2019; Blum et al., 2020). As new methodologies are still under development, current methods test single exposures and single mediators one at a time, which assumes a single, critical EDC and disregards the hormonal milieu that is more likely involved in nausea during pregnancy. One option would be to use dimensional reduction techniques, such as principal component analysis, to limit our potential exposures and mediators; however, as the components may not be easily mapped onto clear exposure profiles or molecular pathways, these results would potentially be difficult to interpret. Future work is needed to develop statistical models that can handle high dimensional exposures and mediators in order to understand the biological relationships between mixtures of chemicals, mixtures of hormone and/or metabolic biomarkers, and health outcomes.

Clinical Implications

There are some potential clinical implications of our research. First, one interesting aspect of this dissertation is the way in which we modeled nausea persistence. While previous studies have tried to understand typical nausea (commonly referred to as morning sickness that

occurs in early pregnancy) or hyperemesis gravidarum (the more severe form of nausea and vomiting during pregnancy), less work has focused on subclinical nausea from which women suffer for long periods of time in pregnancy. In fact, our study suggests that combining women with early occurring (typical) and persistent nausea in one group risks outcome misclassification as these women's symptoms are likely different, with possibly varied causes. Conceptualizing nausea during pregnancy as being along a continuum of typical/transient morning sickness to persistent nausea to hyperemesis gravidarum may be beneficial in teasing out causes, both environmental and physiological, as well as impacts on maternal and fetal health. Thus, substantially more future work will be needed to better understand various types of subclinical nausea within the context of women's and children's health.

The second clinical implication of our research is that non-pharmaceutical interventions that reduce chemical exposures may alleviate nausea symptoms. Current clinical guidelines already recommend dietary modifications, such as consuming smaller portions, avoiding certain foods, or supplementing with vitamins (Bustos et al., 2017; Lee and Saha, 2011; Matthews et al., 2014; Niebyl, 2010); however, there are no current clinical recommendations on reducing use of certain personal care products or avoiding specific chemicals in food packaging materials and plastics. Recent review articles have summarized the literature on intervention studies to reduce EDC exposure (Martin et al., 2022; Park et al., 2022; Sieck et al., 2024; Yang et al., 2023). However, only a few studies have focused on pregnant women, with mixed results. For example, one study of ten low-income pregnant women provided women with organic foods for three days prepared using stainless steel and reported no changes in phthalate metabolites at the end of the study (Barrett et al., 2015). A different randomized controlled trial of 230 pregnant women (152 in intervention and 78 in control) provided workshops on reducing EDC exposures and did not identify changes in paraben levels following the intervention (El Ouazzani et al., 2021). However, one study of 35 pregnant women provided education on reducing exposure through diet and personal care product use as the intervention and reported reductions in phthalate metabolite biomarkers (Wu et al., 2021), while a different study in only eight non-pregnant women reported lower urinary paraben and triclosan levels when women were provided with replacement products that did not contain parabens, benzophenones, triclocarban, triclosan, or BPA (Koch et al., 2014). Paradoxically, a study of 100 adolescent females reported higher ethylparaben levels after an intervention to alter personal care product use; however, this may have been due to

mislabeled replacement products and may not be as relevant for pregnant populations (Harley et al., 2016). Interventions to reduce chemical exposures through modifying diet or personal care product use may be necessary to better target interventions to women to potentially decrease nausea symptomology or persistence; however, care will need to be taken not to inadvertently increase exposures to other chemicals.

Conclusions

In this dissertation, we confirmed our hypothesis that non-persistent EDCs from both food packaging materials and personal care products are associated with nausea during pregnancy—an understudied pregnancy symptom that affects the majority of women during pregnancy and impacts quality of life and long-term health. However, despite identifying relationships of both EDCs and mid-pregnancy hormones with persistent nausea during pregnancy, we did not identify a plausible biological pathway connecting EDCs to persistent nausea through gestational hormones. Thus, future work is needed to identify the biological pathways of our identified overall association, as well as identify determinants of chemical exposure that can inform interventions to reduce EDC exposure during pregnancy.

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