A PROPOSAL TO INVESTIGATE THE ROLES OF MATERNAL INFLAMMATION AND DIET IN ASSOCIATIONS OF MATERNAL PARABEN CONCENTRATIONS WITH GESTATIONAL LENGTH

By

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ABSTRACT

A PROPOSAL TO INVESTIGATE THE ROLES OF MATERNAL INFLAMMATION AND DIET IN ASSOCIATIONS OF MATERNAL PARABEN CONCENTRATIONS WITH GESTATIONAL LENGTH

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The proposed research will fill a public health need by providing information about the roles inflammation and dietary interventions play in associations of maternal paraben concentrations with shorter gestation. Specifically, this study proposes to investigate the maternal inflammatory pathways linking parabens with shorter gestation, as well as the potential intervening effect of a maternal anti-inflammatory diet on shorter gestation in response to parabens. The central hypothesis is that higher paraben concentrations are associated with shorter gestation due to elevated maternal inflammation and that an anti-inflammatory maternal diet mitigates these relationships. The proposed study will test this central hypothesis in 482 pregnant women enrolled in the Illinois Kids Development Study (I-KIDS), which is an ongoing prospective pregnancy and birth cohort with the primary goal of evaluating the impacts of prenatal chemical exposures on infant neurodevelopment. The feasibility of testing these hypotheses has been determined by conducting several preliminary studies in a sub-sample of 294 I-KIDS women. Overall, findings from this study will inform future research and clinical practice about the biological targets of parabens during pregnancy and guide prenatal healthcare professionals to make effective dietary recommendations to their pregnant patients.

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KEY TO ABBREVIATIONS

AHEI-2010	Alternative Healthy Eating Index-2010
BMI	Body mass index
BPA	Bisphenol A
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CRP	C-reactive protein
DAG	Directed acyclic graph
EDC	Endocrine disrupting chemicals
E-DII	Empirical Dietary Inflammatory Index
FFQ	Food Frequency Questionnaire
IFN	Interferon
I-KIDS	Illinois Kids Development Study
IL	Interleukin
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
PCA	Principal Component Analysis
SG	Specific gravity
TGF	Transforming growth factor
TNF	Tumor necrosis factor
U.S.	United States

A. OBJECTIVES

Shorter gestation is one of the leading risk factors associated with poor fetal development and adverse maternal and child outcomes. Even early-term infants (37-39 weeks), not just those born pre-term (<37 weeks), have higher risk of adverse birth and postnatal outcomes compared to full-term infants (39-41 weeks). Pregnant women are ubiquitously exposed to environmental pollutants, including parabens, which are anti-microbial agents found in personal care products and cosmetics. In pregnant populations with lower pre-term birth prevalence, we and others have observed that higher maternal paraben concentrations are associated with shorter gestation. While the precise mechanisms of parabens are poorly understood, several studies suggest that parabens may alter immune cell function, as well as mRNA expression or circulating levels of individual cytokines. This is concerning because trimester-specific inflammatory profiles play a critical role in regulating gestational length. Specifically, the second trimester is uniquely anti-inflammatory to support fetal development, but little is known about the impacts of parabens on maternal inflammatory patterns during the second trimester, and no study has evaluated whether associations of parabens with gestational length are due to inflammation.

A healthy maternal diet is critical for proper fetal development, and the adverse consequences of some persistent chemical exposures (e.g. mercury, lead, and air pollution) have been shown to be mitigated by improving certain components of the maternal diet. However, previous studies only focused on individual nutrients rather than on overall maternal diet quality, making it difficult to provide relevant public health

messages to pregnant women. To address this, our preliminary findings suggest that better overall maternal diet, measured by an index that focuses on diet quality, is protective against shorter gestation in response to parabens. This protective effect may be due to the anti-inflammatory nature of healthy diets, as numerous studies have shown that a healthy maternal diet is associated with reduced maternal inflammation and improved gestational length. However, it is unknown whether a maternal low-inflammatory diet, specifically, can mitigate the negative effects of parabens on gestational length.

Therefore, the overarching goal is to investigate the role of maternal inflammation in the relationship between parabens and shorter gestation, and evaluate how an anti-inflammatory maternal diet interacts with paraben concentrations. The central hypothesis is that parabens are associated with shorter gestation due to elevated maternal inflammation and that an anti-inflammatory maternal diet mitigates these relationships. The central hypothesis will be tested in 482 women enrolled in the Illinois Kids Development Study (I-KIDS), a prospective pregnancy and birth cohort evaluating the impacts of prenatal chemical exposures on infant neurodevelopment in two specific aims that are summarized in **Figure 1**.

B. SPECIFIC AIMS

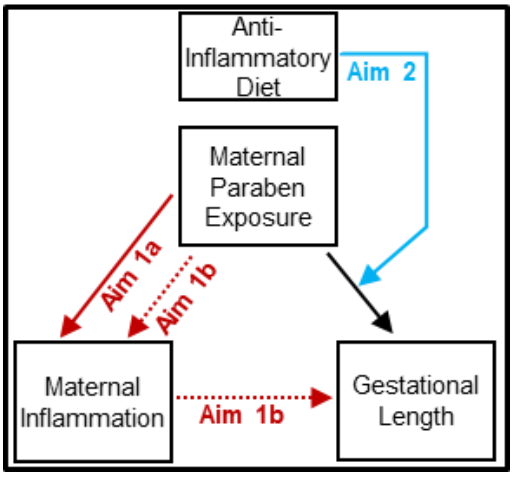

Figure 1. Summary of Specific Aims 1 and 2. Specific Aim 1a will evaluate associations of parabens with maternal inflammation, while Specific Aim 1b will assessed whether associations of parabens with gestational length can be explained by changes in maternal inflammation. Specific Aim 2 will determine whether associations of parabens with gestational length are different by maternal inflammatory diet quality.

B.1. Specific Aim 1.

Specific Aim 1 will evaluate associations of maternal gestational paraben concentrations with maternal inflammation (Aim 1a). Maternal concentrations of four parabens (butyl, ethyl, methyl, propyl) have already been measured in a pool of 5 cross-pregnancy urine samples. A panel of 21 critical pro- and anti- inflammatory cytokines will be analyzed in maternal fasting plasma samples collected at median 17 (range: 13 - 22) weeks gestation, and principal component analysis (PCA) will be used to identify patterns of cytokine concentrations to characterize maternal second trimester inflammatory profiles. Our primary hypothesis is that higher paraben concentrations are associated with elevated maternal inflammation. If Aim 1a suggests that parabens are pro-inflammatory, we may pursue an exploratory Aim 1b to assess whether associations of parabens with shorter gestation can be partly explained by elevated maternal inflammation in response to

parabens. The research accomplished in Specific Aim 1 will determine whether parabens can alter critical maternal inflammatory profiles implicated with shorter gestation.

B.2. Specific Aim 2.

Specific Aim 2 will evaluate differences in associations of maternal paraben concentrations with shorter gestation by a maternal inflammatory diet index. To assess the anti-inflammatory potential of maternal diets in I-KIDS, the validated Empirical Dietary Inflammatory Index (E-DII) will be calculated using average values from three-month food frequency questionnaires administered at 10-14 and 34-36 weeks gestation. Maternal paraben concentrations will be measured as described in Specific Aim 1. Our primary hypothesis is that an anti-inflammatory maternal diet mitigates known associations of parabens with shorter gestation. Completion of Specific Aim 2 will ascertain the intervening effects of an anti-inflammatory maternal diet in response to parabens.

C. BACKGROUND/SIGNIFICANCE

This project will investigate the roles of maternal inflammation and diet quality in the associations of maternal paraben concentrations with shorter gestation in a population of pregnant women. Specifically, these studies will assess whether parabens are associated with altered maternal second trimester inflammation and whether a maternal anti-inflammatory diet protects against shorter gestation in response to parabens. These studies will address a knowledge gap about the gestational molecular targets of parabens and will provide a clinically relevant dietary intervention for mitigating adverse outcomes in response to parabens. The scientific premise is that parabens are pro-inflammatory compounds associated with shorter gestation and that mothers consuming an anti-inflammatory diet are protected against the adverse effects of parabens.

Shorter gestation is the leading risk factor for adverse fetal growth and development. Pre-term birth (<37 weeks gestation) is a leading cause of infant death worldwide and the United States (U.S.) pre-term birth prevalence is ~10% (1). Pre-term birth is associated with life-long adverse health outcomes in offspring, including poor birth outcomes, respiratory problems, cognitive problems, metabolic syndrome, and cardiovascular disease (2). Although infants born pre-term are at greatest risk of complications, even early-term infants born between 37-39 weeks have higher risk of adverse outcomes compared to full-term infants born between 39-41 weeks (3). For example, compared to infants born at 39-41 weeks gestation, those born before 39 weeks have higher risk of poor cognitive and motor development during the first year of life, and higher risk of respiratory problems and hypoglycemia right after birth (4, 5). However, relatively few

studies have focused on evaluating risk factors for shorter gestation in predominately full-term populations.

Inflammation plays a major role in shortening gestation. Pregnancy is characterized by distinct trimester-specific inflammatory profiles that regulate pregnancy progression and fetal development (6). Elevated maternal inflammation during the first and third trimesters is critical for implantation and parturition, respectively (6). Conversely, the second trimester is an anti-inflammatory state that supports fetal development by ensuring maternal tolerance of fetal antigens (7). Dysregulated maternal inflammation, especially during the second trimester, may be linked to adverse birth outcomes, including earlier birth (8, 9). Therefore, identifying factors associated with altered maternal inflammation is critical for developing interventions against shorter gestation.

Parabens may be pro-inflammatory compounds associated with shorter gestation. Parabens (butyl, ethyl, methyl, propyl) are anti-microbial agents used in personal care products, cosmetics, some food products, and medications (10). Over 99% of U.S. pregnant women have measurable levels of at least one paraben in their urine (11). This is concerning because parabens are endocrine disrupting chemicals (EDCs) that target reproductive tissues and hormones (12-16). Additionally, higher paraben concentrations are associated with shorter gestation (17-20), which agrees with our preliminary findings. Some observational studies in pregnant women suggest that parabens alter inflammatory markers (21-23), and experimental studies show that parabens impact immune cell function and pro-inflammatory cytokine expression (24-26). However, to our knowledge,

no study has evaluated associations of maternal paraben concentrations with maternal second trimester inflammation (Aim 1a) or assessed if inflammation partly explains associations of paraben concentrations with shorter gestation (Aim 1b).

An anti-inflammatory maternal diet may protect against shorter gestation in response to parabens. Appropriate maternal nutrition is critical for fetal development, while poor diet quality during key gestational periods is associated with adverse pregnancy outcomes, including shorter gestation (27). Observational studies and randomized controlled trials in non-pregnant populations found that healthier diets are associated with lower inflammation (28-31), likely due to the anti-inflammatory nature of healthy diets. Importantly, studies in pregnant populations suggest that the adverse effects of some chemical exposures can be mitigated by improving maternal diets (32-34). However, these studies focused on individual nutrients rather than overall diet quality, which does not reflect dietary patterns in pregnant women and is difficult to translate into actionable food-based interventions. Our preliminary studies suggest that better overall diet quality mitigates associations of parabens with shorter gestation, but it is unknown whether an anti-inflammatory diet, specifically, protects against shorter gestation in response to parabens (Aim 2).

This research will be among the first epidemiological studies to investigate the roles of maternal inflammation and diet in associations of maternal paraben concentrations with shorter gestation. This proposal will provide insight into potential biological targets of parabens and inform an effective dietary intervention against parabens.

D. PRELIMINARY STUDIES/PROGRESS REPORT

D.1. Specific Aim 1.

D.1.1. Rationale.

Shorter gestation is a leading risk factor for adverse fetal growth and development, and inflammation appears to play a major role (8, 9, 35). The second trimester of pregnancy is an anti-inflammatory period responsible for fetal growth (6, 7), and several studies suggest that elevated second trimester inflammation may predict shorter gestation (8, 9, 36). Anti-microbial agents like parabens are thought to disrupt gestational inflammatory pathways (**Table 1**) (21-23). For example, a prospective study of Northern Puerto Rican women from the PROTECT birth cohort (n=141) found negative associations of butyl and propyl parabens with pro-inflammatory CRP (21). A small study of Michigan women from the MMIP birth cohort (n=56) only found a negative association between butyl paraben and pro-inflammatory IL-6 (22). However, a nested case-control study of Boston women from the LIFECODES cohort (130 pre- and 352 full-term births) found negative associations of ethyl paraben with pro-inflammatory interleukin (IL) 1β (IL-1β) and tumor necrosis factor (TNF) α (TNFα), but positive associations of methyl and propyl parabens with pro-inflammatory IL-6 and C-reactive protein (CRP), respectively (23).

Results from these studies are supported by mechanistic studies and an observational study in a non-pregnant population. One *in vitro* study found that exposing peripheral human lymphocytes to parabens (butyl, ethyl, methyl, propyl) inhibited the release of lysosomal enzymes compared to the control group at concentrations as low as 0.06 mmol/L, which could disrupt critical immune pathways (25). Additionally, a study in

zebrafish found higher mRNA levels of TNFα (pro-inflammatory cytokine) after exposure to methyl and propyl parabens, but lower mRNA levels of IL-8 (pro-inflammatory cytokine) after exposure to propyl paraben at 1μM or 10μM compared to the control group (24). In a cross-sectional analysis of data from U.S. adults, higher paraben concentrations were associated with reduced inflammatory bowel disease symptoms, which authors suggested could connect parabens to alterations in gut inflammatory pathways (37). The precise cellular mechanisms linking parabens to inflammation are unknown, but may be related to the endocrine disrupting properties of parabens since immune cells express hormone receptors (38).

Table 1. Gestational associations of parabens with select maternal inflammatory markers.

Cytokine	Cohort	Butyl	Ethyl	Methyl	Propyl
CRP	LIFECODES	Ø	Ø	Ø	POSITIVE
	PROTECT	NEGATIVE	NA	Ø	NEGATIVE
	MMIP	NA	NA	NA	NA
IL-1β	LIFECODES	Ø	NEGATIVE	Ø	Ø
	PROTECT	Ø	NA	Ø	Ø
	MMIP	Ø	Ø	Ø	Ø
IL-6	LIFECODES	Ø	Ø	POSITIVE	Ø
	PROTECT	Ø	NA	Ø	Ø
	MMIP	NEGATIVE	Ø	Ø	Ø
TNFα	LIFECODES	Ø	NEGATIVE	Ø	Ø
	PROTECT	Ø	NA	Ø	Ø
	MMIP	Ø	Ø	Ø	Ø
IL-10	LIFECODES	Ø	Ø	Ø	Ø
	PROTECT	Ø	NA	Ø	Ø
	MMIP	NA	NA	NA	NA

For cytokines, red and green denote pro- and anti-inflammatory, respectively. POSITIVE or NEGATIVE indicate the direction of significant associations between parabens and cytokines. NA, association not assessed. Ø, association was not significant at $P<0.05$. References for studies from LIFECODES (23), PROTECT (21), and MMIP (22).

Studies in pregnant women provide preliminary evidence that parabens may alter maternal inflammation in populations with high pre-term birth rates (21-23). However, additional research needs to evaluate these relationships in lower risk populations, since early-term infants are also at risk for adverse outcomes (3). Furthermore, two of the previous studies described above evaluated associations of parabens with inflammation across pregnancy (22, 23). Given that the second trimester is uniquely anti-inflammatory, evaluating associations of parabens with maternal inflammation during this time is especially important. Lastly, two studies focused on evaluating the impact of parabens on a limited number of individual cytokines (21, 23). Because inflammation is characterized by a complex network of pro- and anti-inflammatory compounds that interact to create and maintain a favorable fetal environment, characterizing patterns of several cytokine concentrations may better represent maternal inflammation status than individual cytokine concentrations (39). We will address these limitations in a low pre-term birth population and by assessing maternal second trimester inflammation using a composite of anti- and pro-inflammatory cytokines. The feasibility of completing **Specific Aim 1** using I-KIDS data is supported by the following preliminary studies.

D.1.2. Preliminary study 1: urinary paraben concentrations.

Paraben data needed to address the proposed aims are already available for all 482 women, and our preliminary findings suggest that 100% of I-KIDS women had measurable (non-zero) levels of urinary methyl and propyl parabens, while 66% and 99% had measurable levels of butyl and ethyl parabens, respectively (**Table 2**). Median urinary paraben concentrations in I-KIDS were highest for methyl and lowest for butyl paraben,

potentially because women have higher exposure to methyl paraben due to its widespread use in consumer products (40). Median urinary paraben concentrations in I-KIDS women were somewhat lower than those in U.S. reproductive-aged women from the 2013-14 and 2015-16 National Health and Nutrition Examination Surveys (NHANES) (41, 42).

Table 2. Urinary paraben concentrations in I-KIDS.

	I-KIDS women with measurable levels[1]	I-KIDS 2013-2018 (n=482)	NHANES 2013-2016 (n=743)[2]
Paraben	%	Median (25^{th}, 75^{th} percentile) in ng/mL	
Butyl	66	0.1 (0, 0.3)	0.1 (0.1, 0.5)
Ethyl	99	1.3 (0.5, 6.8)	2.2 (0.7, 14.0)
Methyl	100	50.2 (18.2, 133.8)	86.5 (19.0, 266.4)
Propyl	100	8.4 (2.2, 27.7)	15.4 (2.9, 69.5)

[1]Non-zero concentrations; [2]Urinary paraben concentrations of 18-40-year-old U.S. females from 2013-14 and 2015-16 NHANES survey years. NHANES, National Health and Nutrition Examination Survey.

D.1.3. Preliminary study 2: parabens and gestational length.

We preliminarily evaluated associations of four parabens with gestational length in the first 294 I-KIDS women who have paraben and gestational length data (**Figure 2**). Overall, we found that parabens tended to be associated with shorter gestation. Specifically, every 2-fold increase in ethyl, methyl, and propyl paraben was non-significantly associated with 0.3 (95% CI: -0.05, 0.6), 0.5 (95% CI: -0.02, 1.0), and 0.4 (95% CI: -0.1, 0.8) day decreases in gestational length, respectively.

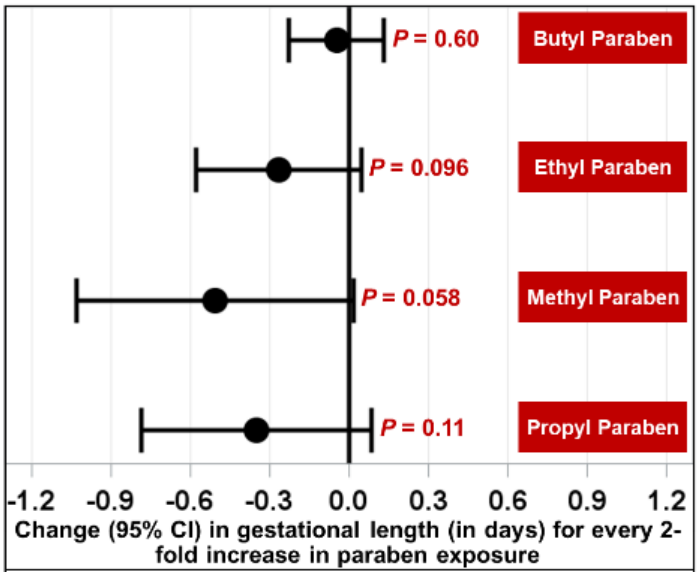

Figure 2. Associations of parabens with gestational length. Parabens were assessed in a pool of five cross-pregnancy urines, specific gravity-adjusted, and ln-transformed. Multivariable linear regression models controlled for maternal age, pre-pregnancy body mass index (BMI), race/ethnicity, education, parity, smoking since conception, second-hand smoke exposure, conception season, diet quality, and fetal sex. Data were back-transformed to represent change in gestational length (95% CI) in days for every 2-fold increase in paraben concentration. CI, confidence interval. n=294.

D.1.4. Conclusions.

As previously discussed, several studies suggest that parabens may impact maternal inflammation (21-26, 37). Given that higher second trimester maternal inflammation may be predictive of shorter gestation (8, 9, 36), the primary objective of Aim 1a is to evaluate whether maternal paraben concentrations are associated with elevated maternal early second trimester inflammation. If Aim 1a suggests that parabens are pro-inflammatory, we may pursue an exploratory Aim 1b to ask whether associations of parabens with shorter gestation are partly explained by elevated maternal inflammation in response to parabens.

D.2. Specific Aim 2.

D.2.1. Rationale.

Maternal diet is an important determinant of fetal health, and in epidemiological studies, poor maternal diet quality during key gestational periods is associated with adverse birth outcomes, including shorter gestation (27). Pregnant women are ubiquitously exposed to parabens, which we and others have shown are associated with shorter gestation (17-20). The adverse consequences of some chemical exposures (e.g. mercury, lead, and air pollution) have been shown to be mitigated by improving maternal diet. For example, in pregnant Boston women, higher mercury levels were associated with poorer child cognitive performance at age 3, which was attenuated in women who consumed >2 weekly servings of fish – a major source of polyunsaturated omega-3 fatty acids important for brain development (32). Another U.S. study evaluated the importance of maternal calcium intake on the release of bone-stored lead into maternal circulation, and found that pregnant women with high calcium intake (>2000 mg/day) had lower blood lead levels than those with low intake (<600 mg/day) (33). Lastly, a prospective cohort study of Boston pregnant women with fertility problems found that associations of air pollution with decreased probability of live birth were attenuated with higher supplemental folate intake (~1000 µg/day) (34). Together, these studies suggest that maternal diet can intervene against the adverse consequences of chemical exposures, especially against exposures that we cannot avoid. However, these studies only focused on individual nutrients rather than on overall diet quality (32-34). This is a limitation since better overall maternal diet quality is protective against adverse gestational outcomes (27, 43), potentially due to the combined anti-inflammatory properties of whole foods in healthy diets (44-46).

Given previous studies (including our own preliminary findings) showing associations of parabens with shorter gestation (17-20), and the known protective effects of healthy maternal diets (27), the current study will investigate whether an anti-inflammatory maternal diet can mitigate associations of parabens with shorter gestation. The feasibility of completing Specific Aim 2 using I-KIDS data is supported by the following preliminary studies.

D.2.2. Preliminary study 1: maternal diet quality and gestational length.

Maternal diet quality in I-KIDS is measured using the Alterative Healthy Eating Index 2010 (AHEI-2010), which is a dietary index based on published research related to foods and nutrients predictive of chronic disease risk (47). The AHEI-2010 is scored out of 110 points using 11 dietary components, and a higher AHEI-2010 score signifies healthier diet quality predictive of lower chronic disease risk. In I-KIDS, the score is calculated using data collected from a three-month food frequency questionnaire (described later) administered at 10-14 and 34-36 weeks gestation. Consistent with other studies assessing associations of diet quality with pre-term birth risk (27), our preliminary findings suggest that a better maternal AHEI-2010 score is associated with longer gestation. Specifically, in fully adjusted models, each 10 point increase in AHEI-2010 (at 10-14 weeks) was associated with 1.2 day (95% CI: 0.2, 2.2) longer gestation (**Table 3**). These preliminary findings highlight the importance of high maternal diet quality for gestational length, and confirm that these associations exist in the I-KIDS population.

Table 3. Associations of AHEI-2010 with gestational length.		
Gestational age at diet assessment	Model 1: Unadjusted β (95%CI); *P*	Model 2: Adjusted[#] β (95%CI); *P*
10-14 weeks	0.9 (0.02, 1.8); 0.04	1.2 (0.2, 2.2), 0.02
34-36 weeks	0.3 (-0.6, 1.2); 0.48	0.4 (-0.6, 1.4); 0.42
Average	0.8 (-0.2, 1.7); 0.11	1.1 (-0.02, 2.2); 0.05
Gestational length change (days) for each 10-point increase in AHEI-2010. [#]Controlling for maternal age, pre-pregnancy BMI, race/ethnicity, education, parity, smoking since conception, conception season, and fetal sex. AHEI-2010, Alternative Healthy Eating Index 2010; CI, confidence interval. n=294.		

D.2.3. Preliminary study 2: parabens, gestational length, and diet quality.

As presented in Figure 2 and described in D.1.3., our preliminary data suggest that higher maternal paraben concentrations during pregnancy are associated with shorter gestational length. Our preliminary findings also suggest that the negative impacts of maternal parabens on gestation length may be mitigated in women with better diet quality. **Figure 3** demonstrates that previously observed overall associations only persisted in women who consumed a lower quality diet (AHEI-2010 score < median; red diamonds), such that 0.5 (95% CI: 0.1, 0.9) and 0.7 (95% CI: -0.003, 1.4) day decreases in gestation length were observed for every 2-fold increase in urinary ethyl and methyl paraben concentrations, respectively. In women who consumed a better-quality diet (AHEI-2010 score ≥ median), parabens were no longer associated with gestational length (green diamonds).

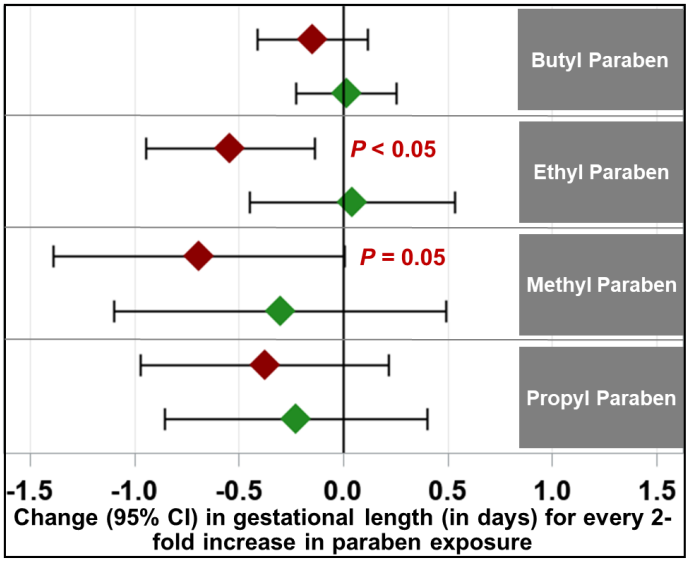

Figure 3. Associations of parabens with gestational length stratified by AHEI-2010. Parabens assessed in a pool of five cross-pregnancy urines were specific gravity-adjusted and ln-transformed. Multivariable linear regression models controlled for maternal age, pre-pregnancy BMI, race/ethnicity, education, conception season, smoking since conception, diet quality, parity, second-hand smoke exposure, fetal sex, and a multiplicative interaction between parabens and AHEI-2010. Data were back-transformed to represent change in gestational length (95%CI) in days for every 2-fold increase in paraben concentrations in women with AHEI-2010 < median (red diamond) and AHEI-2010 ≥ median (green diamond). AHEI-2010, Alternative Healthy Eating Index 2010; CI, confidence interval. n=294.

D.2.4. Conclusions.

We have shown that maternal diet is an important predictor of gestational length, and that a healthier maternal diet may protect against shorter gestation in response to parabens. These protective effects by better diet quality may be due to anti-inflammatory components of a healthy diet (30, 31). Therefore, the objective of Aim 2 is to test the hypothesis that an anti-inflammatory maternal diet mitigates known associations of parabens with shorter gestation.

E. RESEARCH DESIGN AND METHODS

E.1. Introduction.

The proposed studies will test the central hypothesis that elevated maternal inflammation partially explains associations of higher paraben concentrations with shorter gestation and that an anti-inflammatory maternal diet is protective against shorter gestation in response to parabens. To investigate this hypothesis, the proposed studies will expand on the aims of I-KIDS, an ongoing prospective pregnancy cohort with the primary goal of evaluating the impacts of gestational EDC exposures on child health. I-KIDS participants were recruited at the first prenatal visit from two local obstetric clinics in Champaign-Urbana, IL. Eligible women were 10-14 weeks gestation, 18-40 years old, fluent in English, not carrying multiples, not in a high risk pregnancy, residing within a 30-minute drive of the University of Illinois Urbana-Champaign campus, willing to provide a fasting blood sample at 16-18 weeks gestation, not planning on moving out of the area before the child's first birthday, and were not mothers of a child already enrolled in the study.

The proposed studies will utilize data from the first 482 women who enrolled in I-KIDS between 12/2013 and 09/2018 and remained in the study through the birth of their infant. Characteristics of these 482 women are as follows: 59% are ≥30 years of age, 81% are college educated, 80% are non-Hispanic white, 88% are married, 55% have an annual family income ≥$60,000, and 54% have a BMI<25 kg/m^2 (**Table 4**). Additionally, these women have a median AHEI-2010 of 56 (range: 28-83; out of 110), and median gestational age at birth of 39 weeks (range: 30-42).

Table 4. Demographic and lifestyle characteristics of 482 I-KIDS pregnant women (2013-2018).		
Characteristic	**Category**	**n=482**
Maternal age		
	< 30 years old	197 (40.9)
	≥ 30 years old	285 (59.1)
Race/ethnicity		
	Non-Hispanic White	385 (80.0)
	Others	96 (20.0)
Education		
	Some college or less	90 (18.7)
	College grad or higher	392 (81.3)
Marital status		
	Married	426 (88.4)
	Living as married/single	56 (11.6)
Employment status		
	Unemployed	67 (13.9)
	Employed	415 (86.1)
Annual household income		
	< $60,000	138 (28.8)
	$60,000-$99,999	182 (38.1)
	≥ $100,000	158 (33.1)
Conception season		
	Winter	122 (25.4)
	Spring	133 (27.7)
	Summer	107 (22.2)
	Fall	119 (24.7)
Parity		
	No live births	246 (51.0)
	≥ 1 live birth	236 (49.0)
Pre-pregnancy BMI		
	< 25 kg/m^2	256 (53.6)
	≥ 25 kg/m^2	222 (46.4)
Smoking in 1^{st} trimester		
	No	423 (87.7)
	Yes	24 (5.0)
	Missing	35 (7.3)
AHEI-2010		
	Score < 56	240 (49.9)
	Score ≥ 56	241 (50.1)
Missing: race/ethnicity, conception season, AHEI-2010 (n=1); annual household income, pre-pregnancy BMI (n=4).		

E.2. Research design and methods for Specific Aim 1.

The overall objective of Specific Aim 1 is to evaluate associations of maternal paraben concentrations with maternal inflammation (Aim 1a) and determine if maternal inflammation partially explains associations of maternal paraben concentrations with

shorter gestation (Aim 1b).

E.2.1. Outcome variable(s): maternal second trimester inflammation.

Maternal fasting blood samples were collected between 16-18 weeks gestation (early second trimester). Women fasted for 10-12 hours prior to the blood draw, and 30-35 mL of blood was collected by a certified phlebotomist at Carle Physician Group Christie Clinic in Champaign-Urbana, IL. To assure consistency across participants, all blood samples remained at room temperature for exactly 2 hours prior to processing. Samples were collected into glass heparin-containing vacutainer tubes, centrifuged at room temperature for 20 min, and the resulting plasma was aliquoted into cryovial tubes before immediate storage at -80°C. Plasma immune markers will be analyzed with a multiplex assay (Millipore Milliplex™ Map Immunology) using Luminex xMap® Technology at the University of Michigan Diabetes Research Center Clinical Core Chemistry Laboratory, which is a state-of-the art facility with extensive expertise in performing these types of analyses. The benefit of using a multiplex assay is that multiple analytes can be measured in a single, small volume sample. Samples will be assessed for a comprehensive panel of early second trimester maternal pro- and anti-inflammatory cytokines as shown in **Figure 4**, which includes 12 analytes associated with shorter gestation and nine other critical inflammatory cytokines (48, 49). As will be described later in F.1.2., we will utilize statistical methods to identify patterns of cytokine concentrations (as previously reported in non-pregnant populations (50)) that will approximate maternal second trimester inflammation status.

Inflammatory cytokines associated with shorter gestation		Other cytokines involved in inflammatory pathways	
IFNγ	IL-17A	IFN-α2	IL-27
IL-1β	TNFα	IL-1α	IL-31
IL-1Ra	TNFβ	IL-9	IL-33
IL-6	TGFα	IL-13	
IL-10	IL-18	IL-22	
IL-12	CRP	IL-23	

Figure 4. List of cytokines proposed for analysis. All 21 cytokines listed are involved in inflammatory pathways, while 12 have been implicated with shorter gestation (as specified in the figure). CRP, C-reactive protein; IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

E.2.2. Exposure variable(s): maternal urinary paraben concentrations.

I-KIDS collects five first morning urine samples across pregnancy at 10-14, 16-18, 22-24, 28-30, and 34-36 weeks gestation. Field blanks are obtained for every 10 samples. Urine samples are collected using bisphenol A (BPA)- and phthalate-free materials approved by the Centers for Disease Control and Prevention (CDC). After collection, urine samples are vortexed and specific gravity is measured. Samples are then aliquoted and immediately stored at -80°C. Additionally, a pool of all 5 urines is created for each participant by adding 900µL of urine from each gestational timepoint into one 5 mL polypropylene cryovial tube. Because parabens are non-persistent chemicals with short half-lives in the body (51), urinary paraben concentrations for this study were measured in pooled samples to provide a reliable biomarker of maternal paraben exposure across pregnancy (52, 53). Urine has been shown to be most reliable for assessing non-persistent chemical concentrations (54), and pooling urine samples is both cost effective and appropriate for evaluating non-persistent chemicals. Urinary concentrations of four

parabens (butyl, ethyl, methyl, and propyl) were analyzed at the CDC Division of Laboratory Sciences using established protocols (55). To account for urine dilution, all paraben concentrations will be specific gravity (SG) adjusted using the equation $P_c = P[(SG_m - 1)/(SG_i - 1)]$, where P_c is the SG-adjusted paraben concentration, P is the measured paraben concentration (ng/mL), SG_m is the median SG of I-KIDS mothers, and SG_i is the SG of each urine sample (56).

E.2.3. Covariates.

Information regarding maternal characteristics in I-KIDS was collected using interviews and surveys. The current study will utilize maternal data collected at the baseline visit (10-14 weeks), which included information about demographics (e.g. race, age), health (e.g. body mass index), lifestyle (e.g. smoking, alcohol use), diet, depression, perceived stress, and interpersonal support. Health-related covariates, including diagnoses of medical conditions (e.g. pre-eclampsia) and gestational weight gain will be obtained from electronic medical records. Aim-specific covariates used in statistical models are described in F.1.5.

E.2.4. Expected results and alternative strategies for Aim 1.

Based on previous studies implicating parabens with gestational inflammatory pathways, we expect that parabens will be positively or negatively associated with specific individual pro- and anti-inflammatory cytokines, respectively. To provide additional insights into the paraben-inflammation relationship, we will utilize a statistical approach to identify patterns of cytokine concentrations to better predict overall maternal inflammation status. We

expect to observe significant associations of parabens with distinct clusters of cytokines that represent apparent pro- and anti-inflammatory states. If we observe that parabens are associated with maternal inflammation, we will pursue an exploratory Aim 1b to assess whether associations of parabens with shorter gestation can be partly explained by elevated maternal inflammation. Given the known role of second trimester inflammation in predicting shorter gestation, we would expect that maternal inflammation will partly mediate associations of parabens with gestational length. Although we were underpowered to evaluate sex-specific associations of parabens with gestational length in our preliminary studies, we did observe a tendency for stronger associations in female infants (data not shown). Therefore, in Aims 1a and 1b, we may also consider sensitivity analyses to evaluate these associations stratified by fetal sex.

E.3. Research design and methods for Specific Aim 2.

The overall objective of Specific Aim 2 is to evaluate differences in associations of maternal paraben concentrations with shorter gestation by maternal inflammatory diet score.

E.3.1. Outcome variable(s): gestational length.

In preliminary studies, gestational age at birth was calculated using the first day of a woman's last menstrual period and the delivery date obtained during a hospital study visit. For the proposed studies, birth date will be obtained from electronic medical records.

E.3.2. Exposure variable(s): maternal urinary paraben concentrations.

Our approach for assessing maternal paraben concentrations is described in E.2.2.

E.3.3. Effect modifying variable(s): maternal inflammatory diet index.

All women completed a semi-quantitative food frequency questionnaire (FFQ) that was adapted from the full-length Block FFQ (NutritionQuest) to assess diet in pregnancy (57-59). I-KIDS women completed the FFQ at 10-14 and 34-36 weeks gestation. Because the FFQ asks about food intake during the previous three months, dietary information collected during these timepoints represents early and mid-to-late pregnancy maternal diet. The collected dietary information will be used to calculate the E-DII, which is an empirical score created using the Nurses' Health Study (NHS) that assesses overall inflammatory potential of whole diets (60). The E-DII has been validated in different representative U.S. populations, including the NHS-II and the Health Professionals Follow-Up Study, and is created using foods/food groups rather than individual nutrients, which makes this score a more relevant predictor of inflammatory dietary patterns than other published inflammatory diet scores (60-62). Specifically, the E-DII is calculated as the weighted sum of 9 anti-inflammatory (e.g. dark yellow vegetables, fruit juice) and 9 pro-inflammatory (e.g. processed meat, refined grains) foods/food groups shown to be predictive of 3 critical plasma inflammatory markers (CRP, TNFαR2, and IL-6) (60, 62). All dietary components necessary to calculate the E-DII are included in the Block FFQ completed by I-KIDS participants. For the proposed study, the E-DII will be calculated separately at 10-14 and 34-36 weeks gestation and averaged to represent maternal anti-inflammatory diet quality across pregnancy.

E.3.4. Covariates.

Collection of maternal sociodemographic and health-related characteristics is described in E.2.3.

E.3.5. Expected results and alternative strategies for Aim 2.

Because our preliminary findings showed that associations of maternal paraben concentrations with shorter gestation are attenuated in women with better diet quality, and because previous studies suggest that better diet quality is anti-inflammatory, we expect to observe differences in associations of parabens with shorter gestation by maternal E-DII. Although the E-DII has been validated in non-pregnant populations, no study (to our knowledge) has evaluated E-DII in pregnancy. Therefore, if we do not observe differences in associations of parabens with gestational length by maternal E-DII, we may also consider other inflammatory dietary indices that have been validated in pregnancy (e.g. Dietary Inflammatory Index (63)) or use published methods to create our own pregnancy-specific index (future directions). While we plan to evaluate maternal E-DII across gestation (average of 10-14 and 34-36 weeks), we will also consider maternal E-DII separately at the two timepoints because our preliminary data suggests that early pregnancy diet may be more predictive of gestation length than late pregnancy diet. As with Aim 1, we may also perform sensitivity analyses to assess sex-specific associations, although we may be underpowered to detect such differences in the current study.

F. PROPOSED ANALYSIS AND PRESENTATION OF TABLES/FIGURES.

F.1. Statistical analysis.

F.1.1. Overview.

Our **central hypothesis** that parabens (X_1) are associated with shorter gestation (*Y*) due to elevated maternal inflammation (X_2) and that an anti-inflammatory maternal diet (*W*) mitigates associations of X_1 with *Y* will be tested in an anticipated total of 482 women. The posited linear models are as follows: [1] $E(X_2|X_1) = \gamma_0 + \gamma_1 X_1$ and [2] $E(Y|X_1, X_2) = \beta_0 + \beta_1 X_1 + \beta_2 X_2$ for **Aim 1**, as well as [3] $E(Y|X_1, W) = \delta_0 + \delta_1 X_1 + \delta_2 W + \delta_3 X_1 W$ for **Aim 2**. X_2 and *Y* will be analyzed as continuous measures using multivariable linear regression models after considering transformations to mitigate skewness found in distributions. To ensure model assumptions are met, regression models will be checked for non-constant residual variance, influential points, and multicollinearity by standard methods. X_1 will be approximated from SG-adjusted urinary paraben levels and will be analyzed as continuous measures or in quantiles to evaluate non-linear relationships. *A priori* statistical considerations using directed acyclic graph (DAG) will be used to identify potential confounders (see section F.1.5. for more details). All tests will be two-sided at type I error α=0.05.

F.1.2. PCA to characterize inflammation (Aim 1a).

Maternal second trimester inflammation profiles will be created using PCA. PCA is an unsupervised pattern identification method that linearly transforms a set of partially correlated variables into a joint set of uncorrelated factors (or components) that are sorted in descending order based on the contribution to the total data variance (64, 65). The

resulting components representing concentrations of 21 measured cytokines will provide critical information about distinct inflammation patterns in I-KIDS women during the second trimester of pregnancy. Components generated from PCA will be used as continuous outcome variables in generalized linear regression models to evaluate associations between maternal parabens concentrations and cytokines patterns by testing the hypothesis $H_{01}: \gamma_1 = 0$ [1] (64-66). Additionally, components may be used as continuous mediator variables in associations of maternal paraben concentrations with gestational length (exploratory Aim 1b).

F.1.3. Mediation by maternal inflammation (exploratory Aim 1b).

We may examine whether associations of maternal paraben concentrations with shorter gestation can be partly explained by maternal inflammation using the VanderWeele method (67). This regression-based approach assumes a flexible structural model for both *Y* and X_2 and relaxes the assumption that there is no interaction between X_1 and X_2. This approach estimates what proportion of the total relationship between X_1 and *Y* is explained (mediated) by the natural indirect effect X_1 → X_2 → *Y* (proportion mediated = $\gamma_1\beta_2/(\beta_1 + \gamma_1\beta_2)$). This will be accomplished by testing the joint hypothesis $H_0: \gamma_1\beta_2 = 0$ by separately testing the hypotheses $H_{01}: \gamma_1 = 0$ [1] and $H_{02}: \beta_2 = 0$ [2].

F.1.4. Modification by maternal diet (Aim 2).

We will evaluate the role of maternal anti-inflammatory diet (measured by the maternal E-DII) as an effect modifier of associations between maternal paraben concentrations and gestational length. E-DII will be dichotomized at the median as done in our preliminary

studies with AHEI-2010 to maximize power and improve interpretation. We will test the hypothesis $H_{03}: \delta_3 = 0$ [3], which examines the multiplicative interaction between maternal E-DII and parabens.

F.1.5. Covariates.

Given that I-KIDS collects an extensive amount of data, there are numerous covariates available to use for evaluating the associations proposed in Specific Aims 1 and 2. As mentioned in F.1.1., covariate selection for final statistical models will rely primarily on *a priori* considerations and previously published data, which will inform a DAG. **Figure 5** presents a DAG that includes all covariates that are collected in I-KIDS that may be relevant for the proposed analyses. Statistical methods, including evaluating associations of potential covariates with parabens, inflammation, and gestational length, as well as testing correlations between covariates that measure similar constructs, will be conducted to limit overadjustment of statistical models.

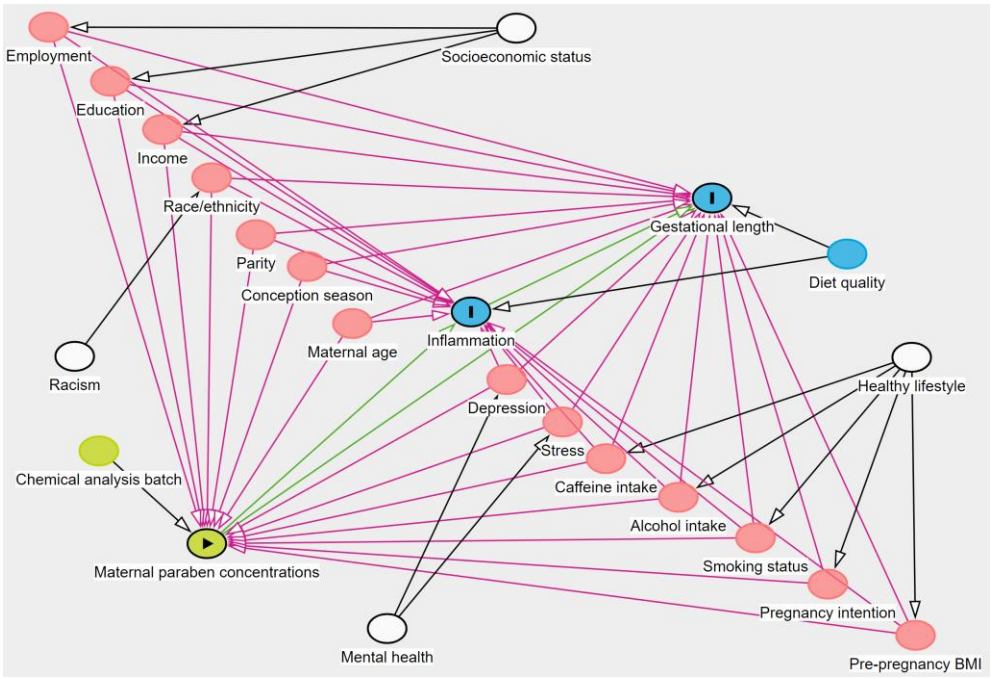

Figure 5. DAG with hypothesized associations of potential covariates with parabens, inflammation, and gestational length. The DAG includes a comprehensive list of covariates that are already available in I-KIDS that may be relevant confounders for evaluating associations of parabens with inflammation (Aim 1a), the mediation of associations between parabens and gestational length by inflammation (Aim 1b), and the modification of associations between parabens and gestational length by E-DII (Aim 2).

F.1.6. Statistical power.

We made our assessments of power on the basis that 482 women will be available to evaluate the relationships posited in linear models [1, 2, 3] having controlled for up to 4 covariates (68-70). For these calculations we translated the model parameters to correlations that were derived from I-KIDS data or published in the literature (71). Plausible correlation ranges for the proposed relationships were as follows: +0.13 to +0.15 for X_1 with X_2 and -0.10 to -0.12 for X_1 and X_2 with Y (23, 72). Based on these values and sample size n=482, we have 81.2% power for testing H_{01} [1] to detect correlations of at least +0.13 for the relationship $X_1 \rightarrow X_2$ (Aim 1a). Additionally, we have

over 97.3% power for testing H_{02} [2] to detect correlations of at least -0.10 for the relationships $X_1 \rightarrow Y$ and $X_2 \rightarrow Y$. As a result, we will have 79.1% power for testing the joint hypothesis H_0 to detect the natural indirect effect $X_1 \rightarrow X_2 \rightarrow Y$ and calculate the proportion mediated (Aim 1b). We will also have over 80% power to for testing H_{03} [3] for the relationship $X_1 \rightarrow Y$ modified by *W* (Aim 2). Power calculations are summarized in **Table 4**, where the shaded row represents the final estimated power for the proposed studies.

Table 5. Power estimation for evaluating associations in 482 I-KIDS women.

1	2	3	4	5	6	7
$r\ X_1 \rightarrow Y$	$r\ X_2 \rightarrow Y$	$r\ X_1 \rightarrow X_2$	Estimated % mediated	Power for testing H_{01}	Power for testing H_{02}	Power for testing joint hypothesis H_0
-0.12	-0.1	0.12	0.08685	0.747	0.97193	0.72610
-0.12	-0.1	0.13	0.09301	0.812	0.97032	0.78821
-0.12	-0.1	0.14	0.09901	0.866	0.96863	0.83895
-0.12	-0.1	0.15	0.10486	0.908	0.96688	0.87825
-0.1	-0.1	0.12	0.10714	0.747	0.97498	0.72837
-0.1	-0.1	0.13	0.11504	0.812	0.97379	0.79103
-0.1	-0.1	0.14	0.12281	0.866	0.97256	0.84236
-0.1	-0.1	0.15	0.13043	0.908	0.97129	0.88226
-0.11	-0.12	0.12	0.11821	0.747	0.99147	0.74069
-0.11	-0.12	0.13	0.12707	0.812	0.99095	0.80497
-0.11	-0.12	0.14	0.13579	0.866	0.99041	0.85781
-0.11	-0.12	0.15	0.14439	0.908	0.98984	0.89911

Data are presented in the following order: columns 1-3, correlations between parabens, inflammation, and gestational length derived from the literature or calculated in preliminary analyses in I-KIDS; column 4, estimated percentage mediated based on correlations in columns 1-3; columns 5-7, estimated power for testing hypotheses based on correlations in columns 1-3. X_1, parabens; X_2, inflammation; *Y*, gestational length.

F.2. Presentation of tables and figures.

F.2.1. Tables and figures related to both specific aims.

Table 1. Characteristics of I-KIDS pregnant women and those in analytic sample.

- This table will present characteristics of all I-KIDS pregnant women in the full

cohort compared to the 482 women who will be included in the final analytic sample. The participant characteristics presented in this table will include final covariates selected for statistical models evaluating associations of urinary paraben concentrations with maternal inflammation. We will use the chi-square goodness-of-fit test to determine if the final analytic sample is biased given the inclusion/exclusion criteria to select the final 482 women.

Figure 1. Urinary paraben concentrations in I-KIDS (n=482).

- This figure will present urinary concentrations of butyl, ethyl, methyl, and propyl parabens. Additionally, this figure will include urinary paraben concentrations of same-age women from NHANES to compare whether urinary paraben concentrations in I-KIDS are similar to those of a representative sample of U.S. women. Results from this figure will provide important information about the distribution of paraben concentrations in I-KIDS.

Figure 2. Correlations between urinary paraben concentrations (n=482).

- This figure will present Pearson correlation coefficients between butyl, ethyl, methyl, and propyl parabens to determine how correlated parabens are with each other. Results from this figure will provide insights into which combinations of parabens women are likely exposed to simultaneously.

Table 2. Urinary parabens and their associations with maternal sociodemographic and health-related characteristics (n=482).

- This table will present geometric mean (standard error) or median (95% CI) concentrations of butyl, ethyl, methyl, and propyl parabens by maternal sociodemographic and health-related characteristics. Parabens will be operationalized as continuous variables, while maternal sociodemographic and health-related characteristics will be operationalized as categorical variables. The non-parametric Kruskal Wallis test will be used to assess whether maternal characteristics are associated with parabens. Results from this table will inform about potential confounders to include in statistical models evaluating associations of paraben concentrations with gestational length and maternal inflammation.

Table 3. Gestational length and its associations with maternal sociodemographic and health-related characteristics (n=482).

- This table will present geometric mean (standard error) or median (95% CI) gestational age at birth by maternal sociodemographic and health-related characteristics. Gestational length will be operationalized as a continuous variable (in weeks or days), while maternal sociodemographic and health-related characteristics will be operationalized as categorical variables. The non-parametric Kruskal Wallis test will be used to assess whether maternal characteristics are associated with gestational length. Results from this table will provide additional information about maternal characteristics that are associated with shorter or longer gestational length. These results will also inform about potential confounders to include in final statistical models evaluating associations of paraben concentrations with gestational length.

Table 4. Associations of urinary parabens with gestational length (n=482).

- This table will present results from generalized linear regression models evaluating associations of four urinary parabens with gestational length. The table will include unadjusted and adjusted β-estimates (95%CI) that represent change (in days) in gestational length for every percent increase in paraben concentrations. Parabens will be operationalized as continuous variables, as well as categorical variables (i.e. quantiles) to assess potential non-linear associations. These results will further provide evidence in the full analytic sample for overall associations of parabens with gestational length, which are the basis of the proposed aims.

F.2.2. Tables and figures related to Specific Aim 1.

Figure 1. Correlations between plasma cytokines (n=482).

- This figure will present Pearson correlation coefficients between 21 cytokines that are components of critical inflammatory pathways. Results from this figure will provide insights about cytokines from common inflammatory pathways. These results will also provide preliminary evidence for PCA analyses.

Figure 2. Loading factors of plasma cytokines generated from PCA (n=482).

- This figure will present several panels of bar graphs with PCA results. The figure will include loading factors for each plasma cytokine in each identified principal component to characterize patterns of cytokines. The PCA will be constrained to components that have eigenvalues >1.0 and together explain >80% of the variance. This figure will be important for interpreting results from statistical models

evaluating associations of paraben concentrations with maternal inflammation and the mediation of associations between parabens and gestational length by inflammation.

Table 1. Plasma cytokine principal components and their associations with maternal sociodemographic and health-related characteristics (n=482).

- This table will present geometric mean (standard error) or median (95% CI) of each identified principal component by maternal sociodemographic and health-related characteristics. Identified cytokine principal components will be operationalized as continuous variables, while maternal sociodemographic and health-related characteristics will be operationalized as categorical variables. The non-parametric Kruskal Wallis test will be used to assess whether maternal characteristics are associated with cytokine components. Results from this table will provide important information about maternal characteristics that are associated with cytokines patterns. These results will also inform about potential confounders to include in final statistical models evaluating associations of paraben concentrations with maternal inflammation.

Table 2. Associations of urinary parabens with plasma cytokine principal components (n=482).

- This table will present results from generalized linear regression models evaluating associations of four urinary parabens with several identified cytokine principal components. The table will include unadjusted and adjusted β-estimates (95%CI)

that represent change in principal component score for every percent increase in paraben concentration. Parabens will be operationalized as continuous variables, as well as categorical variables (i.e. quantiles) to assess potential non-linear associations. These results will determine whether parabens alter critical early second trimester maternal inflammatory profiles. Results from this table will also determine whether inflammation will be assessed as a mediator for associations between parabens and gestational length.

Figure 3. Associations of urinary parabens with gestational length mediated by plasma cytokine principal components (n=482).

- Given findings from the previous table, this figure will present associations of urinary parabens with gestational length mediated by inflammation. Specifically, this figure will include β-estimates and 95%CI for the natural direct and indirect effects. The natural direct effect represents associations of paraben concentrations with gestational length. The natural indirect effect represents associations of paraben concentrations with gestational length explained by the association between parabens and maternal inflammation.

F.2.3. Tables and figures related to Specific Aim 2.

Table 1. E-DII and its associations with maternal sociodemographic and health-related characteristics (n=482).

- This table will present geometric mean (standard error) or median (95% CI) of the E-DII by maternal sociodemographic and health-related characteristics. The E-DII

will be dichotomized at the median, while maternal sociodemographic and health-related characteristics will be operationalized as categorical variables. A chi-square test will be used to assess whether maternal characteristics are associated with E-DII. Results from this table will identify the sub-samples of pregnant women that are more likely to consume pro- vs. anti-inflammatory diets.

Figure 1. Associations of E-DII with plasma cytokine principal components (n=482).

- This figure will present associations of E-DII with plasma cytokine principal components. These results will determine whether the E-DII calculated in I-KIDS is associated with patterns of cytokines identified in PCA, and will validate whether the E-DII is correlated with gestational inflammation.

Table 2. Associations of urinary parabens with gestational length modified by the E-DII (n=482).

- This table will present results from generalized linear regression models evaluating associations of four urinary paraben concentrations with gestational length modified by the E-DII. The table will include unadjusted and adjusted β-estimates (95%CI) that represent change in gestational length (in days) for every percent increase in paraben concentration in women with E-DII scores ≥ median vs. < median. Parabens will be operationalized as continuous variables given power concerns. These results will determine whether a healthier, anti-inflammatory diet can intervene against the negative impacts of parabens on gestational length.

G. OVERALL CONCLUSIONS

G.1. Strengths and future directions.

We have foreseen some potential limitations/pitfalls and addressed several alternative strategies to our approach in sections E.2.4. and E.3.5. The proposed study has several strengths. One major strength is the ability to leverage biospecimens (maternal urine and blood) and extensive covariate data already being collected for the ongoing I-KIDS parent study, thereby allowing for a highly efficient study design. While this study is ambitious, all participants have been recruited and all prenatal data about participant characteristics are available to address our aims. Given that we currently work closely with the I-KIDS team, we do not expect major challenges for obtaining the remaining data needed to address these aims (e.g. electronic medical records). While our results may not be generalizable to highly at-risk populations given the low pre-term birth prevalence in I-KIDS, these findings will provide novel information about risk factors for shorter gestation in an understudied at-risk population. Once these aims are accomplished, findings will be published and utilized to develop new hypotheses about the adverse effects of parabens in pregnancy and the intervening effect of an anti-inflammatory maternal diet on shorter gestation. No large-scale epidemiological studies have investigated the proposed relationships, which is a challenge, but also an opportunity to contribute new knowledge to the field.

G.2. Scientific rigor.

The scientific premise for this research is provided in earlier sections. Various measures will be taken to minimize bias and to assure scientific rigor and reproducibility. First, our

participant selection relies on our ability to obtain the necessary measures for proposed analyses and not on any other factors, such as race/ethnicity. The selection of pregnant women years 18-40 was done to minimize risk associated with pregnancies at younger or older ages and accomplish our scientific goals. Given that we will be evaluating overall associations and differences in associations by maternal diet quality, we will report overall and diet-specific relationships. While we may be underpowered to assess sex-specific associations in each aim, we will consider sex as a biological variable by performing sex-specific sensitivity analyses in each aim that will provide potentially important information for future studies. We have made substantial effort to develop protocols for reducing variability in paraben assessment (e.g. pooled sample, urine density adjustment), as well as holistically assessing inflammation and diet quality (e.g. development of clusters or indices). Importantly, we have adequate power to address our aims, thus enabling us to delve into potential biological pathways and dietary interventions of our hypothesized associations. Finally, we plan to report our results in a timely and transparent manner.

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