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EVALUATING THE RELATIONSHIP BETWEEN PRENATAL EXPOSURE TO
ACETAMINOPHEN AND PHTHALATES AND EARLY ATTENTION AND LANGUAGE
DEVELOPMENT

BY

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DISSERTATION

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ABSTRACT

The potential effects of prenatal acetaminophen exposure on neurodevelopment have become a topic of interest in recent years with an increasing number of studies finding evidence that it may be related to attention problems and poorer language development. Phthalates have also been linked to deficits in attention and language development, as well as other neurodevelopmental outcomes, although the evidence for these associations has been mixed. Acetaminophen is one of the few medications pregnant women are told they can safely take, and exposure to phthalates is ubiquitous, thus exposure to both is common in pregnant women. Both compounds have been shown to be antiandrogenic and, thus, they may also share a mechanism of action. Together this suggests that acetaminophen and phthalates could have cumulative effects on neurodevelopmental outcomes. The goal of this dissertation was to examine the association of prenatal acetaminophen and phthalate exposure individually and their potential cumulative effects with attention in infancy and early childhood, as well as with language development in early childhood. Chapter 3 describes the Illinois Kids Development Study (IKIDS), an ongoing prospective birth cohort within which this study was conducted. Chapter 4 examines the associations of maternal urinary biomarkers obtained during pregnancy of diethyl phthalate (DEP), di-(2-ethylhexyl) phthalate (DEHP), and two replacement chemicals for DEHP, diisononyl phthalate (DINP) and di-2-ethylhexyl terephthalate (DEHTP) with measures of infant cognition obtained via infrared eye tracking using a visual recognition memory (VRM) paradigm. Results were mixed but generally indicate that prenatal DEHTP may be related to improved attention while DINP may be related to slower information processing. Chapter 5 evaluates the associations of maternal self-report of acetaminophen use during pregnancy by trimester, in addition to cumulative exposure across pregnancy, with measures of infant cognition in the VRM paradigm, as well as the potential cumulative effects of prenatal phthalate and acetaminophen exposure on infant cognition. There was little evidence that

prenatal acetaminophen exposure had a negative impact on infant cognition; however, results indicated that there may be a cumulative negative effect of first trimester acetaminophen exposure and DINP exposure on information processing speed. Chapter 6 assesses the associations of prenatal urinary biomarkers of phthalate exposure and maternal self-report measures of acetaminophen use during pregnancy with parental report of attention-related outcomes on a standardized survey of child behavior at 2 and 3 years. Associations generally indicated that prenatal exposure to DEHP was associated with fewer attention problems at 2 years, and while DEHTP was associated with fewer attention problems at both 2 and 3 years of age, MEP was associated with more attention problems at both 2 and 3 years. Associations with prenatal acetaminophen exposure consistently showed that both second trimester and cumulative exposure across pregnancy were associated with increased attention problems at both 2 and 3 years. Potential cumulative effects of prenatal phthalate and acetaminophen exposure on attention at 2 and 3 years of age were also evaluated, and there was evidence for cumulative effects of exposure to acetaminophen during the second trimester and MEP on attention-related behaviors at 2 years. Chapter 7 evaluates the associations of biomarkers of prenatal phthalate exposure and maternal self-report of acetaminophen use during pregnancy with measures of language development at 2 and 3 years of age. Results showed adverse associations of prenatal phthalate exposure with multiple measures of language development at 2 years, and male children were generally more impacted, but only DEHTP was also associated with poorer language development at 3 years. Acetaminophen exposure was associated with lower vocabulary scores and increased odds of female children having lower scores on measures of language development at 2 years, but both second trimester exposure and cumulative exposure to acetaminophen across pregnancy were consistently associated with multiple poorer language outcomes at 3 years and these associations were not sex specific. Potential cumulative effects of prenatal exposure to phthalates and acetaminophen on language development were also assessed, and there was evidence indicating there may be cumulative

effects of acetaminophen exposure during the second trimester with DEHP or DEHTP on measures of language development at 2 years. The results of this study add to the growing body of literature indicating that prenatal exposure to phthalates or acetaminophen may adversely affect attention and language development and suggest that prenatal exposure to both may have cumulative effects on neurodevelopment that have not been considered previously.

Dedicated to The Illinois Kids Development Study participants

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CHAPTER 1. INTRODUCTION

The prenatal period is considered an important window of development during which the developing fetus is particularly susceptible to exposures that may or may not also be of concern for adults. During this time, the brain is developing rapidly and is therefore particularly sensitive to any perturbation (Howdeshell, 2002; Grandjean and Landrigan, 2014). Shortly after the neural tube begins to form in utero, neurogenesis begins (Howdeshell, 2002; De Graaf-Peters and Hadders-Algra, 2006). Throughout neurogenesis, newly formed neurons migrate towards their destination and organize into specific brain structures, beginning with structures of the hindbrain, followed by the mesencephalon, diencephalon, and finally the telencephalon. Some differentiation begins as migration continues, but the majority begins once migration is complete, followed by synaptogenesis and myelination, both of which continue after birth (Howdeshell, 2002). If any one of these processes is interrupted or altered, there may be long-term consequences for the developing individual, such as in the case of fetal alcohol spectrum disorders (Guerri et al., 2009).

The timing of exposure to ethanol during prenatal development is known to be important in how the offspring will be affected; earlier exposure can suppress the migration of neural crest cells, while later exposure can suppress the migration of cortical neurons, neural proliferation, and neural differentiation in the hippocampus (Van De Bor, 2019). While the placenta offers some protection to the developing fetus, it is not impermeable, and many chemicals are able to cross either as the parent compound or its metabolites (Conings et al., 2019). Additionally, the fetus may inadequately metabolize or be completely unable to metabolize chemicals that do cross the placenta, which may make the chemicals that do cross the placenta more damaging to the fetus (Grandjean and Landrigan, 2014). Prenatal exposure to a variety of compounds, such as lead and other environmental contaminants, alcohol consumption, and certain medications, has been associated with a variety of cognitive and behavioral deficits, language

impairments, and neurodevelopmental disorders (Grandjean and Landrigan, 2014; Streissguth et al., 1987). While some compounds have been studied extensively in the context of how prenatal exposure to them may negatively impact development, others, such as acetaminophen, have not.

1.1. IMPACT OF MATERNAL ACETAMINOPHEN USE DURING PREGNANCY ON NEURODEVELOPMENT

Acetaminophen, also called paracetamol, is the most common drug ingredient in the United States, and currently the only analgesic considered safe for women to use throughout pregnancy (Servey and Chang, 2014). Studies have found that 50-65% of pregnant women in North America and Europe report taking acetaminophen, or a medication containing it, at least once during their pregnancy (Brandlistuen et al., 2013; Thorpe et al., 2013; Servey and Chang, 2014; Ji et al., 2018). While little is known about the safety of acetaminophen use during pregnancy due to the lack of clinical trials in pregnant women and the very few reported cases of adverse effects in the developing child (Black and Hill, 2003; Servey and Chang, 2014), recently there has been a surge of interest in examining whether maternal acetaminophen use during pregnancy could be associated with adverse neurodevelopmental outcomes. Multiple studies have shown that prenatal exposure to acetaminophen is associated with neurodevelopmental outcomes and with attention-deficit/hyperactivity disorder (ADHD) and ADHD-like behaviors, in particular (Table 1.1). For instance, one study, the Avon Longitudinal Study of Parents and Children (ALSPAC), in the United Kingdom found that children of mothers who reported use of acetaminophen during pregnancy were more likely to have oppositional-defiant disorder symptoms at 7 years and 9 months of age (Ruisch et al., 2018). In a subset of the Norwegian Mother and Child Cohort (MoBA) study, Brandlistuen et al. (2013) used data from 2,919 same-sex sibling pairs for a sibling-controlled cohort study of prenatal exposure to acetaminophen. They categorized maternal self-report of medication use as short term (less than 28 days) or long term (28 days or more), and they found that short-term prenatal

acetaminophen use was associated with poorer gross motor function, increased externalizing behavioral problems (such as aggression and problems with attention) (American Psychological Association, 2018), and increased negative temperament at age 3. Multiple reports from this cohort have found that long-term use was also associated with delayed age to begin walking, poor communication skills, increased activity levels at 3 years of age, and an increased rate of ADHD diagnosis (Brandlistuen et al, 2013; Vlenterie et al., 2016; Ystrom et al., 2017). More recently, Trønnes et al. (2020) found that maternal acetaminophen use during all three trimesters of pregnancy was associated with an increased relative risk of internalizing and externalizing behaviors in 5-year-old children in this cohort.

In the Danish National Birth Cohort (DNBC), Liew et al. (2014) examined the association between maternal acetaminophen use both in 3-week increments through 20 weeks of gestation and by trimester, and the following ADHD-related outcome measures: (1) hyperkinetic disorder diagnosis at or after age 5, (2) children who filled two or more prescriptions for ADHD medications, and (3) assessment of ADHD-like behaviors using the standardized Strengths and Difficulties Questionnaire (SDQ) sent to parents when the child was 7 years old. They found children whose mothers took acetaminophen during pregnancy had increased risk for a hyperkinetic disorder diagnosis, ADHD medication use, and ADHD-like behaviors, with stronger associations when acetaminophen was taken more frequently during pregnancy (Liew et al., 2014). In a follow-up study, Liew et al. (2016a) found that maternal acetaminophen use during pregnancy was also associated with attention problems at age 5, based on the Test of Everyday Attention for Children administered by trained psychologists, and the Behavior Rating Inventory of Executive Function (BRIEF) completed by both parents and the children's' preschool teachers. Specifically, both any maternal use of acetaminophen during pregnancy and first trimester use were associated with poorer scores on overall attention and selective attention (Liew et al. 2016a). As with their previous study, they also found that longer use of acetaminophen during pregnancy showed a stronger association with poor attention, but only in

the parent ratings; additionally, in this study, associations were strongest with use in the first trimester (Liew et al. 2016a). Recently, Inoue et al. (2020) conducted another follow-up study in this cohort when children were 11-years-old utilizing both parent- and child-reported measures of behavioral outcomes on the SDQ. They found that whether they examined the parent- or child-reported scores, prenatal acetaminophen exposure was associated with more internalizing and externalizing problems (Inoue et al., 2020).

Studies in other cohorts have reported similar findings to those of Liew et al. (2014, 2016a). Liew et al. (2019) also found that maternal acetaminophen use during pregnancy was associated with ADHD diagnosis in the Nurses' II Health Study, and Ji et al. (2018) found that maternal blood acetaminophen metabolite levels measured 1-3 days postpartum were positively associated with ADHD diagnosis in the Boston Birth Cohort. In a follow-up study, Ji et al. (2020) found that higher concentrations of acetaminophen and its metabolites in both maternal and umbilical cord blood samples were associated with greater odds of ADHD or autism spectrum disorder (ASD) diagnosis. In the Auckland Birthweight Collaborative study in New Zealand, Thompson et al. (2014) found that children of mothers who reported using acetaminophen during pregnancy were more likely to exhibit emotional and conduct problems at age 7 and hyperactivity/inattention and conduct problems at age 11. Thompson et al. (2014) also reported that children whose mothers took acetaminophen while they were pregnant had more ADHD symptoms, particularly of the hyperactive-impulsive type, but only at age 7. Stergiakouli et al. (2016) found similar results in the ALSPAC in the United Kingdom. They were able to determine that acetaminophen use during the second and third trimesters was associated with hyperactivity and conduct problems and use at 32 weeks of gestation was associated with emotional problems at age 7 (Stergiakouli et al., 2016). Tovo-Rodrigues et al. (2018) also found that boys in the Pelotas cohort in Brazil whose mothers reported taking any medication containing acetaminophen during pregnancy were more likely to have emotional and hyperactivity/inattention problems at ages 6 and 11, yet in a follow-up analysis, Tovo-Rodrigues

et al. (2020) found no association of prenatal acetaminophen exposure and behavior at 24 or 48 months in the same cohort.

Several studies have also found that maternal acetaminophen use during pregnancy is associated with developmental outcomes related to ASD (Table 1.1). One of the essential criteria for an ASD diagnosis is a persistent deficit in an individual's ability to engage in social interactions, which can include delays or impairments in language development (DSM-5, 2013). In the INfancia y Medio Ambiente (INMA) cohort in Spain, Avella-Garcia et al. (2016) found that at 4-5 years of age boys whose mothers reported using acetaminophen during pregnancy had an increased risk of ASD symptoms, and the association was stronger with more frequent reported use. In the DNBC, Liew et al. (2016c) found that maternal acetaminophen use during pregnancy was associated with an increased risk of ASD diagnosis, according to hospital records, especially with increasing duration of exposure. They observed that this association was strongest when women reported acetaminophen use across all three trimesters or for more than 20 weeks during pregnancy; and for children also diagnosed with a hyperkinetic disorder in addition to ASD (Liew et al, 2016c).

Both Skovlund et al. (2017) and Bornehag et al. (2018b) examined the relationship between maternal acetaminophen use during pregnancy and children's language development (Table 1.1). In the MoBA Study, Skovlund et al. (2017) found that maternal use of acetaminophen during pregnancy was associated with reduced communication skills at age 3, particularly with reported use across more than one trimester. Bornehag et al. (2018b) found that girls in the Swedish Environmental, Longitudinal, Mother and child, Asthma and Allergy (SELMA) Study whose mothers reported any use of acetaminophen at the time of enrollment, 8-13 weeks of gestation, were more likely to exhibit language delays at 30 months of age. This association was stronger the more acetaminophen tablets mothers reported taking since conception and was also seen when examining the relationship between maternal urinary acetaminophen concentrations at the time of enrollment and language delays at 30 months of

age (Bornehag et al., 2018b). From what has been studied thus far, key features seen in ADHD and ASD, such as hyperactivity and communication problems, seem to be associated with maternal use of acetaminophen during pregnancy. However, to date, most studies have evaluated maternal use of acetaminophen by maternal self-report methods administered only once or twice during pregnancy, which may not provide an accurate measure of acetaminophen use across the entire pregnancy. Additionally, most studies have focused on evaluations of behavior and development in middle childhood, and few have examined cognitive and behavioral development in infancy and early childhood.

1.2. THE IMPACT OF PRENATAL PHTHALATE EXPOSURE ON NEURODEVELOPMENT

Phthalates are a family of chemicals added to a variety of consumer products as plasticizers and solvents, including polyvinyl chloride products, personal care products, medical tubing, and children's toys (Centers for Disease Control and Prevention, 2017). Due to their widespread use in products, human exposure to phthalates is ubiquitous. According to the most recent data from the U.S. National Health and Nutrition Examination Survey (NHANES), an ongoing study run by the U.S. Centers for Disease Control (CDC) which aims to evaluate the health and nutrition status of the general U.S. population, it is estimated that 100% of the U.S. population has detectable levels of at least one phthalate metabolite in their system (CDC, 2019). Recently, Johns et al. (2017) found phthalate metabolites in 96-99% of the 409 urine samples they tested from pregnant women enrolled in a prospective birth cohort study at Brigham and Women's Hospital in Boston, Massachusetts. Because exposure to phthalates is so prevalent, including in pregnant women, there is concern that they may affect fetal development; however, results thus far have been mixed.

Studies of the associations between prenatal exposure to phthalates and ADHD have been inconsistent with some reporting increased risk, some reporting decreased risk and some reporting no association with ADHD or ADHD-like behaviors (Table 1.2). For example, Engel et al. (2010) examined the relationship between prenatal exposure to phthalates and child

development in the Mount Sinai Children's Environmental Health Center cohort. For their analyses, they both considered individual phthalate metabolites and grouped phthalate metabolites as high molecular weight or low molecular weight and examined their relationship with child behavior between 4 and 9 years of age using multiple maternal report measures. Specifically, they used the BREIF to evaluate executive function and the Behavior Assessment System for Children-Parent Rating Scales (BASC-PRS) to evaluate problematic behavior, such as externalizing and internalizing behaviors and attention problems (Engel et al., 2010). Neither the high molecular weight phthalate sum (Σ HMWP) nor the individual metabolites in the Σ HMWP group were related to any behavioral outcomes on the BREIF or the BASC-PRS, but the low molecular weight phthalate sum (Σ LMWP) and the individual metabolites in that group were related to some outcomes (Engel et al., 2010). They observed that higher prenatal exposure to Σ LMWP was associated with more attention problems, conduct problems, aggression problems, and externalizing problems in children 4 to 9 years old. For individual phthalates, monobutyl phthalate (MBP) was linked to aggression and externalizing problems; monoethyl phthalate (MEP) to attention problems, conduct problems, and externalizing problems; and monomethyl phthalate (MMP) to attention problems, as measured by the BASC-PRS (Engel et al., 2010). However, in the Taiwan Maternal and Infant Cohort Study, ADHD-like behaviors were related to some high molecular weight phthalate metabolites, such as mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethylhexyl) phthalate (MEHP) (Lien et al., 2015). In this cohort, mothers completed the Chinese version of the Child Behavior Checklist (CBCL) when the child was 8 years old. Like Engel et al. (2010), Lien et al. (2015) observed that higher prenatal exposure to MBP was associated with increased aggressive behavior and externalizing problems. Lien et al. (2015) also found that higher prenatal exposure to MEOHP was associated with more aggressive behavior and externalizing problems, and higher MEHP was linked to more externalizing problems. Kobrosly et al. (2014) also examined the relationship between prenatal exposure to phthalates via a maternal urine sample and child behavior, but

they used the CBCL between ages 6 and 10 in the Study for Future Families across four sites in the U.S. They observed that higher prenatal exposure to mono-benzyl phthalate (MBzP) was related to more oppositional/defiant behavior and conduct problems, but only in boys (Kobrosly et al., 2014). Kobrosly et al. (2014) also observed that mono-isobutyl phthalate (MiBP) was related to increased attention problems, oppositional/defiant problems, and conduct problems in all children, whereas MBP was only related to conduct problems. In only boys, MiBP was also related to greater rule-breaking behavior and externalizing behaviors (Kobrosly et al., 2014). In the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort in California, women provided urine samples to evaluate prenatal exposure to LMWP, HMWP, and Σ DEHP and child behavior at several ages (Hyland et al., 2019). In this majority low-income Mexican American cohort, researchers assessed executive function, general cognition, attention, behavior, and social cognition in children at 7, 9, 10.5, 12, 14, and 16 years of age (Hyland et al., 2019). At 9 and 12 years of age, they observed that higher prenatal exposure to the LMWP sum was associated with more errors of omission in the Conners' Continuous Performance Test (CPT II) and higher child-reported hyperactivity and attention problems on the Self-Report of Personality (Hyland et al., 2019). They also found that higher prenatal exposure to multiple phthalate metabolites was related to increased errors of commission (providing an incorrect response to a stimulus) in the CPT II at 9 and 12 years, suggesting that attentional and impulsivity problems associated with prenatal exposure to phthalates in childhood persist into adolescence (Hyland et al., 2019). Recently, Colicino et al. (2021) also found prenatal phthalate exposure was associated with increases in hyperactivity, as measured via the BASC-3 at 4-6 years of age in the Programming Research in Obesity, Growth Environment and Social Stress study in Mexico. In MoBa, researchers obtained ADHD diagnosis information from medical records of the children born in 2003 or later for a case-control analysis (Engel et al., 2018). They considered various phthalate metabolites on their own as well Σ DEHP and the sum of diisononyl phthalate metabolites (Σ DiNP). Engel et al. (2018) found that children in the highest quintile of

prenatal exposure to Σ DEHP were almost three times more likely to have a diagnosis of ADHD than children in the lowest quintile of prenatal exposure (Engel et al., 2018).

While the results summarized above suggest there is a relationship between prenatal exposure to phthalates and ADHD-like behaviors, others have found results suggesting that prenatal exposure to phthalates is associated with reduced ADHD-like behaviors. For example, in INMA in Spain, researchers examined prenatal exposure to phthalates and ADHD diagnostic criteria in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) at age 4, and ADHD-like behaviors as measured via the SDQ and Short form Conners' Parent Rating Scales at age 7 (Gascon et al., 2015). At age 4 both the sum of di(2-ethylhexyl) phthalate (DEHP) metabolites (Σ DEHP) and the diethyl phthalate (DEP) metabolite, MEP, were associated with a decreased risk of inattention symptoms. At age 7, only Σ DEHP was associated with a decreased risk of inattention symptoms, suggesting that prenatal exposure to DEHP or DEP is actually associated with reduced risk of inattention (Gascon et al., 2015). In another study using the SDQ to evaluate child behavior, Philippat et al. (2017) examined the relationship with prenatal exposure to phthalates in the Study of Pre- and Early Postnatal Determinants of the Child's Development and Health (EDEN) in France. At 3 years old, prenatal exposure to diisobutyl phthalate (DiBP) and di-n-butyl phthalate (DBP) were associated with lower hyperactivity-inattention scores on the SDQ, also suggesting that prenatal exposure to DiBP and DBP may reduce the risk of behaviors related to ADHD (Philippat et al., 2017).

In addition to multiple studies suggesting prenatal exposure to phthalates is related to increased or decreased risk of ADHD-like behaviors, yet others have found evidence to suggest there is no relationship. In the Polish Mother and Child Cohort, the relationship between prenatal exposure to several phthalate metabolites and child behavior via the SDQ was evaluated (Jankowska et al., 2019). Mothers completed the SDQ when their child was 7 years old, and Jankowska et al. (2019) found no relationship between any of the phthalate metabolites they examined and ADHD-like behavior at age 7 as measured by the SDQ. Kim et al. (2017)

examined the relationship between attention at 6 years of age and prenatal exposure to DBP and DEHP in the Environment and Development of Children cohort in South Korea. When children were 6 years old, their selective attention was evaluated using a visual continuous performance test, and Kim et al. (2017) found no relationship between prenatal exposure to either phthalate and selective attention. In another cohort in South Korea, the Children's Health and Environmental Chemicals in Korea, Kim et al. (2018) also examined the relationship between prenatal exposure to phthalates and ADHD-like behaviors, including attention, via the CBCL. They also did not find a relationship between prenatal exposure to phthalates, and attention at 13-24 months of age (Kim et al., 2018). However, despite the discrepancies across individual phthalate studies, a recent meta-analysis of studies looking at the relationships between a variety of environmental factors and ADHD-like behaviors found that the current evidence suggests there is a probable link between phthalate exposure and ADHD diagnosis (Nilsen and Tolve, 2020). In fact, the study reported that all children, girls and boys are at 3.12-3.54 greater odds of an ADHD diagnosis with prenatal exposure to phthalates (Nilsen and Tolve, 2020).

There are also mixed results regarding the relationship between prenatal exposure to phthalates and ASD-related outcomes (Table 1.2). Multiple studies have found no evidence for a link between the two. Singer et al. (2017) evaluated the relationship between prenatal exposure to several phthalate metabolites and child behavior using parental report measures at 12 months and 24 months of age. Both parental report measures used, the Infant Behavior Questionnaire (12 months) and the Toddler Behavior Assessment Questionnaire (24 months), ask questions about the child's social interactions, such as smiling and laughing. They found no relationship between any of the phthalate metabolites considered and any outcomes they evaluated in their sample of 279 children. Minatoya et al. (2016) examined MEHP levels in a maternal blood sample taken during the second trimester and child behaviors using the Bayley Scales of Infant and Toddler Development, Second Edition (BSID-II) at 6 and 18 months of age

in the Sapporo Cohort of the Hokkaido Study on Environment and Children's Health in Japan. They, too, found no relationship between MEHP and outcomes related to personal and social development as measured by the BSID-II in their sample of 127 children. In the Study for Future Families, Kobrosly et al. (2014) also looked at social problems as reported by parents on the CBCL when the child was between 6 and 10 years of age in their sample of 153 participants. While they found some associations between prenatal phthalate exposure and outcomes on the CBCL related to ADHD, but they did not find any relationship between prenatal exposure to phthalates and social problems that might be indicative of ASD (Kobrosly et al., 2014). In the CHAMACOS Cohort in California, Hyland et al. (2019) evaluated social cognition via three different assessments at different ages in 334 participants. At 9 years, researchers administered the Evaluación neuropsicológica del niño and the Affect Recognition subtest of A Developmental Neuropsychological Assessment at 12 years, both of which ask children to identify the mental state of people in several photos. At 14 years, parents completed the Social Responsiveness Scale, which specifically asks questions about how frequently the child exhibits behaviors related to ASD, such as avoiding social situations. Hyland et al. (2019) found no relationship between social cognition outcomes at 9, 12, or 14 years and prenatal exposure to phthalates. Shin et al. (2018) may have provided some of the strongest evidence that there is no relationship between ASD-related outcomes and prenatal exposure to phthalates. In their Markers of Autism Risk in Babies – Learning Early Signs cohort, a cohort at high risk of developing ASD, they evaluated the relationship between many common phthalates and ASD in 201 children (Shin et al., 2018). Children were evaluated for potential ASD diagnosis by licensed clinical psychologists at age 3, and Shin et al. (2018) found no relationship between any phthalate metabolites measured in maternal urine samples during pregnancy and ASD diagnosis.

While there seems to be an abundance of evidence suggesting no link between prenatal exposure to phthalates and ASD diagnosis or outcomes related to ASD, other studies have

provided contradictory evidence suggesting there may be a link. Most, but not all of these, studies have indicated that higher prenatal exposure to phthalates is associated with increased risk of ASD-related behaviors. For instance, in 7-year-old children in the INMA cohort, Gascon et al. (2015) found that only Σ DEHP metabolites were related to social competence as measured by the SDQ in their sample of 367 participants; however, they observed that higher exposure was correlated with improved social competence. In another sample from the Columbia Center for Children's Environmental Health – New York City (n = 319), parents completed the CBCL when their child was between 33 and 43 months of age, and the relationship between behavior and prenatal exposure to several phthalate metabolites was assessed (Whyatt et al., 2012). They found that higher concentrations of both mono-n-butyl phthalate (MnBP) and MBzP were related to more withdrawn and internalizing behaviors in all children, as well as more emotionally reactive behavior in boys only. Increasing MiBP concentrations were linked to more emotionally reactive behavior in all children. In a much smaller sample (n = 86), Kim et al. (2018) observed that only higher prenatal exposure to MEP was related to more internalizing behaviors as measured by the CBCL when the child was between 1 and 2 years of age. Engel et al. (2010) observed in 404 participants in the Mount Sinai Children's Environmental Health Center cohort, higher prenatal exposure to HMWP was associated with poorer adaptability between 4 and 9 years of age, as reported by parents via the BASC-PRS. Lien et al. (2015) also found that at 8 years of age, higher prenatal exposure to MEOHP was related to more social problems as reported by parents on the CBCL in the 430 children in the Taiwan Maternal and Infant Cohort Study. While Jankowska et al. (2019) did not find evidence for prenatal exposure to phthalates and ADHD-related behaviors in their sample of 134 participants, they did observe that higher prenatal exposure to MEP was associated with an increased risk of peer relationship problems as measured by the SDQ when the child was 2 years old. Philippat et al. (2017) also administered the SDQ, but when the child was 3 and 5 years of age. In the EDEN cohort, while MEP was not correlated with any outcomes, higher

concentrations of MnBP and MBzP were both related to more relationship problems (Philippat et al., 2017). MnBP was also related to more emotional problems, and MCPP was related to less prosocial behavior (Philippat et al., 2017). While these studies mostly indicate that prenatal exposure to phthalates is related to ASD-like behaviors, the results have not been consistent; thus, when considered together with the studies that have found no evidence for the relationship, it is not clear whether the link exists or not. Therefore, more research is needed to clarify whether there is a relationship between prenatal exposure to phthalates and ASD-related outcomes.

As Dzwilewski and Schantz (2015) noted, few studies have examined the potential relationship between prenatal phthalate exposure and language development, and of those that have, even fewer directly assessed language development. As with evidence for ADHD and ASD related behaviors, the evidence that prenatal exposure to phthalates negatively effects language development is not clear or consistent. On the Intelligence and Development Scales, Jankowska et al. (2019) were able to obtain some information about children's language skills at age 2 in the Polish Mother and Child Cohort, and, interestingly, they found that higher prenatal exposure to MEP was associated with better language skills. Jones et al. (2018) also observed a relationship between phthalate exposure and language development using the BSID, Third Edition (BSID-III). In the Growing Up in Singapore Towards Healthy Outcomes cohort, a hair sample was obtained from pregnant women between 26 and 28 weeks of gestation for metabolomic analysis, and children were assessed using the BSID-III at 24 months of age (Jones et al., 2018). In their analysis, Jones et al. (2018) found that higher levels of phthalic acid in maternal hair, suggestive of exposure to phthalates, were correlated with poorer expressive language abilities, but not receptive language abilities, or cognitive or motor abilities. However, Polanska et al. (2014) found no relationship between prenatal phthalate exposure and language development at 2 years of age using the BSID-III. Based on maternal blood samples, Minatoya et al. (2016) also found no relationship between prenatal exposure to MEHP and

communication and language development as measured using the BSID-II at 6 or 18 months of age.

Although studies that indirectly evaluated the relationship between prenatal phthalate exposure and language development have yielded mixed results, the few studies that have directly assessed language development have provided more consistent evidence. Bornehag et al. (2018a) examined language development in two different cohort studies: the SELMA study, and The Infant Development and the Environment Study (TIDES) in the U.S. In the SELMA study, 1,133 women provided a first morning urine sample at their first prenatal visit, and their child's language development was assessed as part of a routine evaluation at 30 months of age (Bornehag et al., 2018a). In TIDES, a multi-center cohort located in four different cities across the U.S., 370 women provided a urine sample at their first prenatal visit, and parents completed a translated version of the questionnaire used in the SELMA study when the child was between 24 and 63 months of age (Bornehag et al., 2018a). After controlling for multiple covariates, Bornehag et al. (2018a) found no relationship between prenatal phthalate exposure and language development in the TIDES cohort, but in the SELMA cohort, they observed that higher prenatal MBP, MBzP, and MEP were all associated with greater language delay at 30 months. In the Odense Child Cohort in Denmark, Olesen et al. (2018) found similar results. In this cohort, women completed the MacArthur Bates Communicative Development Inventories (CDI) for their child every three months between 16 and 36 months of age (Olesen et al., 2018). The CDI is a standardized parental report form that is widely used in research to evaluate a child's development of communication skills in the earliest stages of communicative development (Fenson et al., 2007). In their final sample of 518 children, Olesen et al. (2018) observed that boys in the highest quartile of prenatal exposure to the phthalate metabolites measured had lower vocabulary and language complexity scores than boys in the lowest quartile of exposure. Specifically, they found that higher MEP and Σ DEHP were associated with poorer vocabulary, and higher MEP, MBzP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-

carboxypentyl) phthalate (MECPP), MECPP, and Σ DEHP were associated with poorer language complexity scores in boys, and they observed no relationship between phthalates and language development in girls (Olesen et al., 2018). While there has been little work evaluating whether prenatal exposure to phthalates influences language development, the evidence supporting the existence of a relationship appears to outweigh that not supporting a relationship.

1.3. ASSESSING COGNITIVE DEVELOPMENT IN INFANCY & PREDICTIVE VALIDITY OF MEASURES

While many studies have been conducted to evaluate the relationship between prenatal exposures and child behavior and development, most have examined this relationship in early to middle childhood. Few studies to date have evaluated aspects of development in infancy which may be predictive of developmental problems or risk for diagnosis of ADHD or ASD, and even fewer have examined this relationship for the earliest stages of language development. There are many methods for assessing early child development available both for use in screening assessments and in research; however, they are not necessarily equally useful, reliable, or valid measures. The Bayley Scales of Infant and Toddler Development (BSID) has long-been considered the “gold standard” for directly assessing child development between the ages of 3 and 24 months (Yue et al., 2019). The BSID and its first revision (BSID-II) broadly divided measures into two categories: the Mental Developmental Index (MDI) to assess language and cognitive development, and the Psychomotor Developmental Index (PDI) to evaluate gross and fine motor development (Anderson et al., 2010). The fact that the MDI and PDI were so broad were considered the major limitations of the first two versions because the scores did not allow users to parse out which aspect of mental or motor development was the reason for a poor score (Anderson et al., 2010). The most recent version, the BSID-III, further separates scores into cognitive, language, and motor domains, and sub-scores can be utilized to examine specific areas of development, such as expressive communication, but several studies have found that the BSID-III underestimates developmental delay and is only weakly

predictive of later neurodevelopment (Anderson et al., 2010; Spencer-Smith et al., 2015; Gould et al., 2019). Another common assessment, the Ages and Stages Questionnaire (ASQ), could be argued to be a better measure of development as a parental report questionnaire. The ASQ is available to assess children from birth through 60 months of age, with different versions for specific age ranges, and evaluates a variety of developmental domains including fine motor skills, personal-social skills, and problem solving (Ringwalt, 2008). It is often used because it is quick and can be done by a child's caregiver, rather than needing to be done by a professional. There is substantial evidence that the ASQ has high specificity, reliability, and validity, but there is also evidence that it is more accurate the older the child is, and in some cases, it may not sufficiently predict later developmental outcomes (Lindsay et al., 2008; Velikonja et al., 2016; Mendonça et al., 2016; Charkaluk et al., 2017; Lamsal et al., 2018; Charafeddine et al., 2019). While these are only two potential measures, they are both widely used in both research and clinical practice, even though neither assessment is a good predictor of later developmental outcomes.

The field of developmental psychology has long relied upon observing the looking behaviors of infants to understand cognitive development. There is also compelling evidence that the looking behaviors of infants are good predictors of later development. In a meta-analysis of the usefulness of habituation and recognition memory paradigms for predicting child IQ, McCall and Carriger (1993) found that across several studies in various populations, infant performance on habituation and visual recognition memory (VRM) paradigms in their first 12 months was predictive of IQ when measured between 3 and 8 years of age, regardless of paradigm, lab, or population characteristics. Rose et al. (1992) also found that VRM performance at both 7 months and 1 year of age were correlated with multiple cognitive outcomes at 6 years, and children who were considered at risk for developmental disabilities had poorer VRM performance at both 7 months and 1 year of age. Rose et al. (2012) went even further and demonstrated that this relationship persisted through 11 years of age. In recent

years, the development of eye-tracking technology has made looking behaviors easier to measure and allowed researchers to obtain both more objective measures of infant cognition as well as outcome measures that are better predictors of later cognitive development. Downes et al. (2018) used eye-tracking to evaluate attentional control in infants born full-term or preterm when they were 12 months of age and observed that infants born preterm showed poorer attention than those born full-term. They also assessed infants using the BSID-III and found no difference in scores between preterm and full-term infants, which, taken with the concerns about the BSID underestimating developmental delays, suggested to the authors that eye-tracking technology may be a better method for evaluating infant cognitive development than the BSID (Downes et al., 2018). Jones and Klin (2013) used eye-tracking to measure attention across the first two years of life in infants and found that infant looking behavior was related to later ASD diagnosis. They observed that typically developing infants spent at least half of the assessment looking at the eyes of the individual shown in the video across development, and while infants later diagnosed with ASD initially showed this behavior at 2 months of age, the amount of time they spent looking at the eyes quickly decreased after 2 months of age (Jones and Klin, 2013). These two studies provide compelling evidence that eye-tracking methods can provide meaningful information about infant cognitive development. Furthermore, Chhaya et al. (2018) have demonstrated that eye tracking methods are not only possible, but also valid in lower resource, rural settings. In a small sample of 6-to-12-month-old infants in rural Uganda, they successfully modified and implemented the Fagan Test of Infant Intelligence using eye tracking technology (Chhaya et al., 2018). Their results provide evidence that not only is eye tracking feasible in communities with the technology readily available, but also in rural and low-resource areas. Based on these studies, eye-tracking measures would be an ideal alternative to measures such as the BSID for both assessing cognitive development in infancy and predicting cognitive performance later in childhood in large-scale, longitudinal epidemiological studies.

In the present study, eye-tracking was used to evaluate attention during infancy. The average amount of time an infant looked at the stimuli before looking away (average run duration) and the number of saccades, or gaze shifts, between the pair of stimuli per second (shift rate) were used to measure the infant's information processing speed. Shorter run durations and higher shift rates are indicative of faster information processing speed. Time to reach familiarization criteria (trial duration or time to familiarization) was used to assess attention, and shorter familiarization trials suggest better attention. The proportion of time the infant spends looking at a novel stimulus (novelty preference) was used to evaluate recognition memory, and higher novelty preference scores indicate better recognition memory. Previous work using the paradigm that was used has demonstrated that shift rate during familiarization is a strong predictor of novelty preference in infants, with higher shift rates being associated with higher novelty preference scores (Rose et al., 2001; Rose et al., 2003; Rose et al., 2004). Previous work has also shown that processing speed and attention are highly correlated; individuals with faster processing speeds tend to be more attentive and they become familiarized with a stimulus quicker, as indicated by a more rapid decrease in looking to the target stimulus over time (Rose et al., 2003; Ruff, 1975; Rose et al., 2004). Additionally, studies have found that outcomes evaluated in infancy using looking time measures are related to early language development, as well as IQ, memory, and expressive and receptive language and vocabulary at older ages (Rose et al., 2004; Rose et al., 2009). Because eye tracking can provide more detailed and precise information than the traditional methods of evaluating looking behavior employed in developmental psychology, the proposed study is expected to provide results in infancy that could be predictive of later attentional development and ADHD risk in a prospective birth cohort.

1.4. EVALUATING EARLY LANGUAGE DEVELOPMENT

Language is an important and complex part of cognitive development. Typically developing children begin to show evidence of language comprehension around 8 months of

age, and language production begins soon after, around 12 months of age. This is followed by a spurt of rapid development beginning around 16-18 months of age, and girls tend to develop language earlier and faster than boys (Huttenlocher et al., 1991; Bauer et al., 2002; Fernald et al., 2006; Rose et al., 2009; Eriksson et al., 2012). In addition to sex, environmental factors contribute to the differences in language development. Hadley et al. (2011) observed that of child-directed speech, the percentage of verbs providing unambiguous information about tense used by a parent was most predictive of language development at 30 months of age, indicating that the language environment provided by caregivers has a strong influence on language development, and Rowe et al. (2012) found that amount of child-directed speech was consistently predictive of child vocabulary at later ages. Additionally, different qualities of child-directed speech, such as the diversity and sophistication of vocabulary used, were predictive of child vocabulary development at later ages (Rowe et al., 2012). Another environmental factor known to influence language development is socioeconomic status (SES). Children from lower SES homes tend to have smaller vocabularies and less steep developmental trajectories than children from higher SES homes, and they consistently remain behind their higher SES counterparts in language development (Lee, 2011; Fernald et al., 2013). In addition to SES, family history, prematurity, number of siblings, and motor skills are also predictive of early language development (Zubrick et al., 2007). There is also evidence that the prenatal environment has an influence on language development. Whitehouse et al. (2012) found that higher cord blood testosterone levels, likely indicative of fetal blood circulation during third trimester development, were related to an increased risk of language delay in boys but a decreased risk of language delay in girls. Unfortunately, despite the rich information evaluating language development can provide, very few epidemiological studies have directly assessed language development in relation to prenatal environmental factors such as drug or chemical exposures.

Language development is often indirectly evaluated by cognitive tests such as the BSID, but direct assessments of language development are also available via structured testing or parental report measures (Dzwilewski and Schantz, 2015; Fenson et al., 1992; Fenson et al., 2007). Structured testing of language development has similar disadvantages to those of structured cognitive assessments such as the BSID: they require trained staff to administer; are done in a very controlled environment likely unfamiliar to the child; are time consuming, and require the child's cooperation (Fenson et al., 1992; Fenson et al., 2007). Parental report measures are thought to provide a more accurate reflection of a child's language development because unlike in structured tests done in the lab with a trained administrator, parents are able to observe their child across situations (Fenson et al., 1992; Fenson et al., 2007). While parental report measures may be biased, previous work has demonstrated that parents are fairly objective in their assessments of their own child (Reznick and Schwartz, 2001; Fenson et al., 1992; Fenson et al., 2007). One such measure is the MacArthur-Bates Communicative Development Inventories (CDI). The CDI has undergone norming studies and is reliable and valid for evaluating communicative development in both typically developing children and children with developmental disorders (Fenson et al., 2007; Bauer et al., 2002; Lee, 2011; Fernald and Marchman, 2012; Fernald et al., 2013). The CDI provides information about children's development of communication abilities from ages 8 to 30 months, focusing on language comprehension and early language production for younger children and vocabulary and grammar acquisition for older children (Fenson et al., 2007). Multiple studies using the CDI have demonstrated its usefulness for predicting later cognitive abilities. Marchman and Fernald (2008) found that children who were categorized as having both low productive vocabulary size and slow reaction time in a looking time task at 25 months had poorer working memory and lower IQ at 8 years. Lee (2011) also evaluated productive vocabulary size and found that children in the top tertile of vocabulary in their sample at 24 months had better language and literacy scores at 3-11 years of age.

Other parental report measures of language development have also proven informative in evaluating language development in the years between where the CDI leaves off at 30 months and before children begin formal schooling, such as the Speech and Language Assessment Scale (SLAS). The SLAS is a short questionnaire evaluating a child's language development, including articulation, vocabulary, conversational skills, and communication skills considered necessary for children to be considered ready to attend school (Hadley and Rice, 1993). This questionnaire was shown to be reliable and valid by the authors, and other studies have shown that it correlates with other standardized evaluations of language development (Hadley and Rice, 1993; Ratner and Silverman, 2000; Newman et al., 2006). In a study comparing parental ratings on the SLAS and CDI to other standardized assessments of language development in stuttering and non-stuttering children at 35 months of age, Ratner and Silverman (2000) found that both mothers and fathers reported their child's language development similarly on the SLAS and CDI. Scores on both measures were correlated with the child's performance on other standardized assessments (Ratner and Silverman, 2000). Scores on the SLAS were also highly correlated with previous CDI measures (Newman et al., 2006). Not only were SLAS scores at 4-6 years of age consistent with the child's performance in other standardized language assessments at the same age, but CDI scores at 24 months were highly correlated with SLAS scores at 4-6 years, suggesting that the SLAS and CDI are evaluating similar outcomes at different ages (Newman et al., 2006). In summary, although language development has not been directly evaluated in many epidemiological studies, reliable and valid methods to do so exist, and it is important to assess the development of language in large cohorts to further our understanding of the impact of environmental exposures on this critical aspect of cognitive development.

1.5. PRENATAL EXPOSURE TO ACETAMINOPHEN AND PHTHALATES MAY RESULT IN CUMULATIVE ENDOCRINE DISRUPTING EFFECTS

Public awareness of and concern about endocrine disrupting chemicals (EDCs) has increased dramatically in recent years leading to increased research exploring how they may be impacting human health and development. Some compounds, such as phthalates, have become widely recognized as EDCs, while others such as acetaminophen are just beginning to be considered as EDCs. Acetaminophen is widely used and generally recognized as safe for use in pregnancy because use at the recommended dosage has not been associated with birth defects seen with maternal use of other classes of medications used for pain relief, such as nonsteroidal anti-inflammatory drugs (NSAIDs) or opioids; however, there is growing evidence that even low doses of acetaminophen may have endocrine disrupting effects on prenatal development (Golding et al., 2019; Interrante et al., 2017). In two Danish cohorts, maternal use of acetaminophen during pregnancy was associated with congenital cryptorchidism and decreased anogenital distance (AGD) in male infants (Kristensen et al., 2011; Lind et al., 2017). Additionally, reduced AGD in both male and female offspring has been observed in rodent models of prenatal exposure to acetaminophen (Kristensen et al., 2011; Holm et al., 2016). Together, these results suggest that acetaminophen may have antiandrogenic actions. Acetaminophen has also been shown to directly reduce testosterone production, insulin-like factor 3 (ILF-3)¹, and prostaglandin E2 (PGE₂)² in *in vitro* cultures of human fetal testes (Kristensen et al., 2012; Mazaud-Guittot et al., 2013). Additionally, prenatal exposure to acetaminophen in an *in vivo* rodent model has been shown to reduce follicle reserve and fertility in female offspring (Holm et al., 2016). A few studies also found that brain development and behavior are altered in rodent models of prenatal acetaminophen exposure. Hay-Schmidt et al.

¹ ILF-3 is necessary for appropriate testicular descent.

² PGE₂ regulates aromatase expression. This enzyme is necessary for converting androgens to estrogens.

(2017) found that brain masculinization was impaired. That is, the number of neurons was reduced in the sexually dimorphic region of the preoptic area in the anterior hypothalamus of male offspring, and male reproductive behaviors in adulthood were altered. Adult males exposed to acetaminophen prenatally showed an abnormal territorial marking pattern, less aggressive territorial behaviors towards other males, and altered sexual behavior during mating (Hay-Schmidt et al., 2017). In summary, there is growing evidence that acetaminophen can act as an antiandrogenic EDC.

Phthalates are widely considered to be EDCs, and they too have been demonstrated to have antiandrogenic actions. Remarkably, the effects seen in studies of prenatal exposure to acetaminophen seem to mirror those of studies examining the effects of prenatal exposure to phthalates. Phthalates have also been shown to reduce testosterone and ILF-3 and two other enzymes important in the synthesis of steroid hormones, including androgens, specifically cholesterol side chain cleavage enzyme (CYP11A) and steroidogenic acute regulatory protein (StAR), in rodent models (Parks et al., 2000; Howdeshell et al., 2007; Hannas et al., 2011). Recent studies have also reported that prenatal exposure to phthalates results in reduced AGD in both animals and humans, as well as smaller reproductive organs and increased incidence of reproductive malformations in male offspring (Parks et al., 2000; MacLeod et al., 2009; Swan et al., 2015; Howdeshell et al., 2007). Furthermore, exposure to mixtures of antiandrogenic phthalates appear to have these effects in an additive, dose-dependent manner (Howdeshell et al., 2007; Hannas et al., 2011). Exposure to both acetaminophen and phthalates during prenatal development is highly likely because nearly all humans have detectable levels of several phthalate metabolite in urine samples, and acetaminophen is the primary analgesic recommended as safe for pregnant women to use throughout pregnancy (CDC, 2019; Servey and Chang, 2014). Because acetaminophen appears to have antiandrogenic actions similar to those seen in studies of prenatal exposure to phthalates, these two compounds may be exerting their effects on the same pathways or via similar mechanisms. If this is the case, it is reasonable

to expect that prenatal exposure to both acetaminophen and phthalates will disproportionately affect male infants, and have an additive effect, resulting in greater effects than exposure to either one alone. Therefore, the proposed study will examine the relationship between prenatal exposure to acetaminophen and phthalates, both individually and together, and early cognitive and language development.

1.6. TABLES AND FIGURES

Table 1.1. Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) <i>Journal</i> Population/Location <i>Sample size</i>	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Streissguth et al. (1987) <i>Teratology</i> The Seattle Longitudinal Study United States <i>n = 421</i>	Maternal self-report of frequency of use in first 5 months of pregnancy	Vigilance paradigm (attention) 3 months	No association
Brandlistuen et al. (2013) <i>International Journal of Epidemiology</i> Norwegian Mother and Child Cohort (MoBa) Study Norway <i>n = 2919</i>	Maternal self-report of frequency of use at GW 17 and 30, 6 months postpartum	ASQ ¹ , age to walk, CBCL ² , EAS ³ 3 years	Long-term use only ↓ Communication skills ↑ Internalizing behaviors ↑ Activity Short-term use only ↓ Emotionality Long and short-term use ↑ Externalizing behaviors

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Liew et al. (2014) <i>JAMA Pediatrics</i> Danish National Birth Cohort (DNBC) Denmark <i>n</i> = 64,322	Maternal self-report of frequency of use at GW 12 and 30, 6 months postpartum	SDQ ⁴ , hospital diagnosis of hyperkinetic disorder, 2+ prescriptions filled for ADHD medications 7 years	↑ ADHD-like behavior ↑ Hospital diagnosis of hyperkinetic disorder ↑ 2+ prescriptions filled for ADHD medications
Thompson et al. (2014) <i>PLOS ONE</i> Auckland Birthweight Collaborative New Zealand <i>n</i> = 871	Maternal self-report of any use during pregnancy shortly after birth	SDQ, CRS:R-L ⁵ 7 years 11 years	Both ages ↑ ADHD-like behavior

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal	Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Avella-Garcia et al. (2016) <i>International Journal of Epidemiology</i>	Spanish Infancia y Medio Ambiente (INMA) Spain <i>n</i> = 2644	Maternal self-report of frequency of use at GW 12 and 32	BSID ⁶ Mean age 14.84 months MCSA ⁷ , CPSCS ⁸ , CAST ⁹ , K-CPT ¹⁰ , ADHD-DSMIV ¹¹ Mean age 4.8 years	14.84 months No association 4.8 years—Boys ↑ ASD symptoms 4.8 years—Girls No association 4.8 years—Boys and Girls ↑ Hyperactivity symptoms ↑ Impulsivity symptoms ↓ Attention
Liew et al. (2016a) <i>International Journal of Epidemiology</i>	Lifestyle During Pregnancy Study sub-cohort of DNBC Denmark <i>n</i> = 1491	Maternal self-report of frequency of use at GW 12 and 30, 6 months postpartum	TEACH-5 ¹² , BRIEF ¹³ 5 years	↓ Attention

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Liew et al. (2016c) <i>Autism Research</i> DNBC Denmark <i>n</i> = 64,322	Maternal self-report of frequency of use at GW 12 and 30, 6 months postpartum	ASD diagnosis Mean age 12.7 years	↑ ASD diagnosis
Stergiakouli et al. (2016) <i>JAMA Pediatrics</i> Avon Longitudinal Study of Parents and Children (ALSPAC) United Kingdom <i>n</i> = 7796	Maternal self-report of any use at GW 18 and 32, maternal and partner self-report of use when child was 61 months old	SDQ 7 years	Use at GW 18 ↑ Conduct problems ↑ Hyperactivity symptoms Use at GW 32 ↑ Emotional symptoms ↑ Conduct problems ↑ Hyperactivity symptoms ↑ Total difficulties
Vlenterie et al. (2016) <i>International Journal of Epidemiology</i> MoBa Norway <i>n</i> = 51,200	Maternal self-report of frequency of use at GW 17 and 30, 6 months postpartum	ASQ, CBCL, EAS 18 months	↓ Communication

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Skovlund et al. (2017) <i>Pharmacoepidemiology and Drug Safety</i> MoBa Norway <i>n</i> = 51,679	Maternal self-report of any use at GW 17 and 30, 6 months postpartum	Language competency, ASQ 3 years	↓ Communication
Ystrom et al. (2017) <i>Pediatrics</i> MoBa Norway <i>n</i> = 112,973	Maternal self-report of any use at GW 17 and 30, 6 months postpartum	ADHD diagnosis	↑ ADHD diagnosis
Bornehag et al. (2018b) <i>European Psychiatry</i> Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study Sweden <i>n</i> = 754	Maternal self-report of frequency of use and urinary biomarkers at GW 8-13	Nurse evaluation and parental report questionnaire on language use 30 months	Boys No association Girls ↑ Language delay

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal	Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Ji et al. (2018) <i>Brain Sciences</i>	Boston Birth Cohort United States <i>n = 1180</i>	Maternal plasma biomarkers 1-3 days postpartum	ADHD diagnosis, ASD diagnosis, diagnosis of another developmental delay/intellectual disability	↑ ADHD diagnosis
Tovo-Rodrigues et al. (2018) <i>BMC Psychiatry</i>	Pelotas Birth Cohort Brazil <i>n = 3722</i>	Maternal self-report of use at the time of child's birth	SDQ 6 years 11 years	Age 6—Boys ↑ Hyperactive symptoms ↑ Emotional symptoms Age 6—Girls No association Age 11—Boys and Girls No association
Golding et al. (2019) <i>Paediatric and Perinatal Epidemiology</i>	ALSPAC United Kingdom <i>n = 12,418</i>	Maternal self-report of use between GW 18 and 32 at GW 32	Carey temperament, EAS, SDQ, Rutter, DAWBA ¹⁴ Assessed multiple times between 6 months and 17 years of age	Pre-school-age ↓ Temperament ↑ Activity ↓ Attention Grade school-age No association

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Inoue et al. (2020) <i>American Journal of Epidemiology</i> DNBC Denmark <i>n</i> = 40,934	Maternal self-report of frequency of use at GW 12 and 30, 6 months postpartum	SDQ 11 years	↑ Internalizing problems ↑ Externalizing problems ↑ Total difficulties
Ji et al. (2020) <i>JAMA Pediatrics</i> Boston Birth Cohort United States <i>n</i> = 996	Umbilical cord plasma biomarkers	ADHD diagnosis, ASD diagnosis, ADHD and ASD diagnosis, diagnosis of other developmental disorder, no diagnosis	↑ ADHD diagnosis ↑ ASD diagnosis
Liew et al. (2019) <i>American Journal of Epidemiology</i> Nurses' Health Study II United States <i>n</i> = 8856	Maternal self-report via biennially mailed questionnaires after child's birth	Maternal report of ADHD diagnosis	↑ ADHD diagnosis

Table 1.1. (cont.) Characteristics of the studies of maternal acetaminophen use during pregnancy and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Tovo-Rodrigues et al. (2020) <i>Paediatric and Perinatal Epidemiology</i> Pelotas Birth Cohort Brazil <i>n = 3737</i>	Maternal self-report of use at the time of child's birth	BDI ¹⁵ 24 months CBCL 48 months	24 months No association 48 months No association
Trønnes et al. (2020) <i>Paediatric and Perinatal Epidemiology</i> MoBa Norway <i>n = 32,934</i>	Maternal self-report of any use at GW 17 and 30, 6 months postpartum	ASQ, CBCL, EAS 5 years	↑ Internalizing behavior ↑ Externalizing behavior ↓ Shyness

¹ASQ = Ages and Stages Questionnaire

²CBCL = Child Behavior Checklist

⁶CBCL = Child Behavior Checklist

³EAS = Emotionality, Activity and Shyness Temperament Questionnaire

⁴SDQ = Strengths and Difficulties Questionnaire

⁵CRS:R-L = Conners' Behavioural Rating Scale: Revised - Long Format

⁶BSID = Bayley Scales of Infant and Toddler Development

⁷MCSA = McCarthy Scales of Children's Abilities

⁸CPSCS = California Preschool Social Competence Scale

⁹CAST = Childhood Autism Spectrum Test

¹⁰K-CPT = Conner's Kiddie Continuous Performance Test

¹¹ADHD-DSMIV = Attention-Deficit/Hyperactivity Disorder Criteria of the
Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition form list

¹²TEACH-5 = Test of Everyday Attention for Children at Five

¹³BRIEF = Behavior Rating Inventory of Executive Function

¹⁴DAWBA = Development and Well-being Assessment

¹⁵BDI = Battelle's Developmental Inventory

Table 1.2. Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Engel et al. (2010) <i>Environmental Health Perspectives</i> Mount Sinai Children's Environmental Health Center United States <i>n</i> = 177	BRIEF ¹ , BASC-PRS ² 4 - 9 years	MBzP, MEHP, MECPP, MEHHP, MEOHP, MCPP, MMP, MEP, MiBP	↑ HMWP ↓ adaptive behavior skills
			↑ LMWP ↑ aggression ↑ attention problems ↑ conduct problems ↑ externalizing problems
		HMWP (MBzP, MEHP, MECPP, MEHHP, MEOHP, MCPP)	
		LMWP (MMP, MEP, MiBP)	↑ MEP ↑ attention problems ↑ conduct problems ↑ externalizing problems
			↑ MBP ↑ aggression ↑ externalizing problems
			↑ MMP ↑ attention problems
Whyatt et al. (2012) <i>Environmental Health Perspectives</i> Columbia Center for Children's Environmental Health (CCCEH) United States <i>n</i> = 319	CBCL ³ 33 - 43 months	MiBP, MBzP, MnBP, MEHP, MEHHP, MEOHP, MECPP	↑ MiBP ↑ emotionally reactive behavior
		ΣDEHP (MEHP, MEHHP, MEOHP, MECPP)	

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Kobrosly et al. (2014) <i>Environmental Health Perspectives</i> Study for Future Families United States <i>n</i> = 153	CBCL, DSM-IV ⁴ categories 6 - 10 years	MEHP, MEHP, MEOHP, MiBP, MBP, MBzP, MEP Σ DEHP (MEHP, MEHP, MEOHP)	Boys ↑ MBzP ↑ oppositional/defiant behavior ↑ conduct problems Girls No association All children ↑ MiBP ↑ attention problems ↑ oppositional/defiant problems ↑ conduct problems ↑ MBP ↑ conduct problems
Gascon et al. (2015) <i>International Journal of Hygiene and Environmental Health</i> INfancia y Medio Ambiente birth cohort (INMA) Spain <i>n</i> = 657	McCarthy Scales of Children's Abilities, Bilingual version of California Preschool Social Competence Scale, ADHD 4 years SDQ ⁵ , Short form Conners' Parent Rating Scales 7 years	MEHP, MEHP, MEOHP, MBzP, MEP, MiBP Σ DEHP (MEHP, MEHP, MEOHP)	4 years ↑ Σ DEHP ↑ social competence ↓ risk inattention symptoms ↑ MEP ↓ risk inattention symptoms 7 years ↑ Σ DEHP ↓ risk inattention symptoms ↓ risk ADHD symptoms

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal	Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Lien et al. (2015) <i>Environmental Health Perspectives</i>	Taiwan Maternal and Infant Cohort Study Taiwan <i>n</i> = 163	WISC-III ⁶ , CBCL 8 years	MMP, MEP, MBP, MBzP, MEHP, MEHHP, MEOHP	↑ MBP ↑ MEOHP ↑ MEHP ↑ aggressive behavior ↑ externalizing problems ↑ social problems ↑ aggressive behavior ↑ externalizing problems ↑ externalizing problems
Kim et al. (2017) <i>Environmental Research</i>	The Environment and Development of Children cohort South Korea <i>n</i> = 175	Korean Educational Development Institute's WISC ⁷ , Continuous performance test 6 years	DBP metabolites DEHP metabolites	No association

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Philippat et al. (2017) <i>Environmental Health Perspectives</i> Study of Pre- and Early Postnatal Determinants of the Child's Development and Health (EDEN) France <i>n</i> = 546	SDQ 3 years 5 years	MEP, MnBP, MiBP, MBzP, MCOP, MCP, MCNP Σ DEHP (MECP, MEHP, MEHP, MEOHP)	3 years $\uparrow$ MnBP $\uparrow$ internalizing behavior $\uparrow$ relationship problems $\uparrow$ emotional problems $\uparrow$ MBzP $\uparrow$ internalizing behavior $\uparrow$ relationship problems $\uparrow$ MiBP $\downarrow$ hyperactivity-inattention scores $\uparrow$ MCP $\downarrow$ hyperactivity-inattention scores $\uparrow$ MCP $\downarrow$ prosocial behavior scores 5 years No association

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Bornehag et al. (2018a) <i>JAMA Pediatrics</i> Swedish Environmental Asthma Longitudinal Mother and Child, and Allergy (SELMA) study Sweden	SELMA Language assessment 30 months TIDES Language assessment 24 - 63 months	MEP, MBP, MBzP, MiBP, MEHP, MEHHP, MEOHP, MECPP TIDES	SELMA ↑MBP ↑MBzP ↑MEP TIDES No association
<i>n = 1133</i> The Infant Development and the Environment Study (TIDES) United States <i>n = 370</i>			
Engel et al. (2018) <i>Environmental Health Perspectives</i> Norwegian Mother and Child Cohort (MoBa) Norway <i>n = 297 cases</i> <i>n = 553 controls</i>	ADHD cases and randomly selected controls enrolled in MoBa from the Norwegian Patient Registry	MEP, MiBP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MMCHP, OH-MiNP, oxo-MiNP, cx-MiNP ΣDEHP (MEHP, MEHHP, MEOHP, MECPP, MMCHP) ΣDiNP (OH-MiNP, oxo-MiNP, cx-MiNP)	↑ ΣDEHP ↑ risk of ADHD

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Kim et al. (2018) <i>Science of the Total Environment</i> Children's Health and Environmental Chemicals in Korea (CHECK) cohort Korea <i>n</i> = 86	Social Maturity Scale, CBCL 13 - 24 months	MiBP, MnBP, MEHP, MEHHP, MEOHP, MEP	↑ MEP ↑ internalizing problems
Olesen et al. (2018) <i>Neurotoxicology and Teratology</i> Odense Child Cohort Denmark <i>n</i> = 518	MB-CDI ⁸ 20 - 35 months	MEP, MiBP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MiNP, MHiNP, MHiOP, MCIOP ΣDEHP (MEHP, MEHHP, MEOHP, MECPP) ΣDiNP (MiNP, MHiNP, MHiOP, MCIOP)	All children No association Boys ↑ MEP ↓ vocabulary ↓ complexity ↑ ΣDEHP ↓ vocabulary ↓ complexity ↑ MBzP ↓ complexity ↑ MEHHP ↓ complexity ↑ MEOHP ↓ complexity ↑ MECPP ↓ complexity Girls No association

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Shin et al. (2018) <i>Environmental Health</i> Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) cohort United States <i>n</i> = 201	ASD diagnosis by a licensed clinical psychologist using the Autism Diagnostic Observation Schedules 3 years	MEP, MiBP, MHiBP, MBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MCP, MNP, MCOP, MCNP	No association

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Hyland et al. (2019) <i>Environmental Health Perspectives</i>	BRIEF, WISC-IV ⁹ , BASC-2 ¹⁰ , CADS ¹¹ 7 years	MEP, MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECCP, MCOP, MCNP, MCPP	Boys ↑ Σ LMWP ↓ perseverative errors
Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study United States <i>n</i> = 334	BRIEF, NEPSY ¹² tower, WCST ¹³ , Evaluación neuropsicológica del Niño (social cognition), CADS, CPT II ¹⁴ 9 years	Σ LMWP (MEP, MBP, MiBP) Σ HMWP (MBzP, MCPP, MCOP, MCNP) Σ DEHP (MEHP, MEHHP, MECCP, MEOHP)	Girls No associations All children ↑ Σ DEHP ↑ social cognition (9 y)
	WISC-IV, BASC-2, BASC-2 SRP ¹⁵ 10.5 years		↑ Σ LMWP ↑ errors of omission (9 y, 12 y) ↑ hyperactivity (16 y, self-report) ↑ attention (16 y, self-report)
	BRIEF, WCST, NEPSY-II Affect Recognition Subtest ¹⁶ , CADS, CPT II 12 years		↑MEP ↑ errors of commission (9 y, 12 y)
	SRS-2 ¹⁷ , BASC-2, BASC-2 SRP 14 years		↑MBzP ↑ errors of commission (9 y, 12 y)
	BASC-2, BASC-2 SRP 16 years		↑MCPP ↑ errors of commission (9 y, 12 y)
			↑MCOP ↑ errors of commission (9 y, 12 y)

Table 1.2. (cont.) Characteristics of the studies of prenatal exposure to phthalates and neurodevelopmental outcomes related to ADHD and ASD referenced in Chapter 1.

Study Author (Date) Journal Population/Location Sample size	Measure of Acetaminophen Exposure	Cognitive Measurements Age(s) at Assessment	Results
Jankowska et al. (2019) <i>Environmental Research</i> Polish Mother and Child Cohort Poland <i>n = 134</i>	SDQ, IDS ¹⁸ 7 years	MEP, MiBP, MnBP, OH-MnBP, MBzP, MEHP, OH-MEHP, oxo-MEHP, OH-MiNP, oxo-MiNP, MnOP	↑ MEP ↑ risk peer relationship problems
Colicino et al. (2021) <i>Environmental Research</i> Programming Research in Obesity, Growth Environment and Social Stress (PROGRESS) study Mexico <i>N = 514</i>	BASC-3 ¹⁹ 4-6 years	MEP, MBP, MiBP, MHBP, MHiBP, MBzP, MCP, MEHP, MEHP, MEOHP, MECPP, MCOP, MONP, MCNP, MECPTP	All children ↑ MECPTP ↑ hyperactivity Boys ↑ MECPTP ↑ behavioral symptoms index (BSI) score ↑ depression score Girls ↑ MECPTP ↑ hyperactivity ↑ MBzP ↓ hyperactivity

¹BRIEF = Behavior Rating Inventory of Executive Function

²BASC-PRS = Behavior Assessment System for Children-Parent Rating Scales

³CBCL = Child Behavior Checklist

⁴DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

⁵SDQ = Strengths and Difficulties Questionnaire

⁶WISC-III = Wechsler Intelligence Scale for Children, third edition

⁷WISC = Wechsler Intelligence Scale for Children

⁸MB-CDI = MacArthur-Bates Communicative Development Inventories

⁹WISC-IV = Wechsler Intelligence Scale for Children, fourth edition

¹⁰BASC-2 = Behavior Assessment System for Children, second edition

¹¹CADS = Conners' ADHD/DSM-IV Scales, parent versions

¹²NEPSY= A Developmental NEUROPSYchological Assessment

¹³WCST = Wisconsin Card Sort Task

¹⁴CPT II = Conners' Continuous Performance Test, version 5

¹⁵BASC-2 SRP = Behavior Assessment System for Children Self-Report of Personality, second edition

¹⁶NEPSY-II Affect Recognition Subtest = A Developmental NEUROPSYchological Assessment, Affect Recognition Subtest, second edition

¹⁷SRS-2 = Social Responsiveness Scale, second edition

¹⁸IDS = Intelligence and Development Scales

¹⁹BASC-3 = Behavior Assessment System for Children Self-Report of Personality, third edition

CHAPTER 2. SPECIFIC AIMS

As reviewed, acetaminophen is widely used among pregnant women, and although use at the recommended dosage has not been implicated in major birth defects or malformations, there is growing concern that it could be affecting child development in more subtle ways (Feldkamp et al., 2010). In particular, there is emerging evidence that greater maternal use of acetaminophen during pregnancy may be related to poorer neurodevelopmental outcomes, particularly increased attention-deficit/hyperactivity disorder (ADHD) risk, and delays in language development (Fan et al., 2017). Previous work has demonstrated that attentional deficits can be detected in infancy and that these deficits may be early indicators of later ADHD diagnosis (Miller et al., 2018). However, only one study, published over thirty years ago, has examined the relationship between prenatal exposure to acetaminophen and attention in infancy (Streissguth et al., 1987). Furthermore, only two studies have examined the relationship between prenatal exposure to acetaminophen and language development, and neither used a validated parental report measure (Bornehag et al., 2018b; Skovlund et al., 2017). Prenatal exposure to phthalates has also been linked to deficits in attention and language development, suggesting that phthalates and acetaminophen may impact neurodevelopment in similar ways, but no research thus far has considered both exposures together. It is critical that research considers how the exposure to acetaminophen and phthalates together may influence neurodevelopment because exposure to both is common (Ji et al., 2018; Centers for Disease Control, 2019). The present study examined the relationship between prenatal exposure to acetaminophen and attention in infancy using eye tracking methods, and the relationship between prenatal exposure to acetaminophen and early language development using a validated parental report measure, as well as whether co-exposure to acetaminophen and phthalates has cumulative effects on these outcomes.

The Illinois Kids Development Study (IKIDS) is an ongoing prospective birth cohort study at the University of Illinois in which a wide variety of information is collected about maternal demographics, health, and behavior during pregnancy and infant and child development after birth. Pregnant women are interviewed at six time points during their pregnancy, including about prescription and non-prescription drug use, and provide a first-morning urine sample at five time points during pregnancy, providing a comprehensive picture of exposure to a variety of factors, including phthalates, during pregnancy. They then bring their children to the lab multiple times during infancy for cognitive assessments and complete multiple questionnaires and interviews about their child's development throughout infancy and early childhood. This study used this rich dataset to assess associations between developmental exposure to acetaminophen alone, as well as in combination with phthalates, with measures of attention and early language development.

The Specific Aims of this study were to:

SPECIFIC AIM 1: Assess the relationship between prenatal exposure to acetaminophen exposure and children's attention at 7.5 months of age using eye tracking technology and at 2 and 3 years of age using a validated parental report measure. Looking behavior was evaluated in infants between 7 and 8 months of age using a visual recognition memory (VRM) assessment designed by Rose et al. (1992) and adapted for use with infrared eye tracking by our research team (Dzwilewski et al., 2020). Infants were shown pairs of human faces and geometric patterns, and eye tracking was used to capture precise information about the infant's looking behavior. Novelty preference was used to evaluate recognition memory. Average run duration and shift rate were used to measure the infant's information processing speed. Time to familiarization was used to measure attention. The focus of this study is on the measure of attention, and exposure to acetaminophen was evaluated both by trimester and across pregnancy. At 2 and 3 years of age, attention was evaluated using the subscale for

attentional difficulties of the Child Behavior Checklist (CBCL) parent report. Prenatal exposure to acetaminophen was evaluated both by trimester and across pregnancy. **It was hypothesized that children with higher prenatal exposure to acetaminophen would show deficits in attention, and that boys would be disproportionately affected. Additionally, it was expected that greater exposure during the second trimester would be associated with greater deficits in boys.** The second trimester was expected to be particularly susceptible to impacts of prenatal acetaminophen exposure because acetaminophen was suspected to be acting via antiandrogenic action mechanisms, and the second trimester is thought to be the time of peak androgen production by the male fetus (Auyeung et al. 2013).

SPECIFIC AIM 2: Evaluate the association of prenatal exposure to acetaminophen and language development in 2- and 3-year-old children using validated parental-report measures. Children later diagnosed with ADHD or autism spectrum disorder (ASD) often exhibit delayed language development and problems in language development (American Psychiatric Association, 2013; Yew and O'Kearney, 2017; Sciberras et al., 2014; Rohrer-Baumgartner et al., 2016; Goh et al., 2020; Karasinski, 2015). However, early language development is a critical aspect of cognitive development in typically developing children as well, with multiple studies indicating it is predictive of later language development as well as IQ, reading ability, and school success (Zubrick et al., 2007; Nelson et al., 2016). When their child was 2 years old, mothers completed the MacArthur-Bates Communicative Development Inventories (CDI) to provide information about the child's vocabulary, grammatical complexity, and productive language abilities (Fenson et al., 2007). When their child was 3 years old, mothers completed the Speech and Language Assessment Scale (SLAS), providing information about their child's conversational assertiveness in their speech, responsiveness in communication, semantics, syntax, articulation, and talkativeness (Hadley and Rice, 1993). **It was hypothesized that children with higher acetaminophen exposure during prenatal**

development would have poorer outcomes on these measures of language development at 2 and 3 years of age, and that boys would be disproportionately affected, especially with higher exposure during the second trimester.

SPECIFIC AIM 3: Examine the potential for cumulative effects of prenatal exposure to both phthalates and acetaminophen on attention at 7.5 months, 2 years, and 3 years, and language development at 2 and 3 years. Exposures to medications typically co-occurs with other exposures, yet most studies examining the relationship between prenatal exposure to medications or chemicals and development tend to focus on a single drug or chemical (Nordeng et al., 2014). Given that prenatal exposure to phthalates and prenatal exposure to acetaminophen have been shown to be associated with similar neurodevelopmental outcomes, this study examined the potential interactive effects of prenatal exposure to both acetaminophen and phthalates using the VRM paradigm to assess attention at 7.5 months of age, the CBCL to evaluate attention at 2 and 3 years, and the CDI and SLAS to evaluate language development at 2 and 3 years, respectively. **It was hypothesized that children with higher exposures to both compounds prenatally would show greater deficits in both attention and language development. Because both phthalates and acetaminophen appear to have antiandrogenic action, boys were expected to show greater deficits than girls.** Additionally, this was the first study, to our knowledge, to examine how these two common exposures may be interacting to influence neurodevelopment.

CHAPTER 3: COHORT PROFILE: ILLINOIS KIDS DEVELOPMENT STUDY (IKIDS)³

The potential for prenatal exposure to chemical and non-chemical environmental factors, including EDCs, to adversely impact child development is a growing public health concern. Phthalates are known EDCs and are used in many consumer products, resulting in multiple routes of exposure (oral, dermal, and inhalation) in humans, and these chemicals, or their metabolites, are consistently detected in human urine, blood, amniotic fluid, cord blood, and breastmilk (Centers for Disease Control and Prevention, 2019; Hogberg et al., 2008; Shekhar et al., 2017; Miodovnik et al., 2011). Pregnant women and infants are thus vulnerable to the effects of these EDCs. Recently, evidence has emerged suggesting that acetaminophen may also have endocrine disrupting actions, similar to those seen with phthalate exposure (Kristensen et al., 2011; Kristensen et al., 2012; Kristensen et al., 2016). Acetaminophen is one of the few medications pregnant women are told are safe to use during pregnancy, but recent studies have demonstrated that it is able to cross the placenta and that it may not be as safe for use in pregnancy as it was previously thought to be (Conings et al., 2019; Servey and Chang, 2014; Golding et al., 2019). Prenatal exposure to phthalates or acetaminophen has been associated with poorer neurodevelopmental outcomes, however few studies have directly evaluated the impact of these exposures on infant cognitive development (Ejaredar et al., 2015; Miodovnik et al., 2014; Avella-Garcia et al., 2016; Masarwa et al., 2018). Furthermore, published studies have largely focused on global measures of cognition, rather than measures of specific cognitive domains. Another concern is the possible cumulative or interactive impact of phthalates and acetaminophen, but there is limited information about their individual effects on cognition during infancy and early childhood, and little to no information regarding joint effects.

³ Updated from a previous version to include demographic information collected from participants through March 2020 as well as acetaminophen data. Upon completion of cohort recruitment, a version of this manuscript will be submitted for publication to BMJ Open with authorship as follows: Dzwilewski, K.*, Merced Nieves, F.*, Woodbury, M., Strakovsky, R., Aguiar, A., & Schantz, S.

*denotes equal contribution.

The Illinois Kids Development Study (IKIDS), an ongoing prospective pregnancy and birth cohort, was established with the main goal of assessing the impact of prenatal exposure to environmental factors on specific domains of cognition and to obtain these results earlier in life than had been done before. Another goal was to follow children prospectively and assess whether associations of exposures with cognitive outcomes observed in infancy predict continued risk later in childhood. IKIDS has gathered a wealth of information on prenatal exposures, health, diet, demographics, and lifestyle variables to obtain a holistic view of the prenatal environment. Prenatal exposure to phthalates was assessed by measuring metabolites of interest in an individual urine sample at 16-18 weeks of gestation and in a pooled sample of five maternal urines collected approximately 4-6 weeks apart from 10-14 to 34-36 weeks of gestation, providing an average measure of exposure across pregnancy. The time point of 16-18 weeks of gestation may be of particular importance for the influence of EDCs as it is the time of peak androgen production in male fetuses. Maternal medication use, including the use of medications containing acetaminophen, was also collected approximately every 4-6 weeks from 10-14 gestation until the infant's birth, by an interview administered by a trained researcher. In each interview, women were asked to list names of each prescription and over the counter medication they had used; the reason for taking the medication; the frequency of use, and the dates they began and stopped taking the medication.

Our research group is using innovative measures adapted from developmental psychology which use looking behaviors to assess basic building blocks of cognition including physical reasoning, recognition memory, attention, and information processing speed early in infancy. These measures of looking behavior have been shown to be reliable and stable measures of basic cognitive processes and are predictive of cognitive abilities later in childhood (Rose et al. 1995; Rose et al., 2012; Downes et al., 2018; Jones and Klin, 2013; Chhaya et al., 2018). We have adapted these measures using state-of-the-art infrared eye tracking technology that provides much more precise estimation of gaze location than traditional methods and

automates data collection and processing for use in large-scale epidemiological studies. In this paper, we describe the study design, data collection, baseline characteristics, and exposure characteristics of the IKIDS cohort.

3.1. COHORT DESCRIPTION

3.1.1. Recruitment and Eligibility

IKIDS is a prospective pregnancy and birth cohort located in east central Illinois, USA. Participants were recruited between December 2013 and March 2020 at two local obstetric clinics (Christie Clinic and Carle Physician Group) and gave birth at two local hospitals (OSF Heart of Mary Medical Center and Carle Foundation Hospital). The research laboratory is located at the University of Illinois at Urbana-Champaign.

Recruiting clinics gave out informational brochures regarding the study to new pregnant obstetrics patients at their first prenatal visits. Patients were asked to fill out a reply card indicating their interest in being contacted about participation in the study. Those who declined to be contacted were asked to provide basic demographic information for the purpose of comparing participants with non-participants. Those who indicated permission to be contacted were called over the telephone to receive more information about participation and determine eligibility. Women were eligible to participate if they had not reached 15 weeks of gestation, were between the ages of 18 and 40 years, did not have a child already participating in IKIDS, were fluent in English, were not carrying multiples, were not in a high-risk pregnancy, resided within a 30-minute drive of the University of Illinois campus, were able to provide a fasting blood sample and five urine samples throughout pregnancy, and were able to bring their infant to campus for 4 visits during the first year of life. Those who were eligible but chose not to participate were asked to provide demographic information for comparison to participants (Figure 3.1).

3.1.2. Prenatal Data Collection

A timeline of prenatal study visits and data collection is provided in Figure 3.2. Women who chose to participate were enrolled in the study between 10 and 14 weeks of gestation. Baseline data regarding demographics, pregnancy and health history, pregnancy symptoms, medication use, and lifestyle factors were obtained at a home visit shortly following enrollment. Physical measurements were also obtained, including waist and hip circumference, weight, body mass index (BMI), and bioelectrical impedance (BIA) measurements of lean mass, fat mass, and visceral fat were obtained.

Participants were asked to provide first morning urine samples at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation for analysis of phthalate metabolites. Specific gravity was measured and urine samples aliquoted within 48 hours after collection, and aliquots were stored at -80 Celsius for later analysis. A fasted blood sample was collected at 16-18 weeks of gestation and processed 2 hours later to obtain serum, plasma, buffy coat, and whole blood which were stored at -80 Celsius for later analysis. Toenail and hair samples were collected at 34-36 weeks of gestation.

At each of the five urine sample time points, interviews were conducted to update health, pregnancy symptoms, medication use, and lifestyle information. For medication use, women reported the name of the medication, the frequency of use, and the start and end dates of use when applicable. Maternal surveys regarding depression symptoms (Edinburgh Postnatal Depression Survey [EPDS]; Cox et al. 1987), perceived stress (Perceived Stress Scale [PSS]; Cohen et al. 1983), and social support (Interpersonal Support Evaluation List [ISEL]; Cohen et al. 1985) were collected at 10-14, 16-18, and 34-36 weeks of gestation. At these time points, mothers were also asked to fill out 24-hour diaries regarding food and drink sources and containers, personal care product use, and household product use for the purpose of identifying sources of phthalate exposure. A modified Block food frequency questionnaire (FFQ; Brunst et al. 2016; Snook Parrott et al. 2009), which included additional questions specific to seafood

consumption and pregnancy supplement use, was completed at 10-14 and 34-36 gestational weeks, and a questionnaire about neighborhood and social stress (Krieger et al. 2016) was completed at 16-18 weeks.

3.1.3. Postnatal Data Collection

A timeline of data collection during infancy is provided in Figure 3.3. A cord blood sample was collected at the time of delivery by obstetrics providers. Samples were processed as soon as possible between 2 and 24 hours after collection to obtain serum, plasma, buffy coat, and whole blood, which were stored at -80 Celsius for later analysis. Meconium/stool samples were collected by parents or hospital staff, stored at -4 Celsius, and later processed for microbiome analysis. IKIDS researchers visited each mother-infant pair at the hospital within 48 hours following the birth of the infant. An update interview was conducted regarding the last few weeks of pregnancy (health, symptoms, medications, and lifestyle) and labor and delivery. Maternal surveys including the EPDS, PSS, and ISEL, as well a survey of stressful life events during pregnancy (Barrett et al. 2014), were completed. Researchers measured newborn head circumference, second- and fourth-digit length, anogenital distance, and recumbent length.

Mothers were invited to bring their infants to the research laboratory for follow-up assessments at 1-5 weeks, 4-5 months, and 7-8 months of age. Interviews were conducted at each visit regarding infant health, medications, feeding practices, and use of plastics and baby care products. Non-nutritive sucking (NNS) data was obtained at each visit using methods described by Zimmerman and colleagues (Barlow et al. 2012). Assessments of visual recognition memory (VRM) at 1-5 weeks of age, physical reasoning (4-5 months), and VRM again at 7-8 months, as well as psychological reasoning also at 7-8 months, were performed using infant looking behavior assessment techniques. Infants who completed the memory and attention assessment at 7-8 months of age were invited to return for an additional visit that used electroencephalography (EEG) to collect visual event-related potential (ERP) data during a similar visual assessment paradigm. At 4-5 months, infant weight, recumbent length, head

circumference, second- and fourth-digit length, and anogenital distances were measured. Mothers were asked to complete the Ages and Stages Questionnaire (ASQ; Squires et al. 2009), EPDS, PSS, and ISEL at both 4-5 and 7-8 months. Mothers completed the Peabody Picture Vocabulary Test – Fourth Edition (PPVT-IV; Dunn et al. 2007) at either the 4-5 month or 7-8 month visit to obtain maternal verbal IQ. The Pediatric Review and Observation of Children's Environmental Support and Stimulation (PROCESS) Inventory (Casey et al. 1988) was completed at the 7-8 month visit to assess children's home and caregiving environment. A stool sample for microbiome analysis was obtained at 4-5 months and a toenail sample for exposure analysis was collected at 7-8 months.

Figure 3.4 is a timeline of data collection during early childhood. When children reached 2 (26.5 - 28.5 months) and 3 (36 - 38 months) years of age, mothers were mailed a packet of questionnaires to complete which included the EPDS, PSS, ISEL, Parent Relationship Questionnaire (PRQ, part of the Behavior Assessment System for Children, 3rd edition; Kamphaus et al. 2015), age-appropriate ASQ, Child Behavior Checklist – 1.5-5 years (CBCL/1.5-5; Achenbach et al. 2000) – an assessment of emotional and behavioral problems, MacArthur-Bates Communicative Development Inventory – Words and Sentences (CDI; Fenson et al. 2007; age 2 only) – a measure of early language development, Speech and Language Assessment Scale (SLAS, Hadley et al. 1993; age 3 only), and Block Kids FFQ (Cullen et al. 2008; age 2 only). Toenails samples were obtained and an update interview regarding child health and product use was conducted over the phone.

Mothers were again invited to bring their child to the research laboratory for a follow-up visit when the child was 46-48 months of age. Mothers were asked to complete an update interview regarding the child's health and product use as well as the EPDS, PSS, ISEL, PRQ, ASQ, CBCL/1.5-5, SLAS, Block Kids FFQ, and Behavior Rating Inventory of Executive Function – Preschool (BRIEF-P; Gioia et al. 2003). Children underwent a battery of cognitive tasks to assess psychological reasoning, working memory, attention, inhibition, and development of

number concepts. They also completed the Sentence Recall portion of the Clinical Evaluation of Language Fundamentals Preschool – 2nd Edition (CELF Preschool 2; Wiig et al. 2006) to assess language development. A urine sample, stool sample, toenail sample, and hair sample were also obtained and stored for future analysis.

Maternal medical records for the prenatal period and labor and delivery were obtained, as well as child medical records from the time of birth to one year of age. All participants provided written informed consent for participation and HIPAA waivers for medical record release. The study was approved by the Institutional Review Board at the University of Illinois at Urbana-Champaign.

3.1.4. Retention and Demographics

Between December 2013 and March 2020, 10,117 women received informational brochures and returned reply cards at their obstetric clinic. Of those, 1806 were ineligible for participation, 5486 declined to be contacted, and 2825 were eligible and indicated interest in being contacted about participation. Of those 2825, 576 were determined to be ineligible at the screening call, 506 declined participation, and 1065 could not be contacted or dropped contact before eligibility screening was completed. By March 2020, 678 pregnant women had been enrolled in the IKIDS cohort. Of the 678 women enrolled, as of March 2020, birth data had been finalized for 527 infants who were enrolled in the study; birth data had not been finalized for 14 infants enrolled in the study. Ninety-two mothers withdrew or became ineligible before or at the time of birth, and 45 had not yet given birth as of March 2020.

Twenty-eight mothers withdrew from the study or became ineligible before baseline data collection. Demographic information for the remaining 650 women, as well as those with infants who completed the VRM task at 7.5 months ($n = 411$); those completed the CDI when their child was 2 years old ($n = 213$), and those who completed the SLAS when their child was 3 years old ($n = 192$), is given in Table 3.1. The mean $\pm$ SD maternal age at enrollment was 30.11 ± 4.41 years and only marginally higher at each point of data collection included in the proposed

analyses (7.5 months: 30.47 ± 4.14 ; 2 years: 30.80 ± 3.70 ; 3 years: 30.61 ± 3.77).

Approximately eighty percent of mothers and 77% of fathers were white and non-Hispanic in the full sample, and 82-85% of mothers and 79-85% of fathers were white and non-Hispanic in each of the subsamples of the cohort, which completed follow-up visits at 7.5 months, 2 years, and 3 years of age. In the full sample, about 85% of mothers were married and another 9% co-habited with a partner, and 89-96% were married and 4-7% co-habited with a partner in each of the subsamples. Thirty-four percent of mothers had attained a bachelor's degree and another 43% held a master's or doctorate-level degree in the full cohort, and 37-38% had completed a bachelor's degree and 47-51% had completed a master's or doctorate degree in each of the subsamples. Fifty-one percent of mothers were nulliparous in the full cohort and 51-54% were nulliparous in each of the subsamples. In both the entire sample and each of the subsamples, nearly 99% of mothers had some form of health insurance. Only about 5% of mothers reported smoking during the first trimester of pregnancy, and about 30% reported consuming more than one alcoholic beverage on average over a 4-week period in the first trimester in both the entire sample and each of the subsamples. Approximately 61% of mothers in the full cohort reported no alcohol consumption at all during the first trimester, and 58-60% reported no alcohol consumption at all during the first trimester in each of the subsamples. In summary, the demographics for mothers whose child had data for at least one time point included for the proposed analyses were largely similar to those of the cohort as a whole, although slightly higher percentages were white, non-Hispanic, married, and had attained at least a bachelor's degree for each subsample.

Demographic information for children enrolled in the study is presented in Table 3.2. About 71% of enrolled children were white and non-Hispanic and 48% were male in the full cohort. In each of the subsamples that provided data for at least one time point, approximately 72-77% of children were white and non-Hispanic, and 44-56% were male. Sixty-nine percent of enrolled infants were delivered vaginally, 13% by planned Cesarean section, and 13% by

unplanned Cesarean section in the full sample. In each of the subsamples, 71-73% of children were delivered vaginally, 11-12% by planned Cesarean section, and 12-14% by unplanned Cesarean section. The mean $\pm$ SD gestational age at birth was 39.29 ± 1.49 weeks for the full sample, and it was not substantially different for any of the subsamples.

3.1.5. Exposure Analysis

As previously mentioned, participants provided first morning urine samples at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation. Samples were collected from the first morning urine void in cups provided by the study and known to be phthalate-free. Specific gravity was measured using a handheld refractometer. Urine was aliquoted within 48 hours for each sample, and aliquots were stored at -80 Celsius until exposure analysis. A pooled sample consisting of 0.9 mL of urine from each of the five samples was created for each participant and stored at -80 Celsius until exposure analysis. Because phthalates have very short half-lives and exposure varies greatly across time within individuals, a single sample is unreliable for making predictions about exposure levels across pregnancy (Fisher et al. 2014). The pooling of samples has been shown to be cost effective and to provide a more stable estimate of average exposure during gestation (Brookmeyer 1999; Liu et al 2003; Saha-Chaudhuri et al. 2013; Sham et al. 2002; Weinberg et al. 1999).

Exposure biomarker analysis was performed on the pooled samples and on individual samples collected at 16-18 weeks of gestation, a potential critical developmental window for impacts of EDC exposure due to peak androgen production in male fetuses around this time (Auyeung et al. 2013). Samples were analyzed at the Centers for Disease Control (CDC) Division of Laboratory Sciences in Atlanta, Georgia, USA. Phthalate and phthalate replacement chemical metabolites were measured in each urine sample by online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry using a modification of the methods described by Silva and colleagues (2007).

Number of samples analyzed thus far, median biomarker levels and interquartile ranges (IQRs) and limits of detection (LOD), as well number and percentage of samples with no signal detected are presented in Table 3.3 for phthalates and phthalate replacement chemicals. Biomarker concentrations used to calculate median and IQR were corrected for dilution using specific gravity, according to the following equation:

$$\text{adjusted value} = \text{biomarker concentration} * \frac{(\text{sample specific gravity} - 1)}{(\text{IKIDS median specific gravity} - 1)}$$

The most recent median (95% confidence interval) measures for females from the National Health and Nutrition Examination Survey (NHANES) for each of the biomarkers are included for comparison to a national biomonitoring sample (CDC, 2019). Median biomarker levels were similar between IKIDS and the national NHANES sample for most analytes. The IKIDS pooled sample median concentration of mono-carboxyisooctyl phthalate (MCOP), a metabolite of diisononyl phthalate (DINP), was slightly higher than in NHANES. Pooled sample median concentrations of both measured metabolites of di-2-ethylhexyl terephthalate (DEHP) were significantly higher than in NHANES. Although some biomarker measures differed from this national sample, most are representative of biomarker levels in the US.

Because acetaminophen has a very short half-life of just 1.5-2.5 hours and exposure is not daily as with phthalates, the urine samples used for evaluating phthalate exposure across pregnancy could not be used in the same way to evaluate acetaminophen exposure (Prescott, 1980; Nitsche et al., 2017). Instead, participants were asked to report their use during interviews. In the same timeframes that participants provided first morning urine samples (10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation) and within 24 hours of the child's birth, participants were interviewed about their use of medication. At the time of the first interview between 10 and 14 weeks of gestation, participants were asked to list all medications they had used beginning three months prior to conception through the time of the interview as well as their reason for taking the medication; the date they began taking the medication; the date they

stopped taking the medication, if they were no longer taking it; and their frequency of use. At the time of each interview following the first one, participants were asked to recall the same information for the period between their last interview and the current interview. From the data collected via these interviews, medications containing acetaminophen as an active ingredient were identified, and the period in which they were used and the reason for their use were also extracted from the data. From there, using the frequency of use and dates of use reported by participants, the number of times participants took acetaminophen during the first, second, and third trimesters were calculated, and cumulative use was the sum of the number of times acetaminophen was taken during each of the three trimesters (Table 3.4). In line with reports from previous studies, more than half of IKIDS participants (61.0%) used a medication containing acetaminophen at least once during pregnancy (Brandlistuen et al., 2013; Thorpe et al., 2013; Servey and Chang, 2014; Ji et al., 2018). Use during the first trimester of pregnancy was 50.3%, but fewer participants reported use at each following interview, with the fewest reporting any use within the third trimester (31.1%). Of the participants reporting any use of medications containing acetaminophen, most reported using acetaminophen itself rather than other medications containing acetaminophen, and the majority of participants took acetaminophen for pain (Table 3.4).

3.2. STRENGTHS AND LIMITATIONS

The IKIDS study has gathered extensive biospecimen and covariate data spanning from the end of the first trimester of pregnancy through the child's fourth birthday. The sociodemographic aspects of the cohort are relatively homogeneous, but largely reflect the local population. Collected covariate data include key demographic information, as well as extensive health and lifestyle data. There are also archived biospecimens (urine, blood, hair, nails, and stool) that can be used to measure additional exposures and health outcomes. Longitudinal assessments of physical growth and cognition across infancy and early childhood provide

substantial data to assess exposure effects across a variety of domains and developmental time points.

Although the relatively short half-lives of phthalates in the human body makes accurate estimation of exposure difficult, the use of pooled samples for urine biomarker analysis is a cost-effective approach to more accurately estimate average exposure across the prenatal period. While this does not allow for time-specific assessment of effects, additional aliquots have been archived for future analyses at specific time points across the three trimesters of pregnancy. Additionally, biomarker data for replacement chemicals such as DINP, DEHP and DINCH are available for analysis of the potential adverse effects of these chemicals about which relatively little is known. In evaluating prenatal exposure to acetaminophen, interviewing participants multiple times during their pregnancy requires participants to recall information over much shorter periods of time than previous studies, likely resulting in a more accurate recall of medication use, and allowing for consideration of acetaminophen exposure across different windows of gestation.

State-of-the-art methods are being used to collect data on specific cognitive domains during the first year of life - much earlier than has been done in previous environmental epidemiology studies. While these measures have only been used in relatively small studies in developmental psychology and thus have not been standardized, the large number of infants in this cohort demonstrates their scalability for studies of this size and allows for characterization of infants' performance on these tasks (Dzwilewski et al., 2020). These assessments provide measures of specific building blocks of cognition that tend to be stable within individuals across time and follow-up throughout early childhood will allow assessment of whether these measures will be useful as early predictors of long-term impacts associated with prenatal and early life exposures.

As previously mentioned, few environmental epidemiological studies have directly evaluated language development. While direct measurements of language development can be

time consuming and costly, parental report measures, such as those used in the present study, are evaluations of language development that require less time and are more cost-effective. Therefore, they are reasonable to use in large epidemiological studies. Parental report measures of language development have been shown to both accurately represent the child's development at that time and be predictive of later language development. Consequently, these methods are invaluable tools for evaluating the relationship between prenatal exposures and this specific aspect of cognitive development.

3.3. TABLES AND FIGURES

Table 3.1. Parental demographics for all IKIDS participants and each subsample who provided data for the outcomes of interest at 7.5 months (attention - VRM), 2 years (attention – CBCL, language - CDI), and 3 years (attention – CBCL, language - SLAS).⁴

Parental Demographics	Full cohort (n = 650)	Participants whose infant completed VRM at 7.5 mo. (n = 260)	Participants who completed CBCL at 2 yr. (n = 254)	Participants who completed CDI at 2 yr. (n = 300)	Participants who completed CBCL at 3 yr. (n = 215)	Participants who completed SLAS at 3 yr. (n = 206)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Maternal race & ethnicity						
White, Non-Hispanic	519 (79.8)	220 (84.6)	217 (85.43)	254 (84.7)	189 (87.9)	182 (88.8)
White, Hispanic	22 (3.3)	11 (4.2)	8 (3.2)	9 (3.0)	7 (3.3)	7 (3.4)
Black, Non-Hispanic	47 (7.2)	13 (5.0)	9 (3.5)	10 (3.3)	5 (2.3)	5 (2.0)
Asian, Non-Hispanic	35 (5.3)	14 (5.4)	19 (7.5)	26 (8.7)	13 (6.1)	11 (5.4)
Other	26 (4.0)	1 (0.4)	1 (0.4)	1 (0.3)	1 (0.5)	1 (0.5)
Unknown/Missing	1 (0.1)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Paternal race & ethnicity						
White, Non-Hispanic	502 (77.2)	219 (84.2)	221 (87.0)	254 (84.7)	187 (87.0)	180 (87.8)
White, Hispanic	28 (4.3)	9 (3.5)	10 (3.9)	12 (4.0)	11 (5.1)	9 (4.4)
Black, Non-Hispanic	57 (8.7)	19 (7.3)	9 (3.5)	14 (4.7)	7 (3.3)	9 (4.4)
Asian, Non-Hispanic	29 (4.4)	11 (4.2)	12 (4.7)	18 (6.0)	9 (4.2)	7 (3.4)
Other	32 (4.9)	1 (0.4)	2 (0.8)	2 (0.6)	1 (0.5)	1 (0.5)
Unknown/Missing	2 (0.3)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Maternal marital status						
Married	552 (84.9)	238 (91.5)	236 (92.9)	279 (93.0)	205 (95.4)	196 (95.2)
Living as married	56 (8.6)	15 (5.8)	13 (5.1)	16 (5.3)	8 (3.7)	9 (4.4)
Separated/Divorced/Widowed	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Single	41 (6.3)	7 (2.7)	5 (2.0)	5 (1.7)	2 (0.9)	1 (0.5)
Maternal education						
High school or less	32 (4.9)	4 (1.5)	1 (0.4)	2 (0.7)	0 (0.0)	0 (0.0)
Some college or technical school	117 (18.0)	33 (12.7)	28 (11.0)	33 (11.0)	23 (10.7)	22 (10.7)
Bachelor's degree	221 (34.0)	97 (37.3)	95 (37.4)	115 (38.3)	80 (37.2)	77 (37.4)
Master's or doctorate degree	280 (43.0)	126 (48.5)	130 (51.2)	150 (50.0)	112 (52.1)	107 (51.9)
Paternal education						
High school or less	72 (11.0)	12 (4.6)	7 (2.8)	9 (3.0)	8 (3.7)	8 (3.9)
Some college or technical school	144 (22.1)	42 (16.2)	42 (16.5)	48 (16.0)	35 (16.3)	32 (15.5)
Bachelor's degree	210 (32.3)	92 (35.4)	95 (37.4)	110 (36.7)	82 (38.1)	81 (39.3)
Master's or doctorate degree	220 (33.8)	113 (43.5)	110 (43.3)	133 (44.3)	90 (41.9)	85 (41.3)
Unknown/Missing	4 (0.6)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3.1. (cont.) Parental demographics for all IKIDS participants and children with data for each timepoint of interest.⁴

Parental Demographics	Full cohort (n = 650)	Participants whose infant completed VRM at 7.5 mo. (n = 260)	Participants who completed CBCL at 2 yr. (n = 254)	Participants who completed CDI at 2 yr. (n = 300)	Participants who completed CBCL at 3 yr. (n = 215)	Participants who completed SLAS at 3 yr. (n = 206)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Maternal parity						
0	331 (50.9)	143 (55.0)	139 (54.7)	171 (57.0)	112 (52.1)	104 (50.7)
1	202 (31.0)	76 (29.2)	79 (31.1)	87 (29.0)	73 (34.0)	71 (34.6)
≥2	117 (18.0)	41 (15.8)	36 (14.2)	42 (14.0)	30 (14.0)	31 (15.0)
Household income						
\$0-\$49,999	141 (21.7)	46 (17.7)	41 (16.4)	49 (16.3)	30 (14.0)	30 (11.5)
\$50,000-\$99,999	304 (46.8)	124 (47.7)	115 (45.3)	136 (45.3)	105 (48.8)	99 (48.1)
≥\$100,000	200 (30.8)	88 (33.8)	98 (38.6)	115 (38.3)	79 (36.7)	76 (36.9)
Unknown/Missing	5 (0.7)	2 (0.8)	0 (0.0)	0 (0.0)	1 (0.5)	1 (4.9)
Maternal health insurance						
Private or provided by employer	551 (84.7)	236 (90.8)	232 (91.7)	275 (91.7)	200 (93.0)	190 (92.7)
Medicaid	73 (11.2)	17 (6.5)	15 (5.9)	18 (6.0)	10 (4.7)	11 (5.4)
Military or VA health insurance	13 (2.0)	5 (1.9)	6 (2.4)	6 (2.0)	4 (1.9)	4 (2.0)
Provided by employer & Medicaid or All Kids	5 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Uninsured	6 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown/Missing	2 (0.3)	2 (0.8)	1 (0.4)	1 (0.3)	1 (0.5)	0 (0.0)
Maternal tobacco smoking during pregnancy reported at 10-14 weeks gestation						
Yes	35 (5.3)	1 (0.4)	0 (0.0)	0 (0.0)	1 (0.5)	1 (4.9)
No	576 (88.6)	240 (92.3)	237 (93.3)	283 (94.3)	196 (91.2)	187 (90.8)
Unknown/Missing	39 (6.0)	19 (7.3)	17 (6.7)	17 (5.7)	18 (8.4)	18 (8.3)

Table 3.1 (cont.) Parental demographics for all IKIDS participants and children with data for each timepoint of interest.⁴

Parental Demographics	Full cohort (n = 650)	Participants whose infant completed VRM at 7.5 mo. (n = 260)	Participants who completed CBCL at 2 yr. (n = 254)	Participants who completed CDI at 2 yr. (n = 300)	Participants who completed CBCL at 3 yr. (n = 215)	Participants who completed SLAS at 3 yr. (n = 206)
	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>
Maternal alcohol consumption						
Gestational weeks 0-4:						
None	397 (61.1)	46 (17.7)	180 (70.9)	209 (69.7)	147 (68.4)	142 (68.9)
Less than 4 drinks per week	194 (29.8)	18 (6.9)	53 (20.9)	68 (22.7)	42 (19.5)	40 (19.4)
4-10 drinks per week	51 (7.8)	2 (0.8)	19 (7.5)	46 (15.3)	23 (10.7)	21 (10.2)
11-21 drinks per week	7 (1.0)	194 (74.6)	2 (0.8)	4 (1.3)	3 (1.4)	3 (1.5)
Gestational weeks 5-8:						
None	629 (96.9)	225 (86.5)	251 (98.8)	296 (98.7)	209 (97.2)	201 (97.6)
Less than 4 drinks per week	17 (2.6)	3 (1.2)	2 (0.8)	3 (1.0)	4 (1.9)	4 (2.0)
4-10 drinks per week	3 (0.4)	2 (0.8)	1 (0.4)	1 (0.3)	2 (0.9)	1 (0.5)
11-21 drinks per week	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gestational weeks 9-14:						
None	637 (98.1)	258 (99.2)	252 (99.2)	298 (99.3)	213 (99.1)	205 (99.5)
Less than 4 drinks per week	11 (1.6)	2 (0.8)	2 (0.8)	2 (0.7)	2 (0.9)	1 (0.5)
4-10 drinks per week	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
11-21 drinks per week	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown/Missing	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>
Maternal age (years) at baseline	30.10 (4.41)	30.66 (3.95)	30.82 (3.66)	30.83 (3.73)	30.63 (3.78)	30.65 (3.72)
Maternal verbal IQ (PPVT standard score)*	107.88 (11.33)	108.47 (11.39)	108.42 (11.12)	107.91 (10.77)	109.01 (10.91)	109.19 (11.03)

⁴Parental demographics for all enrolled participants with demographic information collected (n=650) and participants with an infant enrolled in the study (n=521). All Kids is an Illinois program to provide free or reduced-cost comprehensive health insurance based on family size and household income. *Maternal verbal IQ: n=365, (mean = 107.88, SD = 11.33) not yet collected for 156 mothers. PPVT-IV: Peabody Picture Vocabulary Test - Fourth Edition.

Table created by Dzwilewski, K., Merced Nieves, F., and Strakovsky, R. Updated and modified by Woodbury, M.

Table 3.2. Child demographics for children enrolled in IKIDS with data available for each analysis.⁵

Child Demographics	All enrolled infants (n = 527)	Infants with VRM data at 7.5 mo. (n = 260)	Children with CBCL data at 2 yr. (n = 254)	Children with CDI data at 2 yr. (n = 300)	Children with CBCL data at 3 yr. (n = 215)	Children with SLAS data at 3 yr. (n = 206)
	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>	<u>N (%)</u>
Child sex						
Male	254 (48.2)	128 (49.2)	119 (46.9)	149 (49.7)	101 (47.0)	94 (45.6)
Female	273 (51.8)	132 (50.8)	135 (53.2)	151 (50.3)	114 (53.0)	112 (54.4)
Child race & ethnicity						
White, Non-Hispanic	373 (70.9)	205 (78.9)	203 (79.9)	233 (77.7)	175 (81.4)	169 (82.0)
White, Hispanic	28 (5.3)	14 (5.4)	15 (5.9)	17 (5.7)	14 (6.5)	11 (5.3)
Black, Non-Hispanic	22 (4.1)	11 (4.2)	6 (2.4)	7 (2.3)	4 (1.9)	4 (1.9)
Asian, Non-Hispanic	19 (3.6)	8 (3.1)	10 (3.9)	14 (4.7)	6 (2.8)	4 (1.9)
Other	84 (15.9)	21 (8.1)	20 (7.9)	29 (9.7)	16 (7.4)	18 (8.7)
Unknown/Missing	1 (0.1)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Delivery type						
Vaginal	361 (68.5)	179 (68.8)	186 (73.2)	222 (74.0)	158 (73.5)	149 (72.3)
Planned Cesarean section	71 (13.4)	33 (12.7)	28 (11.0)	32 (10.7)	23 (10.7)	22 (10.7)
Unplanned Cesarean section	67 (12.7)	39 (15.0)	32 (12.6)	36 (12.0)	25 (11.6)	26 (12.6)
Unknown/Missing	28 (5.3)	9 (3.5)	8 (3.2)	10 (3.3)	9 (4.2)	9 (4.4)
	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>	<u>Mean (SD)</u>
Child gestational age at birth (weeks)	39.29 (1.49)	39.32 (1.51)	39.41 (1.53)	39.57 (1.54)	39.41 (1.60)	39.33 (1.61)
Child weight at birth (kg)	3.48 (0.43) ¹	3.51 (0.44) ²	3.50 (0.43) ³	3.49 (0.43) ⁴	3.50 (0.44) ⁵	3.49 (0.44) ⁶

¹Child weight at birth missing for n = 86²Child weight at birth missing for n = 41³Child weight at birth missing for n = 36⁴Child weight at birth missing for n = 44⁵Child weight at birth missing for n = 31⁶Child weight at birth missing for n = 28⁵Table created by Dzwilewski, K., Merced Nieves, F., and Strakovsky, R. Updated and modified by Woodbury, M.

Table 3.3. Phthalate and phthalate replacement chemical metabolites measured and median measures for both gestational week 16-18 and pooled urine samples.⁶

Parent compound	Parent abbr.	Metabolite compound	Metabolite abbr.	LOD (µg/L)	16-18 weeks gestation sample n = 480		Pooled sample n = 482		NHANES Females (2015-2016)
					Median (IQR) (µg/L)	N (%) with no detection	Median (IQR) (µg/L)	N (%) with no detection	Median (95% CI) (µg/L)
Benzylbutyl phthalate	BBzP	Mono-benzyl phthalate	MBzP	0.3	4.80 (9.30)	1 (0.21)	5.60 (8.55)	0 (0.0)	4.20 (3.40, 5.00)
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP	1.2	21.7 (32.3)	4 (0.83)	26.30 (33.59)	0 (0.0)	31.6 (25.6, 41.1)
Di-n-butyl phthalate	DBP	Mono-n-butyl phthalate	MBP	0.4	11.8 (12.8)	0 (0.00)	13.20 (9.27)	0 (0.0)	10.6 (9.60, 11.9)
		Mono-(3-hydroxybutyl) phthalate	MHBP	0.4	1.10 (1.35)	12 (2.50)	1.29 (1.10)	6 (1.2)	0.90 (0.80, 1.00)
Di-(2-ethylhexyl) phthalate	DEHP	Mono-(2-ethylhexyl) phthalate	MEHP	0.8	1.00 (1.40)	31 (6.46)	1.33 (1.29)	16 (3.3)	1.00 (0.90, 1.10)
		Mono-(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	0.4	4.65 (6.00)	4 (0.83)	5.80 (4.94)	0 (0.0)	5.40 (4.90, 5.70)
		Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.2	3.80 (4.40)	3 (0.63)	4.61 (3.48)	0 (0.0)	3.50 (3.20, 3.90)
		Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.4	7.75 (8.50)	1 (0.21)	9.10 (7.84)	0 (0.0)	8.40 (7.70, 9.30)
Di-isononyl phthalate	DINP	Mono-isononyl phthalate	MINP	0.9	0.50 (0.80)	39 (8.13)	0.75 (1.07)	5 (1.0)	< LOD
		Mono-carboxyisooctyl phthalate	MCOP	0.3	7.00 (15.4)	0 (0.00)	11.51 (20.50)	0 (0.0)	6.90 (5.30, 8.50)
		Mono-oxononyl phthalate	MONP	0.4	1.75 (2.40) [†]	1 (0.21)	2.64 (2.90) [‡]	0 (0.0)	1.70 (1.50, 2.00)
Di-isobutyl phthalate	DIBP	Mono-hydroxyisobutyl phthalate	MHIBP	0.4	2.95 (3.35)	1 (0.21)	3.20 (2.78)	0 (0.0)	2.70 (2.50, 3.10)
		Mono-isobutyl phthalate	MIBP	0.8	8.00 (9.05)	0 (0.00)	8.78 (7.41)	0 (0.0)	8.70 (8.00, 9.30)
Di-2-ethylhexyl terephthalate	DEHTP	Mono-(2-ethyl-5-carboxypentyl) terephthalate	MECPTP	0.2	19.0 (50.7) [†]	0 (0.00)	59.56 (109.63) [‡]	0 (0.0)	15.7 (12.6, 19.6)
		Mono-(2-ethyl-5-hydroxyhexyl) terephthalate	MEHHTP	0.4	3.60 (8.20) [†]	0 (0.00)	8.21 (15.96) [‡]	0 (0.0)	3.80 (3.20, 4.70)

⁶LOD: limit of detection, CI: confidence interval. [†]n=308, [‡]n = 309 (analytical method introduced after initial samples analyzed).

Table created by Dzwilewski, K., Merced Nieves, F., and Strakovsky, R. Modified by Woodbury, M.

Table 3.4. Maternal report of use of medications containing acetaminophen during pregnancy in the IKIDS cohort.⁷

	1st Trimester N (%)	2nd Trimester N (%)	3rd Trimester N (%)	Entire Pregnancy N (%)
None	312 (49.7)	343 (54.6)	433 (68.9)	245 (39.0)
Any	316 (50.3)	285 (45.4)	195 (31.1)	383 (61.0)
Formulation				
Acetaminophen alone	262 (82.9)	231 (81.1)	159 (81.5)	284 (74.2)
Acetaminophen in combination with other active ingredients	24 (7.6)	19 (6.7)	15 (7.7)	22 (5.7)
Acetaminophen in combination with opioid or barbiturate	1 (0.3)	0 (0.0)	2 (1.0)	1 (0.3)
Multiple formulations	29 (9.2)	35 (12.3)	19 (9.7)	76 (19.8)
Indication				
Pain	217 (68.7)	202 (70.9)	135 (69.2)	240 (62.3)
Cold, flu, fever, sinus problems, or other illness	36 (11.4)	26 (9.1)	21 (10.8)	48 (12.5)
Multiple reasons given	27 (8.5)	28 (9.8)	19 (9.7)	61 (15.9)
Other	3 (0.9)	5 (1.8)	5 (2.6)	6 (1.6)
Missing	33 (10.4)	23 (8.1)	15 (7.7)	28 (7.3)

⁷ Medication use data available for 628 participants as of August 2021. Prior to the first interview at 10-14 gestational weeks, 28 participants withdrew from the study or became ineligible. Prior to the second interview at 16-18 gestational weeks, an additional 23 participants became ineligible or withdrew from the study. Between the second interview and the third interview at 22-24 gestational weeks, 8 more participants withdrew or became ineligible. Another 9 participants withdrew or became ineligible prior to the fourth interview at 28-30 weeks of gestation; 2 more prior to the fifth interview at 34-36 weeks of gestation; and 10 more prior to the final interview at the time of the child's birth.

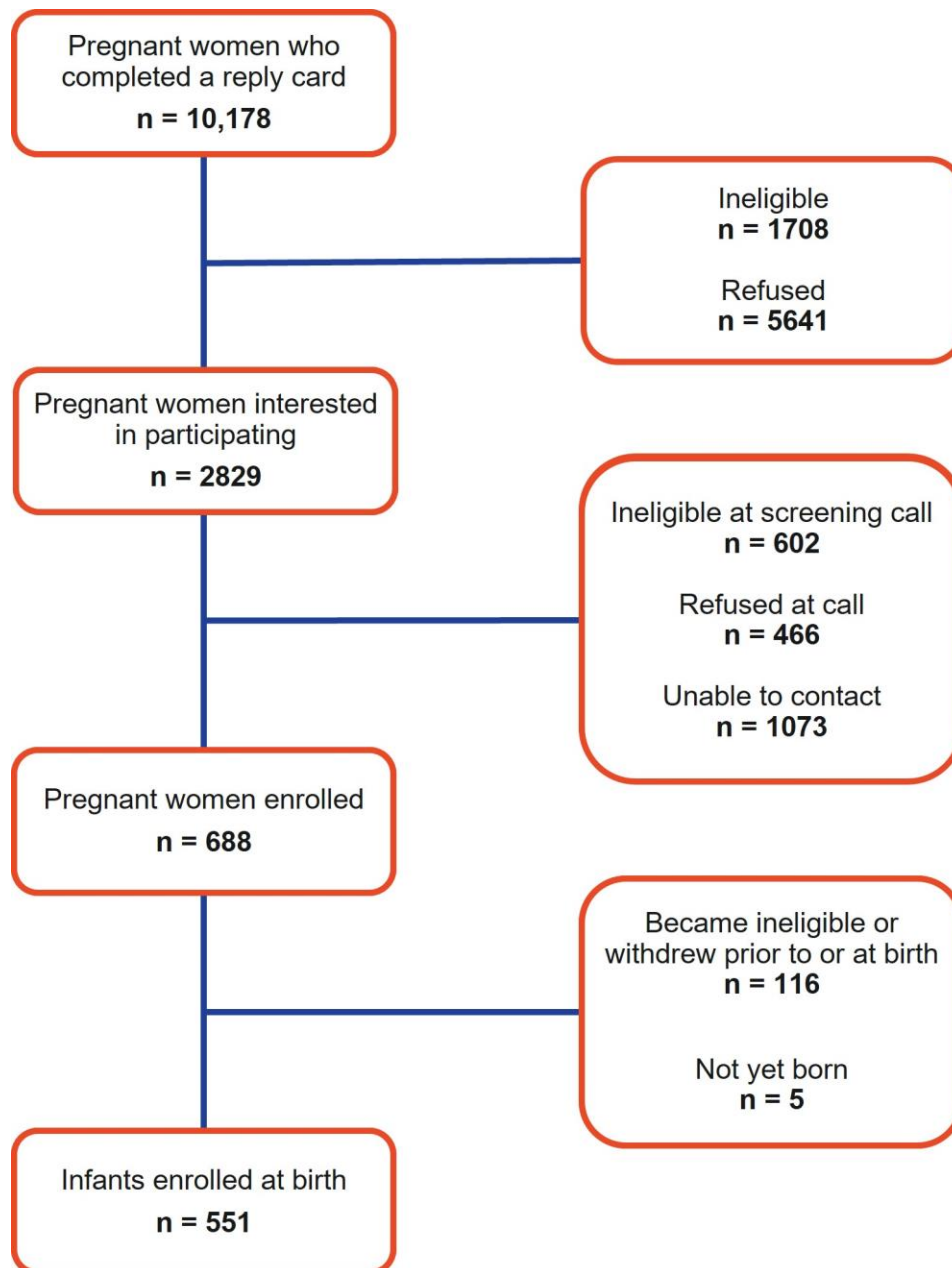


Figure 3.1. Flowchart of recruitment and retention during the prenatal period as of September 2020.⁸

⁸ Original figure created by Merced Nieves, F. Updated and modified by Woodbury, M.

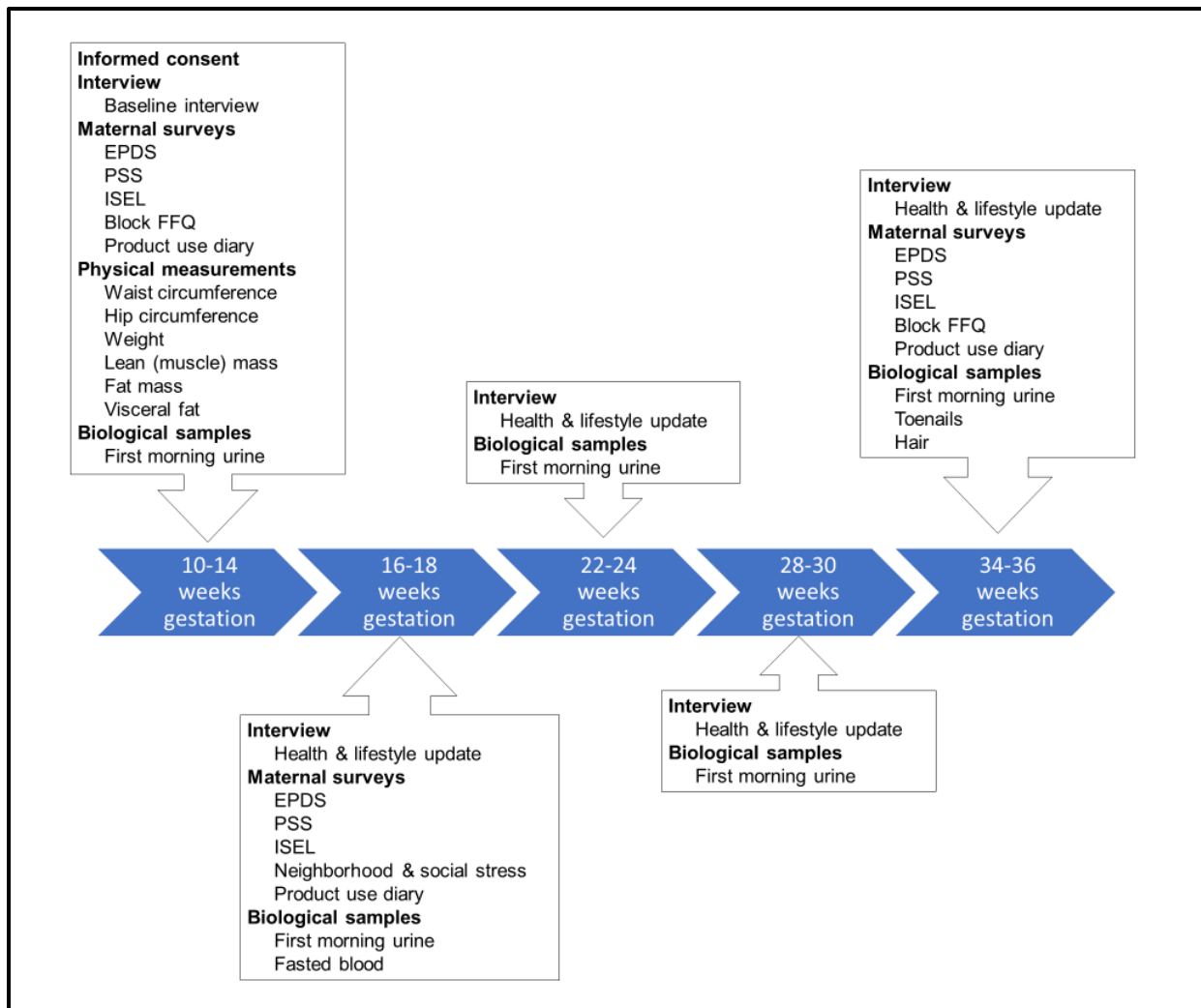


Figure 3.2. Prenatal data collection timeline.⁹

⁹ EPDS: Edinburgh Postnatal Depression Scale, PSS: Perceived Stress Scale, ISEL: Interpersonal Support Evaluation List, FFQ: Food Frequency Questionnaire.

Figure created by Dzwilewski, K., Merced Nieves, F., Strakovsky, R., and Aguiar, A.

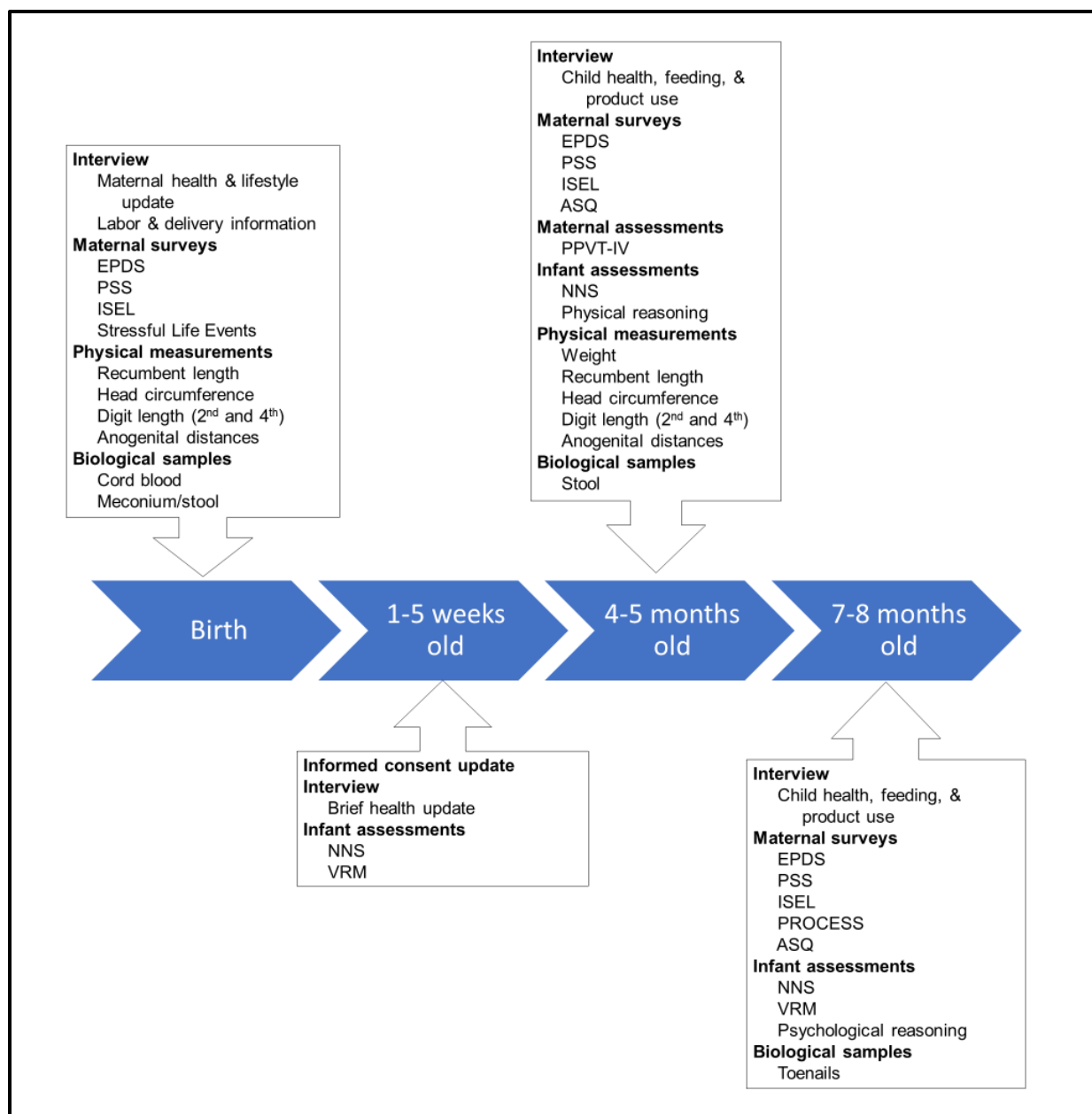


Figure 3.3. Timeline of data collection during infancy.¹⁰

¹⁰ EPDS: Edinburgh Postnatal Depression Scale, PSS: Perceived Stress Scale, ISEL: Interpersonal Support Evaluation List, FFQ: Food Frequency Questionnaire, NNS: non-nutritive sucking, VRM: Visual Recognition Memory, ASQ: Ages & Stages Questionnaire, PPVT-IV: Peabody Picture Vocabulary Test - Fourth Edition, PROCESS: Pediatric Review and Observation of Children's Environmental Support and Stimulation Inventory.

Figure created by Dzwilewski, K., Merced Nieves, F., Strakovsky, R., and Aguiar, A.

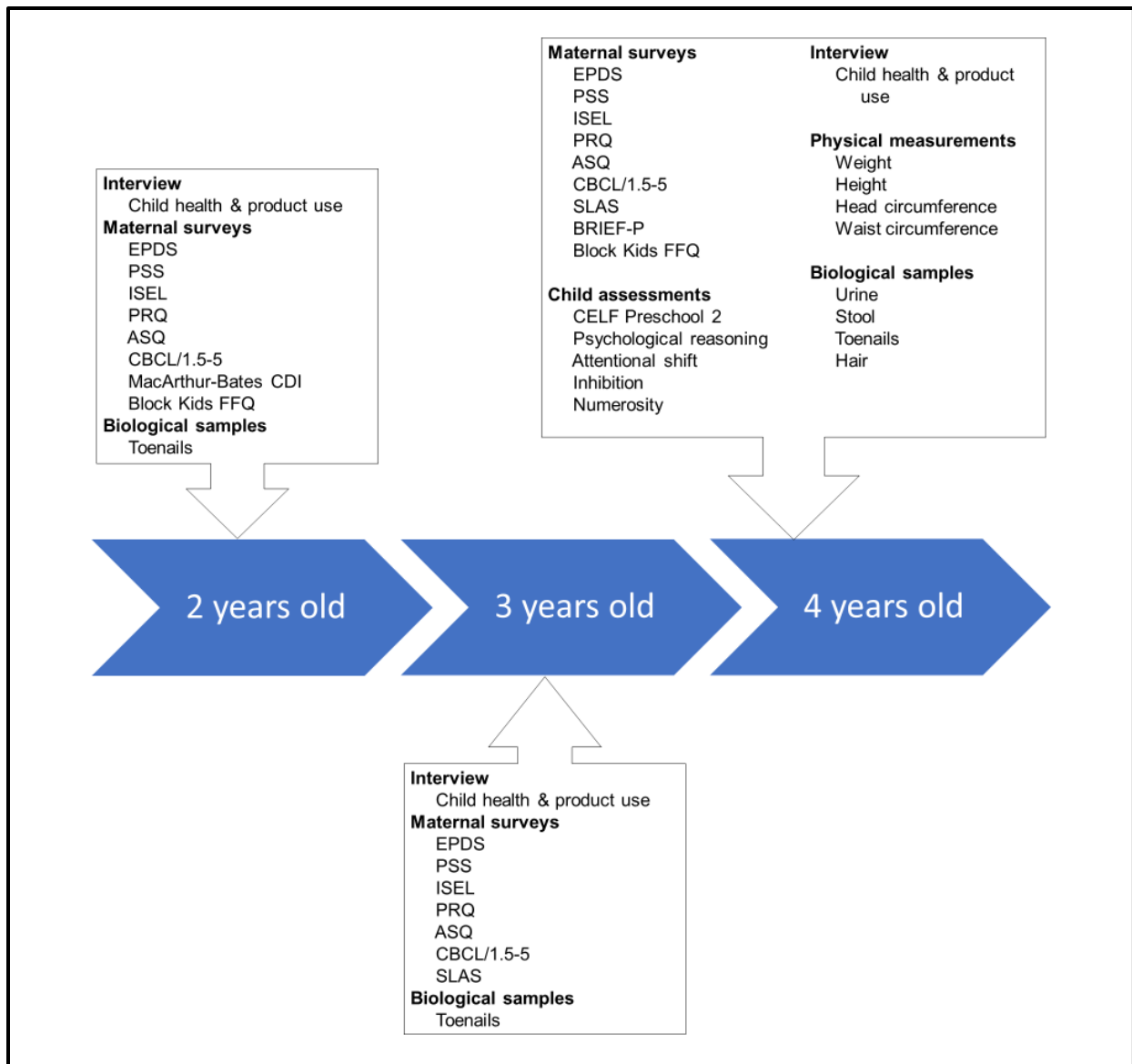


Figure 3.4. Timeline of data collection during early childhood.¹¹

¹¹ EPDS: Edinburgh Postnatal Depression Scale, PSS: Perceived Stress Scale, ISEL: Interpersonal Support Evaluation List, PRQ: Parent Relationship Questionnaire, ASQ: Ages & Stages Questionnaire, CBCL/1.5-5: Child Behavior Checklist – 1.5 - 5 years, CDI: Communicative Development Inventory, FFQ: Food Frequency Questionnaire, SLAS: Speech and Language Assessment Scale, BRIEF-P: Behavior Rating Inventory of Executive Function – Preschool, CELF Preschool 2: Clinical Evaluation of Language Fundamentals – Preschool – 2nd Edition (Recalling Sentences section).

Figure created by Dzwilewski, K., Merced Nieves, F., Strakovsky, R., and Aguiar, A.

CHAPTER 4. ASSOCIATIONS OF PRENATAL EXPOSURE TO PHTHALATES WITH MEASURES OF COGNITION IN 7.5-MONTH-OLD INFANTS¹²

4.1. INTRODUCTION

The potential relationship of prenatal exposure to phthalates with attentional problems is inconsistent (Radke et al., 2020). Most studies investigating this relationship have evaluated attention via parental report measures rather than direct assessment of the child, and few have assessed children under 1 year of age (Engel et al., 2010; Kobrosly et al., 2014; Gascon et al., 2015; Philippat et al., 2017; Hyland et al., 2019; Jankowska et al., 2019; Dzwilewski, Woodbury et al., 2021). Because attention tends to be stable across development, early detection of attentional problems would be useful in identifying children who may benefit from early interventions (Aslin, 2007). Looking time or eye tracking methods have previously been shown to be reliable measures of attention that are also predictive of cognitive function later in childhood, such as IQ and language (Rose et al., 1992; Rose et al., 2012; Jones and Klin, 2013; Chhayya et al., 2018; Downes et al., 2018). The analyses presented here used automated eye tracking methods to assess attention in 7-8-month-old infants in the Illinois Kids Development Study (IKIDS) cohort to evaluate the potential relationship between prenatal exposure to phthalates and cognitive development during infancy. In addition to attention (time to familiarization), outcomes related to working memory (novelty preference), and information processing (run duration, shift rate) were also examined. It was expected that infants with higher exposure to phthalates would show poorer attention, as well as poorer working memory and information processing, and that with the focus on phthalates known to have antiandrogenic action, male infants would show greater deficits than female infants. Additionally, higher

¹² Portions of this chapter have been published in Dzwilewski, K.L.C.*, Woodbury, M.L.*, Aguiar, A.A., Shoaff, J., Merced-Nieves, F., Korrick, S.A.**, & Schantz, S.L.** 2021. Associations of prenatal exposure to phthalates with measures of cognition in 7.5-month-old infants. *Neurotoxicology* 84, 84–95. <https://doi.org/10.1016/j.neuro.2021.03.001>

*denotes equal contribution as first-authors.

**denotes equal contribution as senior authors.

concentrations of the antiandrogenic phthalates measured at 16-18 weeks of gestation, which is thought to be the time of peak androgen production by male fetuses, were anticipated to be associated with greater deficits in cognitive outcomes, again, particularly in male infants, both because of higher androgen production during this time and because this period of brain development may be more sensitive to hormonal disruption (Auyeung et al. 2013).

4.2. METHODS

4.2.1. Study Cohort

A detailed description of the recruitment methods is presented in Chapter 3. As previously described, pregnant women were recruited from two local obstetrics clinics (Christie Clinic and Carle Physician Group) in the Champaign-Urbana, Illinois area beginning in December 2013. Women were eligible to participate if they had not yet reached 15 weeks of gestation, were between the ages of 18 and 40 years, were fluent in English, were not in a high-risk pregnancy, were not carrying multiples, did not have a previous child already participating in the IKIDS cohort, lived within a 30-minute drive of the University of Illinois at Urbana-Champaign, were able to provide a fasting blood sample and five first-morning urine samples throughout pregnancy, and were planning to remain in the area until the child's first birthday. Participants were interviewed at the time of enrollment (between 10 and 14 weeks of gestation) to obtain baseline sociodemographic data, medical and reproductive history, and lifestyle information. Mothers were invited to bring their infants to the University of Illinois campus for follow-up physical and cognitive assessments when the infants were 1-5 weeks, 4-5 months, and 7-8 months of age. This analysis focuses on data collected at the 7–8-month assessment. This study was approved by the Institutional Review Board at the University of Illinois, and all participants gave written informed consent both at the time of enrollment and again after the birth of the participating infant.

4.2.2. Exposure Assessment

Chapter 3 describes in more detail how phthalate exposure was evaluated during pregnancy. Briefly, women provided first morning urine samples at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation, and a pooled sample was created for each participant for analysis of phthalate metabolites. As described in Chapter 3, pooling samples has been established as a method to provide a more stable measure of average exposure across pregnancy for chemicals like phthalates that have variable exposure across time and short half-lives (Brookmeyer 1999; Liu et al 2003; Saha-Chaudhuri et al. 2013; Sham et al. 2002; Weinberg et al. 1999). Additionally, a sample from the 16–18-week time point was analyzed as this is the time of peak androgen production by male fetuses (Auyeung et al., 2013) and, thus, this window of prenatal development may be especially susceptible to endocrine disrupting chemicals (EDCs). Phthalate metabolites were measured by online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry using a modification of the methods described by Silva and colleagues (2007). All concentrations were adjusted using specific gravity to account for differences in urine dilution.

The phthalates evaluated were di-(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DINP), diethyl phthalate (DEP), di(2-ethylhexyl)terephthalate (DEHTP), and a sum of antiandrogenic phthalates (Σ AA). DEP was included as a comparison because it does not have antiandrogenic activity, but it accounts for a large proportion of the metabolites found in maternal urine samples that have been analyzed in the IKIDS cohort. Little is known about the potential endocrine activity of DEHTP, but because it is increasingly used as a replacement for DEHP worldwide (Schwedler et al., 2020), and it accounts for the largest proportion of phthalates metabolites measured in the IKIDS cohort (Pacyga et al., 2021), it was also investigated. Some phthalates, such as DEHP and DINP, are rapidly metabolized into multiple urinary metabolites, therefore, weighted molar sums of their metabolites were calculated as biomarkers of exposure to these phthalates according to the following equations:

$$\Sigma DEHP = \frac{MEHP}{278} + \frac{MEHHP}{294} + \frac{MEOHP}{292} + \frac{MECPP}{308}$$

$$\Sigma DINP = \frac{MiNP}{292} + \frac{MCOP}{322}$$

$$\Sigma DEHTP = \frac{MECPTP}{308} + \frac{MEHHTP}{294}$$

Because urine was sent to the CDC laboratory in batches and new methods for phthalate metabolite measurement were developed between batches, measures of MONP, MECPTP, and MEHHTP were available for only 58% of the participants, and the DINP sum was calculated using just the two metabolites available for all participants in order to retain a larger sample size for analyses. A weighted molar sum of all antiandrogenic phthalate metabolites was also calculated to represent exposure to phthalates with a common mechanism of action according to the following equation:

$$\begin{aligned} \Sigma AA = & \frac{MiBP}{222} + \frac{MBzP}{256} + \frac{MEHP}{278} + \frac{MEHHP}{294} + \frac{MEOHP}{292} + \frac{MECPP}{308} + \left(\frac{MCOP}{322} \times 0.43 \right) \\ & + \left(\frac{MiNP}{292} \times 0.43 \right) + \frac{MBP}{222} + \frac{MHiBP}{238} + \frac{MHBP}{238} \end{aligned}$$

In the anti-androgens sum, the metabolites of DINP were weighted less than other metabolites because mechanistic studies in lab animals have found DINP to have less potent antiandrogenic effects than other antiandrogenic phthalates (Hannas et al., 2011; Howdeshell et al., 2015).

4.2.3. Cognitive Assessment

Details of the cognitive assessment used at 7-8 months of age have previously been reported by Dzwilewski et al. (2020). Infant cognition was evaluated between approximately 32 weeks (224 days) and 37 weeks (259 days) of age. The ages of infants born at less than 37 weeks of gestation ($n = 14$) were calculated from their estimated due dates, instead of their actual date of birth, to account for prematurity. The infant was seated on a parent's lap in a small area surrounded by black curtains to minimize distractions. Stimuli were presented on a large, high-definition television screen (102 x 57 cm) approximately 1.5 meters from the infant. Eye tracking was used to capture precise and detailed information about the infant's looking behavior during the assessment. Looking behavior was tracked using an EyeLink 1000 Plus infrared eye tracker (SR Research Ltd., Mississauga, Ontario, Canada), and the parent was instructed to maintain neutral demeanor and look down or close their eyes during the assessment to avoid influencing the infant's looking behavior. The eye tracker was calibrated prior to the assessment by directing the infant's gaze to three specific points on the screen (top center, bottom left, bottom right) using a short video clip of a child accompanied by laughter or an animated object accompanied by musical tones. After the eye tracker was calibrated, the eye tracking model was validated using the same stimuli in the same configuration that was used for calibration. The average deviation and maximum deviation of estimated gaze locations were recorded.

The visual recognition memory (VRM) assessment that was used was developed based on the assessment used by Rose et al. (1992). It consisted of nine blocks of trials with each block consisting of one familiarization trial followed by two test trials. In each trial, the stimuli were presented in the same left and right positions on the screen separated by approximately 9.5 degrees visual angle, and each stimulus subtended approximately 9.5 degrees of visual angle in both the vertical and horizontal directions. A video clip similar to that used for

calibration and validation was used to draw the infant's attention to the center of the screen before each trial began to ensure that looking was not biased based on where the infant was looking at the start of the trial. The first five blocks consisted of black-and-white photos of human faces, and the final four blocks showed colorful abstract geometric shapes (Figure 4.1). In each familiarization trial, two identical stimuli were presented side-by-side until the infant reached criterion (total looking time accumulated: 20 seconds for faces, 10 seconds for shapes). In each test trial, the familiar stimulus was paired with a novel stimulus until the infant accumulated a total of 1 second looking at the stimuli, plus 5 additional seconds, regardless of looking behavior. The novel stimulus was presented on one side of the screen in the first test trial and on the other side in the second test trial of each block. Each infant was randomly assigned to one of four assessment conditions within blocks of 48 infants within each sex: A, B, C, or D. In conditions A and B, one set of stimuli was used as the familiar stimuli (set 1), and the other set was used in conditions C and D (set 2). Blocking was used to ensure approximately equal numbers of each sex were assigned to each condition across time. In conditions A and C, the novel stimuli were presented on the right side of the screen in the first test trial of each block and on the left in the second (Figure 4.2). In conditions B and D, the novel stimuli were presented on the left side of the screen first and then on the right in each block. As revealed by the blocking, infants show a preference for set 2 stimuli over set 1 stimuli, resulting in differences in performance between infants shown set 1 and those shown set 2 stimuli as familiar (Dzwilewski et al., 2020).

DataViewer software (SR Research Ltd., Mississauga, Ontario, Canada) was used to extract and process data for analysis. DataViewer allows users to define interest areas for each stimulus, which can then be used to extract information about visual fixations, blinks, and saccades within, between, and outside of the interest areas. For these analyses, the interest areas consisted of rectangular areas encompassing each stimulus plus a small area around each stimulus to account for small inaccuracies in eye tracking (Figure 4.3). For each

familiarization trial, the average duration of time spent looking at the stimuli before looking away (run duration), the average time to reach the familiarization criterion (time to familiarization), and the number of saccades between the stimuli per second (shift rate) were extracted for analysis. Additionally, the familiarization trials were divided into two interest periods roughly corresponding to the first and second half of each familiarization trial to examine the changes in run duration and shift rate to provide further insight into the infants' attentional abilities. For each test trial, the proportion of time looking at the novel stimulus was extracted (novelty preference, a measure of recognition memory).

Time to familiarization is considered a measure of attention and is the total time the infant required to reach the familiarization criterion (20 seconds for faces trials, 10 seconds for shapes trials). Infants with better attention are expected to require less time to reach the familiarization criterion.

Shift rate is considered a measure of information processing. Direct saccades, or shifts, between the stimuli during the familiarization trial indicate the infant is attending to and comparing the stimuli to one another. Faster shift rate is suggestive of better information processing. For these analyses, shift rate for the full trial was calculated as shown below:

$$\text{Average shift rate} = \frac{(\# \text{ of shifts between stimuli})}{(\text{total time looking at stimuli})}$$

The total time looking at the stimuli is equivalent to the familiarization criterion. To calculate the shift rate for the first and last portion of familiarization trials, only the number of shifts between stimuli within the first and last 10 seconds of the trial for faces and the first and last 5 seconds of the trial for shapes were included, and the total time of looking at the stimuli was halved, as shown in the example for calculating shift rate for the last portion of shapes trials below:

$$\begin{aligned} &\text{Average shift rate during the last 5 seconds of shapes trials} \\ &= \frac{(\# \text{ of shifts between stimuli during the last 5 seconds of the familiarization trial})}{5} \end{aligned}$$

Novelty preference is considered a measure of working memory and is calculated by the following equation, using values extracted from the test trials:

Novelty preference

$$= \frac{(time\ looking\ at\ novel\ stimulus)}{[(time\ looking\ at\ novel\ stimulus) + (time\ looking\ at\ familiar\ stimulus)]}$$

Values greater than 0.5 indicate infants spent more time looking at the novel stimulus than the familiar stimulus in test trials, while values less than 0.5 indicate the opposite.

Run duration is considered a measure of information processing speed where infants who process information quickly are expected to show shorter run durations. This measure is extracted from the familiarization trials and was calculated using the following equation:

$$Average\ run\ duration = \frac{(total\ time\ looking\ at\ stimuli)}{(number\ of\ runs\ between\ the\ two\ stimuli)}$$

where the total time looking at the stimuli is equivalent to the familiarization criterion. To measure the average run duration for the first portion of familiarization trials and the last portion of familiarization trials, the number of runs between stimuli was extracted for the first and last 10 seconds (faces) and the first and last 5 seconds (shapes) of the familiarization trials, and the total time looking at the stimuli was divided by 2, as shown in the example equation below for calculating the average run duration for the first portion of faces trials.

Average run duration in the first 10 seconds of faces trials

$$= \frac{10}{(number\ of\ runs\ between\ the\ two\ stimuli\ during\ the\ first\ 10\ seconds)}$$

4.2.4. Covariates

Based on *a priori* knowledge and using directed acyclic graphs (DAGs, Figure 4.4), the following variables were selected for inclusion in the models as covariates: maternal race and ethnicity (white, non-Hispanic vs. all others), maternal education (< bachelor's degree vs bachelor's degree or higher), maternal age, maternal parity (nulliparous vs. ≥ 1), maternal verbal

IQ (VIQ), annual household income (< \$50,000, \$50,000-\$99,999, ≥ \$100,000), infant gestational age at birth, and infant's age at assessment. Stimulus set (1 vs. 2) was also included as a potential modifier in VRM analyses because performance on the VRM task varies by familiar stimulus set (Dzwilewski et al., 2020). Infant sex was included in analyses as a potential modifier. Covariate factors were examined using means, standard deviations, and proportions where appropriate.

4.2.5. Statistical Approach

Multivariable generalized linear regression models were used to evaluate associations between each of five continuous maternal urinary biomarkers of phthalate exposure (MEP, sum of DEHP metabolites [Σ DEHP], sum of DINP metabolites [Σ DINP], sum of DEHTP metabolites [Σ DEHTP], and sum of antiandrogenic metabolites [Σ AA]), both at 16-18 weeks of gestation and across pregnancy (pooled sample), and each continuous outcome measure averaged across the 5 sets of faces trials and 3 of the 4 sets of shapes trials. Only 3 sets of the shapes trials were included in analyses because previous work has demonstrated that infants show an overwhelming preference for the pink and blue image in block 8 that results in a failure to show a novelty preference when that image is used as the familiar image (Dzwilewski et al., 2020). Based on previous evidence of phthalate effect modification by child sex, interactions of sex-by-exposure were also assessed for each exposure-outcome model (Doherty et al., 2017; Ipapo et al., 2017; Kim et al., 2011; Kobrosly et al., 2014; Tellez-Rojo et al., 2013). Stimulus set-by-exposure interactions were also considered to examine potential differences in associations depending on which stimulus set (1 or 2) was the familiar one (Dzwilewski et al., 2020). Finally, sex-by-stimulus set-by exposure interactions were also explored. Interaction terms with p-values < 0.10 were retained in the final models. Results for models with interactions are presented using stratum-specific estimates in addition to the main effect of exposure. Exposure associations were considered significant if p-values were less than 0.05, and beta estimates

were scaled to an interquartile range (IQR) increase in each exposure biomarker for ease of comparison across broad exposure biomarker ranges. Sensitivity analyses to explore the potential impact of maternal smoking (removing women who reported smoking) or alcohol consumption (yes/no) during pregnancy, as well as analyses that removed potential influential observations (Cook's $D > 0.1$) were also conducted. All statistical analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (Copyright © 2013, SAS Institute Inc., Cary, NC, USA).

4.3. RESULTS

4.3.1. Participation and Demographics

Urinary phthalate metabolites were available for 482 (74%) of the 650 pregnant women enrolled in the IKIDS cohort between 2013 and March 2020. Of these, as of March 2020, 474 (98%) remained active in the study after the birth of their infant and were eligible to participate in the VRM assessment, of which 374 mothers and their infants (79%) did participate. Eye tracking was attempted for 365 of those, with 9 infants being too fussy or sleepy to attempt eye-tracking. Useable eye-tracking data were not obtained for 37 infants due to eye tracking failure (software malfunction or inability to track infants' corneal reflection; $n = 15$) or infant fussiness, inattention, or sleepiness ($n = 22$). Of the 328 infants with data available, 260 completed all five faces trials, and 194 also completed all shapes trials. The breakdown of infants who completed each trial type (faces and shapes) and had phthalate data (all phthalates and the subset with Σ DEHTP exposure data available measured in both the pooled and 16-18 weeks of gestation samples) and complete covariate data available is shown in Figure 4.5. In general, the infants included in the present analyses were representative of the full cohort, although slightly more mothers were white, non-Hispanic; married or living as married; held at least a bachelor's degree; were nulliparous; reported an annual household income of at least \$50,000; and reported no alcohol

use during the first trimester. Characteristics of infants who completed only the faces trials were largely similar to those who also completed the shapes trials as well

4.3.2. Exposure Characterization

Due to the skewed nature of phthalate exposure concentrations, exposures were characterized using minimums, maximums, medians, and interquartile ranges. Pregnancy urine biomarker concentrations reflecting prenatal phthalate exposures for the full cohort are shown in Table 3.3. Biomarker concentrations for the subset included in these analyses were generally very similar to those for the full cohort, with the exception of mono-(2-ethyl-5-carboxypentyl) terephthalate (MECPTP), a metabolite of DEHTP, which was substantially higher in the pooled sample of the subset included in these analyses than that of the full cohort. Similar to the full cohort, MCOP and both DEHTP metabolites in the pooled sample were higher than what was reported in the latest biomonitoring data for reproductive age women from the National Health and Nutrition Examination Survey (CDC, 2019). As expected, all concentrations showed a right-skewed distribution.

4.3.3. Cognitive Assessment Outcomes

Due to the method of calculating shift rate for the first and last portion of trials, not all infants had values for these outcomes. Shift rate is calculated as the number of saccades directly between the interest areas across the amount of time required for familiarization criterion (20 seconds for faces, 10 seconds for shapes), meaning that direct saccades may not occur within the short periods of time considered for the first portion and last portion of familiarization trials. Therefore, only 243 infants had shift rate for the first and last portion of faces trials, and 180 had shift rate for the first and last portion of shapes trials (Table 4.1). Novelty preference was slightly higher for the shapes trials compared to the faces trials, and time to familiarization and average run duration were longer for faces trials than shapes trials (Table 4.1). As previously reported by Dzwilewski et al. (2020), stimulus set was a significant

predictor of novelty preference, with infants who saw set 1 showing higher novelty preference scores. Average shift rate for the full familiarization trials was slightly higher for shapes trials than faces trials, and shift rate also differed between the first and last portions of familiarization trials with faster shift rates in the last portion of faces trials, but faster shift rates in the first portion of the shapes trials (Table 4.1). Descriptive statistics of the indices of run duration and shift rate for each trial type revealed that infants tended to show longer run durations in the first portion of familiarization trials, although this was less evident in shapes trials.

More outcomes were correlated with one another for the faces trials than the shapes trials, as shown in Tables 4.2 and 4.3. Briefly, longer run durations in the full familiarization trials were associated with faster shift rates for the first and second portion of familiarization trials. Longer run durations in the full familiarization trials were also associated with longer run durations in both the first and last portions of familiarization trials, and faster shift rates in the full familiarization trials were associated with faster shift rates in the first and last portions of familiarization trials. Longer run durations for the full trial were associated with shorter times to familiarization, and longer run durations during the full trials were associated with faster shift rates for the last portion of trials. Additionally, for the faces trials only, longer times to familiarization were also associated with shorter run durations in the first portion of trials and faster shift rates in both the first and last portion of trials.

As previously reported (Dzwilewski et al., 2020; Dzwilewski, Woodbury et al. 2021), infants who saw stimulus set 2 as familiar had a higher novelty preference and longer run durations as compared to those who saw stimulus set 1 as familiar for the faces trials; however, this was not the case for the shapes trials, and most of the other outcomes did not differ by set.

Phthalate exposure and cognitive outcomes at 7-8 months

Time to familiarization

Significant associations of the orthophthalates with time to familiarization were few and inconsistent across phthalate biomarkers or across the face and shape trials (Figure 4.6 and Table 4.4). An IQR increase in Σ DINP at 16-18 weeks was associated an increase in time to familiarization in the faces trials, but only for infants who saw set 1 as familiar (Figure 4.6E). In the shapes trials, an IQR increase in Σ DINP in the pooled sample was associated with a decrease in time to familiarization, regardless of which set the infant saw as familiar (Figure 4.6P). An IQR increase in MEP was only associated with increased time to familiarization in male infants who saw set 1 as familiar in the shapes trials, but this association was only statistically significant for the pooled sample (Figure 4.6R). Per IQR increase, the terephthalate, Σ DEHTP, was associated with longer time to familiarization in the faces trials, but not the shapes trials. This relationship was only significant for the pooled sample (Figure 4.6J). Associations with time to familiarization were not influenced by including alcohol use during the first trimester as a covariate in models, excluding women who reported smoking ($n = 20$), or removing potentially influential data points (Cook's $D > 0.05$).

Shift rate

Because the results of analyses of shift rate during only the first portion or last portion of familiarization trials were similar to those for the full familiarization trial, only the results from analyses of the full trial are presented here for simplicity. Only Σ DINP and MEP had any associations with shift rate and the results were inconsistent across exposure biomarkers (16-18 weeks or pooled samples) and across the faces and shapes trials (Figure 4.7 and Table 4.6). In the faces trials, an IQR increase in Σ DINP at 16-18 weeks was associated with an increase in shift rate in male infants who saw set 1 as familiar (Figure 4.7E), whereas in the shapes trials,

an IQR increase in Σ DINP in the pooled sample was associated with an increase in shift rate in male infants, regardless of the set they saw as familiar (Figure 4.7P).

For MEP, associations with shift rate were only apparent when stratified by both infant sex and stimulus set (Figure 4.7 and Table 4.6). In the faces trials, an IQR increase in MEP in both the 16-18 weeks (Figure 4.7G) and pooled samples (Figure 4.7H) was associated with a decrease in shift rate in female infants who saw set 1 stimuli as familiar, but an increase in shift rate in males who saw set 1. However, the association for males was only significant for MEP in the pooled sample (Figure 4.7H). In the shapes trials, MEP was associated with an increase in shift rate in females who saw set 2 stimuli as familiar, but this also only reached significance for the pooled sample (Figure 4.7R). Together, these results suggest that specific phthalates, Σ DINP and MEP, may be related to faster shift rate in male infants but slower shift rate in female infants shown set 1 stimuli as familiar. No associations were altered by including maternal alcohol use during the first trimester as a covariate, removing women who reported smoking, or removing potentially influential observations.

Run duration

The results of analyses examining the first or last portion of familiarization trials were largely similar to those for the full trial, and because associations for the full familiarization trial tended to be stronger, only those results are presented here for simplicity. In general, prenatal exposure to orthophthalates considered antiandrogenic, including the Σ AA phthalates, was related to longer run durations, particularly for infants who saw set 2 stimuli as familiar, but this was primarily true for the faces trials, with the exception of Σ DINP (Table 4.5, 4.3). An IQR increase in Σ DINP was consistently associated with longer run durations in infants who saw set 2 as familiar across both biomarkers of exposure (16-18 weeks or pooled sample), and across both faces and shapes trials. An IQR increase in the terephthalate Σ DEHP at 16-18 weeks was also associated with longer run durations for the shape trials (Figure 4.8S). Associations of all phthalate biomarkers with run duration were not substantially altered by

sensitivity analyses that adjusted for maternal alcohol intake during the first trimester, removing mothers who smoked, or removing potential high leverage observations from the analysis.

Novelty preference

In the faces trials, exposures to orthophthalates considered antiandrogenic were generally associated with lower novelty preference for faces, regardless of the biomarker (16-18 weeks or pooled). However, this relationship approached statistical significance only for Σ AA phthalates (Figure 4.9A) and Σ DEHP (Figure 4.9C) in the 16-18 weeks sample and was only observed for the face trials (Table 4.4). For Σ DINP, this association only approached significance for the pooled sample in female infants who saw set 1 and only on the faces trials (Figure 4.9F, Table 4.6). An IQR increase in MEP, which is not considered antiandrogenic, at 16-18 weeks of gestation was associated with an increase in novelty preference on the face trials in males who saw set 1 (Figure 4.9G). In contrast, an IQR increase in MEP was associated with a decrease in novelty preference in females who saw set one. A similar pattern of associations was present for the pooled sample. For the shapes trials, an IQR increase in MEP was associated with an increase in novelty preference in males regardless of which set of stimuli were novel and the association was significant for the 16–18-week sample (Figure 4.9Q). An IQR increase in the terephthalate, Σ DEHTP, in the pooled sample was associated with a significant decrease in novelty preference but only in infants who saw set 1 as familiar and only in the face trials (Figure 4.9J, Table 4.6). While antiandrogenic orthophthalates and the terephthalate Σ DEHTP, were generally associated with reduced novelty preference, MEP appeared to be associated with increased novelty preference in male infants. These associations were not altered by including maternal alcohol use during the first trimester as a covariate, removing women who reported smoking, or removing potentially influential observations.

4.4. DISCUSSION

4.4.1. Time to familiarization

Associations with time to familiarization were largely inconsistent across trial type (faces or shapes) and biomarker of exposure (16-18 weeks or pooled) with some associations suggestive of better attention and others suggestive of worse attention (Figure 4.6). The most consistent associations were for Σ AA and Σ DEHP, with associations with Σ AA indicating it may be related to improved attention (Figure 4.6A, B), while associations with Σ DEHP suggested it may be related to poorer attention (Figure 4.6I, J). Some other studies have also found that higher phthalate exposures were associated with better neurodevelopmental outcomes, including lower risk of inattention (Gascon et al., 2015; Philippat et al., 2017), whereas other found associations with poorer outcomes, such as increases in attention problems or increased risk of attention-deficit/hyperactivity disorder (ADHD; Kobrosly et al., 2014; Engel et al., 2018). While there is substantial evidence indicating that DEHP exposure has dramatically increased around the world, particularly in the mainland U.S. and Puerto Rico and especially in children (Schwedler, et al., 2020), to date, only a couple of studies have explored its potential impact on neurodevelopment. In a cohort in Puerto Rico, Zimmerman et al. (2021) found that prenatal DEHP exposure was associated with changes in non-nutritive suck behavior in infants which, while the exact relationship is currently unclear, has been linked to later neurodevelopmental outcomes such as IQ and language development (Wolthuis-Stigter et al., 2015; Wolthuis-Stigter et al., 2017). In a Mexico City cohort, higher prenatal exposure to one of the major metabolites of DEHP, MECPTP, was found to be associated with more problem behaviors, such as hyperactivity in children at 4-6 years of age (Colicino, et al., 2021). These results, combined with the results of Pacyga et al. (2021) and Long et al. (2021) suggesting that DEHP may interfere with sex hormones in both pregnant and non-pregnant women, are cause for concern and indicate that further study of this chemical is needed.

While the results did not support an association between prenatal exposure to orthophthalates and attention in infants, it is important to point out that measurement of time to familiarization as defined in this task may not be optimal for assessing visual attention. Because infants were required to look for a specified length of time (20 seconds for faces, 10 seconds for shapes) rather than ending the familiarization trial when they naturally looked away for longer breaks, indicating that they had become familiarized (i.e., they had processed the stimuli and were becoming bored with it), it is possible that infants who were more attentive became familiarized with the stimuli sooner than those who were less attentive, and then lost interest and started to look away for longer stretches of time, resulting in longer times to familiarization. This possibility is supported by the fact that run duration was negatively correlated with time to familiarization (Table 4.3). That is, shorter run durations, which are considered indicative of faster information processing, were associated with longer times to familiarization. To address this possibility, more detailed analyses of the looking patterns of infants during familiarization is required. In assays of behavior in animals, such as in the 5-choice serial reaction time task, animals are trained to associate a stimulus with a specific response to receive a reward, often food. Across trials, animals learn this association and their performance improves with the animal more quickly responding after the stimulus (Higgins and Breysse, 2008). Likewise, in this assessment, if infants learn that they need to look at the stimuli for a specific amount of time before the images will change, it is expected that their looking behavior would change across blocks of trials. Attentive infants who require less time than the criterion to become familiarized with the stimuli would be expected to show shorter times to familiarization as the task progresses. Future studies should examine each block of trials (1 familiarization trial followed by 2 test trials) to understand how infant looking behavior changes during the task in order to evaluate this possibility. In summary, the meaning of shorter or longer times to familiarization on this task remains open to interpretation at this time.

4.4.2. Shift rate

Only Σ DINP and MEP were associated with shift rate. Σ DINP exposure was associated with faster shift rates in males (Figure 4.7E, P), suggesting that it may be related to better information processing in males, while higher MEP exposure was associated with slower shift rates in females who saw set 1 stimuli as familiar (Figure 4.7G, H), indicating that MEP may be related to poorer information processing in females. Few studies have found associations of prenatal exposure to DINP or its metabolites with measures of neurodevelopment, but those that have reported mixed results. For example, while Philippat et al. (2017) found that DINP was associated with less hyperactivity and inattention, they also found that it was associated with lower prosocial behaviors, and they did not find sex-specific effects. Associations of prenatal exposure to MEP with cognition have also been mixed, with studies finding associations with both worse and better attention (Engel et al., 2010; Gascon et al., 2015) as well as increases in peer relationship problems (Jankowska et al., 2019) and hyperactivity (Hyland et al., 2019), although the majority of results seem to indicate MEP may be related to poorer outcomes, in line with the results of the present study.

4.4.3. Run Duration

Overall, the phthalates included in these analyses appear to be largely associated with longer run durations, which is considered a measure of information processing speed (Columbo et al., 1991). This was particularly true in infants shown set 2 stimuli as familiar. The difference between infants seeing the set 2 vs 1 stimuli in the familiarization trials is not unexpected as previous findings have shown that infants have significantly longer run durations when set 2 rather than set 1 stimuli are used in familiarization (Dzwilewski et al., 2020). While there was generally no adverse association of MEP, unlike for shift rate, or Σ DEHP with average run duration, adverse associations were observed in orthophthalates known to have antiandrogenic activity (Σ DEHP, Σ DINP, and Σ AA), suggesting slower information processing is associated with higher prenatal exposure to antiandrogenic phthalates (Figure 4.8). Similar to Σ DINP, Σ DEHP

in the 16-18 weeks sample was also associated with significantly longer run durations during the shapes trials (Figure 4.8O, S), suggesting it may also be related to slower information processing.

4.4.4. Novelty preference

Although estimates were imprecise and included the null, all of the antiandrogenic orthophthalate biomarkers were generally associated with lower novelty preference on the face trials (Figure 4.9), indicative of reduced visual recognition memory (Rose et al., 2001). This pattern of findings is generally consistent with, although not as robust as, those of Ipapo and colleagues (2017) who reported lower novelty preference in infants with higher prenatal phthalate exposure using a similar task. MEP, the only orthophthalate we examined that is not antiandrogenic, showed a different pattern of results, where the main association was only negative for the pooled sample for faces trials (Figure 4.9H) and otherwise more positive, and interestingly, Σ DEHTP followed a similar pattern (Figure 4.9J). However, the association of MEP with novelty preference differed between males and females with male children, especially those who saw set 1 as familiar in the faces trials, showing higher novelty preference (Figure 4.9G, H), and female children who saw set 1 showing lower novelty preference in the faces trials (Figure 4.9G, H). Associations with Σ DEHTP also differed, but only by stimulus set, and only for Σ DEHTP in the pooled sample and only in the faces trials, with infants who saw set 1 showing lower novelty preference (Figure 4.9J). These similarities between associations of MEP and Σ DEHTP and novelty preference are interesting, but, particularly for Σ DEHTP, these analyses are under-powered due to the small sample size, and further studies with larger and more diverse samples are warranted.

4.4.5. Modification of associations by infant sex or stimulus set

There were some differences of associations by infant sex and by stimulus set. Although not all key findings were sex specific, when only one sex was affected, it was typically males,

and, except for run duration, they generally showed improvements in cognitive outcomes with higher exposure to orthophthalates. On the other hand, when females were affected, they always showed poorer outcomes in associations with orthophthalates (Table 4.7). This indicates that prenatal exposure to orthophthalates may differentially affect the cognitive abilities of males and females, disproportionately negatively impacting females in measures of attention, recognition memory, and one measure of information processing speed. As described earlier, previous studies have also found that associations differed by child sex, although generally they have found associations in male children only (Kobrosly et al., 2014; Hyland et al., 2019). For measures of run duration, antiandrogenic orthophthalates were associated with slower information processing speeds in both males and females who saw set 2 stimuli as familiar. The most consistent of these associations was for Σ DINP which was associated with longer run durations in infants who saw set 2, regardless of exposure biomarker and in both faces and shapes trials. Except for effects observed for run duration, the impact of stimulus set was more mixed (Table 4.7).

4.4.6. Summary and conclusions

In summary, these results indicate that cognitive outcomes measured in infancy may be influenced by prenatal phthalate exposure, although associations often differ by stimulus set and child sex. These results consistently suggested that prenatal exposure to antiandrogenic orthophthalates is related to slower information processing at 7-8 months, supporting recent calls by experts for removal of orthophthalates from consumer products (Engel et al., 2021). These results were particularly consistent for Σ DINP, a replacement phthalate for DEHP, suggesting this may be a case of regrettable substitution, and that replacement of orthophthalates in consumer products with different orthophthalates may be just as detrimental to child neurodevelopment, if not more (Engel et al., 2021). In addition, while this was the first study to assess the association of prenatal Σ DEHTP exposure with cognitive outcomes in infancy, Σ DEHTP was only available for a small subset of the population, and Σ DEHTP

exposure levels in this subset were higher compared to those of the full cohort (Table 3.3), thus, the findings for this exposure measure should be interpreted cautiously. As DEHTP has been increasingly used as a replacement for DEHP (Schwedler, et al., 2020), it is important that future studies evaluate the relationship of prenatal exposure to this terephthalate with cognitive outcomes in children. In general, although the models examined herein had only moderate sample sizes, these findings illustrate the value of this automated, domain-specific approach to assessing early cognitive function and further demonstrate the prenatal phthalate exposure may be associated with cognitive deficits that can be detected in infancy.

4.5. TABLES AND FIGURES

Table 4.1. Distributional characteristics of cognitive outcomes from the visual recognition memory task administered to IKIDS infants at 7-8 months for the subset of infants included in analyses.

Outcome measure (units)	Faces <i>n</i> = 246		Shapes <i>n</i> = 182	
	Mean ± SEM	Median	Mean ± SEM	Median
Novelty preference (proportion)	0.57 ± 0.004	0.57	0.60 ± 0.01	0.61
Average time to familiarization (seconds)	52.0 ± 1.37	48.3	33.1 ± 1.21	27.6
Average run duration (seconds)	4.32 ± 0.15	3.71	2.89 ± 0.11	2.56
First portion of trial	6.74 ± 0.11	6.67	3.98 ± 0.06	4.17
Last portion of trial	5.22 ± 0.09	5.12	3.62 ± 0.06	3.35
Average shift rate (shifts/second)	0.41 ± 0.01	0.41	0.48 ± 0.02	0.47
First portion of trial [†]	0.30 ± 0.01	0.34	4.03 × 10 ⁻⁷ ± 1.60 × 10 ⁻⁸	3.33 × 10 ⁻⁷
Last portion of trial [†]	0.06 ± 0.01	0.00	1.96 × 10 ⁻⁷ ± 1.19 × 10 ⁻⁸	1.33 × 10 ⁻⁷

[†]Shift rate for first and/or last portion of familiarization trial only able to be calculated for *n* = 243 for faces and *n* = 180 for shapes

Table 4.2. Correlations among outcome measures for faces trials in the VRM assessment.

	Faces							
	1.	2.	3.	4.	5.	6.	7.	8.
1. Novelty preference (proportion)	--	--	--	--	--	--	--	--
2. Average time to familiarization (seconds)	0.02	--	--	--	--	--	--	--
3. Average run duration for full trial (seconds)	-0.06	-0.48	--	--	--	--	--	--
4. Average run duration for first portion of trial (10s)	-0.06	-0.21	0.53	--	--	--	--	--
5. Average run duration for last portion of trial (10s)	-0.02	0.13	0.34	0.25	--	--	--	--
6. Average shift rate for full trial (shifts/second)	0.08	-0.11	-0.05	-0.09	-0.17	--	--	--
7. Average shift rate for first portion of trial (10s)	-0.03	-0.26	0.05	-0.05	-0.18	0.74	--	--
8. Average shift rate for last portion of trial (10s)	0.08	-0.29	0.19	-0.01	-0.14	0.46	0.43	--

Table 4.3. Correlations among outcome measures for shapes trials in the VRM assessment.

	Shapes							
	1.	2.	3.	4.	5.	6.	7.	8.
1. Novelty preference (proportion)	--	--	--	--	--	--	--	--
2. Average time to familiarization (seconds)	0.18	--	--	--	--	--	--	--
3. Average run duration for full trial (seconds)	-0.15	-0.46	--	--	--	--	--	--
4. Average run duration for first portion of trial (5s)	-0.15	-0.10	0.43	--	--	--	--	--
5. Average run duration for last portion of trial (5s)	0.03	0.15	0.29	0.24	--	--	--	--
6. Average shift rate for full trial (shifts/second)	0.04	-0.01	-0.09	-0.04	-0.12	--	--	--
7. Average shift rate for first portion of trial (5s)	0.004	-0.14	0.13	0.15	-0.04	0.73	--	--
8. Average shift rate for last portion of trial (5s)	-0.05	0.21	0.25	-0.05	-0.03	0.52	0.32	--

Table 4.4. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of biomarkers of prenatal phthalate exposure from the 16-18 gestational weeks sample and the pooled sample with infant cognition in faces trials.¹

Outcome	Exposure	Faces		Shapes	
		16-18 weeks gestation sample ²	Pooled sample ³	16-18 weeks gestation sample ²	Pooled sample ³
		<i>n</i> = 244	<i>n</i> = 246	<i>n</i> = 180	<i>n</i> = 182
Novelty Preference	Σ AA Phthalates	# -0.003 (0.0003, -0.006)	-0.003 (0.001, -0.007)	-0.001 (0.004, -0.007)	-0.0001 (0.007, -0.007)
	Σ DEHP	# -0.001 (0.0001, -0.002)	-0.001 (0.001, -0.004)	-0.0003 (0.001, -0.002)	0.001 (0.005, -0.003)
	Σ DINP	-0.0001 (0.002, -0.002)	-0.003 (0.001, -0.006)	-0.004 (0.001, -0.008)	-0.006 (0.003, -0.014)
	MEP	0.001 (0.004, -0.001)	-0.002 (0.002, -0.007)	0.004 (0.010, -0.002)	0.007 (0.017, -0.003)
	Σ DEHTP	-0.0001 (0.003, -0.003)	-0.005 (0.002, -0.013)	0.001 (0.005, -0.004)	0.004 (0.018, -0.010)
Time to Familiarization	Σ AA Phthalates	# -0.8942 (0.135, -1.924)	# -1.147 (0.140, -2.435)	0.007 (0.819, -0.805)	-0.261 (0.779, -1.301)
	Σ DEHP	-0.179 (0.119, -0.476)	-0.236 (0.521, -0.993)	0.018 (0.246, -0.210)	0.117 (0.703, -0.468)
	Σ DINP	0.417 (0.986, -0.151)	0.524 (1.892, -0.845)	-0.411 (0.248, -1.071)	* -1.458 (-0.242, -2.675)
	MEP	0.188 (1.081, -0.705)	-0.090 (1.421, -1.600)	-0.054 (0.840, -0.947)	-0.431 (1.093, -1.954)
	Σ DEHTP	# 0.877 (1.764, -0.010)	* 2.857 (5.195, 0.519)	-0.147 (0.609, -0.903)	0.809 (3.027, -1.408)
Run Duration	Σ AA Phthalates	# 0.104 (0.2199, -0.012)	0.105 (0.251, -0.041)	-0.028 (0.044, -0.100)	0.015 (0.109, -0.079)
	Σ DEHP	0.019 (0.052, -0.015)	0.058 (0.144, -0.027)	-0.017 (0.003, -0.037)	-0.043 (0.010, -0.095)
	Σ DINP	0.044 (0.108, -0.0201)	0.088 (0.243, -0.067)	** 0.085 (0.143, 0.028)	* 0.128 (0.238, 0.018)
	MEP	-0.044 (0.056, -0.145)	-0.084 (0.087, -0.255)	0.046 (0.125, -0.033)	0.021 (0.159, -0.116)
	Σ DEHTP	-0.053 (0.039, -0.145)	-0.161 (0.083, -0.405)	* 0.064 (0.127, 0.001)	-0.047 (0.142, -0.236)
Shift Rate	Σ AA Phthalates	0.001 (0.009, -0.007)	0.0003 (0.010, -0.010)	0.003 (0.013, -0.008)	0.006 (0.019, -0.008)
	Σ DEHP	0.001 (0.003, -0.002)	0.001 (0.007, -0.005)	0.001 (0.003, -0.002)	0.003 (0.010, -0.005)
	Σ DINP	-0.001 (0.004, -0.005)	# -0.010 (0.001, -0.020)	-0.003 (0.005, -0.012)	-0.001 (0.015, -0.017)
	MEP	-0.003 (0.004, -0.009)	-0.004 (0.008, -0.016)	0.005 (0.016, -0.006)	0.013 (0.033, -0.006)
	Σ DEHTP	0.005 (0.012, -0.003)	-0.009 (0.010, -0.029)	0.003 (0.013, -0.007)	-0.012 (0.017, -0.041)

¹All models were adjusted for maternal race and ethnicity, education, parity, household income, age, and verbal IQ as well as infant sex, gestational age at birth, postnatal age at assessment, and stimulus set for cognitive assessment.

²*n* = 142 completed all faces trials and had Σ DEHTP data available from the 16-18 weeks gestation sample.

³*n* = 143 completed all faces trials and had Σ DEHTP data available from the pooled sample.

⁴*n* = 115 completed all shapes trials and had Σ DEHTP data available from the 16-18 weeks gestation sample.

⁵*n* = 116 completed all shapes trials and had Σ DEHTP data available from the pooled sample.

Sum of antiandrogenic phthalates (Σ AA Phthalates), di-2-ethylhexyl phthalate sum (Σ DEHP), di-isononyl phthalate sum (Σ DINP), mono-ethyl phthalate (MEP), di-2-ethylhexyl terephthalate sum (Σ DEHTP)

p < 0.10, * *p* < 0.05, ** *p* < 0.01

Table 4.5. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of biomarkers of prenatal phthalate exposure from the 16-18 gestational weeks sample and the pooled sample with infant cognition in both faces and shapes trials, stratified by sex or set.¹

Stratified by Sex		16-18 Weeks Gestation ²		Pooled Sample ³	
<i>Faces Trials</i>		<i>n = 244</i>		<i>n = 246</i>	
Outcome	Exposure	Females	Males	Females	Males
Novelty Preference	Σ DEHTP	^b ** 0.010 (0.016, 0.003)	-0.005 (0.003, -0.013)	--	--
Stratified by Set		Set 1	Set 2	Set 1	Set 2
Time to Familiarization	Σ DINP	^b * 0.847 (1.543, 0.151)	-0.381 (0.564, -1.326)	--	--
Run Duration	Σ DEHP	--	--	^a -0.078 (0.098, -0.254)	* 0.100 (0.196, 0.003)
	Σ DINP	^b -0.028 (0.050, -0.105)	** 0.178 (0.283, 0.072)	^b -0.084 (0.090, -0.258)	** 0.591 (0.889, 0.292)
Shift Rate	Σ DEHP	^a -0.002 (0.002, -0.005)	* 0.002 (0.004, 0.0002)	--	--
Stratified by Sex		<i>n = 180</i>		<i>n = 182</i>	
<i>Shapes Trials</i>		Females	Males	Females	Males
Shift Rate	Σ DEHP	--	--	^a * -0.083 (-0.014, -0.152)	-0.018 (0.016, -0.051)
Stratified by Set		Set 1	Set 2	Set 1	Set 2
Run Duration	Σ DINP	^b -0.011 (0.075, -0.098)	** 0.157 (0.232, 0.083)	^b -0.005 (0.116, -0.126)	** 0.535 (0.749, 0.321)

¹All models were adjusted for maternal race and ethnicity, education, parity, household income, age, and verbal IQ as well as infant sex, gestational age at birth, postnatal age at assessment, and stimulus set for cognitive assessment.

²n = 142 completed all faces trials and had Σ DEHTP data available from the 16-18 weeks gestation sample.

³n = 143 completed all faces trials and had Σ DEHTP data available from the pooled sample.

Sum of antiandrogenic phthalates (Σ AA Phthalates), di-2-ethylhexyl phthalate sum (Σ DEHP), di-isononyl phthalate sum (Σ DINP), mono-ethyl phthalate (MEP), di-2-ethylhexyl terephthalate sum (Σ DEHTP)

Interaction p-value: ^a p < 0.10, ^b p < 0.05

* p < 0.05, ** p < 0.01

Table 4.6. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of biomarkers of prenatal phthalate exposure from the 16-18 gestational weeks sample and the pooled sample with infant cognition in both faces and shapes trials, stratified by sex and set.¹

<i>Faces Trials</i>			16-18 Weeks Gestation Sample		Pooled Sample	
			<i>n</i> = 244		<i>n</i> = 246	
Outcome	Exposure		Set 1	Set 2	Set 1	Set 2
Novelty Preference	MEP	Females	-0.006 (0.002, -0.014)	0.002 (0.005, -0.001)	--	--
		Males	^b # 0.014 (0.030, -0.001)	0.002 (0.009, -0.006)	--	--
Time to Familiarization	MEP	Females	--	--	0.689 (6.382, -5.004)	0.079 (3.873, -3.716)
		Males	--	--	^b ** 8.295 (14.185, 2.405)	# -1.534 (0.223, -3.292)
Run Duration	Σ AA Phthalates	Females	^b -0.055 (0.141, -0.252)	* 0.615 (1.103, 0.127)	--	--
		Males	0.572 (1.475, -0.331)	# 0.135 (0.281, -0.011)	--	--
Shift Rate	Σ DINP	Females	-0.001 (0.005, -0.006)	-0.002 (0.008, -0.011)	--	--
		Males	^a # 0.024 (0.049, -0.001)	-0.005 (0.007, -0.017)	--	--
	MEP	Females	^b # -0.018 (0.003, -0.040)	0.0002 (0.008, -0.008)	^b # -0.025 (0.003, -0.053)	-0.002 (0.017, -0.022)
		Males	0.022 (0.064, -0.020)	-0.014 (0.006, -0.035)	* 0.055 (0.105, 0.004)	-0.003 (0.015, -0.021)

<i>Shapes Trials</i>			16-18 Weeks Gestation Sample		Pooled Sample	
			<i>n</i> = 180		<i>n</i> = 182	
Outcome	Exposure		Set 1	Set 2	Set 1	Set 2
Shift Rate	MEP	Females	--	--	-0.033 (0.042, -0.107)	* 0.056 (0.106, 0.007)
		Males	--	--	^a 0.029 (0.107, -0.049)	0.010 (0.033, -0.014)

¹All models were adjusted for maternal race and ethnicity, education, parity, household income, age, and verbal IQ as well as infant sex, gestational age at birth, postnatal age at assessment, and stimulus set for cognitive assessment.

Sum of antiandrogenic phthalates (Σ AA Phthalates), di-2-ethylhexyl phthalate sum (Σ DEHP), di-isononyl phthalate sum (Σ DINP), mono-ethyl phthalate (MEP), di-2-ethylhexyl terephthalate sum (Σ DEHTP)

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$

Table 4.7. Summary of results of analyses of the relationship of phthalates and VRM outcomes at 7-8 months.

Faces								
	Time to Familiarization		Shift Rate		Run Duration		Novelty Preference	
	16-18 weeks	Pooled	16-18 weeks	Pooled	16-18 weeks	Pooled	16-18 weeks	Pooled
ΣAA phthalates	↓	↓	--	--	↑ F Set 2 ↑ M Set 2	--	↓	--
ΣDEHP	--	--	--	--	--	↑ Set 2	↓	--
ΣDINP	↑ Set 1	--	↑ M Set 1	↓	↑ Set 2	↑ Set 2	--	↓ F Set 1
MEP	--	--	↓ F Set 1	↓ F Set 1 ↑ M Set 1	--	--	↑ M Set 2	↓ F Set 1
ΣDEHTP	↑	↑	--	--	--	--	--	↓ Set 1

Shapes								
	Time to Familiarization		Shift Rate		Run Duration		Novelty Preference	
	16-18 weeks	Pooled	16-18 weeks	Pooled	16-18 weeks	Pooled	16-18 weeks	Pooled
ΣAA phthalates	--	--	--	--	--	--	--	--
ΣDEHP	--	--	--	--	--	--	--	--
ΣDINP	--	↓	--	↑ M	↑ Set 2	↑ Set 2	--	--
MEP	↓ M Set 2	↑ M Set 1 ↓ M Set 2	--	↑ M Set 2	--	--	↑ M	--
ΣDEHTP	--	--	--	--	↑	--	--	--

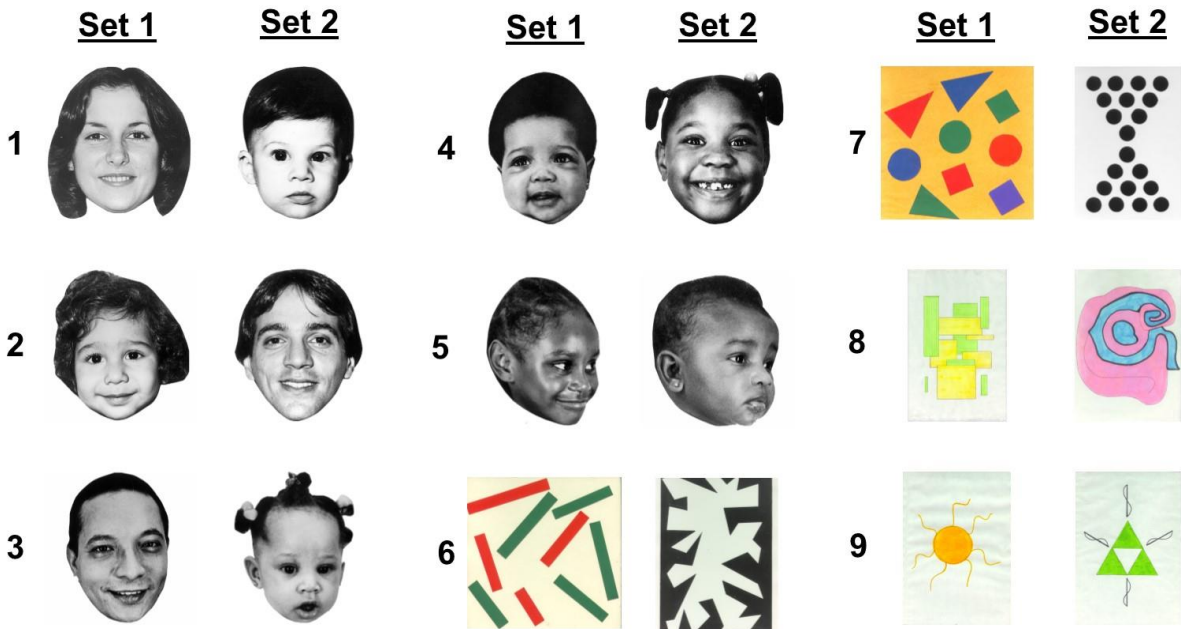


Figure 4.1. Stimulus pairs used in VRM assessment at 7-8 months. All stimuli pairs were presented in the order indicated by the number to the left of each pair for all infants for a total of 9 blocks (5 faces blocks, 4 shapes blocks). Infants who saw set 1 saw the left image of each pair as the familiar stimulus in each block. Infants who saw set 2 saw the image on the right in each pair as the familiar stimuli.

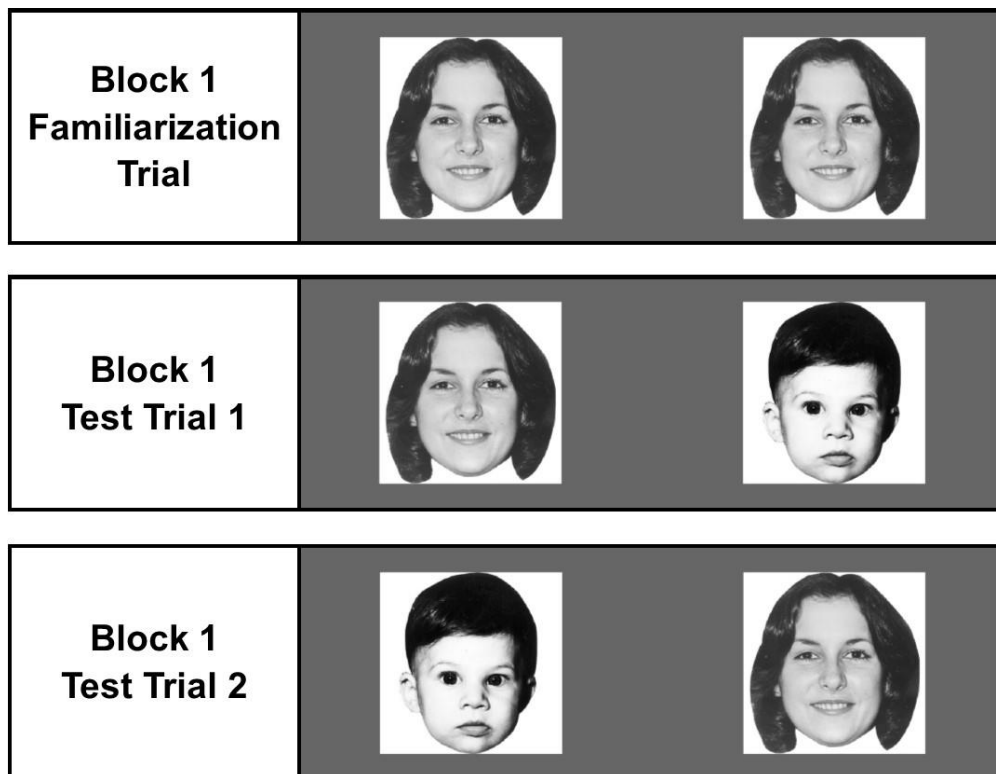


Figure 4.2. An example of the stimulus display across one block for the VRM assessment at 7-8 months. This pattern was repeated across 9 blocks with different stimuli shown in each block. This example shows what an infant assigned to set 1, condition A or C would see in block 1 of the assessment.



Figure 4.3. An example of the interest areas used for the present analysis. Each interest area, shown in orange, contained the entire image plus a small area around the image to account for small errors in eye tracking estimations. These interest areas were used to measure looking behavior in each trial of the VRM assessment at 7-8 months.

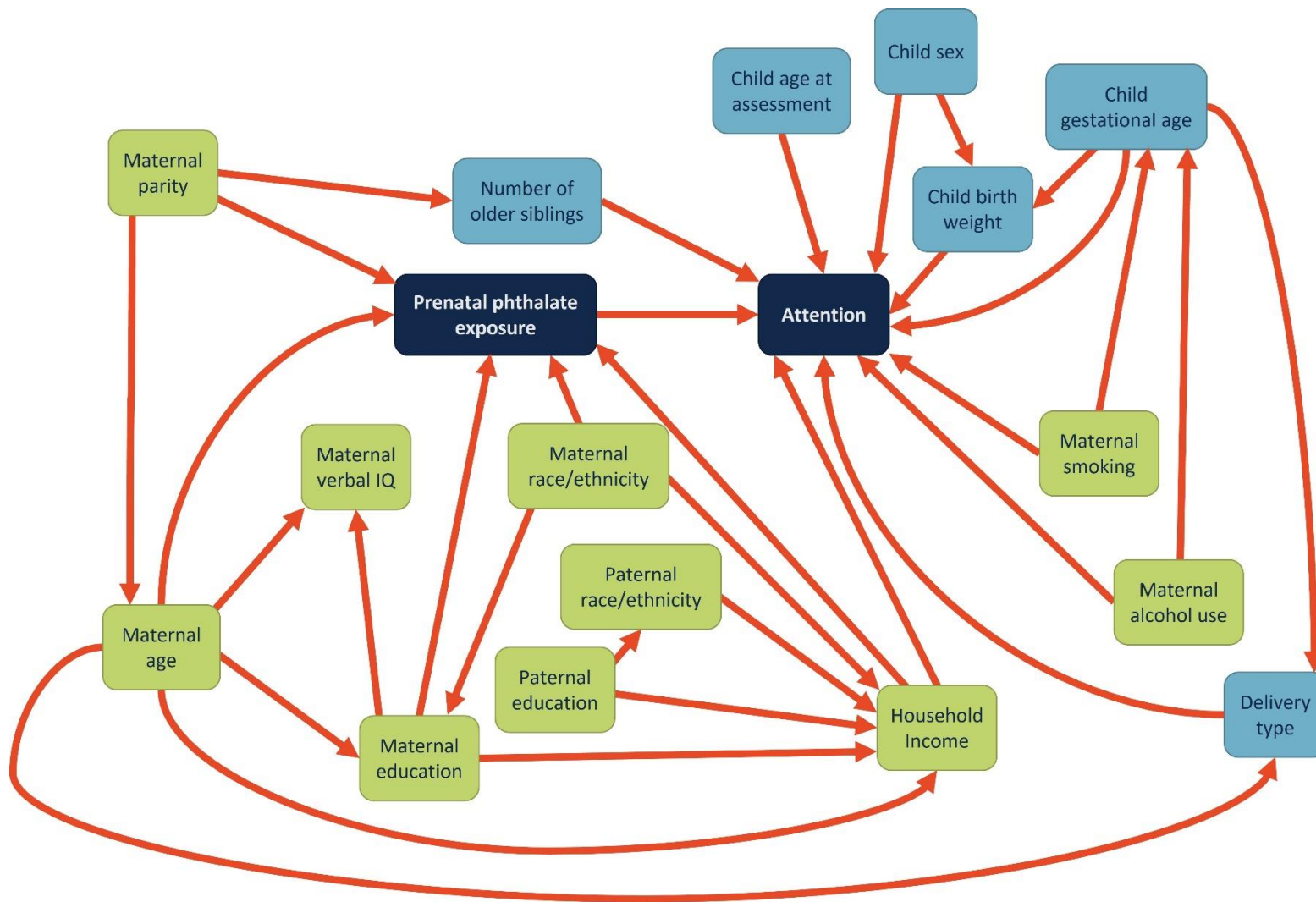


Figure 4.4. Directed acyclic graph (DAG) used to identify potential covariates for inclusion in models examining the association of prenatal phthalate exposure and attention outcomes. Green squares indicate parent-related variables, and light blue squares indicate child-related variables.

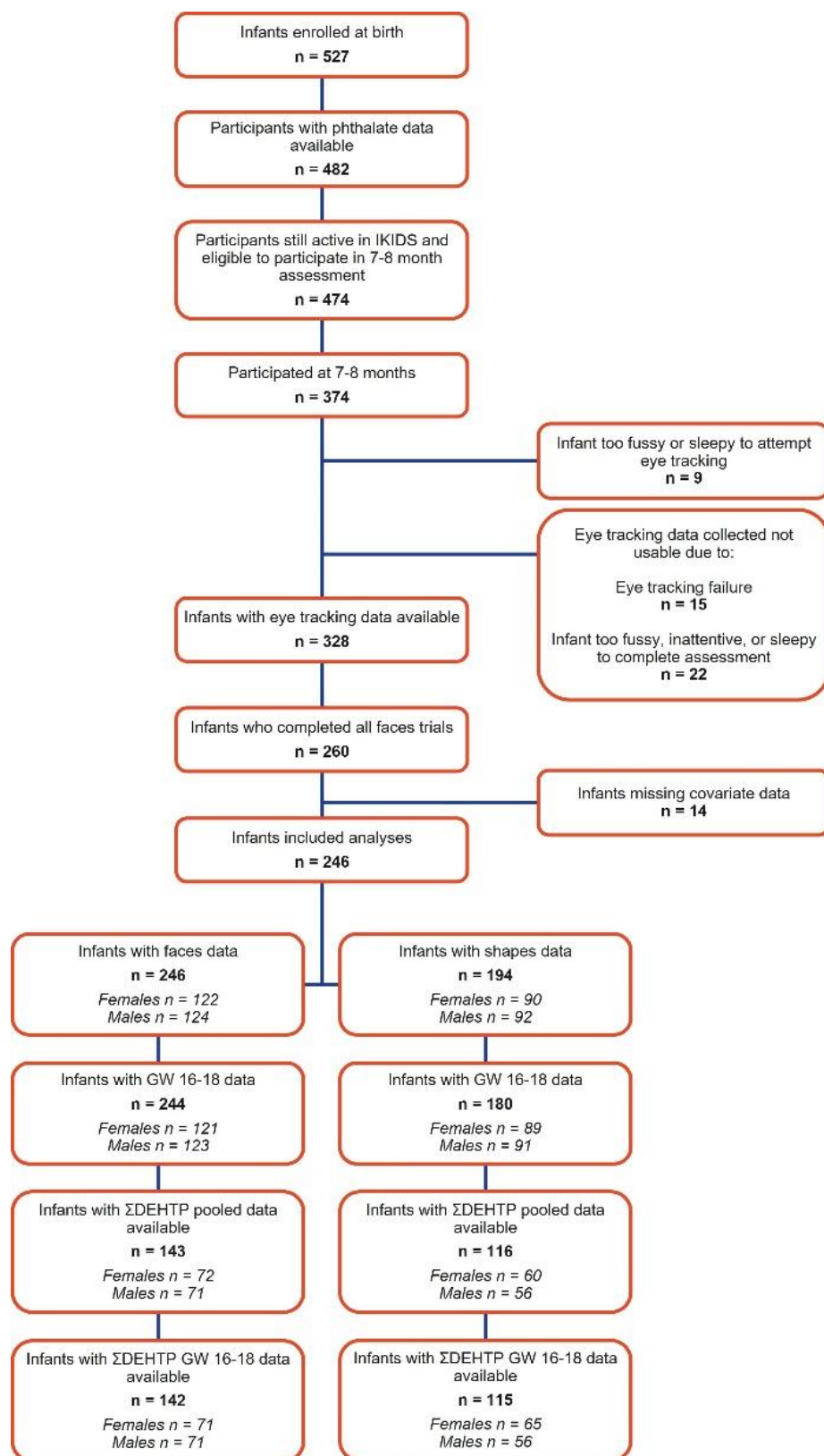


Figure 4.5. Number of infants who completed faces and shapes trials and also had phthalate data and complete covariate data available both overall and by infant sex.

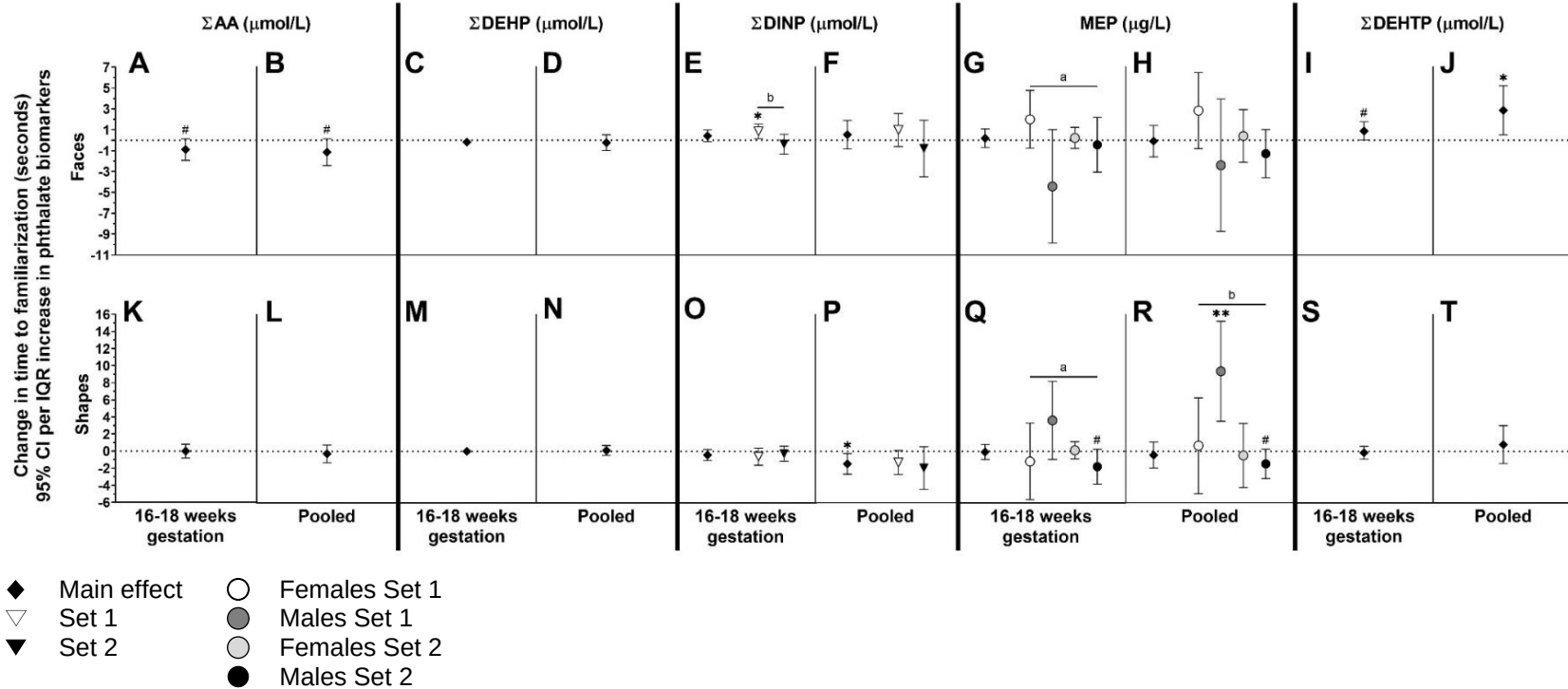


Figure 4.6. The relationship between prenatal phthalate exposure and time to familiarization for both the faces (top) and shapes (bottom) trials in the VRM assessment at 7-8 months of age. Per IQR increase in Σ AA phthalates in (A) the sample from 16-18 weeks of gestation and (B) the pooled sample, infants showed a decrease in time to familiarization in the faces trials. There was no association of Σ AA phthalates for the shapes trials (K, L) or for Σ DEHP in either the faces (C, D) or shapes trials (M, N). Σ DINP at 16-18 weeks of gestation was associated with an increase in time to familiarization in infants who saw set 1 stimuli as familiar in the faces trials (E) but not in the shapes trials (O) while Σ DINP in the pooled sample was associated with a decrease in time to familiarization in all infants in the shapes trials (P) but not the faces trials (F). In the 16-18 weeks of gestation sample, the set-by-sex-by exposure interaction was $p < 0.10$ for both the faces (G) and shapes trials (Q), but nothing was significant for faces, and only males who saw set 2 stimuli as familiar showed a decrease in time to familiarization in the shapes trials. There was no relationship between MEP in the pooled sample and time to familiarization for the faces trials (H), but in the shapes trials, males who saw set 1 stimuli showed an increase in time to familiarization while males who saw set 2 stimuli showed a decrease (R). In both the 16-18 weeks of gestation (I) and the pooled sample (J), Σ DEHTP was associated with an increase in time to familiarization in the faces trials, and it was not associated with time to familiarization in the shapes trials (S, T).

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$

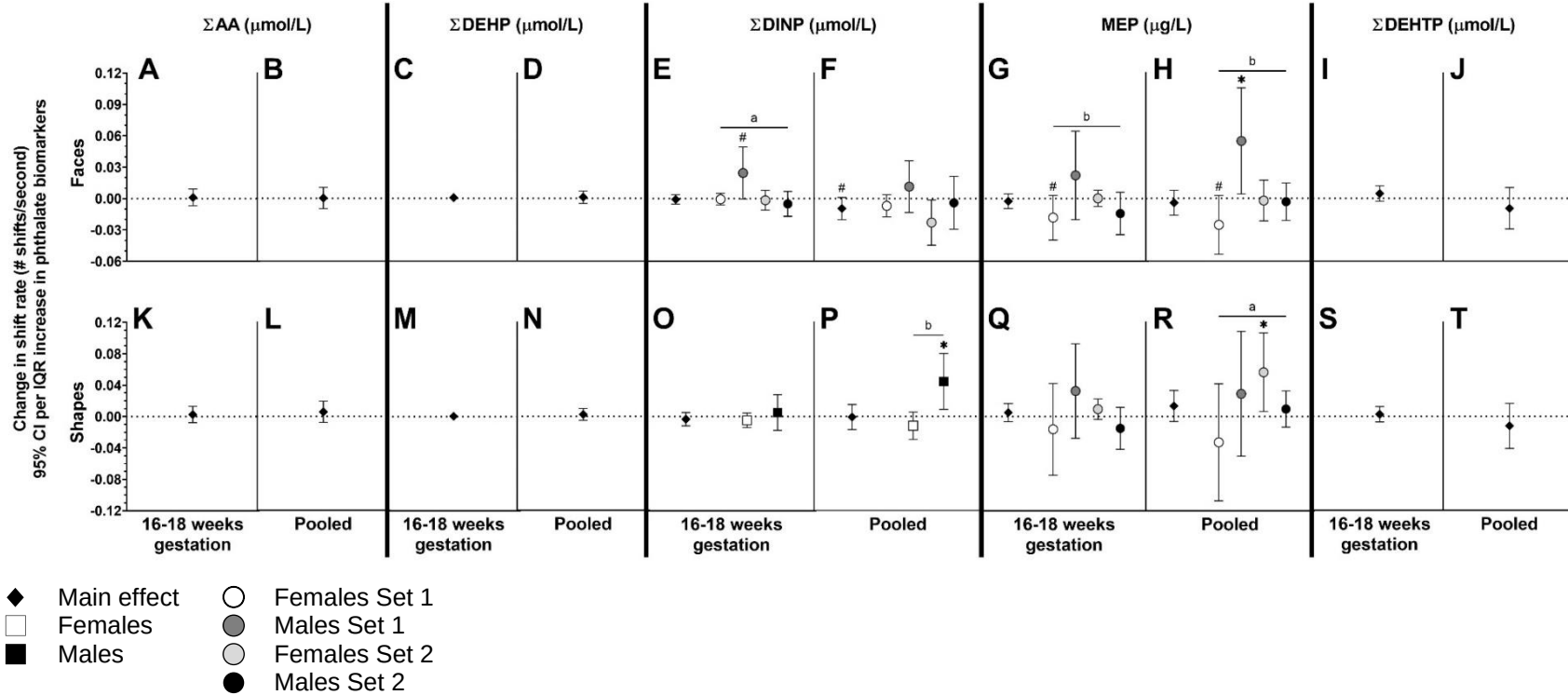


Figure 4.7. Associations of prenatal phthalate exposure and shift rate for the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months. There was no relationship between Σ AA phthalates (A, B, K, L), Σ DEHP (C, D, M, N), or Σ DEHTP (I, J, S, T) in the 16-18 weeks or pooled samples for either the faces or shapes trials. Σ DINP in the 16-18 weeks sample was associated with increased shift rate in males who saw set 1 as familiar for the faces trials (E), but there was no association for the shapes trials (O). Σ DINP in the pooled sample was associated with decreased shift rate in the faces trials (F) but increased shift rate in males in the shapes trials (P). At 16-18 weeks of gestation, MEP was associated with a decrease in shift rate in females who saw set 1 as familiar in only the faces trials (G). There was no relationship between MEP in the 16-18 weeks sample and shift rate in the shapes trials (Q). In the faces trials, MEP in the pooled sample was associated with a decrease in shift rate in females who saw set 1 but an increase in males who saw set 1 (H), and in the shapes trials, it was only associated with an increase in shift rate in females who saw set 2 as familiar (R).

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$

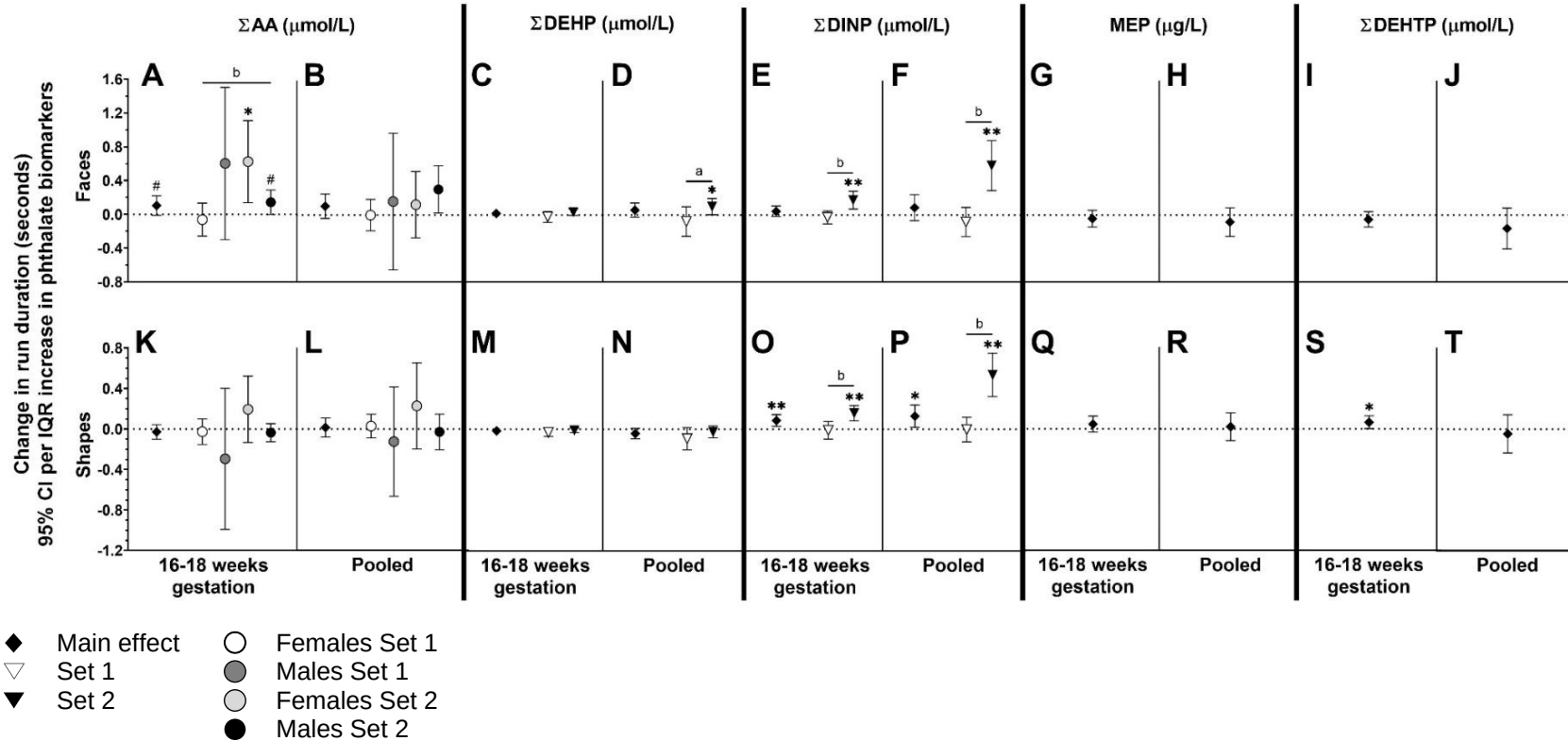
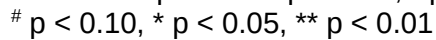


Figure 4.8. Associations of prenatal phthalate exposure and run duration in both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months. Σ AA phthalates in the 16-18 weeks sample was associated with increased run duration in all infants and both male and female infants who saw set 2 stimuli in the faces trials (A). Σ AA phthalates in the 16-18 weeks sample was not associated with any changes in run duration in the shapes trials (K), and Σ AA phthalates in the pooled sample was not associated with changes in run duration in either the faces (B) or shapes trials (L). Σ DEHP in the 16-18 weeks sample was not associated with any changes in run duration (C,M), but in the pooled sample, Σ DEHP was associated with increased run duration in infants who saw set 2, but only in the faces trials (D, N). Σ DINP in both the 16-18 weeks sample and the pooled sample was associated with increased run duration in both the faces (E, F) and shapes trials (O, P) in infants who saw set 2, as well as in all infants during the shapes trials. MEP was not associated with any changes in run duration (G, H, Q, R). Σ DEHTP in the 16-18 weeks sample was associated with increased run duration in all infants only in the shapes trials (I, S). Σ DEHTP in the pooled sample was not associated with any change in run duration (J, T).

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$



CHAPTER 5: ASSOCIATIONS OF PRENATAL EXPOSURE TO ACETAMINOPHEN WITH MEASURES OF COGNITION IN 7.5-MONTH-OLD INFANTS AND THE POTENTIAL MODIFICATION BY PRENATAL PHTHALATE EXPOSURE

5.1. INTRODUCTION

As stated in previous chapters, there is substantial evidence suggesting that prenatal exposure to phthalates or acetaminophen may be related to attentional problems or attention-deficit/hyperactivity disorder (ADHD) diagnosis in children; however, to date, only one study has examined the relationship of prenatal exposure to acetaminophen with attention in children under 1 year of age (Streissguth et al., 1987). While Streissguth et al. (1987) found no evidence supporting this potential link, they only examined exposure to acetaminophen during the first five months of gestation. Most studies since have examined this relationship for children in the early or mid-childhood age ranges (Table 1.1) and have attempted to obtain a more complete estimate of acetaminophen exposure during prenatal development. For example, the Norwegian Mother and Child Cohort (MoBa) study in Norway collected maternal self-report use of acetaminophen at 17 and 30 weeks of gestation as well as 6 months postpartum (Brandlistuen et al., 2013; Vlenetie et al., 2016; Skovlund et al., 2017; Ystrom et al., 2017; Trønnes et al., 2020), and the Danish National Birth Cohort (DNBC) in Denmark used a similar method, asking mothers to report their use of acetaminophen at 12 and 30 weeks of gestation as well as 6 months postpartum (Liew et al., 2014; Liew et al., 2016a; Liew et al., 2016c; Inoue et al., 2020). However, due to the long periods of time over which participants were asked to report acetaminophen use in these studies, there is a significant concern about recall bias. Other studies have examined biomarkers of acetaminophen use in maternal urine or blood samples during pregnancy or shortly after giving birth or in umbilical cord blood samples (Ji et al., 2018; Bornehag et al., 2018b; Ji et al., 2020). These measures may provide more accurate measures of prenatal acetaminophen exposure than self-report, but because acetaminophen is quickly metabolized and use is often sporadic, they likely only provide information about current use at

the time of sample collection (Prescott, 1980; Nitsche et al., 2017). Because of this, the most practical and feasible method of collecting information about acetaminophen use during pregnancy may be via self-report multiple times during pregnancy. Additionally, because acetaminophen use during pregnancy is so common with 50-65% of women reporting use at least once during pregnancy (Brandlistuen et al., 2013; Thorpe et al., 2013; Servey and Chang, 2014; Ji et al., 2018), it is imperative that prenatal exposure is evaluated in combination with other common exposures, such as phthalates. The analyses presented in this chapter used data collected for the Illinois Kids Development Study (IKIDS) to examine the relationship between prenatal exposure to acetaminophen during each trimester of pregnancy, as well as cumulative exposure across pregnancy, and attention at 7-8 months of age using the visual recognition memory (VRM) task described in Chapter 4, as well as the potential for cumulative effects of prenatal exposure to acetaminophen and phthalates. Maternal self-report of acetaminophen use was collected approximately every 4-6 weeks during pregnancy, from around gestational week 12 through the birth of the infant. Similar to the analyses for phthalates reported in Chapter 4, outcomes related to both working memory and information processing were evaluated in addition to attention. Infants with greater exposure to acetaminophen during development were expected to show poorer attention, working memory, and information processing, and because there is evidence to suggest that acetaminophen may have antiandrogenic actions similar to some phthalates (Kristensen et al., 2011; Kristensen et al., 2012; Mazaud-Guittot et al., 2013; van den Driesche et al., 2015; Holm et al., 2016; Lind et al., 2017; Hay-Schmidt et al., 2017; Hurtado-Gonzalez et al., 2018), male infants were expected to show greater deficits than female infants, particularly with greater exposure during the second trimester. The second trimester was specified because acetaminophen is hypothesized to have antiandrogenic effects during prenatal development, and the second trimester is thought to be the time of peak androgen production by male fetuses (Auyeung et al. 2013). Finally, the interactive effects of prenatal phthalate and acetaminophen exposure on these cognitive

outcomes were explored, and infants with higher exposure to both phthalates and acetaminophen were expected to show the poorest outcomes.

5.2. METHODS

5.2.1. Study Cohort

Eligibility and recruitment for IKIDS are described in detail in Chapter 3. In brief, pregnant women were recruited between 10 and 14 weeks of gestation from two local obstetrics clinics. At the time of enrollment, baseline demographic information was collected including sociodemographic factors, reproductive and health history, and lifestyle factors. Follow-up interviews occurred every 4-6 weeks during pregnancy beginning at 10-14 weeks of gestation. After the birth of the infant, mothers were invited to bring their infants to the University of Illinois campus for cognitive assessments three times before the infant's first birthday. The analyses presented in this chapter focus on data collected when infants were 7-8 months of age.

5.2.2. Exposure Assessment

A detailed description of the acetaminophen exposure measurements can be found in Chapter 3. Women were interviewed about their use of medications at the same 5 timepoints that they provided first morning urine samples and also within 24 hours after the birth of the child. Using the information provided, medications containing acetaminophen were identified, as well as the duration of use and reason for use, for six windows of gestation, as well as the entire pregnancy. These data were used to obtain measures of exposure in each trimester of pregnancy as well as cumulative exposure throughout the entire pregnancy. The number of times acetaminophen use was reported in each trimester and across the entire pregnancy were used as exposure measures. The method of evaluating prenatal phthalate exposure was described in Chapter 4. Both the pooled sample and the sample from the 16–18-week time point for di-(2-ethylhexyl) phthalate (Σ DEHP), diisononyl phthalate (Σ DINP), monoethyl phthalate (MEP), di-2-ethylhexyl terephthalate (Σ DEHTP), and an antiandrogenic sum (Σ AA) were

considered for inclusion in models exploring interactions between acetaminophen and phthalate exposure.

5.2.3. Cognitive Assessment

The cognitive assessment used in these analyses was described in detail in Chapter 4. Briefly, infant cognition was evaluated at 7-8 months using the VRM task together with infrared eye tracking. The VRM assessment consisted of nine blocks of trials with each block composed of one familiarization trial followed by two test trials. Infants were shown black and white faces in the first five blocks of trials and colorful abstract geometric shapes in the last four blocks of trials (Figure 4.1). In the familiarization trials, infants viewed two identical images side-by-side until they reached criterion, which was accumulation of 20 seconds of looking at the images for the face trials and 10 seconds for the shape trials. In each test trial, the familiar image was presented with a new image until the infant accrued a total of 1 second of looking at the stimuli, plus 5 additional seconds, regardless of looking behavior. In the first test trial of each block, the novel image was presented on one side and then it was presented on the other side in the second test trial. Each infant was randomly assigned to one of four assessment conditions (A, B, C, D) within blocks of 48 infants and within each sex in order to ensure that approximately equal numbers of each sex were assigned to each of the four conditions across time. In conditions A and B, one set of images was used as the familiar stimuli (set 1), and the other set was used as familiar in conditions C and D. In conditions A and C, the novel images were presented on the right side in the first test trial and on the left side in the second test trial. Infants in conditions B and D saw the novel images on the left side in the first test trial and on the right side in the second (Figure 4.2). The average novelty preference, run duration, time to familiarization, and shift rate were extracted and calculated as described in Chapter 4.

5.2.4. Covariates

Covariates were selected based on directed acyclic graphs (DAGs) and *a priori* knowledge. DAGs were first created to identify variables to consider (Figure 5.1), and correlations of those variables with exposures and outcomes were examined to select covariates for inclusion in models. For the analyses focused on acetaminophen exposure, acetaminophen formulation (none, acetaminophen alone, in combination with other drugs), maternal age, parity (nulliparous vs. ≥ 1), and education ($<$ bachelor's degree vs bachelor's degree or higher), as well as infant gestational age and birthweight were included in all models. Stimulus set (set 1 vs. set 2) and infant sex were included as potential modifiers. Sensitivity analyses were also completed to explore the potential influence of maternal marital status (married/living as married vs. single/separated/divorced/widowed), alcohol use during the first trimester (any vs. none), reason for taking acetaminophen (pain, cold/flu/fever/illness/sinus, other), or household income ($<$ \$50,000, \$50,000-\$99,999, $\geq$ \$100,000) on the associations. As 41 infants with acetaminophen exposure data and outcome data were missing birthweight, and additional sensitivity analysis excluding birthweight as a covariate was conducted.

In the analyses examining the interactive effects of acetaminophen and phthalates, the following covariates were selected for inclusion in all models: maternal parity and verbal IQ, acetaminophen formulation, infant gestational age at birth and sex, and stimulus set. These covariates were selected based on similarities between the DAGs and *a priori* knowledge used to select covariates for separate phthalate and acetaminophen analyses and the outcomes selected for the interaction analyses. While verbal IQ was not included in models evaluating the relationship of prenatal acetaminophen exposure and cognitive outcomes in the VRM assessment, because in addition to being correlated with VRM outcomes, it was also correlated with phthalate exposure, it was selected for inclusion in the interaction models. Sensitivity analyses were also conducted to examine the potential influence of maternal marital status,

alcohol use during the first trimester, cigarette use during the first trimester (any vs. none), and reason for taking acetaminophen on the associations.

5.2.5. Statistical Approach

Multivariable generalized linear regression models were used to evaluate associations between acetaminophen exposure during each trimester (first, second, and third) as well as throughout pregnancy and each continuous outcome (time to familiarization, shift rate, novelty preference, and run duration). The outcome measures were averaged across the 5 sets of faces trials and 3 sets of shapes trials, and sex-by-exposure, set-by-exposure, and sex-by-set-by-exposure interactions were also examined, as described in Chapter 4. Interaction terms with p-values < 0.10 were retained in the final models, and results for models with interactions are shown using stratum-specific estimates in addition to the main effect of exposure. Beta estimates were scaled to an interquartile range (IQR) increase in acetaminophen exposure for ease of comparison across broad ranges of exposure, and exposure associations were considered significant if p-values were less than 0.05. The effect of removing potentially influential data points (Cook's $D > 0.10$) on the results was also examined.

For the acetaminophen-phthalate interaction analyses, a conservative approach was taken. To limit the number of analyses conducted, only outcomes where acetaminophen exposure was associated with a change in the outcome ($p < 0.10$), were considered, and only for phthalates that had at least borderline significant associations with that outcome. Analyses were only conducted for the faces trials because more infants had data for the faces trials. In addition to the exposure interaction, acetaminophen-by-phthalate-by-infant sex and acetaminophen-by-phthalate-by-stimulus set interactions were also explored. Where an interaction term was $p < 0.10$, the interaction was further examined by categorizing phthalate exposure as low ($< 25^{\text{th}}$ percentile), medium ($25^{\text{th}} - 75^{\text{th}}$ percentile), or high exposure ($> 75^{\text{th}}$ percentile), and the association of acetaminophen with the outcome across these three

phthalate exposure groups was explored. The effect of removing potentially influential data points (Cook's $D > 0.10$) on the results was also examined. All statistical analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (Copyright © 2013, SAS Institute Inc., Cary, NC, USA).

5.3. RESULTS

5.3.1. Participation and Demographics

A total of 260 participants had both acetaminophen exposure data and complete outcome data available, however, 41 infants were missing birthweight. Ultimately, 219 of these infants had complete covariate data available and were included in the analyses of the relationship between prenatal acetaminophen exposure and cognitive outcomes in the faces trials. Of these 219, 159 (72.60%) infants also completed all shapes trials. One participant was missing acetaminophen exposure data for the first trimester, and therefore the total exposure for the entire pregnancy as well, resulting in only 218 and 158 participants being included in analyses of first trimester exposure and exposure throughout pregnancy. The subset included in the acetaminophen exposure analyses was largely similar to the full cohort (Table 3.1), although slightly more mothers were white, non-Hispanic, married or living as married, had obtained at least a bachelor's degree, were nulliparous, had an annual household income of \$50,000 or greater, and reported not smoking or consuming alcohol during their first trimester of pregnancy. Child characteristics were also similar between the full cohort and the subset (Table 3.2).

Because birthweight was not selected as a covariate for the acetaminophen-by-phthalate interaction analyses, more infants were able to be included. A total of 247 infants had complete acetaminophen and phthalate exposure, covariate, and outcome data for the second and third trimesters, and 246 infants had complete data for the first trimester and acetaminophen exposure throughout pregnancy. The subset included in the interaction

analyses was also largely similar to that of the full cohort and is described in more detail in Chapter 4.

5.3.2. Exposure Characterization

Regression diagnostics generally supported the use of continuous, untransformed biomarker measures and linear models to characterize the associations. Distributions of acetaminophen exposure were right skewed, and therefore exposures were characterized using minimums, maximums, medians, and interquartile ranges. Acetaminophen exposure, formulation, and reason for use for the full cohort are shown in Table 3.5, and the distribution of exposure was generally similar to that of the subset included in these analyses.

Pregnancy urine biomarker concentrations reflecting prenatal phthalate exposures for the full cohort are shown in Table 3.3. Biomarker concentrations for the subset included in these analyses are described in detail in Chapter 4. They were generally very similar to those for the full cohort and a nationally representative sample (CDC, 2019).

5.3.3. Associations of Exposure and Cognitive Outcomes

Acetaminophen exposure and cognitive outcomes at 7-8 months

The outcomes for the VRM task are described in more detail in Chapter 4. In brief, eight outcomes were evaluated: average novelty preference, average time to familiarization, average run duration in the first and last portions of familiarization trials, average run duration for the full familiarization trials, average shift rate in the first and last portions of familiarization trials, and average shift rate for the full familiarization trials. All outcomes were assessed separately for the faces and shapes trials in the models examining prenatal exposure to acetaminophen. As in Chapter 4, because the results of analyses of shift rate and run duration during only the first portion or last portion of familiarization trials were similar to those for the full familiarization trial, only the results from analyses of the full trials are presented here for simplicity.

Time to familiarization

There was little evidence of an association of acetaminophen exposure with time to familiarization (Table 5.1, Figure 5.2). The only association that approached statistical significance was that of acetaminophen exposure throughout the entire pregnancy and reduced time to familiarization in the shapes trials for male infants only (Table 5.2).

Shift rate

Acetaminophen exposure during prenatal development was associated with increased shift rate, particularly for infants who saw set 1 stimuli as familiar (Figure 5.3). Specifically, acetaminophen exposure during the first trimester was associated with increased shift rate in both faces (Figure 5.3A) and shapes trials (Figure 5.3E), although this relationship was only significant in the shapes trials (Table 5.2). For second trimester exposure, this relationship was only apparent in males who saw set 1 stimuli as familiar and only in the faces trials (Figure 5.3B, Table 5.3). With third trimester exposure, infants who saw set 1 stimuli as familiar showed an increase in shift rate for both faces (Figure 5.3C, Table 5.2) and shapes trials (Figure 5.3G, Table 5.2), but this was only significant in the faces trials. When acetaminophen exposure throughout the entire pregnancy was considered, infants showed an increase in shift rate for both faces (Figure 5.3D) and shapes trials (Figure 5.3H). this was particularly true in infants who saw set 1 stimuli, but the relationship was only significant for the shapes trials (Table 5.2).

Run duration

Associations of prenatal acetaminophen exposure and run duration were generally consistent between the faces and shapes trials. That is, prenatal acetaminophen exposure during the first trimester was associated with longer run durations in both the faces and shapes trials, although this relationship was significant for face trials (Figure 5.4A) and only approached significance for the shape trials (Figure 5.4E, Table 5.2). There were no associations between

acetaminophen exposure during the second (Figure 5.4B, F) or third trimester or across pregnancy (Figure 5.4C, G) and run duration.

Novelty preference

There was little evidence of an association between acetaminophen exposure and novelty preference on either the face or shape trials (Figure 5.5, Table 5.1).

Sensitivity analyses

Sensitivity analyses including alcohol use as a covariate (yes/no), including reported reason for taking acetaminophen (pain, illness, other), excluding participants who were not married or living as married ($n = 13$), or removing potentially influential data points (Cook's $D > 0.05$) did not significantly alter any associations observed between prenatal acetaminophen exposure and cognitive outcomes at 7-8 months. However, because more infants were able to be included in the sensitivity analysis where birthweight was not included as a covariate, there were some differences in the associations for this analysis. For time to familiarization, while there was only a borderline significant association of acetaminophen exposure throughout pregnancy with shorter time to familiarization in male infants in the shapes trials (Figure 5.2), when birthweight was excluded as a covariate, more sex- and set-specific associations approached significance for the shapes trials (Figure 5.6). For shift rate, when birthweight was excluded as a covariate, while associations were generally still primarily observed for infants who saw set 1, the trimesters that had this effect differed compared to the results when birthweight was included as a covariate (Figure 5.7). When birthweight was excluded as a covariate, infants still largely showed longer run durations with acetaminophen exposure, although there were more significant sex-by-set associations for the shapes trials (Figure 5.8). In analyses of associations of prenatal acetaminophen exposure with novelty preference, there were no associations when birthweight was included as a covariate (Figure 5.5); however, when birthweight was excluded, there were significant associations of prenatal acetaminophen exposure with lower novelty

preference (Figure 5.9). Despite these differences, patterns of associations were largely similar to those of the main models when birthweight was excluded as a covariate and more infants were included in the analyses.

Interactive effects of acetaminophen and phthalate exposure on cognitive outcomes at 7-8 months

Because patterns of associations largely differed between acetaminophen and phthalate exposures, it was considered unlikely that the interaction models would show cumulative effects of the two exposures. Therefore, only outcomes where acetaminophen exposure was associated with a change in the outcome at $p < 0.10$ (average shift rate and run duration), were explored in these models. Only the outcomes for the faces trials were studied in these analyses because many associations were consistent between faces and shapes trials, and more infants completed the faces trials, resulting in a larger sample size for these interaction models. When acetaminophen-by-phthalate interactions were $p < 0.10$, they were explored further by categorizing phthalate exposure as low, medium, and high as shown in Table 5.4.

Shift rate

For shift rate, interactions of Σ DINP and MEP in the pooled sample with both second and third trimester acetaminophen exposure were explored. However, no interactions of acetaminophen-by-phthalate exposure interactions met the $p < 0.10$ criterion for further exploration for shift rate.

Run duration

For analyses of run duration, it was determined that a Poisson distribution was a better fit than a normal distribution and was therefore assumed. For the pooled sample, only interactions with Σ DINP met the $p < 0.10$ criterion for further exploration for run duration. The interaction for first trimester acetaminophen exposure-by- Σ DINP-by stimulus set was significant, but post-hoc tests showed there was no difference in the association of acetaminophen with run

duration in infants tested with either set 1 or set 2 across Σ DINP exposure levels. However, within set 2, the effect of acetaminophen exposure on run duration at high Σ DINP was borderline significant, showing a decrease in run duration with higher acetaminophen exposure, and post-hoc tests revealed that the effect of acetaminophen on run duration significantly differed between the low and high Σ DINP groups tested with set 2 (Figure 5.10A, Table 5.6).

For the 16-18 weeks sample, only interactions of Σ DEHP-by-first trimester exposure and Σ DINP-by-first trimester exposure-by-stimulus set met the $p < 0.10$ criterion for further exploration (Table 5.5). When stratified by Σ DEHP level, there was no difference in the association of first trimester acetaminophen exposure with run duration among levels of Σ DEHP exposure. Post-hoc analyses also revealed a significant interaction of first trimester acetaminophen exposure and Σ DINP for set 2. Within set 2, first trimester acetaminophen exposure was associated with an increase in run duration that was larger with increasing level of Σ DINP exposure, suggesting a cumulative effect. Post-hoc tests revealed that while associations of acetaminophen exposure with run duration were not different between the low and medium levels of Σ DINP, associations in both low and medium Σ DINP levels were significantly different from the association of acetaminophen exposure with run duration in the high Σ DINP exposure level (Table 5.5, Figure 5.10B). Sensitivity analyses excluding participants who were not married or living as married ($n = 13$), excluding smokers ($n = 20$), removing potentially influential data points (Cook's $D > 0.08$), including alcohol use as a covariate, or including reported reason for taking acetaminophen as a covariate generally weakened associations of acetaminophen-by phthalate interactions, but they were in the same direction.

5.4. DISCUSSION

5.4.1. Prenatal acetaminophen exposure and infant cognition

Prenatal acetaminophen exposure was generally not associated with time to familiarization (Figure 5.2). This is the opposite of what was hypothesized based on the findings of previous

studies which suggested prenatal acetaminophen exposure is associated with poorer attention (Liew et al., 2014; Liew et al., 2016a; Avella-Garcia et al., 2016; Golding et al., 2019). However, those studies examined children in early or mid-childhood, and the only other study thus far to examine this potential relationship in infants observed no association of prenatal acetaminophen exposure and attention in infants at 3 months of age (Streissguth et al., 1987), suggesting this finding may not be completely unexpected. Utilizing a vigilance paradigm, they found no association of prenatal acetaminophen exposure and attention (Streissguth et al., 1987), which is similar to what was found in the present study. Acetaminophen exposure during a single trimester of prenatal development was not associated with attention; however, exposure throughout pregnancy, which was not evaluated by Streissguth et al. (1987), was associated with better attention in male infants at 7-8 months of age. This difference in findings highlights that neurodevelopmental outcomes often differ by age because both cognitive and behavioral development are dynamic processes. Furthermore, while it was hypothesized that acetaminophen exposure during the second trimester would result in greater effects on attention, there was no association of acetaminophen exposure during the second trimester with time to familiarization. This hypothesis was driven by evidence from previous studies indicating that acetaminophen has antiandrogenic activity (Kristensen et al., 2011; Holm et al., 2016; Lind et al., 2017; Interrante et al., 2017; Golding et al., 2019) and the fact that the second trimester is thought to be the time of peak androgen production by the male fetus (Auyeung et al. 2013), but previous studies that have looked at the timing of prenatal acetaminophen exposure in relation to neurodevelopmental outcomes have not found associations with second trimester exposure. Few previous studies have examined associations with outcomes by trimester of exposure, but Liew et al. (2016a) found that associations of prenatal acetaminophen exposure with poorer attention in 5-year-old children were strongest for first trimester exposure, and Stergiakouli et al. (2016) observed that associations with higher risk of conduct problems, hyperactivity symptoms, and emotional problems in 7-year-old children were strongest with third trimester use. Thus,

while the hypothesis of this study was driven by a potential mechanism, there is little evidence from the few previous epidemiological studies that have examined timing of exposure by trimester that second trimester exposure would have the strongest associations with cognitive outcomes. Additionally, as described in Chapter 4, time to familiarization may not be the most effective method of evaluating attention in this task. As previously described, further analyses of the VRM trials are needed in order to better understand how infants' looking behavior changes throughout the task and determine whether time to familiarization is a useful outcome to assess attention.

To our knowledge, this is the first study to investigate the association of prenatal acetaminophen exposure with cognitive outcomes other than attention in infants. Prenatal acetaminophen exposure was associated with faster shift rates, and this was most apparent for infants who saw set 1 as familiar (Figure 5.3), implying that prenatal acetaminophen exposure may be linked to more efficient information processing. In the case of run duration, only prenatal exposure to acetaminophen during the first trimester was associated with longer run durations (Figure 5.6A, E), suggesting slower information processing, which is the opposite of what the findings for shift rate suggest. The discrepancy in the findings with these two measures of information processing could be due to the two outcomes evaluating different aspects of information processing. While shift rate gives the rate at which infants switch their attention from one stimulus directly to the other and can provide insight into how infants are processing the stimuli, as well as their memory and encoding during the task, run duration is more demonstrative of how long it takes infants to process the stimuli. While faster shift rates would be expected to be correlated with shorter run durations, correlations of the outcomes showed this was not generally the case (Table 4.2, 4.3), and instead, shorter runs were correlated with slower shift rates. Further investigation of this is necessary, and future analyses investigating how the looking behavior of infants changes throughout the assessment (which was beyond the

scope of this analysis) will aid in understanding these results. Finally, acetaminophen exposure was not associated with novelty preference in the primary analysis, but in the sensitivity analysis removing birth weight (which was missing for 41 infants) as a covariate, acetaminophen was associated with poorer novelty preference, suggesting there may be a subtle relationship of prenatal acetaminophen exposure with poorer recognition memory that was only observed when statistical power was increased by including those additional infants.

5.4.2. Cumulative effects of exposure to both phthalates and acetaminophen on infant cognition

While several interactions of prenatal acetaminophen exposure and prenatal phthalate exposure were investigated, most showed no difference in associations of acetaminophen with cognitive outcomes by level of phthalate exposure. Only first trimester acetaminophen exposure-by- Σ DINP-by-stimulus set interactions showed that the association of acetaminophen exposure with run duration may be modified by phthalate exposure, and this was observed for Σ DINP in both the 16-18 weeks and pooled samples (Figure 5.10). For both, in infants who saw set 2, there was an apparent cumulative effect with first trimester acetaminophen exposure associated with longer run durations with increasing Σ DINP exposure level, although the difference was negligible between low and medium Σ DINP levels. However, when results were stratified by Σ DINP exposure levels and stimulus set, the number of infants per group were small; thus, these results must be interpreted carefully. Additionally, the median acetaminophen exposure differed between infants who saw set 1 compared to those who saw set 2, with infants who saw set 2 stimuli as familiar having a higher median exposure, as well as by Σ DINP exposure level with infants categorized as having low Σ DINP exposure having a lower median acetaminophen exposure than infants categorized as having medium or high Σ DINP exposure. While these are important limitations to consider, these results still suggest that there may be a cumulative effect of Σ DINP and acetaminophen exposure that should be further examined in

studies with larger sample size in order to have the appropriate statistical power to detect significant effects.

5.4.3. Summary and conclusions

While it was hypothesized that second trimester acetaminophen, specifically, would be associated with poorer cognitive outcomes, there was very little evidence that this was the case at 7-8 months. As previously stated, this is the first study to investigate the relationship of prenatal acetaminophen exposure and cognitive outcomes other than attention in infancy; however, this was not the first study to evaluate this association for attention in infants. Nearly 35 years ago, Streissguth et al. (1987) investigated the relationship of acetaminophen exposure during the first five months of prenatal development and attention in 3-month-old-children and found no association of prenatal acetaminophen exposure with attention. Combined with their results, the results of the present study suggest that if prenatal acetaminophen exposure is related to poorer attention, this effect may not emerge until children are older, as previous studies that have found an association with poorer attention involved children at least 3 years of age (Table 1.1). As additional analyses in this study also examined the relationship of prenatal acetaminophen exposure and attention in 2- and 3-year-old children, this will be addressed in later chapters.

Several interactions of prenatal acetaminophen exposure and prenatal phthalate exposure were investigated, but only interactions of first trimester acetaminophen exposure-by- Σ DINP-by-stimulus set for run duration showed that associations of acetaminophen exposure with run duration differed by Σ DINP exposure level. As Σ DINP has been shown to have weaker antiandrogenic activity than Σ DEHP or Σ AA phthalates (Hannas et al., 2011; Howdeshell et al., 2015), this was an unexpected, but concerning, finding. DINP has been increasingly used as a replacement for Σ DEHP (Zota et al., 2014; Engel et al., 2021), but as Engel et al. (2021) recently described, evidence is mounting that this may be a case of regrettable substitution;

thus, in combination with results of other studies, these results indicate that DINP should not be considered a “safer” alternative to Σ DEHP.

5.5. TABLES AND FIGURES

Table 5.1. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with infant cognition for the faces and shapes trials of the VRM assessment.¹

Outcome	Exposure	Faces ²	Shapes ³
Novelty Preference	1st Trimester	-0.0001 (0.002, -0.002)	-0.002 (0.002, -0.005)
	2nd Trimester	-0.002 (0.001, -0.005)	-0.001 (0.005, -0.007)
	3rd Trimester	-0.001 (0.0004, -0.002)	-0.001 (0.002, -0.004)
	Full Pregnancy	-0.003 (0.002, -0.007)	-0.004 (0.005, -0.012)
Time to Familiarization	1st Trimester	-0.310 (0.410, -1.029)	-0.304 (0.262, -0.871)
	2nd Trimester	-0.481 (0.545, -1.507)	-0.379 (0.451, -1.208)
	3rd Trimester	-0.202 (0.281, -0.684)	-0.017 (0.424, -0.459)
	Full Pregnancy	-0.796 (0.700, -2.293)	-0.674 (0.576, -1.923)
Run Duration	1st Trimester	* 0.079 (0.157, 0.001)	# 0.047 (0.098, -0.004)
	2nd Trimester	0.007 (0.118, -0.105)	0.009 (0.084, -0.065)
	3rd Trimester	-0.007 (0.046, -0.060)	0.012 (0.052, -0.027)
	Full Pregnancy	0.055 (0.219, -0.109)	0.078 (0.190, -0.035)
Shift Rate	1st Trimester	0.004 (0.010, -0.001)	** 0.010 (0.017, 0.003)
	2nd Trimester	0.003 (0.012, -0.005)	-0.001 (0.010, -0.013)
	3rd Trimester	0.001 (0.005, -0.003)	-0.002 (0.003, -0.008)
	Full Pregnancy	0.008 (0.020, -0.004)	0.011 (0.028, -0.005)

¹All models were adjusted for acetaminophen formulation, maternal age, maternal parity, maternal education, infant birth weight, infant gestational age, infant sex, and stimulus set for cognitive assessment.

²n = 219

³n = 159

p < 0.10, * p < 0.05, ** p < 0.01

Table 5.2. Multivariable linear regression analyses of the sex- and set-specific relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with infant cognition for the faces trials (top) and the shapes trials (bottom) of the VRM assessment.¹

		Faces ²			
Outcome	Exposure	Stratified by Sex		Stratified by Sex	
		Females	Males	Females	Males
Time to Familiarization	1st Trimester	-0.270 (1.270, -1.810)	-0.320 (0.478, -1.118)	0.067 (1.387, -1.252)	-0.383 (0.237, -1.002)
	2nd Trimester	-0.097 (1.177, -1.370)	-1.064 (0.474, -2.602)	-0.097 (1.177, -1.370)	-1.064 (0.198, -2.519)
	3rd Trimester	-0.016 (0.276, -0.818)	-0.271 (0.276, -0.818)	0.377 (1.095, -0.340)	-0.184 (0.316, -0.684)
	Full Pregnancy	-0.181 (1.919, -2.281)	-1.320 (0.631, -3.270)	^a 0.285 (1.964, -1.394) [#]	-1.634 (0.046, -3.315)
		Shapes ³			
Outcome	Exposure	Stratified by Set		Stratified by Set	
		Set 1	Set 2	Set 1	Set 2
Shift Rate	1st Trimester	0.003 (0.010, -0.003)	0.008 (0.019, -0.003)	0.010 (0.018, 0.001)	0.012 (0.026, -0.003)
	2nd Trimester	0.023 (0.049, -0.004)	0.002 (0.010, -0.006)	0.001 (0.052, -0.050)	0.002 (0.010, -0.006)
	3rd Trimester	^b ** 0.018 (0.030, 0.005)	-0.0001 (0.004, -0.004)	0.020 (0.080, -0.039)	-0.002 (0.004, -0.008)
	Full Pregnancy	0.019 (0.040, -0.003)	0.004 (0.018, -0.010)	^a * 0.034 (0.065, 0.004)	0.003 (0.022, -0.016)

¹All models were adjusted for acetaminophen formulation, maternal age, maternal parity, maternal education, infant birth weight, infant gestational age, infant sex, and stimulus set for cognitive assessment.

²n = 219

³n = 159

Interaction p-value: ^a p < 0.10, ^b p < 0.05

[#] p < 0.10, * p < 0.05, ** p < 0.01

Table 5.3. Multivariable linear regression analyses of the set-and-sex-specific relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with infant cognition for the faces (top) and shapes trials (bottom) of the VRM assessment.¹

		Faces ²			
Outcome	Exposure	Set 1		Set 2	
		Females	Males	Females	Males
Shift Rate	1st Trimester	0.007 (0.028, -0.015)	0.002 (0.009, -0.004)	0.014 (0.029, -0.0002)	-0.001 (0.015, -0.018)
	2nd Trimester	^a 0.005 (0.057, -0.047) * 0.04 (0.075, 0.006)		0.004 (0.015, -0.006)	-0.002 (0.011, -0.015)
	3rd Trimester	0.006 (0.043, -0.031)	0.018 (0.031, 0.006)	0.0005 (0.007, -0.006)	-0.0001 (0.004, -0.005)
	Full Pregnancy	0.005 (0.058, -0.048)	0.019 (0.042, -0.004)	0.01 (0.027, -0.008)	-0.004 (0.017, -0.024)

		Shapes ³			
Outcome	Exposure	Set 1		Set 2	
		Females	Males	Females	Males
Shift Rate	1st Trimester	0.0005 (0.033, -0.032)	0.01 (0.018, 0.001)	0.012 (0.031, -0.008)	0.011 (0.032, -0.011)
	2nd Trimester	0.005 (0.057, -0.047)	-0.017 (0.179, -0.213)	-0.003 (0.011, -0.017)	-0.00008 (0.018, -0.019)
	3rd Trimester	0.045 (0.144, -0.055)	0.009 (0.077, -0.058)	-0.005 (0.004, -0.015)	-0.0003 (0.007, -0.007)
	Full Pregnancy	0.009 (0.09, -0.073)	0.038 (0.09, -0.073)	0.001 (0.024, -0.023)	0.006 (0.035, -0.023)

¹All models were adjusted for acetaminophen formulation, maternal age, maternal parity, maternal education, infant birth weight, infant gestational age, infant sex, and stimulus set for cognitive assessment.

²n = 219

³n = 159

Interaction p-value: ^a p < 0.10

* p < 0.05

Table 5.4. Phthalate exposure cutoffs for acetaminophen and phthalate interaction analyses presented in Chapter 5.

Phthalate exposure	Units	16-18 weeks sample		Pooled sample	
		25th percentile	75th percentile	25th percentile	75th percentile
ΣAA phthalates	μmol/L	0.158	0.427	--	--
ΣDEHP	μmol/L	--	--	0.048	0.116
ΣDINP	μmol/L	0.014	0.073	0.021	0.096
MEP	μg/L	--	--	0.066	0.219

Table 5.5. The relationship of first trimester acetaminophen exposure-by ΣDINP exposure with run duration stratified by stimulus set in the VRM assessment at 7-8 months.¹

Exposure Interaction	Interaction p-value	Stratified by Stimulus Set	
		Set 1	Set 2
1st Trimester x ΣDINP Pool	0.022	0.003 (0.055, -0.049)	** -0.102 (-0.046, -0.159)
1st Trimester x ΣDINP PB	0.036	** 1.421 (1.525, 1.317)	** 1.512 (1.607, 1.416)

¹All models were adjusted for acetaminophen formulation, maternal parity, maternal VIQ, infant gestational age, infant sex, and stimulus set for cognitive assessment.

**p < 0.01

Table 5.6. The relationship of first trimester acetaminophen exposure with run duration stratified by stimulus set and Σ DINP exposure in the VRM assessment at 7-8 months.

Set 1			
Stratified by Phthalate Exposure Level			
Exposure Interaction	Low	Medium	High
1st Trimester x Σ DINP Pool	# -4.469 (0.033, -8.971)	-0.061 (0.278, -0.401)	-0.019 (0.176, -0.214)
1st Trimester x Σ DINP PB	** 1.549 (1.756, 1.343)	** 1.399 (1.548, 1.25)	** 1.243 (1.47, 1.017)

Set 2			
Stratified by Phthalate Exposure Level			
Exposure Interaction	Low	Medium	High
1st Trimester x Σ DINP Pool	^b 6.18 (18.163, -5.804)	0.096 (0.887, -0.695)	^b # -0.097 (-0.014, -0.207)
1st Trimester x Σ DINP PB	^b ** 1.38 (1.587, 1.172)	^b ** 1.444 (1.586, 1.303)	^{bb} ** 1.692 (1.847, 1.537)

¹All models were adjusted for acetaminophen formulation, maternal parity, maternal VIQ, infant gestational age, infant sex, and stimulus set for cognitive assessment.

#p < 0.10, **p < 0.01

Post hoc comparison: ^bp < 0.05

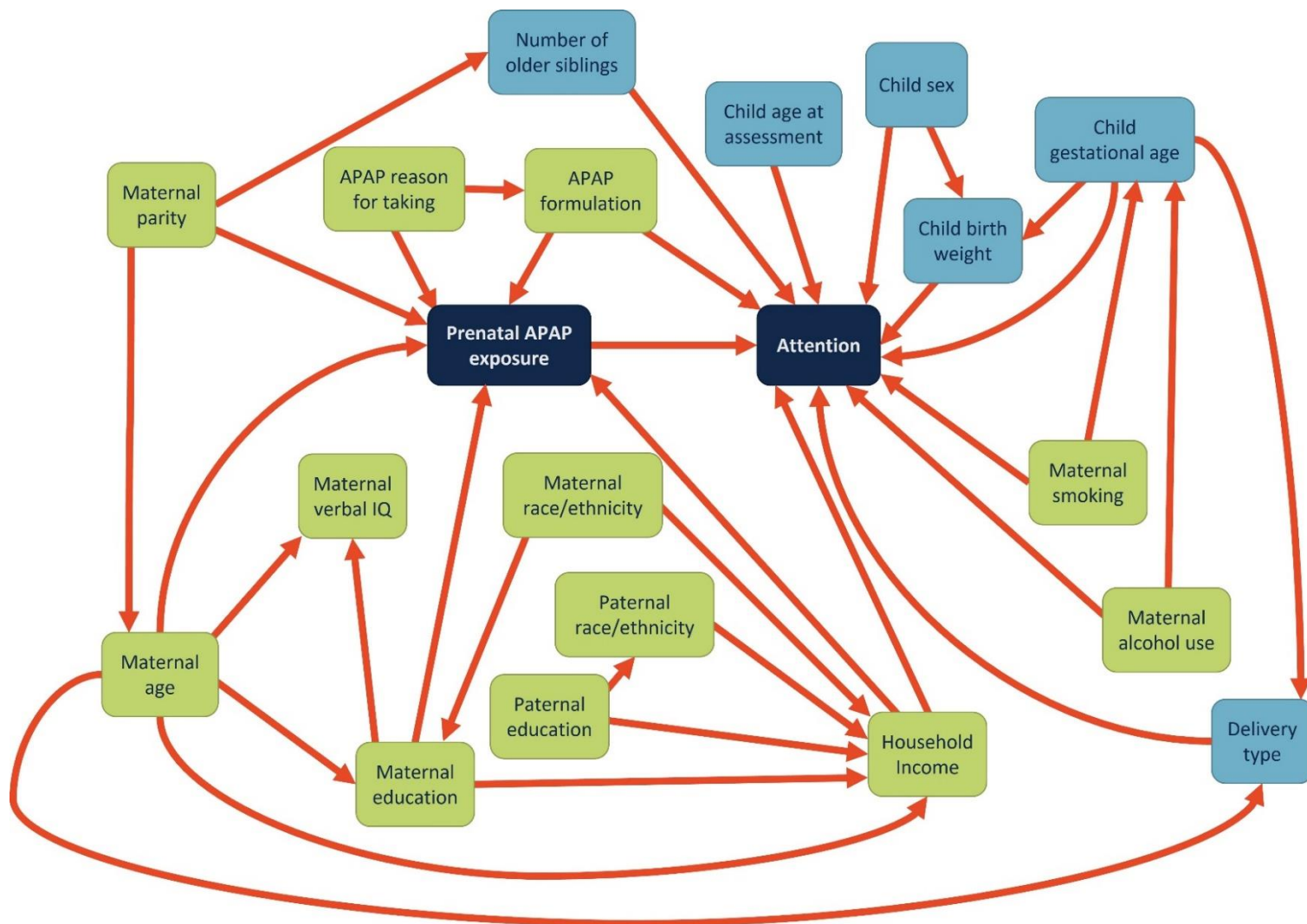


Figure 5.1. Directed acyclic graph (DAG) used to identify potential covariates for inclusion in models examining the association of prenatal acetaminophen exposure and attention outcomes. Green squares indicate parent-related variables, and light blue squares indicate child-related variables.

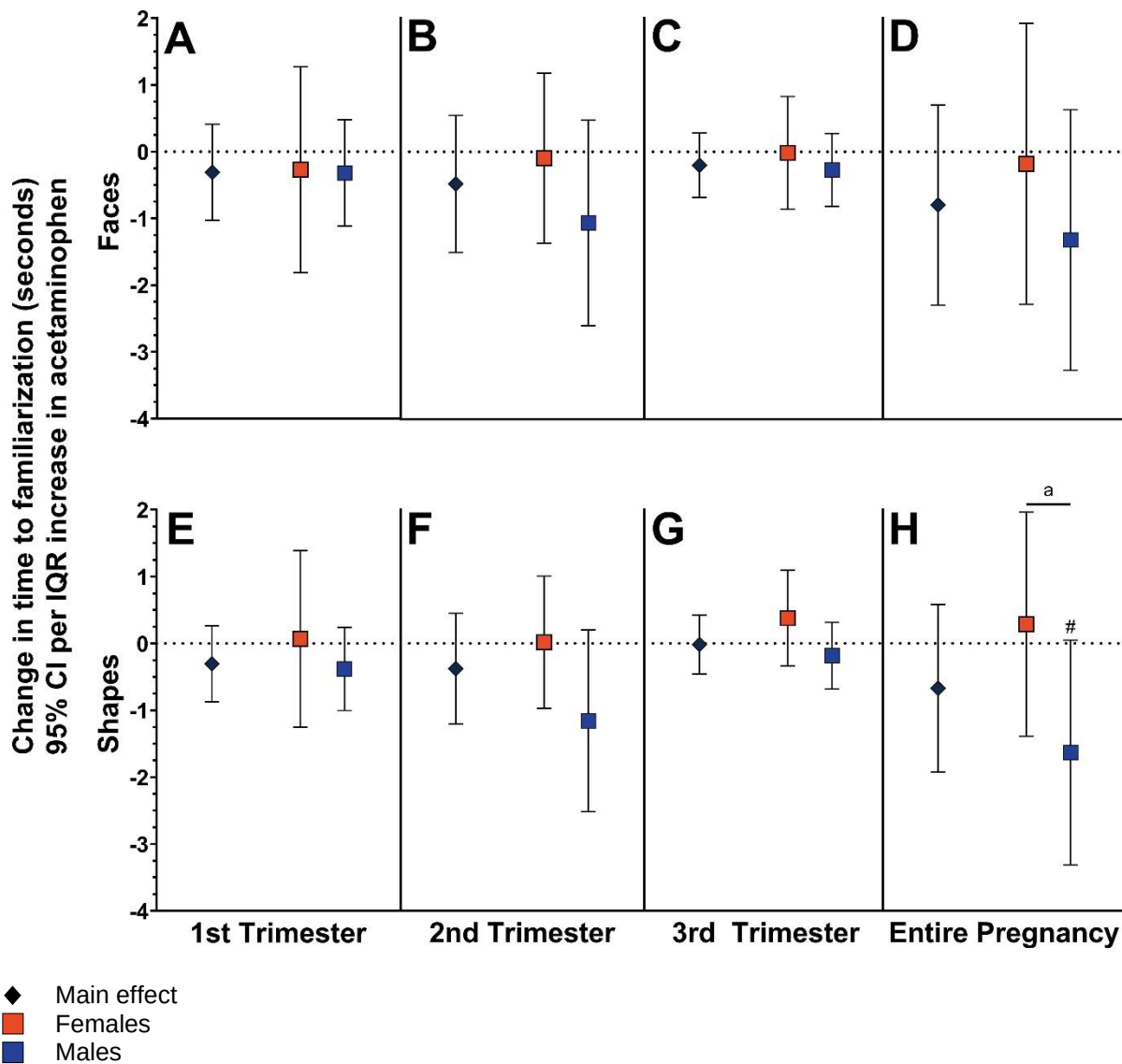


Figure 5.2. Associations of prenatal acetaminophen exposure and time to familiarization for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months. Acetaminophen exposure during the first (A, E), second (B, F), and third trimesters (C, G), as well as throughout pregnancy (D, H) was associated with a decrease in time to familiarization in all infants and males specifically for both the faces and shapes trials. However, the only association that approached significance was for acetaminophen exposure throughout pregnancy and in males for the shapes trials (H).

Interaction p-value: ^b $p < 0.05$

[#] $p < 0.10$

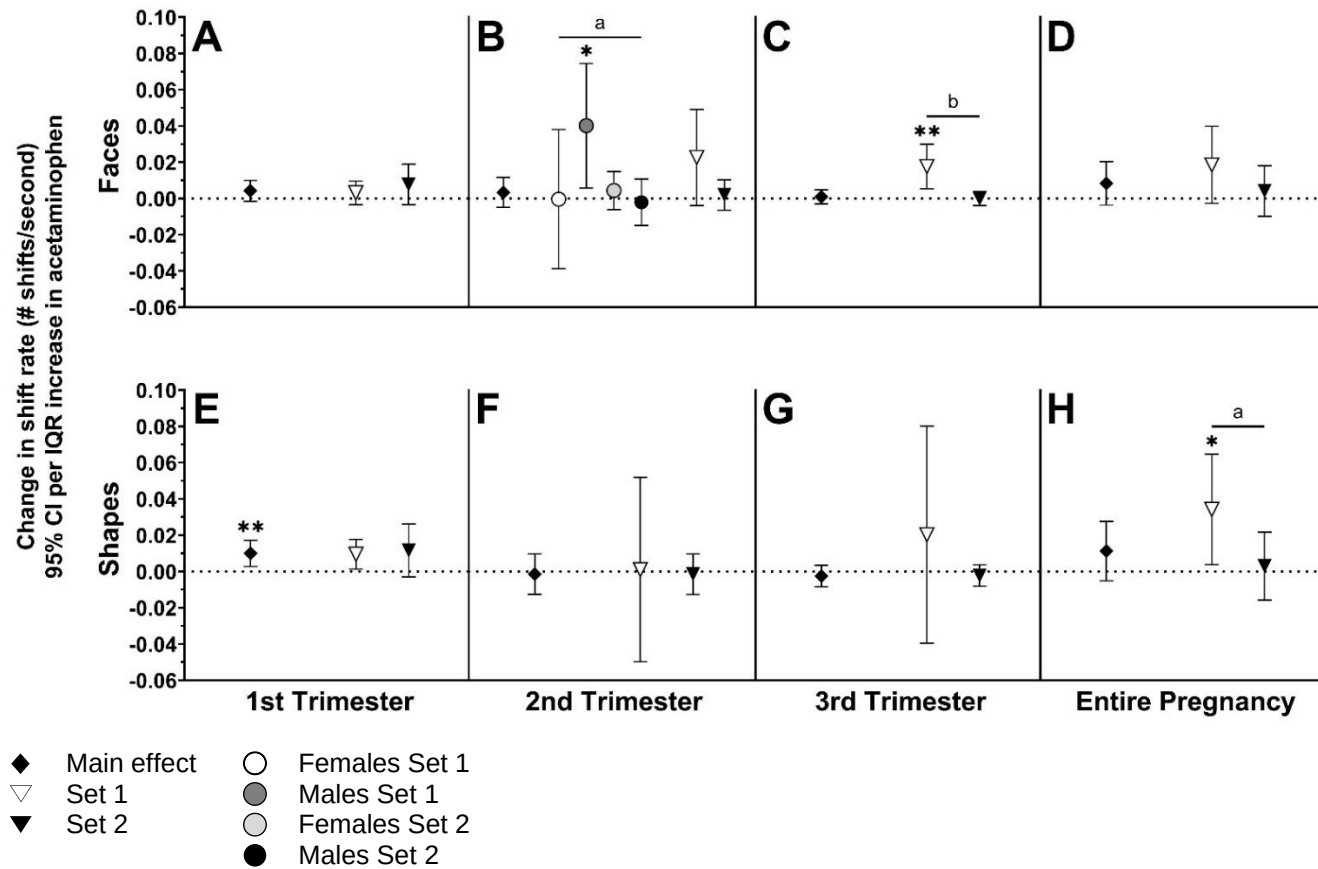


Figure 5.3. Associations of prenatal acetaminophen exposure and shift rate in the full familiarization trials for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months. Prenatal acetaminophen exposure was generally associated with faster shift rates, although associations did not reach significance with first trimester exposure (A) or exposure throughout pregnancy (D) for the faces trials, or with second (F) or third trimester (G) exposure for the shapes trials. Exposure during the first trimester was associated with faster shift rates in all infants (E). Exposure during the second trimester was associated with faster shift rates only in males who saw set 1 stimuli as familiar and only in the faces trials (B). Exposure during the third trimester was associated with faster shift rates only in infants who saw set 1 in the faces trials (C). Acetaminophen exposure throughout pregnancy was also associated with faster shift rates in infants who saw set 1 but only in the shapes trials (H).

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

* $p < 0.05$, ** $p < 0.01$

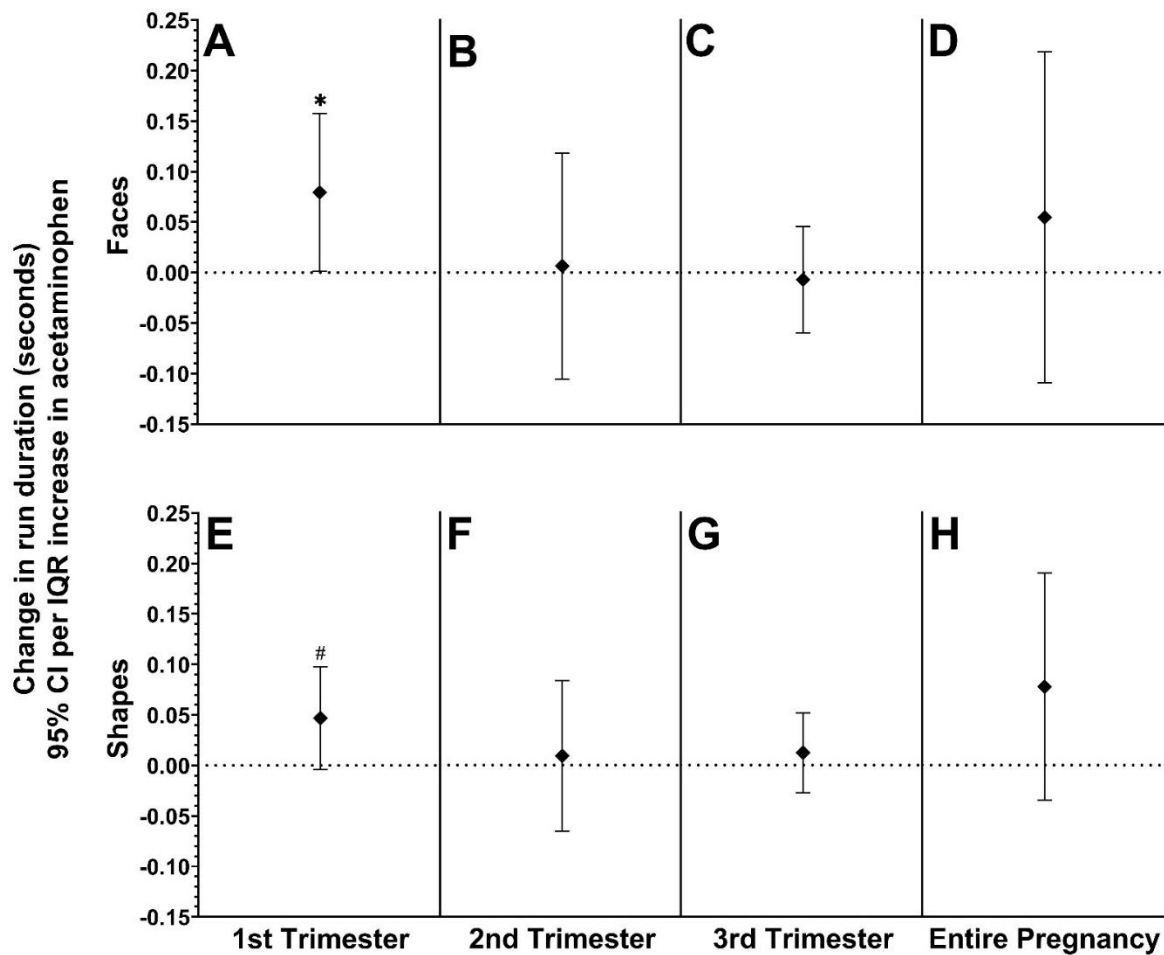


Figure 5.4. Associations of prenatal acetaminophen exposure and run duration for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months. Acetaminophen exposure during the first trimester was associated with longer run durations in both the faces (A) and shapes trials (E). There was no relationship between acetaminophen exposure during the second (B, F) or third trimester (C, G) and run duration. Exposure throughout pregnancy was also associated with longer run durations (D, H), although these associations did not reach statistical significance.

$p < 0.10$, * $p < 0.05$

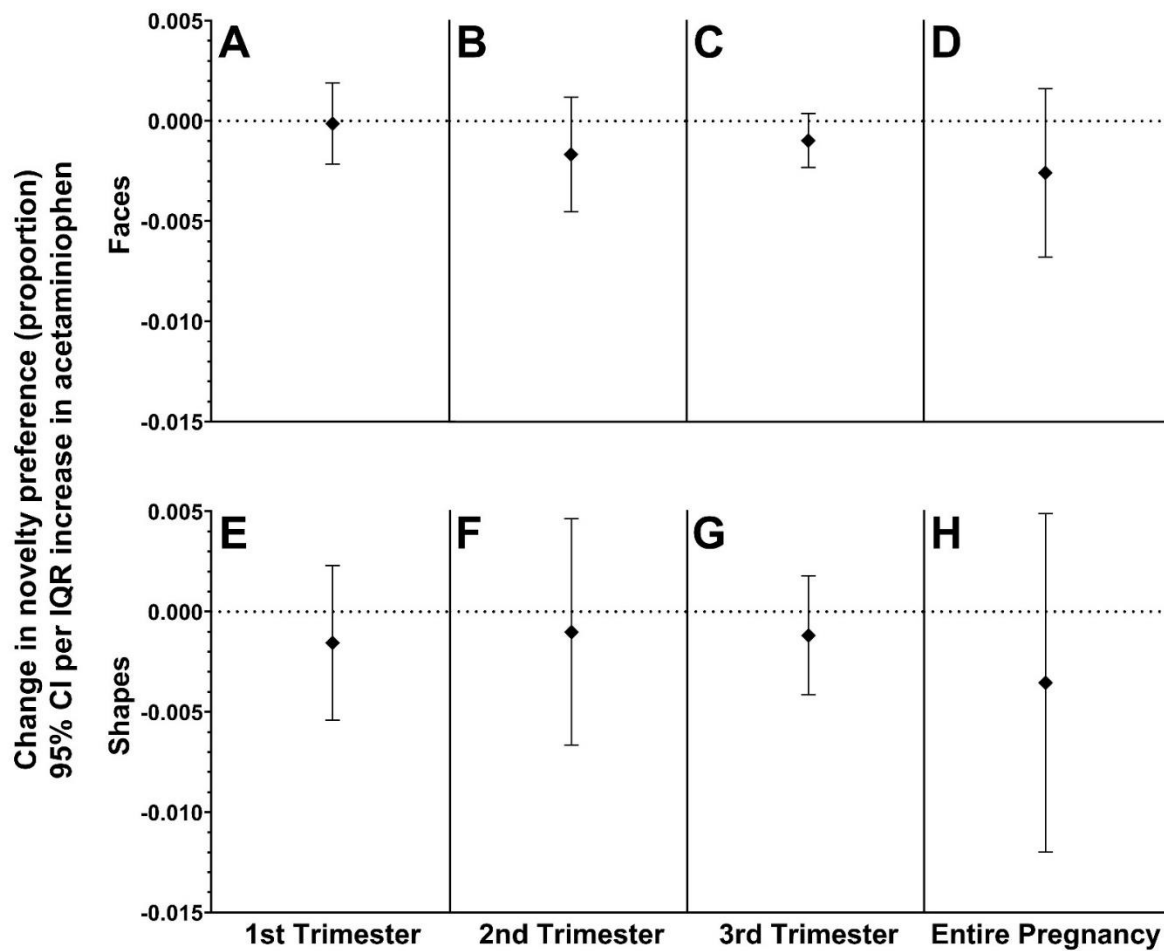


Figure 5.5. Associations of prenatal acetaminophen exposure and novelty preference for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months. Acetaminophen exposure at any time during pregnancy was associated with lower novelty preference, although none of these associations reached statistical significance.

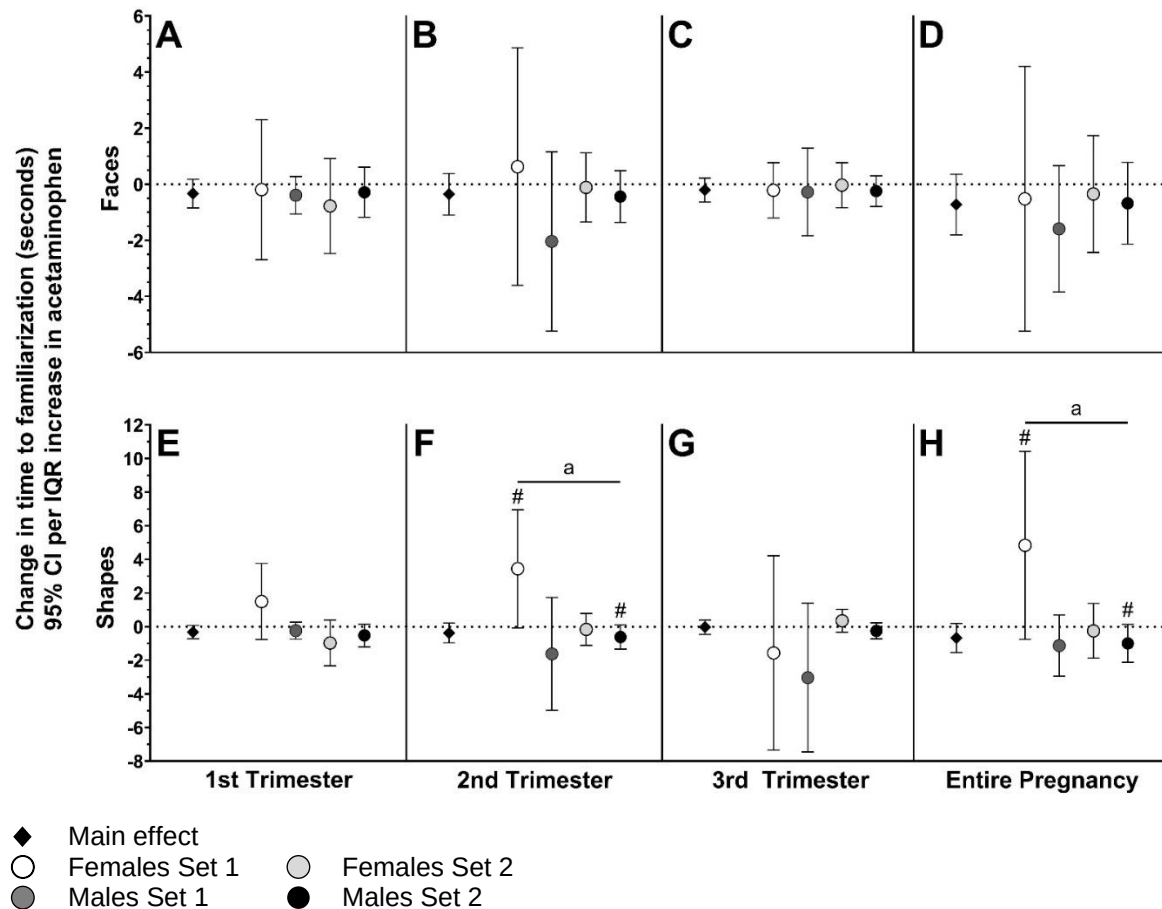
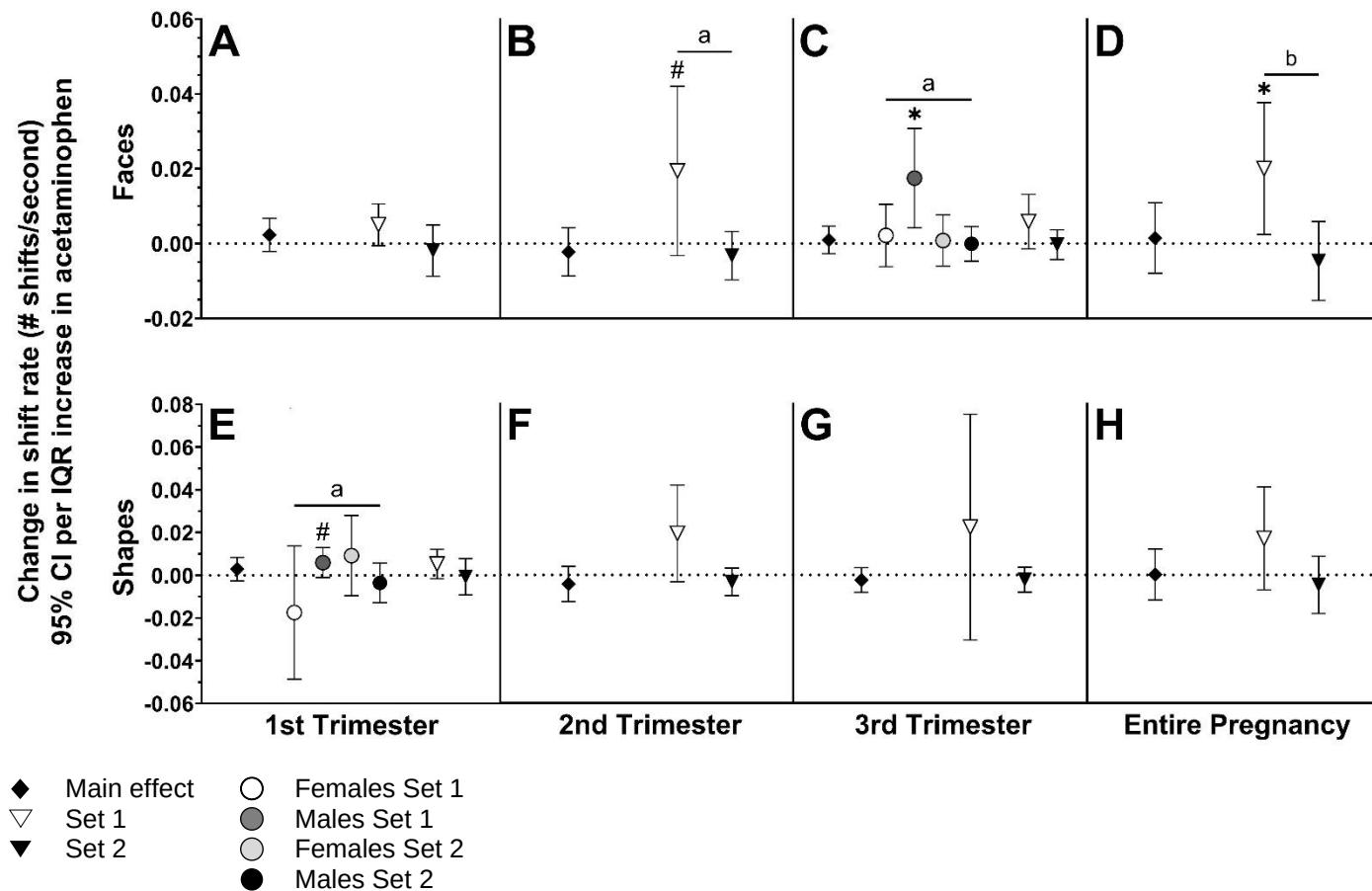


Figure 5.6. Associations of prenatal acetaminophen exposure and time to familiarization for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months when birthweight was excluded as a covariate, allowing for the inclusion of 41 additional children. When birthweight was not included as a covariate, there was no association of acetaminophen exposure during (A) the first trimester, (B) the second trimester, (C) the third trimester, (D) or the entire pregnancy with time to familiarization in the faces trials. There was also no association of (E) first trimester or (G) third trimester acetaminophen exposure and time to familiarization in the shapes trials. Both (F) second trimester exposure and (H) exposure throughout pregnancy were associated with longer time to familiarization in females who saw set 1 but shorter time to familiarization in males who saw set 2 in the shapes trials.

Interaction p-value: ^a $p < 0.10$

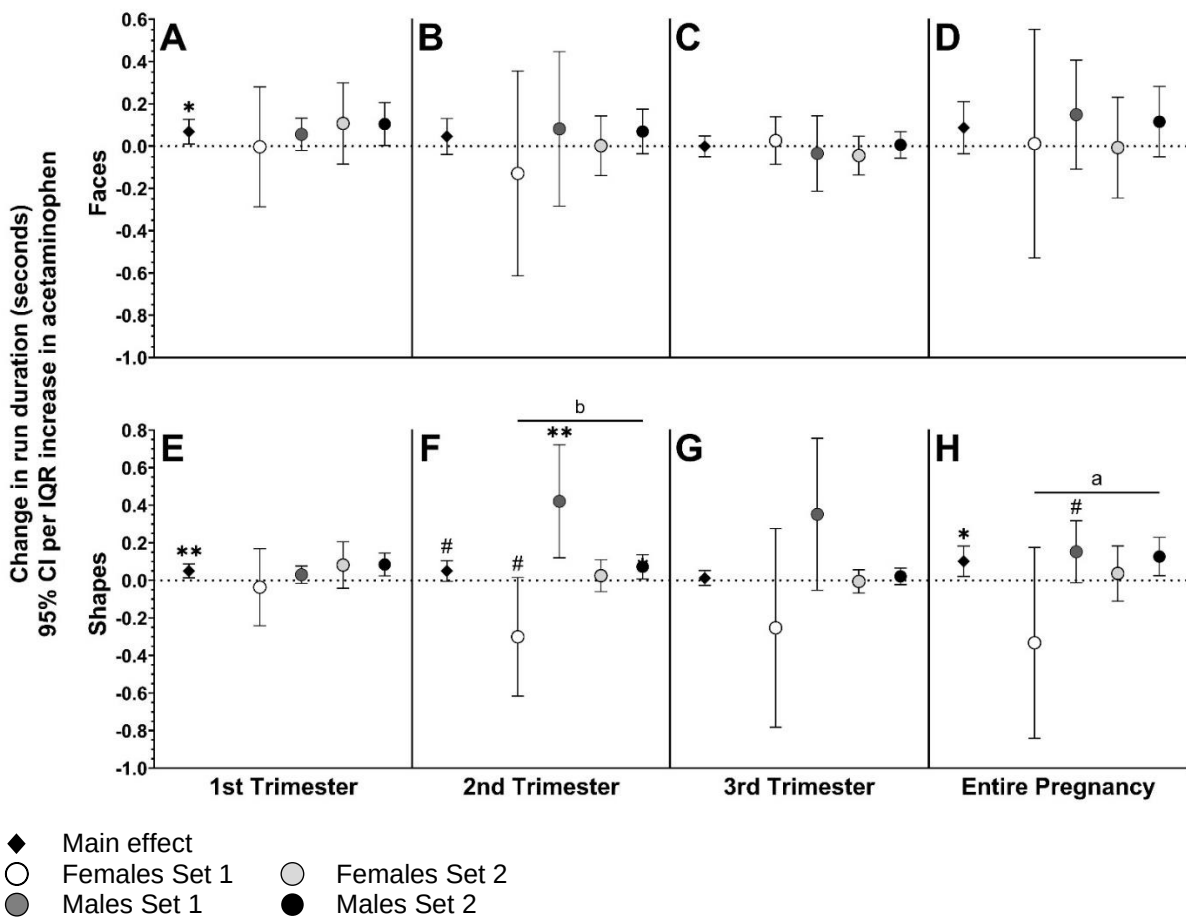
$p < 0.10$



Figures 5.7. Associations of prenatal acetaminophen exposure and shift rate for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months when birthweight was excluded as a covariate, allowing for the inclusion of 41 additional children. When birthweight was not included as a covariate, there was no association of acetaminophen exposure during (A) the first trimester with shift rate in the faces trials or (F) second trimester exposure, (G) third trimester exposure, or (H) exposure throughout pregnancy with shift rate in the shapes trials. In the faces trials, (B) second trimester exposure and (D) exposure throughout pregnancy were both associated with faster shift rates in infants who saw set 1. (C) Third trimester exposure for the faces trials and (E) first trimester exposure for the shapes trials was associated with an increase in shift rate in only males who saw set 1.

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

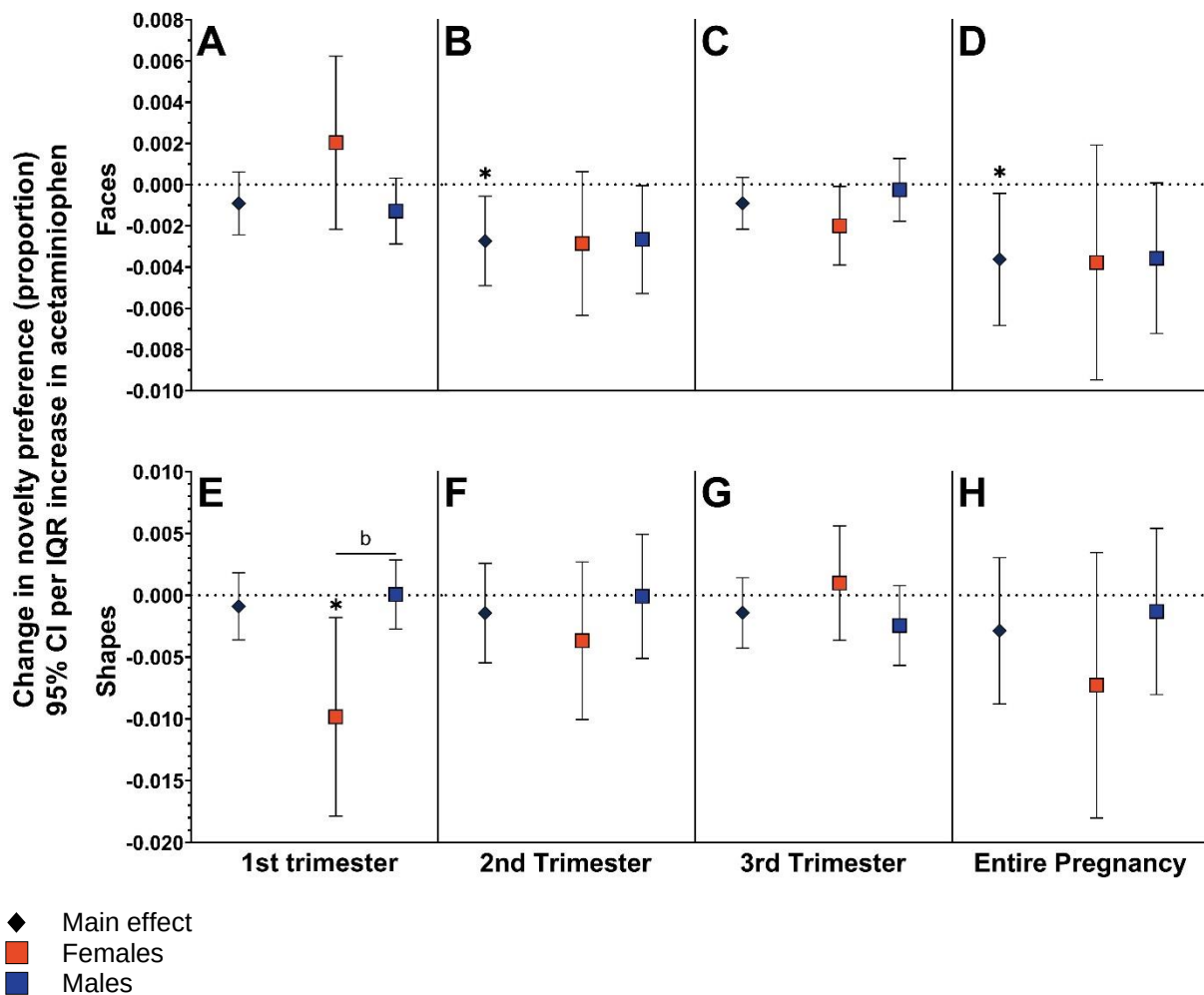
$p < 0.10$, * $p < 0.05$



Figures 5.8. Associations of prenatal acetaminophen exposure and run duration for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months when birthweight was excluded as a covariate, allowing for the inclusion of 41 additional children. When birthweight was not included as a covariate, first trimester exposure in both the (A) faces and (E) shapes trials was associated with longer run durations. (B) Second trimester exposure, (C) third trimester exposure, and (D) exposure throughout pregnancy were not associated with run duration in the faces trials. (F) Second trimester exposure was associated with longer run durations in males and females combined, but shorter run durations in females who saw set 1 and longer run durations in males who saw set 1 in the shapes trials. (G) There was no association of third trimester exposure and run duration in the shapes trials. (H) Exposure throughout pregnancy was associated with longer run durations in males and females combined and males who saw set 1 in the shapes trials.

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$



Figures 5.9. Associations of prenatal acetaminophen exposure and novelty preference for both the faces (top) and shapes trials (bottom) in the VRM task at 7-8 months when birthweight was excluded as a covariate, allowing for the inclusion of 41 additional children. When birthweight was not included as a covariate, there was no association of (A) first trimester or (C) third trimester exposure and novelty preference in the faces trials. (B) Second trimester exposure and (D) exposure throughout pregnancy were associated with lower novelty preference in the faces trials. While there was no association of (F) second trimester exposure, (G) third trimester exposure, or (H) exposure throughout pregnancy and novelty preference in the shapes trials, (E) first trimester exposure was associated with lower novelty preference in females in the shapes trials.

Interaction p-value: ^b $p < 0.05$

* $p < 0.05$

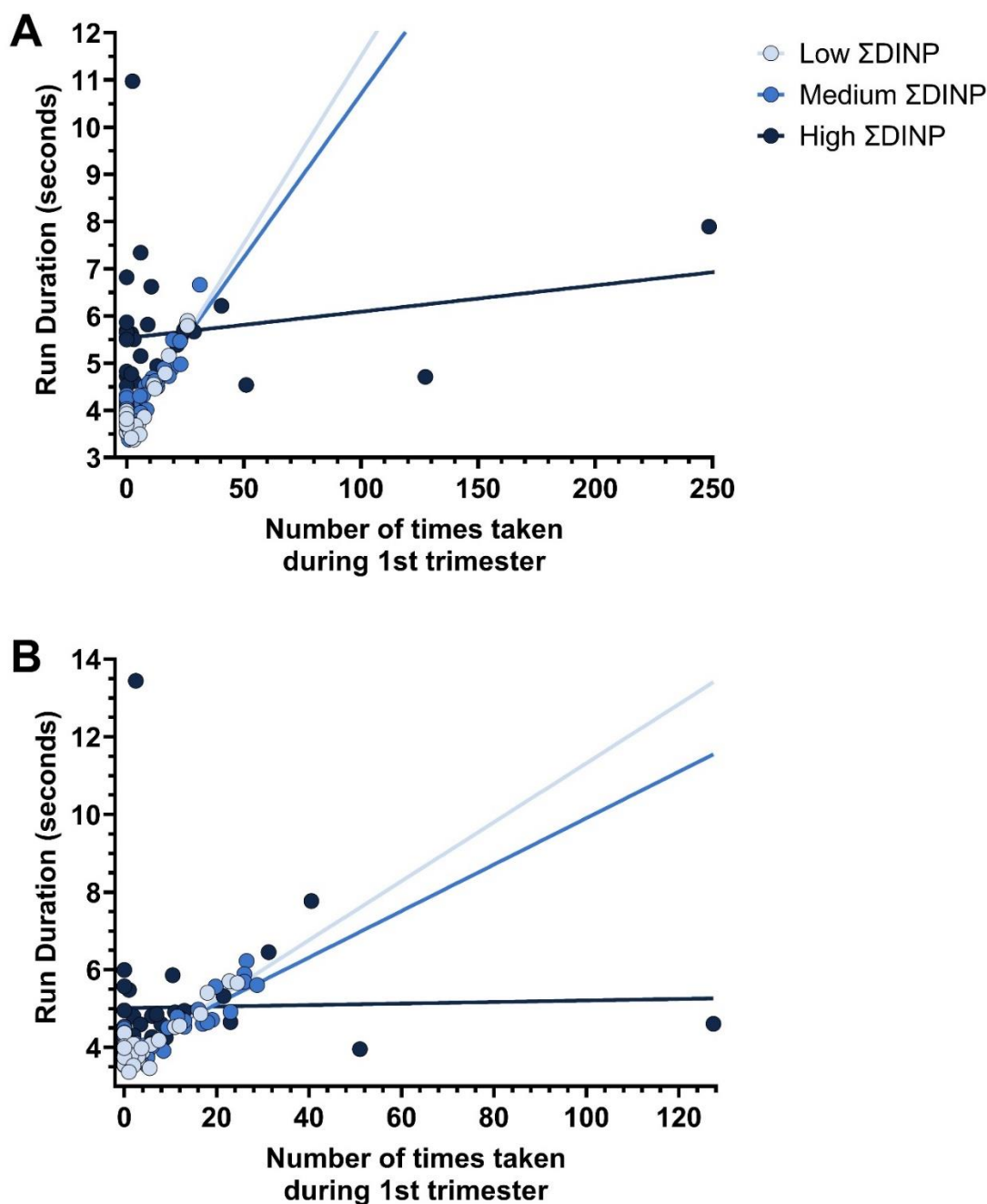


Figure 5.10. The association of acetaminophen exposure during the first trimester and run duration by levels of Σ DINP exposure in both the (A) pooled sample and (B) the 16-18 weeks sample in infants who saw set 2 stimuli as familiar. (A) For Σ DINP in the pooled sample, within infants who saw set 2, there was a borderline significant effect of acetaminophen on run duration at high Σ DINP, and there was a significant difference in the effect of acetaminophen on run duration between low and high Σ DINP groups. (B) In the 16-18 weeks sample, within infants who saw set 2, there was a significant effect of acetaminophen on run duration at low, medium, and high Σ DINP, and there was a significant difference in the effect of acetaminophen on run duration between low and high Σ DINP groups, and medium and high Σ DINP groups.

CHAPTER 6: ASSOCIATIONS OF PRENATAL EXPOSURE TO PHTHALATES AND ACETAMINOPHEN WITH MEASURES OF ATTENTION IN 2- AND 3-YEAR-OLD CHILDREN

6.1. INTRODUCTION

As described in Chapter 1, human exposure to phthalates is very common with studies finding detectable levels of phthalates in 96-100% of participants (CDC, 2019; Johns et al., 2017). There have been many studies exploring the relationship between prenatal exposure to phthalates and attention related outcomes, but results have been inconsistent (Table 1.2). Some studies have found associations of phthalates with increased externalizing behavior (Engel et al., 2010; Lien et al., 2015), attention problems (Engel et al., 2010; Kobrosly et al., 2014; Hyland et al., 2019), and attention-deficit/hyperactivity disorder (ADHD) diagnosis (Engel et al., 2018), but others have found that phthalate exposure was associated with decreases in attention problems (Gascon et al., 2015; Philippat et al., 2017), and still others have observed no association of phthalates with outcomes related to attention (Kim et al., 2017; Shin et al., 2018). Due to these mixed results, more research is necessary.

Use of acetaminophen during pregnancy is common with studies reporting 50-65% of women taking acetaminophen at least once while they are pregnant (Brandlistuen et al., 2013; Thorpe et al., 2013; Servey and Chang, 2014; Ji et al., 2018). As interest in the potential effects of prenatal acetaminophen exposure on child development has increased over the last several years, a number of studies have found associations of prenatal acetaminophen exposure with ADHD (Liew et al., 2014; Thompson et al., 2014; Ystrom et al., 2017; Ji et al., 2018; Liew et al., 2019; Ji et al., 2020), externalizing behaviors (Brandlistuen et al., 2013; Inoue et al., 2020; Trønnes et al., 2020), and attention problems (Liew et al., 2016a; Avella-Garcia et al., 2016; Golding et al., 2019). However, most of these studies have evaluated these associations in children 4 years or older, with only six studies total evaluating the association of prenatal acetaminophen exposure and attention-related outcomes in children under 4 years of age

(Streissguth et al., 1987; Brandlistuen et al., 2013; Avella-Garcia et al., 2016; Vlenterie et al., 2016; Golding et al., 2019; Tovo-Rodrigues et al., 2020). Those results have been more mixed with some finding no association and others finding increases in attentional problems, similar to studies that have investigated this association in older children (Table 1.1). Additionally, most previous studies have relied upon only one to three maternal reports of acetaminophen use during pregnancy covering long periods of time during pregnancy, leaving significant room for recall bias (Table 1.1); thus, further study is needed to examine the relationship of prenatal acetaminophen exposure and attention in early childhood.

This analysis examined the relationship of prenatal phthalate exposure, prenatal acetaminophen exposure, and the cumulative effects of prenatal exposure to both, to attention at 2 and 3 years of age using the Child Behavior Checklist (CBCL). Previous studies have demonstrated that scores on the CBCL scales are stable over time, are useful for measuring behavior longitudinally and have moderate sensitivity and specificity for identifying ADHD-related problem behaviors in children, suggesting it is a useful survey for evaluating associations of prenatal exposures with attention (Biederman et al., 2001; Chang et al., 2016). The focus of these analyses was on the subset of CBCL scales related to attention: specifically, the externalizing behaviors scale, and subscales that measure attention problems, and ADHD-related behaviors, respectively. It was expected that higher prenatal exposure to phthalates, specifically those that have been shown to be antiandrogenic or to acetaminophen, which has also been shown to be antiandrogenic, would be associated with poorer attention at 2 and 3 years. Male children were expected to be more impacted than females, especially with higher exposure in the second trimester (16-18 weeks sample for phthalates), a period during which there is a peak in androgen production in male fetuses which is important for brain development (Auyeung et al. 2013). In the exploration of the potential for interactive effects of prenatal

exposure to acetaminophen and phthalates, children with high exposure to both were expected to have the highest scores, indicating more attention problems.

6.2. METHODS

6.2.1. Study Cohort

Eligibility and recruitment were described in detail in Chapter 3. To summarize, pregnant women were recruited at two local obstetrics clinics when they were between 10 and 14 weeks of gestation. Baseline demographic information was collected at the time of enrollment in the study and included information about sociodemographic factors, reproductive health history, and lifestyle factors. Follow-up interviews were conducted every 4-6 weeks during pregnancy and shortly after the child's birth. After birth, mothers were invited to bring their infants to the University of Illinois campus for cognitive assessments three times before the child's first birthday. Mothers were invited to complete surveys, including the CBCL, and an interview about their child's general health, lifestyle factors, and product usage via phone around their child's second and third birthdays. The analyses presented in Chapter 6 focus on data collected from the CBCL when the child was 2 and 3 years of age.

6.2.2. Exposure Assessment

Prenatal phthalate exposure

Details about exposure assessment are provided in Chapter 3. Women provided five first-morning urine samples during pregnancy at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation for measurement of phthalate metabolites. For each participant, a pooled sample was created from the five separate urines, and both the pooled sample and a sample from the 16–18-week time point were sent to the U.S. Centers for Disease Control (CDC) Division of Laboratory Sciences in Atlanta, Georgia for analysis. The pooled sample allows for an estimation of the average exposure to phthalates across pregnancy, and the 16–18-week period may be especially vulnerable to adverse effects of antiandrogenic endocrine

disrupting chemicals (EDCs), including phthalates, because this is the window of peak androgen production in male fetuses (Auyeung et al., 2013). Phthalate metabolites were measured using online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry (Silva et al., 2007). Concentrations of phthalate metabolites were adjusted using specific gravity to account for differences in urine dilution. Because some phthalates, such as di-(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP), are rapidly metabolized into multiple urinary metabolites, weighted molar sums were calculated for these parent compounds as described in Chapter 4. As described earlier in Chapter 4, the analyses focused on Σ DEHP, Σ DINP, di-2-ethylhexyl terephthalate (Σ DEHTP), an antiandrogenic sum (Σ AA) and monoethyl phthalate (MEP).

Prenatal acetaminophen exposure

Details about the quantification of acetaminophen exposure during pregnancy are also described in Chapter 3. Information about medication use during pregnancy was collected in the same 5 time periods the urine samples were collected, as well as immediately after the child's birth for a total of six windows of gestation. Mothers reported the name of all prescription and over the counter (OTC) medications used since the last interview, the dates of use, frequency of use, and reason for use. Medications containing acetaminophen were identified, and the number of times acetaminophen was taken was calculated for each trimester of pregnancy as well cumulative use across the entire pregnancy.

6.2.3. Attention-Related Outcomes

As described in Chapter 3, parents are invited to participate in follow-up visits via mail and phone once the child reaches both 2 (26.5 - 28.5 months) and 3 (36 - 38 months) years of age. At both ages, parents who agreed to participate were sent a packet of questionnaires to complete regarding the child's overall development, behavior, and language development, as

well as maternal depression, maternal stress, maternal social support, and their relationship with the child. Parents were sent the preschool version of the CBCL at both ages.

The CBCL consists of 100 questions divided into two composite scales (internalizing behaviors and externalizing behaviors), both of which are further divided into syndrome subscales. Parents rate each question on a 3-point Likert scale (“not true” = 0 points, “somewhat true” = 1 point, and “often true” = 2 points), and point values are summed to provide scores for both the internalizing and externalizing behaviors composite scales and their corresponding syndrome subscales. Higher scores correspond with more problems in the area of behavior addressed by each question, subscale, and composite scale. In these analyses, the Attention Problems subscale, the ADHD Problems subscale, and the Externalizing Behaviors composite score were used to examine attentional problems at 2 and 3 years of age. The Attention Problems subscale is composed of five questions, and scores can range from 0 to 10. The ADHD Problems subscale is composed of six questions regarding behaviors consistent with the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) diagnostic criteria for ADHD (Achenback & Rescorla, 2001), three of which (items 5, 6, and 59) are also part of the Attention Problems subscale. The Externalizing Behaviors composite score is composed of the sum of the scores on the Attention Problems and Aggressive Behavior subscales. The Aggressive Behavior subscale includes items 8 and 16 which are also part of the ADHD Problems subscale. Externalizing Behavior scores can range from 0 to 48 or can be converted to a standardized T-score with a range of 28 to 100 where scores above 70 are considered clinical (Achenback & Rescorla, 2001). For these analyses, the standardized T-scores were used. Because the scales included in the present analysis include some of the same items, the three outcomes that were measured were not independent of one another.

6.2.4. Covariates

Models of prenatal phthalate exposure

As described in previous chapters, covariates were selected based on directed acyclic graphs (DAGs, Figure 4.4) and *a priori* knowledge. DAGs were first created to identify variables to consider, and correlations of those variables with exposures and outcomes were examined to select covariates for inclusion in models. Because CBCL data was collected at both 2 and 3 years of age, correlations of potential covariates with exposure for both subsets of children and outcome variables at both 2 and 3 years were considered in the selection process. For the analyses of the relationship between prenatal phthalate exposure and attention-related outcomes at 2 and 3 years of age on the CBCL, maternal age, maternal education (< bachelor's degree vs bachelor's degree or higher), household income (< \$50,000, \$50,000-\$99,999, ≥ \$100,000), and number of older siblings (0, ≥1) were ultimately selected for inclusion in all models with child sex also included as a potential modifier. Sensitivity analyses were conducted to explore the potential influence of maternal marital status (where participants not married or living as married were excluded), alcohol use during the first trimester (any vs. none), and maternal cigarette use during the first trimester (where participants who reported smoking or whose response was missing were excluded) on observed associations.

Models of prenatal acetaminophen exposure

Based on DAGs (Figure 5.1) and examination of correlations among exposure variables, outcome variables, and potential covariates, acetaminophen formulation (no acetaminophen taken, acetaminophen alone, in combination with other drugs), maternal age, household income, delivery type (Cesarian section vs. vaginal delivery), and number of older siblings were included in all models with child sex again included as a potential modifier in analyses of prenatal acetaminophen exposure and attention-related outcomes at 2 and 3 years of age. Additionally, the potential influence of marital status, maternal alcohol use, and maternal

cigarette use on associations in were evaluated in sensitivity models. Because birth weight was missing for so many children with CBCL data available, sensitivity analyses including child weight at birth as a covariate were also conducted, rather than including it as a covariate in the primary models.

Models of interactive effects of prenatal phthalate and acetaminophen exposure

For the analyses of the interactive effects of prenatal acetaminophen and phthalates on attention-related outcomes at 2 and 3 years, covariates were selected in a similar manner as described in Chapter 5. Covariates were selected based on similarities between the DAGs and *a priori* knowledge used to select covariates for the separate exposure analyses. Ultimately, acetaminophen formulation, maternal age, household income, delivery type, number of older siblings, and child sex were selected for inclusion in all models. Sensitivity analyses were conducted to examine the potential influence of maternal marital status, maternal alcohol use, maternal cigarette use, and child weight at birth on associations.

6.2.5. Statistical Approach

Multivariate generalized linear regression models were used to examine the relationships between each individual biomarker of exposure (Σ AA phthalates, Σ DEHP, Σ DINP, MEP, and Σ DEHTP in both the 16-18 weeks of gestation and pooled samples; acetaminophen use during the first, second, and third trimester, and throughout the entire pregnancy) and each outcome (Attention Problems, ADHD Problems, Externalizing Behavior T-score). Sex-by-exposure interactions were examined as well, and interaction terms with p-values < 0.10 were retained in the final models. Results where interactions were retained are shown with both the main effect of exposure and stratum-specific estimates. Beta estimates were scaled to an interquartile range (IQR) increase in exposure for ease of comparison across wide ranges of exposure, and associations were considered significant if $p < 0.05$. The effect of removing potentially influential data points (Cook's D > 0.05) on the observed associations was also

examined. All statistical analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (Copyright © 2013, SAS Institute Inc., Cary, NC, USA).

As in previous analyses of the potential interactive effects of prenatal exposure to both phthalates and acetaminophen (Chap. 5), a relatively conservative approach was taken where only the phthalate exposures associated with any outcome(s) at age 2 or 3 were included. Acetaminophen-by-phthalate-by-child sex interactions were also explored. Where an interaction term was $p < 0.10$, the interaction was further examined by categorizing phthalate exposure as low ($< 25^{\text{th}}$ percentile), medium ($25^{\text{th}} - 75^{\text{th}}$ percentile), or high exposure ($> 75^{\text{th}}$ percentile) and assessing whether the impact of increasing acetaminophen exposure differed at each of these three levels of phthalate exposure.

6.3. RESULTS

6.3.1. Participation and Demographics

The subset of participants who completed the CBCL when the child was 2 years of age was somewhat different from the full cohort (Table 3.1). The subset had more parents, especially fathers, who were white, non-Hispanic, and substantially more parents, both mothers and fathers, held at least a bachelor's degree. More participants in the subset were also married or living as married, nulliparous, had an annual household income of \$100,000 or greater, and reported not smoking or consuming alcohol during the first trimester of pregnancy. Slightly more children in the subset were female and were born vaginally than in the full cohort (Table 3.2). The subset of participants that completed the CBCL when the child was 3 years old was largely similar to the subset that completed the CBCL when the child was 2 years old (Table 3.1). The subset with data available at 3 years had more participants with an annual household income of \$50,000-\$99,999 and had a higher mean maternal verbal IQ than either the subset with data available at 2 years or the full cohort (Table 3.2).

6.3.2. Exposure Characterization

Prenatal phthalate exposure

A total of 254 children had CBCL data available at 2 years, and 215 had data available at 3 years. For the analyses of the relationship between prenatal phthalate exposure and attention-related outcomes, all 254 children with CBCL data at 2 years had pooled phthalate data available, and only 1 was missing phthalate data at 16-18 weeks of gestation ($n = 253$). Of these children with both phthalate and CBCL data available at 2 years of age, 160 had Σ DEHTP data available for both the pooled sample and the 16-18 weeks sample. Of the 215 children with CBCL data available at 3 years, all had pooled phthalate data available, but 1 was missing phthalate data at 16-18 weeks of gestation. Of these 215 children, 120 also had Σ DEHTP data available for both the pooled sample and the 16-18 weeks sample. At 3 years, not all children had all CBCL scores because some questions were skipped by the parent who completed the CBCL ($n=117-119$ for Σ DEHTP and 209-211 for other phthalates). Regression diagnostics generally supported the use of continuous, untransformed biomarker measures and linear models to characterize associations. As in Chapter 4, due to the skewed nature of phthalate exposure concentrations, exposures were characterized using minimums, maximums, medians, and interquartile ranges. Phthalate exposure in both the 16-18 weeks of gestation and pooled samples in both the subset of children with data available at 2 years and the subset with data available at 3 years was similar to phthalate exposure in the full cohort.

Prenatal acetaminophen exposure

Of the 254 children with CBCL data available at 2 years, 8 were missing delivery type, leaving 246 with outcome and full covariate data available for the analyses of the relationship between prenatal acetaminophen exposure and attention-related outcomes at 2 years of age. Of the 215 children with data available at 3 years, 12 were missing delivery type, leaving 203 children with complete covariate data available for analyses of the Attention Problems subscale,

202 for analyses of the ADHD Problems subscale, and 201 for analyses of the Externalizing Behaviors T-score. Because delivery type was also included as a covariate in the acetaminophen-by-phthalate exposure interaction models, the same number of children were included in those analyses for both 2 and 3 years (Table 6.6). As in Chapter 5, acetaminophen exposure was characterized using minimums, maximums, medians, and interquartile ranges because the distributions of exposure were right-skewed. Regression diagnostics generally supported the use of continuous, untransformed exposure measures to characterize the associations of prenatal acetaminophen exposure and attention-related outcomes. There was no difference in acetaminophen exposure between the subset with data available at 2 years and the full cohort, nor between the full cohort and the subset with data available at 3 years (Table 3.4).

6.3.3. Attention-Related Outcomes

As described earlier in the chapter, the outcome measures used are not independent of one another because there is overlap in the items used to calculate each score. Unsurprisingly, the outcome measures were all highly positively correlated with one another at both ages (Table 6.1). As shown in Figure 6.1, the children in this cohort on average have scores well-below the point considered to be of clinical concern (indicated by the dotted line in Figure 6.1) on the outcomes related to attention. Very few children have attention problems (Figure 6.1A), ADHD-related behaviors (Figure 6.1B), or externalizing behavior problems (Figure 6.1C) at either 2 or 3 years of age in the clinical range. As expected, at both ages, males tended to have higher scores on all attention-related outcomes than females. Interestingly, the mean scores on all three outcomes were lower at 3 years compared to 2 years for all children, females, and males.

6.3.4. Associations of Exposure and Attention-Related Outcomes

Prenatal phthalate exposure and attention-related outcomes at 2 and 3 years

The patterns of associations for each phthalate were similar across outcome measures, and ages, suggesting the behaviors evaluated are relatively stable between the ages of 2 and 3 years. Only two phthalates in the 16-18 weeks of gestation sample and three in the pooled sample had associations with changes in attention or ADHD problems or problems with externalizing behaviors at 2 or 3 years. Σ AA phthalates in either the 16-18 weeks of gestation or pooled sample was not associated with any changes in externalizing behavior, ADHD problems, or attention problems (Table 6.2, 6.10). An IQR increase in Σ DEHP only in the pooled sample was associated with a decrease in externalizing behavior (Figure 6.2A), a decrease in ADHD problems (Figure 6.2K), and a decrease in attention problems (Figure 6.2U), but only at 2 years of age (Table 6.2). The pattern of associations for Σ DEHP was similar at 3 years but none were significant (Figure 6.2B, L, V; Table 6.3). In contrast, an IQR increase in Σ DINP in the pooled sample was associated with an increase in ADHD problems at 3 years (Figure 6.2N; Table 6.3). There was no relationship of Σ DINP and any of the attention-related outcome at 2 years (Figure 6.2C, M, W; Table 6.2), and while there were increases in externalizing behavior (Figure 6.2D) and attention problems (Figure 6.2X) at 3 years per IQR increase in Σ DINP exposure, neither reached significance (Table 6.3).

Σ DEHTP was the only phthalate associated with changes in attention related behavior at 2 or 3 years for both the 16-18 weeks of gestation and pooled samples. Per IQR increase in Σ DEHTP in the 16-18 weeks sample, there was a significant decrease in males only in externalizing behavior at 2 (Figure 6.2G) and 3 years (Figure 6.2H), ADHD problems at 2 (Figure 6.2Q) and 3 years (Figure 6.2R), and attention problems at 2 years only (Figure 6.2AA; Table 6.2, 6.10). The pattern was consistent for males and attention problems at 3 years, but it was not significant (Figure 6.2AB; Table 6.3). In the pooled sample, the sex-by- Σ DEHTP

interaction was significant for both externalizing behavior and attention problems at 2 years, with an IQR increase in Σ DEHTP associated with increases in externalizing behaviors (Figure 6.2I) and attention problems (Figure 6.2AC) in females and decreases in males, although these associations did not reach significance (Table 6.2). The pattern was consistent for ADHD problems at 2 years, but the sex-by-exposure interaction did not reach $p < 0.10$ (Table 6.2). At 3 years, the pattern of associations for Σ DEHTP in the pooled sample and attention-related behaviors was similar to that observed at 2 years, but it was much less pronounced, and none reached significance (Figure 6.2J, T, AD; Table 6.3).

MEP in the 16–18-week sample was associated with changes at both 2 and 3 years. At 2 years, an increase in MEP in the 16–18-week sample was associated with an increase in ADHD problems (Figure 6.2O) and attention problems (Figure 6.2Y) in males only (Table 6.2). At 3 years, there was also an increase in attention problems in male children only per IQR increase in MEP exposure (Figure 6.2Z; Table 6.3). Males also appeared to have an increase in ADHD behavior at 3 years (Figure 6.2P) per IQR increase in MEP exposure, but these associations did not reach statistical significance (Table 6.2, Table 6.3). Sensitivity analyses removing women who were not married or living as married ($n = 13$ at 2 years, $n = 8$ at 3 years), women who reported smoking during their first trimester ($n = 17$ at 2 years, $n = 19$ at 3 years), or potentially influential data points (Cook's $D > 0.05$) did not substantially change the results. In general, Σ DEHP and Σ DEHTP were associated with decreases in attention-related behavior problems in male children at both 2 and 3 years, while MEP was associated with increases in attention-related behavior problems in male children at 2 and 3. Σ DINP was associated with increases in attention-related behaviors only at 3 years, and Σ DEHTP may be associated with increases in attention-related behaviors in female children only at 2 years.

Prenatal acetaminophen exposure and attention-related outcomes at 2 and 3 years

Acetaminophen exposure during prenatal development was generally associated with increases in attention-related behavior problems on the CBCL at both 2 and 3 years. No sex-by-exposure interaction met the $p < 0.10$ criterion in these analyses, thus only main effects are presented (Table 6.4). An IQR increase in acetaminophen exposure during the second trimester was associated with a significant increase in externalizing behaviors (Figure 6.3C, D), ADHD problems (Figure 6.3K, L), and attention problems (Figure 6.3S, T) at both 2 and 3 years (Table 6.4). Similar to second trimester exposure, there was a significant increase in externalizing behavior (Figure 6.3G, H), ADHD problems (Figure 6.3O, P), and attention problems (Figure 6.3W, X) per IQR increase in total acetaminophen exposure during the entire pregnancy (Table 6.4). Sensitivity analyses including child birthweight as a covariate (removed due to missing birthweight $n = 36$ at 2 years, $n = 31$ at 3 years) or removing women who were not married or living as married ($n = 13$ at 2 years, $n = 8$ at 3 years), women who reported smoking during their first trimester ($n = 17$ at 2 years, $n = 19$ at 3 years), or potentially influential data points (Cook's $D > 0.05$) did not substantially influence results.

Interactive effects of acetaminophen and phthalate exposure on attention-related outcomes at 2 and 3 years of age

Interactions of prenatal acetaminophen and phthalate exposure were explored when the acetaminophen exposure—outcome association had a p -value < 0.10 (ADHD problems at 3 years for first trimester exposure; externalizing behavior, ADHD problems, and attention problems at 2 and 3 years for second trimester and entire pregnancy exposure) and only for the phthalates that also had an effect with p -values of < 0.10 (MEP and Σ DEHTP in the 16-18 weeks sample, and Σ DEHP, Σ DINP, and Σ DEHTP in the pooled sample). No acetaminophen-by-phthalate-by-sex interactions reached the $p < 0.10$ criterion for further exploration, but several acetaminophen-by-phthalate exposure interactions did for MEP, Σ DEHTP, and Σ DINP,

for attention problems and ADHD problems, and these interactions were explored further by categorizing phthalate exposure as low, medium, and high (Table 6.5). With two exceptions, when these interactions were stratified by phthalate exposure level, there was no difference in the effect of acetaminophen on the outcomes among the levels of phthalate exposure (Table 6.6). However, for ADHD problems at 2 years, when stratified by levels of MEP exposure in the 16-18 weeks sample, there was a borderline significant difference between children categorized as having low MEP exposure and those categorized as having medium MEP exposure. For both acetaminophen exposure during the second trimester (Figure 6.4A) and exposure throughout pregnancy (Figure 6.4B), children in the medium MEP exposure group had a larger increase in ADHD problems with increasing acetaminophen exposure compared to those in the low MEP group (Table 6.6). In sensitivity analyses excluding child birthweight as a covariate (removed due to missing birthweight $n = 36$ at 2 years, $n = 31$ at 3 years), or removing participants who were not married or living as married ($n = 13$ at 2 years, $n = 8$ at 3 years), who reported smoking during the first trimester ($n = 17$ at 2 years, $n = 19$ at 3 years), or potentially influential data points (Cook's $D > 0.05$), there were no major changes to the observed associations.

6.4. DISCUSSION

6.4.1. Prenatal phthalate exposure and attention

The impact of prenatal exposure to phthalates on attention at 2 and 3 years of age was mixed, similar to what was observed for measures of attention at 7-8 months. Σ DEHP in the pooled sample (Figure 6.2A, K, U) and Σ DEHTP in the 16-18 weeks sample (Figure 6.2G, Q, AA) were both associated with decreases in attention problems on all three measures, but only in males. Σ DINP in the pooled sample was associated with an increase in attention problems in all children but only at 3 years (Figure 6.2N), and MEP in the 16-18 weeks sample was associated with increases on the attention problems and ADHD subscales, but only in males (Figure 6.2O, Y, Z). The association of Σ DEHP with decreases on all three scales was

somewhat unexpected, as previous studies have found that DEHP or its metabolites were associated with increases in externalizing behavior in a cohort in Taiwan and increased risk of ADHD in a cohort in Norway (Lien et al., 2015; Engel et al., 2018). However, similar to our results, Gascon et al. (2015) observed that prenatal exposure to DEHP was associated with decreased risk of inattention symptoms at both 4 and 7 years of age and decreased risk of ADHD symptoms at 7 years in a cohort in Spain.

There were some similarities of these results at 2 and 3 years to the results of the measure of attention (time to familiarization) on the interquartile range (VRM) task at 7-8 months (see Chapter 4). At both 7-8 months and 3 years, Σ DINP, MEP and Σ DEHTP were all associated with measures of attention, although the directions of the associations differed (Figure 4.6, 6.2). There were also associations of MEP and Σ DEHTP, but not Σ DINP, with measures of attention at 2 years (Figure 6.2). At 7-8 months, all associations differed by trial type with associations of Σ DEHTP only present for the faces trials (Figure 4.6I, J) and associations of MEP only present for the shapes trials (Figure 4.6Q, R). In the faces trials, Σ DINP was associated with increased time to familiarization, indicating poorer attention (Figure 4.6E), but it was associated with decreased time to familiarization, indicating better attention in the shapes trials (Figure 4.6P). Then at 3 years, Σ DINP was associated with increased ADHD problems (Figure 6.2N). Associations of MEP were primarily for shorter time to familiarization at 7-8 months (Figure 4.6), suggesting better attention, but at 2 years, it was associated with increases in ADHD and attention problems in male children only (Figure 6.2O, Y). Σ DEHTP was associated with increased time to familiarization, indicative of poorer attention at 7-8 months (Figure 4.6), but at both 2 and 3 years, Σ DEHTP was associated decreased ADHD and attention problems in male children (Figure 6.2G, H, Q, R). Σ DEHTP was also associated with lower externalizing behavior scores in male children as well, but only at 2 years (Figure 6.2AA). In summary, while Σ DINP, MEP and Σ DEHTP were all associated with outcomes of attention at

7-8 months as well as those measured at 2 and 3 years, the associations were not consistent across outcome measures or ages.

This is one of the first studies to examine the effects of prenatal exposure to DEHP or its metabolites on behavioral development in children and appears to be the first to focus on attention-related outcomes. A recent study by Colicino et al. (2021) in a cohort in Mexico found that one of the primary metabolites of DEHP, MECPTP, was associated with increases in problem behaviors, depression, and hyperactivity in children between 4 and 6 years of age, but they observed no association of MECPTP with externalizing behaviors. The discrepancy between Colicino et al. (2021) and the present study could be due to the differences in sample size and methods of outcome assessment used in the two studies, or it may be due to the fact that children of different ages were assessed (Table 1.2). However, because attention tends to be stable across ages, it is more likely that the differences in sample size and the surveys used contributed to the discrepancy between the two studies (Aslin, 2007). Interestingly, in the IKIDS cohort, while male children had higher (worse) scores on all attention related outcomes than females at both 2 and 3 years, the mean scores of the outcomes were lower at 3 years compared to 2 years for both males and females. This may be because of the 291 children total with CBCL data available at 2 or 3 years, only 170 (58%) had CBCL data at both ages. It is likely the differences in scores at 2 and 3 years are because CBCL measures were obtained for different subsets of children at each age due to a combination of attrition and age because not all children with CBCL data available at 2 years were 3 years old at the time of these analyses. Because this is one of the first studies to evaluate the effects of prenatal exposure to DEHP on any neurodevelopmental outcomes in children and DEHP is increasingly used as a replacement for DEHP (Schwedler, et al., 2020), more research on this chemical is urgently needed.

Although a few previous studies have examined the effects of prenatal exposure to DINP or its metabolites on neurodevelopmental outcomes, most have not found any relationship with attention-related outcomes (Table 1.2). The exception is Hyland et al. (2019) which found that prenatal exposure to the DINP metabolite, MCOP was associated with an increase in errors of commission on the Conners' Continuous Performance Test (CPT-II) at both 9 and 12 years of age, suggesting that prenatal DINP exposure may be related to impulsive behavior, a key aspect of ADHD. This result aligns with the results observed here with increased DINP exposure being associated with an increase in ADHD behaviors on the CBCL at 3 years. This finding is interesting because DINP is used as a replacement for DEHP, and while DINP was associated with increased ADHD problems at 3 years, DEHP was not associated with any measures of attention at 3 years, and at 2 years, DEHP was actually associated with decreases in all measures of attention problems. In the present study, prenatal exposure to MEP was associated with increases in externalizing behavior, ADHD problems, and attention problems at both 2 and 3 years of age. While the results of previous studies have also been mixed for associations of MEP with neurodevelopmental outcomes, these results add to the majority indicating that prenatal MEP exposure is associated with poorer outcomes related to attention (Table 1.2). While Gascon et al. (2015) found that MEP was associated with a decreased risk of inattention problems at 4 years, Engel et al. (2010), found that prenatal MEP exposure was associated with increased externalizing behavior and attention problems in children 4 to 9 years of age, and Hyland et al. (2019) also observed that MEP was associated with an increase in errors of commission at 9 and 12 years of age, like mono-carboxyisooctyl phthalate (MCOP), a metabolite of DINP. Together, these results suggest that prenatal exposure to DINP, an antiandrogenic phthalate, and MEP, which is not antiandrogenic, may adversely impact attention in early childhood and into middle childhood.

6.4.2. Prenatal acetaminophen exposure and attention

In contrast to the mixed results for phthalates, acetaminophen was consistently associated with increased attention problems at 2 and 3 years with exposure during the second trimester and cumulative exposure throughout pregnancy associated with higher scores on all three scales at both ages (Figure 6.3). At 7-8 months, prenatal acetaminophen exposure in any individual trimester was not associated with attention, but there was an association of cumulative exposure throughout pregnancy with improved attention (shorter time to familiarization) in male children (Figure 5.2H), a result that is not consistent with the findings of increased attention problems at 2 and 3 years. There are a few possibilities for these discrepancies. As discussed in Chapter 4, it may be that the primary measure of attention used at 7-8 months (time to familiarization) is not the best method of assessing attention in infants. As described earlier, in the VRM assessment, infants are required to look at the stimuli for a specific amount of time in the familiarization trial before moving on to the test trials instead of ending the familiarization trial when they looked away from the screen for long periods of time (i.e., became habituated to the stimuli). Due to this design, infants who were more attentive may have become familiarized with the stimuli before accumulating the specified amount of looking time and subsequently looked away for longer periods of time, resulting in longer rather than shorter times to familiarizations for more attentive infants. Future studies will investigate this possibility.

While it was hypothesized that male children specifically would show higher scores on measures of attention at 2 and 3 years, indicating more attention-related problem behaviors, with greater prenatal acetaminophen exposure, this was not observed (Figure 6.3). Instead, both male and female children showed higher scores on all measures of attention at both 2 and 3 years. These results suggest that effects of prenatal exposure to acetaminophen on attention are not sex-specific, indicating that effects on attention may not be related to its antiandrogenic

activity. Other theories of how prenatal acetaminophen exposure may exert its effects on later attentional development include via disruption of the endocannabinoid system or increased oxidative stress, both of which have been linked to ADHD (Navarrete et al., 2020; Bauer et al., 2018). Interestingly, exposure during the second trimester was hypothesized to be associated with more attention problems, and this was indeed the case even though the pattern of results did not provide strong support for the hypothesis that acetaminophen is acting via antiandrogenic mechanism.

A few previous studies have examined the potential effect of prenatal exposure to acetaminophen on child behavior using the CBCL, but only two have found that acetaminophen was associated with an increase in attention-related outcomes, specifically externalizing behavior, at 5 years (Trønnes et al., 2020) and 3 years (Brandlistuen et al., 2013), while another found no relationship between acetaminophen and attention-related behaviors measured via the CBCL at 48 months (Tovo-Rodrigues et al., 2020). However, many other previous studies have found associations of prenatal acetaminophen exposure and attention using other assessment instruments (Table 1.1). In addition to Trønnes et al. (2020), other studies have observed an association between prenatal exposure to acetaminophen and increased externalizing behavior at 11 years (Inoue et al., 2020), poorer attention in early childhood (Liew et al., 2016a; Avella-Garcia et al., 2016; Golding et al., 2019), and ADHD diagnosis or related behaviors (Liew et al., 2014; Thompson et al., 2014; Ystrom et al., 2017; Ji et al., 2018; Liew et al., 2019; Ji et al., 2020) utilizing other scales and methods, such as the Strengths and Difficulties Questionnaire (SDQ) and medical records. While all of these previous studies have used cohorts much larger than that used in the present analyses, the present study was able to measure acetaminophen exposure throughout pregnancy more accurately because participants were surveyed about their medication use six times during pregnancy, while the previous studies surveyed participants two times during pregnancy, at most (Table 1.1). Despite potential concerns with

self-report measures of acetaminophen use during pregnancy leading to recall bias, and the small sample size of this study compared to previous studies, our findings were remarkably consistent. They indicate that prenatal exposure to acetaminophen during the second trimester as well as cumulative exposure throughout pregnancy was significantly associated with increases in attention-related problems measured via a widely used, standardized survey, providing additional evidence that prenatal acetaminophen exposure may be negatively impacting attentional development, and that this association can be reliably detected early in childhood. Given these results and the prevalence of acetaminophen use during pregnancy (Brandlistuen et al., 2013; Thorpe et al., 2013; Servey and Chang, 2014; Ji et al., 2018), the safety of acetaminophen use during pregnancy for child neurodevelopment should be reevaluated.

6.4.3. Cumulative effects of exposure to both phthalates and acetaminophen on attention

The exploration of potential interactive effects of prenatal exposure to both phthalates and acetaminophen provided little evidence that the impact of prenatal exposure to acetaminophen on attention was modified by phthalate exposure, although medium MEP exposure moderately increased the effect of prenatal acetaminophen exposure on ADHD problems (Figure 6.4). While it was hypothesized that combined prenatal exposure to antiandrogenic phthalates and acetaminophen, which also has antiandrogenic activity (Kristensen et al., 2011; Kristensen et al., 2012; Mazaud-Guittot et al., 2013; van den Driesche et al., 2015; Holm et al., 2016; Lind et al., 2017; Hay-Schmidt et al., 2017; Hurtado-Gonzalez et al., 2018), would result in an increase in attention problems, the effects of antiandrogenic phthalates on attention-related outcomes at 2 and 3 years old were inconsistent, and the only indication that the impact of acetaminophen exposure was modified by exposure to phthalates was for a phthalate metabolite that does not have antiandrogenic activity, MEP. While the results of the interaction analyses are interesting and suggest there may be a cumulative effect

of prenatal exposure to MEP and acetaminophen, the relatively small number of participants in this study likely resulted in insufficient statistical power to reliably detect whether prenatal phthalate exposure modified the effect of acetaminophen on attention problems. Categorizing phthalate exposure as low, medium, or high, resulted in small numbers of children included in each category which substantially reduced power compared to analyses of individual phthalate or acetaminophen exposure. In addition, the distributions of acetaminophen exposure within each category of phthalate exposure were often uneven; for example, children categorized as having high MEP exposure at 2 or 3 years also tended to have higher prenatal acetaminophen exposure than children in the low or medium MEP exposure groups, but at 2 years, children categorized as having medium DEHP exposure had higher prenatal acetaminophen exposure than children in the low or medium acetaminophen groups. Therefore, future studies should be conducted to examine the potential for interactive effects of exposure to both phthalates and acetaminophen in larger cohorts with better statistical power (Survey and Chang, 2014; Johns et al., 2017).

6.4.4. Summary and Conclusions

At 2 and 3 years, prenatal phthalate exposure had inconsistent effects on outcomes related to attention with associations of a given phthalate often differing between ages, and associations of phthalates differing by child sex and across the different attention-related outcomes. Associations were also not consistent among phthalates with antiandrogenic activity. However, associations of prenatal acetaminophen exposure with measures of attention at 2 and 3 years were highly consistent indicating associations of prenatal acetaminophen with increases in attention problems as measured by all three scales and at both ages. These results also indicated that second trimester exposure is particularly important in this association. Acetaminophen is one of the few medications, and the only analgesic, pregnant women are told they can take without concern of it negatively impacting fetal development (Survey and Chang,

2014; Cendjas-Hernandez et al., 2020). The results of this study add to the mounting evidence indicating this guidance needs to be reassessed.

6.5. TABLES AND FIGURES

Table 6.1. Correlations among CBCL outcome measures related to attention at both 2 and 3 years.

	2 Years		3 Years	
	1.	2.	1.	2.
1. Attention Problems	--	--	--	--
2. ADHD Problems	0.81	--	0.84	--
3. Externalizing Behavior T-score	0.73	0.85	0.68	0.8

Table 6.2. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal phthalate exposure with attention-related outcomes on the CBCL at 2 years.¹

		16-18 weeks gestation sample		
Outcome	Exposure	Main Effect	Stratified by Sex	
			Females	Males
Attention Problems	Σ AA Phthalates	-0.032 (0.082, -0.145)	-0.030 (0.091, -0.151)	-0.043 (0.295, -0.382)
	Σ DEHP	0.003 (0.038, -0.032)	0.004 (0.040, -0.031)	-0.057 (0.161, -0.275)
	Σ DINP	-0.013 (0.017, -0.042)	-0.019 (0.016, -0.054)	0.001 (0.052, -0.051)
	MEP	0.015 (0.098, -0.068)	^b -0.018 (0.067, -0.102)	** 0.438 (0.748, 0.128)
	Σ DEHTP	-0.017 (0.040, -0.075)	^a 0.002 (0.063, -0.058)	[#] -0.164 (0.003, -0.331)
ADHD Problems	Σ AA Phthalates	-0.066 (0.092, -0.224)	-0.069 (0.101, -0.238)	-0.047 (0.416, -0.510)
	Σ DEHP	0.001 (0.051, -0.048)	0.003 (0.052, -0.047)	-0.045 (0.260, -0.351)
	Σ DINP	-0.023 (0.018, -0.064)	-0.032 (0.018, -0.081)	-0.004 (0.068, -0.076)
	MEP	0.034 (0.150, -0.082)	^b 0 (0.119, -0.120)	* 0.475 (0.912, 0.038)
	Σ DEHTP	-0.059 (0.017, -0.136)	^a -0.035 (0.046, -0.116)	* -0.240 (-0.016, -0.463)
Externalizing Behavior T-Score	Σ AA Phthalates	-0.457 (0.150, -1.064)	-0.560 (0.089, -1.209)	0.285 (2.056, -1.486)
	Σ DEHP	-0.080 (0.109, -0.269)	-0.063 (0.128, -0.254)	-0.703 (0.467, -1.873)
	Σ DINP	-0.074 (0.083, -0.231)	-0.155 (0.035, -0.345)	0.096 (0.372, -0.180)
	MEP	0.035 (0.480, -0.410)	-0.032 (0.430, -0.494)	0.890 (2.580, -0.800)
	Σ DEHTP	-0.099 (0.191, -0.388)	^b 0.039 (0.342, -0.264)	** -1.123 (-0.287, -1.960)
		Pooled sample		
Outcome	Exposure	Main Effect	Stratified by Sex	
			Females	Males
Attention Problems	Σ AA Phthalates	-0.044 (0.065, -0.153)	-0.021 (0.093, -0.706)	-0.320 (0.077, -0.718)
	Σ DEHP	-0.012 (0.096, -0.120)	^b 0.030 (0.144, -0.084)	* -0.331 (-0.016, -0.646)
	Σ DINP	-0.039 (0.049, -0.127)	-0.029 (0.075, -0.134)	-0.063 (0.101, -0.227)
	MEP	0.073 (0.175, -0.029)	0.024 (0.148, -0.100)	0.174 (0.351, -0.003)
	Σ DEHTP	0.033 (0.250, -0.183)	^a 0.235 (0.532, -0.063)	-0.181 (0.126, -0.488)
ADHD Problems	Σ AA Phthalates	-0.096 (0.056, -0.249)	-0.075 (0.083, -0.234)	-0.345 (0.208, -0.899)
	Σ DEHP	-0.013 (0.137, -0.164)	0.031 (0.191, -0.128)	-0.352 (0.090, -0.793)
	Σ DINP	-0.039 (0.083, -0.162)	-0.004 (0.141, -0.150)	-0.125 (0.104, -0.353)
	MEP	0.046 (0.189, -0.096)	-0.010 (0.163, -0.184)	0.162 (0.410, -0.086)
	Σ DEHTP	0.056 (0.347, -0.234)	0.222 (0.624, -0.181)	-0.119 (0.295, -0.534)
Externalizing Behavior T-Score	Σ AA Phthalates	-0.451 (0.134, -1.036)	-0.425 (0.185, -1.035)	-0.754 (1.376, -2.884)
	Σ DEHP	-0.207 (0.372, -0.786)	-0.031 (0.582, -0.645)	-1.540 (0.155, -3.236)
	Σ DINP	-0.077 (0.395, -0.549)	-0.145 (0.415, -0.706)	0.092 (0.973, -0.789)
	MEP	0.116 (0.666, -0.433)	0.043 (0.714, -0.628)	0.265 (1.223, -0.692)
	Σ DEHTP	0.103 (1.197, -0.992)	^b 1.174 (2.678, -0.331)	-1.035 (0.515, -2.586)

¹All models were adjusted for maternal age, maternal education, household income, child sex, and number of older siblings.

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

[#] $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

Table 6.3. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal phthalate with attention-related outcomes on the CBCL at 3 years.¹

		16-18 weeks gestation sample		
		Stratified by Sex		
Outcome	Exposure	Main Effect	Females	Males
Attention Problems	Σ AA Phthalates	-0.016 (0.091, -0.122)	-0.005 (0.110, -0.120)	-0.081 (0.201, -0.363)
	Σ DEHP	-0.001 (0.034, -0.037)	0.004 (0.041, -0.032)	-0.102 (0.056, -0.260)
	Σ DINP	0.019 (0.058, -0.020)	0.022 (0.076, -0.032)	0.016 (0.073, -0.041)
	MEP	0.017 (0.107, -0.074)	^b -0.033 (0.069, -0.135) [#]	0.183 (0.370, -0.005)
	Σ DEHTP	-0.029 (0.023, -0.082)	-0.019 (0.037, -0.076)	-0.089 (0.048, -0.226)
ADHD Problems	Σ AA Phthalates	-0.063 (0.105, -0.231)	-0.057 (0.125, -0.239)	-0.099 (0.345, -0.542)
	Σ DEHP	-0.017 (0.039, -0.073)	-0.007 (0.050, -0.065)	-0.195 (0.053, -0.442)
	Σ DINP	0.025 (0.087, -0.037)	0.021 (0.106, -0.064)	0.029 (0.119, -0.060)
	MEP	0.035 (0.177, -0.107)	-0.018 (0.144, -0.180)	0.211 (0.507, -0.085)
	Σ DEHTP	-0.056 (0.138, -0.505)	^a -0.027 (0.055, -0.110) [*]	-0.224 (-0.024, -0.425)
Externalizing Behavior T-Score	Σ AA Phthalates	-0.559 (0.117, -1.234)	-0.602 (0.129, -1.332)	-0.301 (1.493, -2.095)
	Σ DEHP	-0.144 (0.082, -0.371)	-0.115 (0.117, -0.347)	-0.671 (0.336, -1.678)
	Σ DINP	-0.080 (0.171, -0.330)	-0.262 (0.081, -0.606)	0.122 (0.483, -0.239)
	MEP	0.080 (0.657, -0.496)	-0.043 (0.615, -0.701)	0.490 (1.693, -0.714)
	Σ DEHTP	-0.184 (0.138, -0.505)	^b -0.052 (0.289, -0.393) [*]	-0.968 (-0.138, -1.798)
		Pooled sample		
		Stratified by Sex		
Outcome	Exposure	Main Effect	Females	Males
Attention Problems	Σ AA Phthalates	-0.001 (0.104, -0.106)	0.009 (0.120, -0.101)	-0.105 (0.245, -0.456)
	Σ DEHP	0.025 (0.115, -0.064)	0.035 (0.130, -0.061)	-0.043 (0.215, -0.301)
	Σ DINP	0.066 (0.150, -0.017)	0.077 (0.179, -0.025)	0.045 (0.191, -0.102)
	MEP	0.002 (0.099, -0.094)	-0.052 (0.103, -0.207)	0.037 (0.161, -0.087)
	Σ DEHTP	0.053 (0.256, -0.149)	0.060 (0.316, -0.197)	0.042 (0.381, -0.297)
ADHD Problems	Σ AA Phthalates	-0.062 (0.109, -0.233)	-0.070 (0.110, -0.250)	0.013 (0.566, -0.539)
	Σ DEHP	0.009 (0.149, -0.132)	0.028 (0.178, -0.122)	-0.130 (0.275, -0.535)
	Σ DINP	* 0.135 (0.265, 0.004)	0.145 (0.305, -0.014)	0.112 (0.341, -0.117)
	MEP	-0.027 (0.125, -0.178)	-0.121 (0.122, -0.364)	0.034 (0.227, -0.160)
	Σ DEHTP	-0.046 (0.253, -0.345)	-0.024 (0.355, -0.402)	-0.084 (0.415, -0.583)
Externalizing Behavior T-Score	Σ AA Phthalates	-0.378 (0.288, -1.045)	-0.436 (0.261, -1.134)	0.220 (2.455, -2.016)
	Σ DEHP	-0.129 (0.439, -0.697)	-0.103 (0.503, -0.709)	-0.316 (1.327, -1.960)
	Σ DINP	0.363 (0.896, -0.171)	0.181 (0.831, -0.468)	0.736 (1.667, -0.195)
	MEP	-0.217 (0.397, -0.83)	-0.457 (0.528, -1.442)	-0.063 (0.722, -0.849)
	Σ DEHTP	-0.287 (0.949, -1.522)	-0.237 (1.327, -1.802)	-0.372 (1.691, -2.435)

¹All models were adjusted for maternal age, maternal education, household income, child sex, and number of older siblings.

Interaction p-value: ^a p < 0.10, ^b p < 0.05

[#]p < 0.10, ^{*}p < 0.05

Table 6.4. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with attention-related outcomes on the CBCL at 2 and 3 years.¹

Outcome	Exposure	2 Years		3 Years	
		Main Effect		Main Effect	
Attention Problems	1st Trimester	0.045 (0.112, -0.022)		0.047 (0.103, -0.009)	
	2nd Trimester	** 0.070 (0.115, 0.024)		** 0.093 (0.141, 0.044)	
	3rd Trimester	0.022 (0.054, -0.010)		0.003 (0.029, -0.023)	
	Full Pregnancy	** 0.087 (0.152, 0.023)		** 0.103 (0.169, 0.037)	
ADHD Problems	1st Trimester	0.043 (0.137, -0.051)		# 0.080 (0.163, -0.003)	
	2nd Trimester	** 0.087 (0.152, 0.022)		** 0.142 (0.218, 0.067)	
	3rd Trimester	0.026 (0.07, -0.019)		-0.002 (0.038, -0.042)	
	Full Pregnancy	* 0.107 (0.198, 0.015)		** 0.156 (0.258, 0.055)	
Externalizing Behavior T-Score	1st Trimester	0.156 (0.519, -0.206)		0.171 (0.511, -0.17)	
	2nd Trimester	* 0.322 (0.573, 0.072)		** 0.440 (0.748, 0.132)	
	3rd Trimester	0.115 (0.286, -0.057)		0.101 (0.264, -0.061)	
	Full Pregnancy	* 0.400 (0.753, 0.046)		* 0.500 (0.913, 0.086)	

¹All models were adjusted for acetaminophen formulation, maternal age, delivery type, household income, child sex, and number of older siblings.

#p < 0.10, *p < 0.05, **p < 0.01

Table 6.5. Phthalate exposure cutoffs for acetaminophen and phthalate interaction analyses presented in Chapter 6.

		2 Years			
Phthalate exposure	Units	16-18 weeks sample		Pooled sample	
		25th percentile	75th percentile	25th percentile	75th percentile
ΣDINP	μmol/L	--	--	0.019	0.084
MEP	μg/L	0.060	0.228	--	--
ΣDEHTP	μmol/L	0.036	0.205	0.084	0.480

		3 Years			
Phthalate exposure	Units	16-18 weeks sample		Pooled sample	
		25th percentile	75th percentile	25th percentile	75th percentile
ΣDINP	μmol/L	--	--	0.021	0.091
MEP	μg/L	0.056	0.224	--	--
ΣDEHTP	μmol/L	0.032	0.183	0.082	0.471

Table 6.6. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with attention-related outcomes on the CBCL at 2 (top) and 3 years (bottom) at different levels of phthalate exposure.¹

Outcome		Interaction p-value	Stratified by Phthalate Exposure Level		
2 Years	Exposure Interaction		Low	Medium	High
Attention Problems	2nd Trimester x MEP PB ²	0.001	** 1.567 (2.027, 1.107)	** 1.939 (2.290, 1.589)	** 1.849 (2.287, 1.412)
	2nd Trimester x Σ DEHTP Pool	< 0.001	** 1.704 (2.310, 1.098)	** 1.822 (2.286, 1.358)	** 2.234 (2.849, 1.618)
	Full Pregnancy x MEP PB	0.0002	** 1.471 (1.034, 1.034)	** 1.865 (1.551, 2.178)	** 1.736 (1.313, 2.159)
	Full Pregnancy x Σ DEHTP Pool	0.012	** 1.868 (2.437, 1.299)	** 1.862 (2.289, 1.436)	** 2.013 (2.601, 1.426)
ADHD Problems	2nd Trimester x MEP PB	0.066	^a ** 3.093 (3.745, 2.441)	^a ** 3.849 (4.346, 3.352)	** 3.540 (4.161, 2.920)
	Full Pregnancy x MEP PB	0.021	^a ** 3.049 (3.667, 2.432)	^a ** 3.836 (4.279, 3.393)	** 3.462 (4.059, 2.864)
Outcome		Interaction p-value	Stratified by Phthalate Exposure Level		
3 Years	Exposure Interaction		Low	Medium	High
Attention Problems	2nd Trimester x MEP PB	0.001	** 1.304 (1.781, 0.826)	** 1.524 (1.873, 1.175)	** 1.628 (2.074, 1.181)
	Full Pregnancy x MEP PB	0.001	** 1.195 (1.658, 0.734)	** 1.443 (1.767, 1.120)	** 1.545 (1.985, 1.105)
ADHD Problems	1st Trimester x Σ DEHTP PB	0.003	** 3.420 (4.336, 2.504)	** 2.847 (3.546, 2.149)	** 2.507 (3.595, 1.420)
	1st Trimester x Σ DINP Pool	0.013	** 3.311 (4.025, 2.596)	** 2.792 (3.343, 2.241)	** 2.853 (3.600, 2.107)
	Full Pregnancy x Σ DINP Pool	0.030	** 3.247 (3.933, 2.562)	** 2.800 (3.317, 2.284)	** 2.920 (3.636, 2.204)
	Full Pregnancy x Σ DEHTP PB	0.063	** 3.357 (4.221, 2.492)	** 2.846 (3.492, 2.199)	** 2.298 (3.828, 0.767)

¹All models were adjusted for acetaminophen formulation, maternal age, household income, delivery type, child sex, and number of older siblings.

²PB indicates sample from 16-18 weeks of gestation.

Post hoc comparison: ^a p < 0.10

** p < 0.01

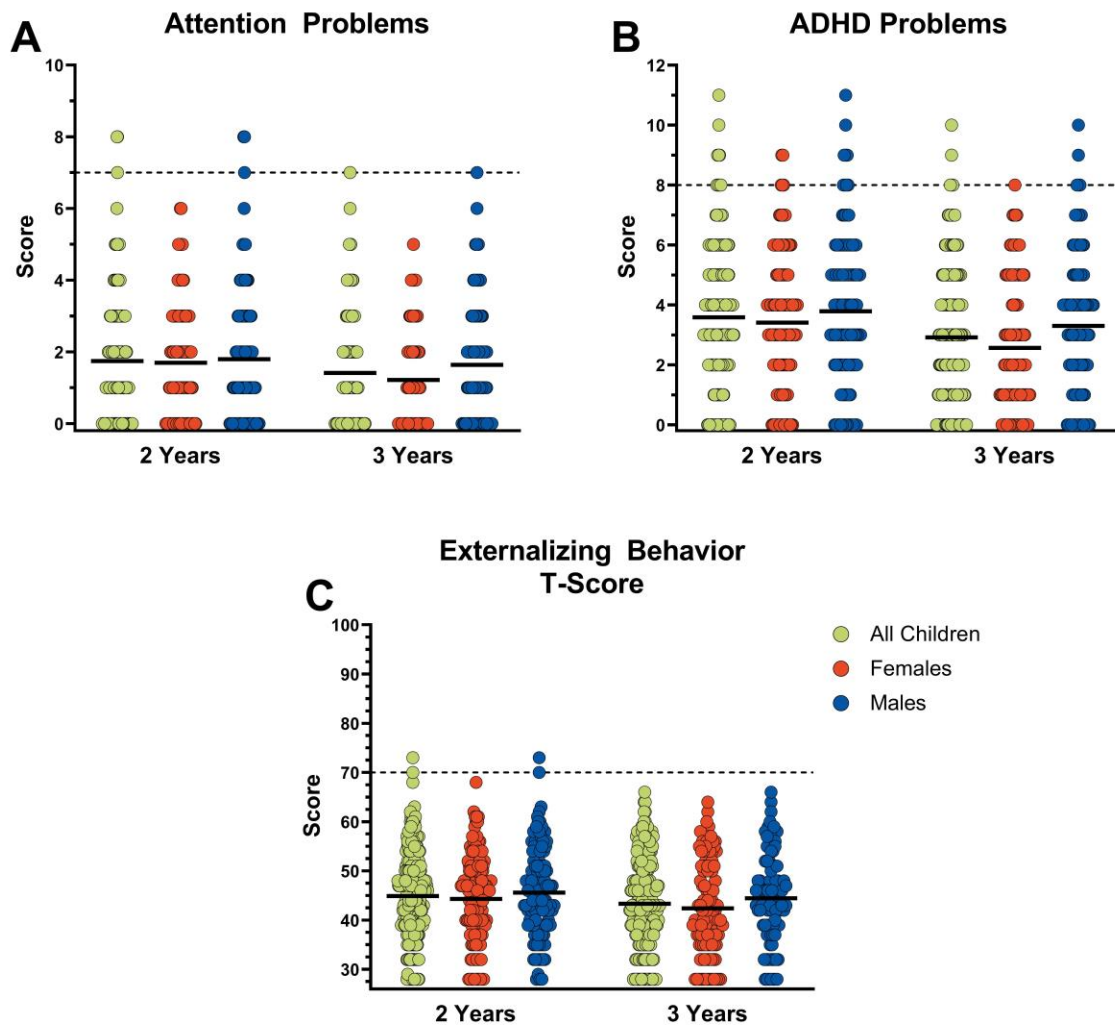


Figure 6.1. Distribution and mean of CBCL scores for all children and by child sex at 2 and 3 years. The y-axis spans the minimum score to the maximum score for each outcome. On average, female children had lower scores compared to male children. (A) The mean attention problems score for all children, females, and males at both 2 and 3 years of age was well-below the point at which scores are considered to be of clinical concern, indicated by the dotted line. At 2 years, only four children had scores at or above that point, all of which were males. At 3 years, only one child, a male, had an attention score at the point of clinical concern. (B) The mean ADHD problems score for all children, females, and males at both 2 and 3 years were below the point at which scores are considered to be of clinical concern. At 2 years, 14 children, 5 females and 9 males, had ADHD problems scores at or above that point, and at 3 years, 6 children, one female and 5 males, had scores at or above that point. (C) The mean externalizing behavior t-score for all children, females, and males at both 2 and 3 years was below the point of clinical concern. At 2 years, two children, both males, had scores at or above this point.

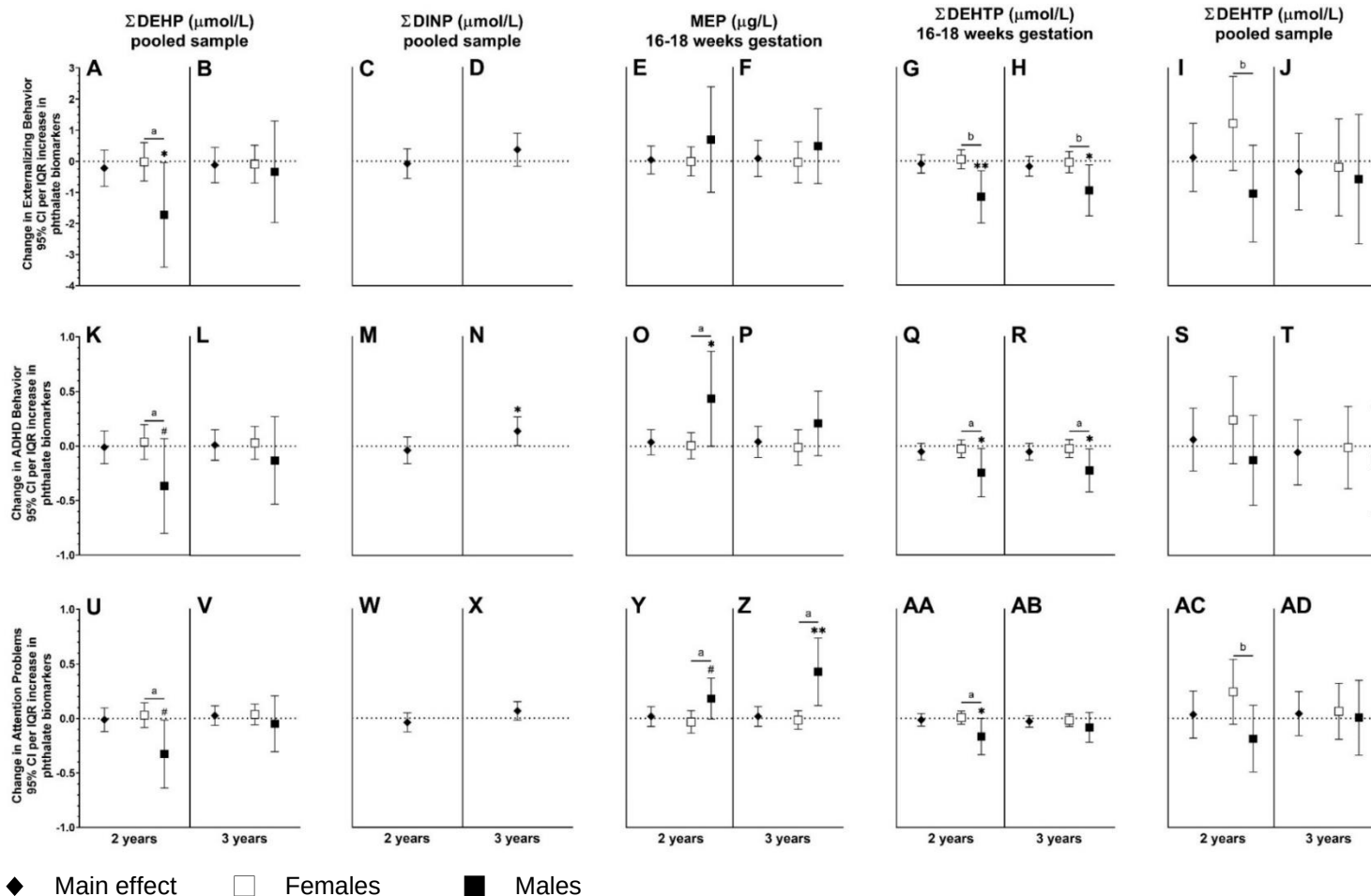


Figure 6.2. Associations of prenatal phthalate exposure and attention-related outcomes on the CBCL at 2 and 3 years of age. Σ DEHP in the pooled sample was associated with decreases in (A, B) externalizing behavior, (K, L) ADHD problems, and (U, V) attention problems in male children at 2 years of age. Σ DINP in the pooled sample was only associated with an increase in ADHD problems at 3 years (N). MEP in the 16-18 weeks sample was associated with an increase in (O) ADHD problems at 2 years and (Y, Z) attention problems in male children at 2 and 3 years. Σ DEHTP in both the 16-18 weeks sample was associated with a decrease in (G, H) externalizing behavior and (Q, R) ADHD problems at 2 and 3 years and (AA) attention problems in male children at 2 years. Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$, # $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

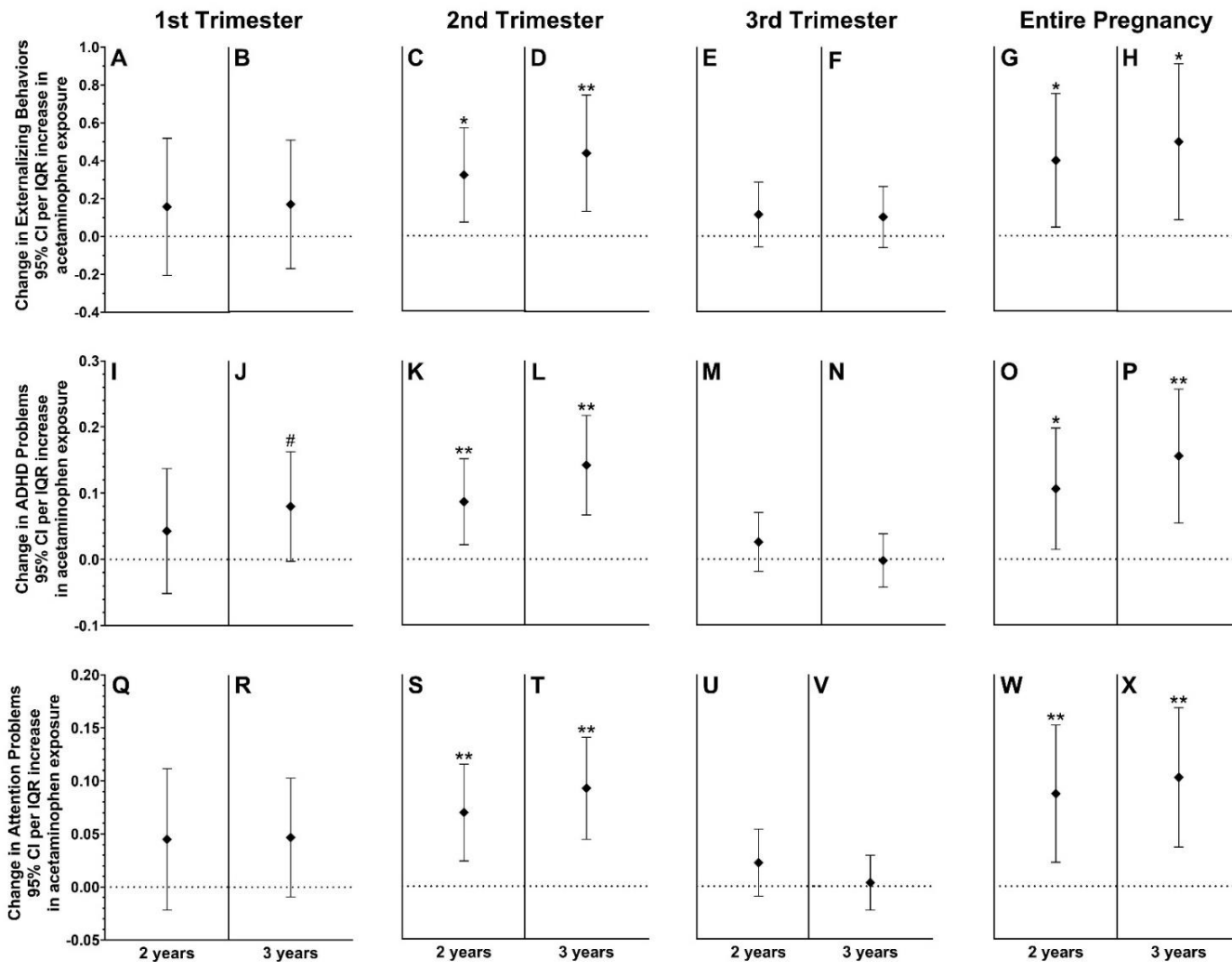


Figure 6.3. Associations of prenatal acetaminophen exposure and attention-related outcomes on the CBCL at 2 and 3 years of age. Acetaminophen exposure during (A, B, I, J, Q, R) the first trimester, (C, D, K, L, S, T) second trimester, (E, F, M, N, U, V) third trimester, and (G, H, O, P, W, X) throughout pregnancy was associated with an increase in externalizing behavior, ADHD problems, and attention problems at both 2 and 3 years.

* $p < 0.05$, ** $p < 0.01$

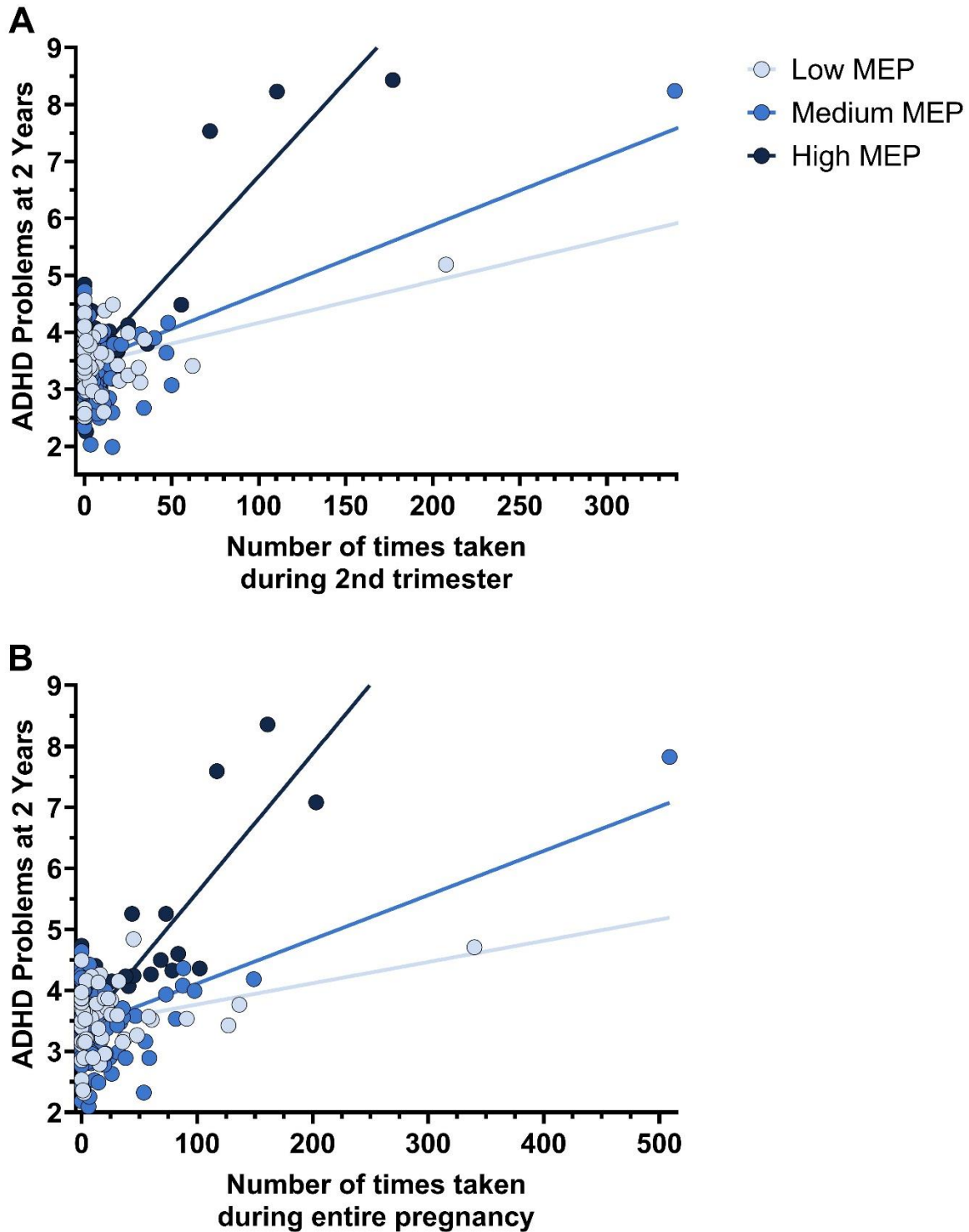


Figure 6.4. The association of acetaminophen exposure during (A) the second trimester and (B) the entire pregnancy and ADHD Problems at 2 years by levels MEP exposure. Acetaminophen exposure in either only the second trimester or throughout pregnancy was associated with an increase in ADHD problems at 2 years within all levels of MEP exposure, but there was only a borderline significant difference between the low and medium groups.

CHAPTER 7: ASSOCIATIONS OF PRENATAL EXPOSURE TO PHTHALATES AND ACETAMINOPHEN WITH MEASURES OF EARLY LANGUAGE DEVELOPMENT IN 2- AND 3-YEAR-OLD CHILDREN

7.1. INTRODUCTION

As described previously, early language development is a critical part of cognitive development that can be predictive of later cognitive abilities (Lee, 2011). Additionally, there is evidence that language development is not only influenced by factors in the child's postnatal environment, such as the quality and quantity of language the child is exposed to, family socioeconomic status, and the level of education of the child's primary caregiver (Hadley et al., 2011; Rowe, 2012; Fernald et al., 2013), but by family history (Zubrick et al., 2007), but also by the prenatal environment. Both Whitehouse et al. (2012) and Hollier et al. (2013) found that higher levels of circulating testosterone during prenatal development were associated with poorer language outcomes in male children. Specifically, Whitehouse et al. (2012) observed that higher concentrations of testosterone during prenatal development were associated with a higher risk of language delay in male children but a lower risk of language delay in female children between 1 and 3 years of age, while Hollier et al. (2013) observed that higher prenatal testosterone exposure was associated with smaller vocabularies in males only at 2 years of age.

Despite these interesting results indicating that differing levels of hormones during prenatal development may impact later language development, few studies have investigated the relationship between prenatal exposure to endocrine disrupting chemicals (EDCs) and language development. The few studies that have been done examining the potential relationship of prenatal exposure to either phthalates or acetaminophen with language development found evidence to suggest there may be delays or deficits related to both of these exposures, but further research is needed to better understand the potential for prenatal exposure to impact language development (Skovlund et al., 2017; Bornehag et al., 2018a;

Bornehag et al., 2018b; Olesen et al., 2018). Both Bornehag et al. (2018b) and Olesen et al. (2018) found evidence suggesting prenatal exposure to specific phthalates may be related to problems in language development. While both studies examined many of the same phthalate metabolites, only Olesen et al. (2018) also considered parent phthalate sums, specifically di-(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP), and found evidence suggesting that antiandrogenic phthalates (mono-benzyl phthalate [MBzP] and Σ DEHP as well as three of its metabolites individually) and monoethyl phthalate (MEP) may be related to language problems in boys only. Bornehag et al. (2018a) looked at two cohorts using different measures of language development and also found that antiandrogenic phthalates (MBP and MBzP) and MEP were associated with increased language delay in both males and females, although they only observed associations for two antiandrogenic phthalates and only in one of the two cohorts. Additionally, they used different methods to evaluate language development. Bornehag et al. (2018b) utilized a clinician's evaluation of language development to determine whether a child had delayed language development, and Olesen et al. (2018) used the MacArthur-Bates Communicative Development Inventory (CDI) to assess children's language development repeatedly from 16 months to 36 months of age.

In a sample of children from the same study cohort used by Bornehag et al. to assess phthalate exposure (2018a), Bornehag et al. (2018b) found evidence to suggest there is a relationship between prenatal exposure to acetaminophen and delayed language development, but only in girls; however, maternal report of acetaminophen use during pregnancy was only collected once early in pregnancy. In another large cohort, Skovlund et al. (2017) used a validated language competency scale and the Ages and Stages Questionnaire (ASQ) to evaluate language and communication development and found acetaminophen exposure during prenatal development was also associated with lower communication skills.

The present study utilized data from the IKIDS cohort collected at both 2 and 3 years of age to examine the relationship between prenatal exposure to phthalates, acetaminophen and the potential cumulative effects of these two common exposures on language development. Greater exposure to either phthalates or acetaminophen was hypothesized to be associated with lower scores at 2 years as measured on the CDI (Fenson et al., 2007), and at 3 years as measured via the Speech and Language Assessment Scale (SLAS; Hadley and Rice, 1993), especially for male children. It was also hypothesized that children with high exposure to both phthalates and acetaminophen would be most severely impacted at both ages, particularly males, and that exposures during the second trimester would be the most sensitive to the actions of these compounds given their antiandrogenic activity (Auyeung et al. 2013).

7.2. METHODS

7.2.1. Study Cohort

Eligibility criteria and recruitment of the IKIDS cohort are described in detail in Chapter 3. In brief, pregnant women were recruited between 10 and 14 weeks of gestation from two local obstetrics clinics. At the time of enrollment, baseline demographic information was collected including sociodemographic factors, reproductive and health history, and lifestyle factors. Follow-up interviews occurred every 4-6 weeks during pregnancy and four times during the infant's first year of life. When children reached 2 years (26.5 - 28.5 months) of age, mothers were contacted and asked to participate in an additional follow-up by phone and mail. This was repeated when children reached 3 years (36 - 38 months) of age. This section focuses on the language assessments done at these two early childhood time points.

7.2.2. Exposure Assessment

Details about exposure assessment are provided in Chapters 3. Women provided five first-morning urine samples during pregnancy at approximately 10-14, 16-18, 22-24, 28-30, and 34-36 weeks of gestation. For each participant, a pooled sample was created from these 5

individual urine collections. Both the pooled sample and a sample from the 16–18-week time point were sent to the CDC Division of Laboratory Sciences in Atlanta, Georgia for analysis of phthalate metabolites. The pooled sample allowed for an estimation of the average exposure across pregnancy, and the 16-18 week was collected at a time point that may be especially vulnerable to adverse effects of EDCs because this is within the window of peak androgen production in male fetuses (Auyeung et al., 2013). Phthalate metabolites were measured by online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry (Silva et al., 2007). All concentrations were adjusted using specific gravity to account for differences in urine dilution. Because some phthalates, such as DEHP and DINP, are rapidly metabolized into multiple urinary metabolites, weighted molar sums were calculated for these parent compounds. As described in previous chapters, the present analyses focused on Σ DEHP, Σ DINP, MEP, di-2-ethylhexyl terephthalate (Σ DEHTP), and an antiandrogenic sum (Σ AA).

Information about acetaminophen use during pregnancy was collected at the same 5 time points the urine samples were collected, as well as immediately after the child's birth, representing use during six time periods of gestation (see Chapter 3). Data from these time periods were combined to examine the relationship between the acetaminophen exposure (number of days used) and language development, both within each trimester of pregnancy and across the entire pregnancy.

7.2.3. Language Development Assessments

As described in Chapter 6, mothers who agreed to participate in the follow-up assessments at 2 and 3 years were sent packets of questionnaires which included questions about their child's overall development, diet, behavior, and language development, as well as maternal depression, maternal stress, maternal social support, and their relationship with the child. At 2 years of age, CDI (Words and Sentences) was used to evaluate the child's language

development. As described in Chapter 1, the CDI is a parental report form which provides outcome measures that have established standardized scores by sex and age, including expressive vocabulary, language complexity, mean utterance length, and whether the child is combining words. The CDI provides a checklist of 680 words for parents to indicate which words their child says to measure vocabulary and pairs of sentences for parents to indicate which of each pair sounds more like how their child speaks to measure complexity. To evaluate mean utterance length, parents were asked to provide three examples of the child's longest sentences. The examples were broken down into morphemes (the smallest unit of meaning) to calculate their mean utterance length. Raw scores were calculated and were converted to percentile scores based on the child's age and sex, and whether the child's percentile score was above or ≤ 15 th percentile and ≤ 25 th percentile was indicated. Analyses were conducted using raw scores in generalized linear regression models and percentile scores categorized as ≤ 15 th percentile and ≤ 25 th percentile in logistic regression models to calculate odds ratios.

The SLAS was used to evaluate language development at 3 years. Mothers were again invited to participate in a follow-up assessment via mail and phone when the child was between 36 and 38 months of age. As at the 2-year follow-up, those who agreed to participate were sent a packet of questionnaires to complete regarding the child's overall development, behavior, and language development, as well as maternal depression, maternal stress, maternal social support, and their relationship with the child. As described in Chapter 3, the SLAS has been found to be reliable and correlated with other language development measures, including the CDI. The SLAS is a short questionnaire with Likert scale questions which evaluate 9 categories of language development: language comprehension, cultural awareness, speaking too loudly or softly, talkativeness, assertiveness, responsiveness, semantics, syntax, and articulation. In the initial study using the SLAS, five items pertaining to comprehension, cultural awareness, and speaking too loudly or softly were found to be unreliable, and studies done since have excluded

these questions and categories from their analyses (Hadley and Rice, 1993; Ratner and Silverman, 2000; Newman et al., 2006). Talkativeness is based on one question; therefore, most studies have also excluded it from analyses (Ratner and Silverman, 2000; Newman et al., 2006). The outcomes measured in the present study were assertiveness (3 questions), responsiveness (2 questions), semantics (3 questions), syntax (2 questions), and articulation (3 questions). The scores for the measures of responsiveness and syntax ranged from 1 to 14, and the scores of assertiveness, semantics, and articulation ranged from 1 to 21.

7.2.4. Covariates

Multivariable generalized linear regression models were used to evaluate associations between the previously described exposures: for phthalates (MEP, Σ DEHP, Σ DINP, Σ DEHTP, and Σ AA), both pooled and at 16-18 weeks; for acetaminophen, (first trimester, second trimester, third trimester, and exposure across pregnancy) and each outcome measure at 2 years using the CDI (expressive vocabulary, mean length of utterance, and sentence complexity) and at 3 years using the SLAS (assertiveness, responsiveness, semantics, syntax, and articulation). As described in previous chapters, when associations of an outcome with acetaminophen were significant, the potential interactive effects of prenatal exposure to both phthalates and acetaminophen were investigated.

The following sociodemographic factors described earlier were also considered for inclusion in models based on *a priori* knowledge and using directed acyclic graphs (DAGs, Figures 7.1, 7.2): maternal age, maternal parity (nulliparous vs. ≥ 1), maternal education (< bachelor's degree vs. bachelor's degree or higher), maternal verbal IQ, child age at time of the assessment, and delivery type (Cesarean section vs. vaginal delivery). In all models evaluating continuous outcomes, child sex was included as a potential modifier. Correlations of potential covariates with both exposure (phthalates or acetaminophen) and outcome (CDI and SLAS variables) were explored. Covariates were selected for inclusion in all models for a given

exposure (phthalates or acetaminophen) when they were correlated with exposure and at least one language outcome. The following covariates were selected for models investigating the relationship of prenatal exposure to phthalates and early measures of language development: maternal age, maternal parity, maternal education, and child delivery type. In models examining this relationship for outcomes on the CDI specifically, maternal verbal IQ was also included as a covariate (n = 35 missing maternal verbal IQ data). Sensitivity analyses were conducted including maternal race and ethnicity (white, non-Hispanic vs. all others) and maternal alcohol use (any vs none) as covariates and excluding women who reported smoking during the first trimester of pregnancy or for whom that data was missing (n = 17 at 2 years, n = 19 at 3 years).

Following a similar process, acetaminophen formulation (none, acetaminophen alone, or in combination with other drugs), maternal age, maternal parity, and maternal education were selected for inclusion in models investigating the relationship between prenatal acetaminophen exposure and language outcomes at both 2 and 3 years. Delivery type was also included as a covariate in models evaluating this relationship for outcomes on the CDI. For these models, sensitivity analyses were conducted including reason for taking acetaminophen (pain, illness, or other), marital status (married/living as married vs. single/separated/divorced/widowed), and maternal use of alcohol (any vs. none), and household income (< \$50,000, \$50,000-\$99,999, ≥ \$100,000), and excluding women who smoking during the first trimester.

In models investigating the potential cumulative effect of prenatal exposure to both acetaminophen and phthalates on outcomes measured at 2 years of age via the CDI, acetaminophen formulation, maternal age, maternal parity, maternal verbal IQ, maternal education, child age at the time of assessment, and delivery type were included in all models, and sensitivity analyses were conducted including reason for taking acetaminophen, paternal race and ethnicity (white, non-Hispanic vs. all others), and maternal alcohol use during the first trimester. In interaction models examining this relationship with outcomes measured at 3 years

via the SLAS, acetaminophen formulation, maternal age, maternal parity, maternal education, household income, and delivery type were included. Sensitivity analyses were conducted including maternal alcohol use during the first trimester, maternal race and ethnicity, and reported reason for taking acetaminophen as covariates in models; excluding participants who reported smoking during the first trimester or whose answer to that question was missing, and excluding participants who were not married or living as married (n = 16 at 2 years, n = 9 at 3 years).

7.2.5. Statistical Approach

Multivariate generalized linear regression models were used to evaluate the relationship between each individual phthalate exposure biomarker (Σ AA phthalates, Σ DEHP, Σ DINP, MEP, and Σ DEHTP in both the 16-18 weeks of gestation and pooled samples; acetaminophen use during the first, second, and third trimester, and throughout the entire pregnancy) and each continuous outcome at 2 years (vocabulary size, mean length of utterance, and sentence complexity) and 3 years (assertiveness, responsiveness, semantics, syntax, and articulation). Sex-by-exposure interactions were also examined, and interaction terms with p-values < 0.10 were retained in the final models. As described in previous chapters, where interactions were retained, both the main effect of exposure and stratum-specific estimates and confidence intervals are reported. Beta estimates were scaled to an IQR increase in exposure for ease of comparison across wide ranges of exposures, and associations were considered significant if p < 0.05.

Additionally, the raw scores were converted to percentile scores which were then categorized as $\leq 25^{\text{th}}$ or $> 25^{\text{th}}$ percentile and $\leq 15^{\text{th}}$ or $> 15^{\text{th}}$ percentile for the child's age and sex for each outcome variable for complementary analyses using multivariable logistic regression. This analysis allowed for characterization of the odds of a child having outcome scores which would classify them as having delayed language ($\leq 15^{\text{th}}$ percentile) or being on the

low end of normal ($\leq 25^{\text{th}}$ percentile) for their sex and age with increasing prenatal exposure, while analysis of the raw scores allowed for estimation of changes in scores with changes in prenatal exposure. For these models, child sex and age were not included because these variables are accounted for when raw scores are converted to percentile scores. Because language development is sexually dimorphic, with females developing language earlier and faster than males, results were also stratified by child sex to investigate the relationship between exposure and each outcome for each sex.

As described in previous chapters, analyses of the potential cumulative effects of exposure to both phthalates and acetaminophen during prenatal development were approached conservatively. For each outcome measure (CDI or SLAS), only in cases where both acetaminophen and phthalates on their own were significantly associated with an outcome were interactions explored. Acetaminophen-by-phthalate-by-child sex interactions were also explored, and where an interaction term was $p < 0.10$, the interaction was further examined by categorizing phthalate exposure as low ($< 25^{\text{th}}$ percentile), medium ($25^{\text{th}} - 75^{\text{th}}$ percentile), or high exposure ($> 75^{\text{th}}$ percentile) to assess the association of acetaminophen exposure with the outcome at each level of phthalate exposure.

For all analyses described above, influential observations were evaluated using Cook's distance, and values greater than 0.06 were removed from models analyzing the raw scores. For all analyses using logistic regression, observations with high leverage (extreme values on a predictor variable), defined as greater than 0.3, were removed. All statistical analyses were performed using SAS software, Version 9.4 of the SAS System for Windows (Copyright © 2013, SAS Institute Inc., Cary, NC, USA).

7.3. RESULTS

7.3.1. Participation and Demographics

The subset of the IKIDS cohort included in the analyses of the CDI were more often white-non-Hispanic, married, held at least a bachelor's degree, were nulliparous at the time of enrollment in the study, had an annual household income of at least \$50,000, reported not smoking or consuming alcohol during their first trimester, and had a vaginal birth (Tables 3.1 and 3.2). In the subset, more fathers were also white, non-Hispanic and had at least a bachelor's degree. The subset with SLAS data available was similar to those of the subset with CDI data available (Tables 3.1 and 3.2). Mothers in this subset were slightly older and had a higher verbal IQ score compared to the full cohort. Phthalate exposure in the full cohort compared to the subsets of participants with CDI or SLAS data available were very similar. There was also no difference in acetaminophen exposure between the subset with CDI data available and the full cohort, nor between the subset with SLAS data available and the full cohort.

7.3.2. Language Outcomes

Due to the design of the CDI, not all children whose parents completed the form when the child was 2 years of age have all three outcome measures. As of March 2021, 300 children had CDI outcome data, with 149 of those being male and 151 being female (Tables 3.1 and 3.2). Children had vocabulary scores ranging from 1 to 680 with a median score of 387. In the sample of participants who completed the CDI at 2 years in the IKIDS cohort, females had significantly more advanced language development than males, as expected (Figure 7.3). On average, females had a significantly larger vocabulary of 424.97 ± 12.26 words compared to males who had a vocabulary of 331.97 ± 13.99 words ($p < 0.0001$). Females also had significantly longer mean length of utterance (M3L, 6.12 ± 0.21 morphemes) than males ($4.58 \pm$

0.19 morphemes) and spoke in significantly more complex sentences (19.76 ± 1.02) than males (12.43 ± 1.03) at 2 years of age ($p < 0.0001$).

In the subset of participants who completed the SLAS when the child was 3 years of age, on average, children had scores above what was considered normal for their age on all outcomes, indicated by the dotted lines in graphs in Figure 7.4. At 3 years of age, children in this subset of the IKIDS cohort had high levels of conversational assertiveness (16.18 ± 0.26) and responsiveness (10.18 ± 0.17) as well as advanced use of syntax (11.04 ± 0.19), articulation (15.30 ± 0.26), and semantics (16.79 ± 0.24). Children in this subset therefore also had high total scores on the SLAS (99.84 ± 1.34). While females again tended to have higher scores on all outcomes compared to males, the differences were not as dramatic as they were at 2 years of age, nor were they significant at $p < 0.05$.

7.3.3. Associations of Exposure and Language Outcomes

Prenatal phthalate exposure and language development at 2 and 3 years

A total of 300 children had CDI data available at 2 years, and 206 children had SLAS data available at 3 years. Of these children, 35 with data available at 2 years were missing maternal verbal IQ, and an additional 9 children were missing delivery type ($n = 256$ with complete covariate data available). At 3 years, only 9 children with SLAS data available were missing delivery type, leaving a total of 197 children with complete covariate data available. Both subsets had pooled phthalate data available, but one child in each subset was missing phthalate exposure data from the 16-18 weeks sample. Of children with CDI and complete covariate data available at 2 years, 80 were missing Σ DEHP exposure data for both the 16-18 weeks and pooled samples, leaving a total of 125 children with both Σ DEHP exposure data and CDI data available. Of the children with SLAS and complete covariate data available, 90 were missing Σ DEHP exposure data for both the 16-18 weeks and pooled samples, resulting in a total of 107 children with both Σ DEHP data and SLAS data available at 3 years. As in

previous chapters, regression diagnostics generally supported the use of continuous, untransformed biomarker measures and linear models to characterize associations, and due to the skewed nature of phthalate exposure concentrations, exposures were characterized using minimums, maximums, medians, and interquartile ranges.

The associations of prenatal phthalate exposure and language outcomes on the CDI at 2 years were generally consistent for a given phthalate both between the 16-18 weeks sample and pooled sample as well as across all three outcomes (Figure 7.5, 7.6, 7.7; Table 7.1). An interquartile range (IQR) increase in Σ AA phthalates exposure in both the 16-18 weeks and pooled sample was associated with a decrease in vocabulary size in males only, but this only approached statistical significance for the pooled sample (Figure 7.5). In contrast, an IQR increase in Σ AA phthalates in the 16-18 weeks sample was associated with a significant increase in the M3L of all children (Figure 7.6). There were no significant associations of Σ AA phthalates with complexity scores (Figure 7.7). Associations for Σ DEHP were similar to those observed for Σ AA phthalates. Per IQR increase in Σ DEHP exposure in both the 16-18 weeks and pooled samples, there was a borderline significant decrease in vocabulary size in males only (Figure 7.5). Like Σ AA phthalates, an IQR increase in Σ DEHP was associated with an increase in M3L in all children, which only reached statistical significance for Σ DEHP in the 16-18 weeks sample (Figure 7.6). For complexity, while the sex-by- Σ DEHP interaction for the pooled sample met the $p < 0.10$ criterion, there were no significant associations of Σ DEHP in either the 16-18 weeks or pooled samples with complexity (Figure 7.7). Unlike Σ AA phthalates and Σ DEHP, an IQR increase in Σ DINP in the pooled sample was associated with an increase in vocabulary size in males only (Figure 7.5). There were no significant associations of Σ DINP in the 16-18 weeks or pooled samples for either M3L or complexity (Figure 7.6, 7.7). Interestingly, an IQR increase in MEP in both the 16-18 weeks and pooled samples was associated with a decrease in vocabulary size (Figure 7.5), M3L (Figure 7.6), and complexity (Figure 7.7) in all

children, these associations were clearest for the pooled samples, and were significant for vocabulary and complexity, but only approached statistical significance for M3L. The child sex-by-MEP exposure in the 16-18 weeks sample met the $p < 0.10$ criterion only for vocabulary, and an IQR increase in MEP was associated with a significant decrease in vocabulary size in males only (Figure 7.5). Finally, an IQR increase in Σ DEHTP in both the 16-18 weeks and pooled samples was associated with a decrease in vocabulary size (Figure 7.5), M3L (Figure 7.6), and complexity (Figure 7.7) in all children. Overall, the clearest associations of prenatal phthalate exposure with CDI outcomes were for MEP and DEHTP both of which were associated with decreases in all three outcomes. Including maternal alcohol use or race and ethnicity, or excluding potentially influential data points or women who reported smoking or for whom that data was missing did not significantly influence the results.

Due to the fact that there were few children in this cohort with percentile scores ≤ 15 th percentile, only logistic regression analyses examining the odds of children having percentile scores ≤ 25 th percentile were investigated. Additionally, when this sample was stratified by child sex, the number of children with scores ≤ 25 th percentile was further reduced, resulting in analyses that were under-powered to reliably calculate odds ratios (ORs) of each sex having percentile scores ≤ 25 th percentile; therefore, only ORs for males and females combined are presented (Table 7.2, Figure 7.8). Ultimately, there were few ORs that approached or reached statistical significance. For vocabulary size, only MEP in the pooled sample was associated with an increased odds of children having a vocabulary score ≤ 25 th percentile (Figure 7.8). Σ DINP in the pooled sample was associated with a decreased odds of having a M3L score ≤ 25 th percentile while Σ AA phthalates in the 16-18 weeks sample was also associated with a decreased odds of having a score ≤ 25 th percentile (Figure 7.8). For complexity, Σ DEHTP in both the 16-18 weeks and pooled samples was associated with an increased odds of having a score ≤ 25 th percentile (Figure 7.8). These results are generally consistent with the findings

from the linear regression models described above. As with the analyses of associations of prenatal phthalate exposure with the raw scores, including maternal alcohol use or race and ethnicity as a covariate, or excluding women who reported smoking or for whom that information was missing or excluding potentially influential data points did not substantially alter the results.

At 3 years of age, only Σ DEHP in the 16-18 weeks sample was associated with any language development outcomes on the SLAS, and no child sex-by-phthalate exposure interactions reached the $p < 0.10$ criterion (Table 7.3, Figure 7.9, 7.10, 7.11, 7.12, 7.13, 7.14). An IQR increase in Σ DEHP in the 16-18 weeks sample was associated with a significant decrease in articulation (Figure 7.13E) and a decrease in conversational assertiveness that approached statistical significance (Figure 7.9E). Removing potentially influential data points or participants who reported smoking or for whom that data was missing did not substantially change the results, nor did including maternal alcohol use or race and ethnicity as covariates in models.

Prenatal acetaminophen exposure and language development at 2 and 3 years

At 2 years, 10 children with CDI data available were missing delivery type, leaving 290 children with complete covariate data available for analyses of the association of prenatal acetaminophen exposure and language development outcomes. Because delivery type was not included in models assessing the association of prenatal acetaminophen exposure and language development outcomes at 3 years, all 206 children with SLAS data available were able to be included in those analyses. Because maternal verbal IQ and delivery type were included in the acetaminophen-by-phthalate exposure interaction models for language development at 2 years, 256 children were included in those analyses. At 3 years, only delivery type was included in the acetaminophen-by-phthalate exposure interaction models, leaving 197 children with SLAS outcome and complete covariate data available for those analyses. As in previous chapters, regression diagnostics generally supported the use of continuous,

untransformed exposure measures to characterize the associations of prenatal acetaminophen exposure and language development outcomes at both 2 and 3 years.

An IQR increase in acetaminophen exposure during the second trimester or throughout pregnancy was associated with a significant reduction in vocabulary size in all infants (Table 7.4, Figure 7.15A, D). However, for M3L, only an IQR increase in acetaminophen exposure during the third trimester was associated with a borderline significant decrease (Table 7.4, Figure 7.15G), and there were no significant associations of prenatal acetaminophen exposure and complexity at 2 years of age (Table 7.4, Figure 7.15I, J, K, L). Including reason for taking acetaminophen, household income, or maternal alcohol use as covariates did not substantially change the results, nor did excluding participants who were not married or living as married, those who reported smoking or for whom that data was missing, or potentially influential data points.

As with the phthalate analyses, there were too few children with percentile scores $\leq$ 15th percentile on the CDI to reliably estimate ORs, thus only ORs for scores $\leq$ 25th percentile are presented; however, unlike the phthalate analyses, there was sufficient power to reliably estimate ORs for prenatal acetaminophen exposure and language outcomes at 2 years on the CDI both when males and females were combined and when children were stratified by sex (Table 7.5). Interestingly, most significant associations were only observed in female children. Female children with higher acetaminophen exposure in the first or second trimester, as well as throughout pregnancy, had greater odds of having vocabulary scores $\leq$ 25th percentile (Figure 7.16A). Only exposure during the second trimester and exposure throughout pregnancy were associated with greater odds of female children having M3L scores $\leq$ 25th percentile (Figure 7.16B). First trimester acetaminophen exposure was associated with greater odds of female children having complexity scores $\leq$ 25th percentile while third trimester exposure was associated with greater odds of male children having complexity scores $\leq$ 25th percentile

(Figure 7.16C). Including household income, maternal alcohol use, or reason for taking acetaminophen as covariates did not significantly change the results, nor did excluding participants who were not married or living as married, those who reported smoking or for whom that data was missing, or potentially influential data points.

Associations of prenatal acetaminophen exposure generally showed that higher exposure during the second trimester of development and cumulative exposure throughout pregnancy was associated with lower language outcome scores on the SLAS at 3 years of age (Table 7.6). An IQR increase in prenatal acetaminophen exposure during the second trimester, as well as throughout pregnancy, was associated with a decrease in conversational assertiveness, responsiveness, semantics, syntax, and total SLAS score in all children (Figure 7.17, 7.18). although the acetaminophen-by-sex interaction was not significant for all outcomes, females were generally more adversely impacted than males. An IQR increase in acetaminophen exposure during the third trimester was associated with a decrease in syntax in all children (Figure 7.18C), but a decrease in male children only in conversational assertiveness (Figure 7.17C). However, an IQR increase in acetaminophen exposure during the second trimester was associated with lower syntax (Figure 7.18B), articulation (Figure 7.18F), and total SLAS score (Figure 7.18J) in female children. In summary, prenatal acetaminophen exposure during the second trimester and cumulative exposure across pregnancy were both associated with lower scores on all SLAS outcomes in all children, with female children generally more affected than males. An exception to this pattern was that third trimester acetaminophen exposure was associated with lower syntax scores in male children. Including maternal alcohol use, reason for taking acetaminophen, or household income did not substantially change the results, nor did removing potentially influential points, participants who reported smoking or for whom that data was missing, or participants who were not married or living as married.

Interactive effects of acetaminophen and phthalate exposure on language development at 2 and 3 years of age

As described in previous chapters, interactions of prenatal acetaminophen and phthalate exposure were explored where the results of a model investigating acetaminophen exposure alone showed an association of acetaminophen exposure and an outcome with a p-value < 0.10. Prenatal phthalate exposures were selected based on which exposures also had an association with an outcome with a p-value < 0.10. Both acetaminophen-by-phthalate exposure interactions and acetaminophen-by-phthalate-by-child sex interactions were explored, and where an interaction was $p < 0.10$, the phthalate exposure was categorized as low, medium, and high exposure as shown in Table 7.7 to investigate whether the association with acetaminophen varied by phthalate exposure level.

Associations of phthalates with language outcomes were generally in similar directions for both the 16-18 weeks and pooled samples and more significant associations were present for the pooled samples, thus only interactions with phthalate exposures in the pooled sample were explored. Additionally, due to the small sample size and small number of children in the sample with percentile scores $\leq$ 25th percentile, these analyses used the raw scores for vocabulary size, M3L, and sentence complexity and did not explore ORs. Few analyses for the CDI outcomes had significant acetaminophen-by-phthalate interactions. No acetaminophen-by-phthalate exposure interactions reached the $p < 0.10$ criterion for vocabulary size, but two each for M3L and complexity did and were further explored. The interaction of cumulative prenatal acetaminophen exposure and Σ AA phthalates was $p < 0.10$ for M3L, but there was no difference in acetaminophen exposure associations with the outcome by low, medium, or high Σ AA phthalate exposure (Table 7.8). The first trimester acetaminophen exposure-by- Σ DEHP-by-child sex interaction also met the $p < 0.10$ criterion for M3L and was further explored. Male and female children had significantly different M3L scores with females having longer M3L

compared to males, but there was no difference in acetaminophen-outcome associations by levels of Σ DEHP exposure in female or male children (Table 7.9). For complexity, the first trimester-by- Σ DEHTP exposure interaction was $p < 0.10$, and when stratified by level of phthalate exposure, there was no difference in the effect of acetaminophen between low and medium Σ DEHTP exposure groups, but the association of acetaminophen with complexity scores indicated that children in the high Σ DEHTP exposure group had lower complexity scores compared to those in the low and medium Σ DEHTP exposure groups (Figure 7.19A, Table 7.8). Third trimester acetaminophen exposure-by- Σ DEHP-by-child sex also met the $p < 0.10$ criterion for complexity. When stratified by child sex and phthalate exposure level, there was no difference in the association of prenatal acetaminophen exposure with complexity scores among Σ DEHP exposure levels in male children, but in female children, acetaminophen exposure was associated with lower complexity scores in the high Σ DEHP exposure group compared to female children categorized as having medium Σ DEHP exposure (Figure 7.19B, Table 7.9). The results of these analyses suggest that prenatal exposure to acetaminophen during the first trimester or third trimester of pregnancy, as well as exposure throughout pregnancy, combined with exposure to Σ DEHTP or Σ DEHP may negatively impact language development at 2 years of age. Excluding potentially influential points or participants who reported smoking or for whom that information was missing, or including maternal alcohol use, reason for taking acetaminophen, or paternal race and ethnicity did not significantly change the results.

For the SLAS the only phthalate exposure that was significantly associated with lower scores was Σ DEHTP in the 16-18 weeks sample, but acetaminophen exposure was associated with lower scores on all outcomes. In interaction models, several acetaminophen-by- Σ DEHTP-by child sex interactions met the $p < 0.10$ criterion and were further investigated. The three-way interaction for third trimester acetaminophen exposure, Σ DEHTP, and child sex was $p < 0.0001$ for assertiveness; however, there was no significant difference in the effect of acetaminophen

on assertiveness scores when stratified by sex and Σ DEHP exposure level as defined in Table 7.7. The interaction of acetaminophen exposure throughout pregnancy, Σ DEHP, and child sex was also $p < 0.10$ for assertiveness, but there was no difference in the association of prenatal acetaminophen exposure and any outcome when stratified by child sex and Σ DEHP levels (Table 7.10). While there were differences that approached significant between levels of Σ DEHP exposure for only one model, these results indicate that prenatal acetaminophen exposure during the third trimester and cumulative exposure throughout pregnancy combined with Σ DEHP exposure between 16 and 18 weeks of gestation may be related to language development at 3 years of age. Including maternal alcohol use, reason for taking acetaminophen, or maternal race and ethnicity as covariates did not significantly influence the results, nor did excluding participant who were not married or living as married, or participants who reported smoking or for whom that information was missing.

7.4. DISCUSSION

7.4.1. Prenatal phthalate exposure and language development

MacArthur-Bates Communicative Development Inventory

Prenatal phthalate exposure was associated with changes in vocabulary size. While MEP and Σ DEHP in both the 16-18 weeks sample and the pooled sample were both associated with significant reductions in vocabulary size, reductions in vocabulary size were only observed in male children for Σ AA phthalates and Σ DEHP, and these associations only approached statistical significance (Figure 7.5). Interestingly, contrary to all other phthalate associations with vocabulary scores, Σ DINP in the pooled sample was associated with an increase in vocabulary size in male children. When scores were categorized as ≤ 25 th percentile, only prenatal MEP exposure was associated with an increased odds of children having a score ≤ 25 th percentile (Figure 7.8). Consistent with these findings, Olesen et al. (2018) previously reported that higher prenatal MEP and Σ DEHP exposure were both

associated with lower vocabulary scores on the CDI. Specifically, they found that MEP and Σ DEHP were both associated with an increased odds of male children having vocabulary percentile scores $\leq$ 15th percentile (Olesen et al., 2018). While Olesen et al. (2018) observed this association with phthalates measured in a maternal urine sample taken during the third trimester of pregnancy, the results of associations with phthalates in the present study were observed for both the 16-18 weeks and pooled sample when the outcome was considered as continuous, but only in the pooled sample when the outcome was dichotomized as above or below the 25th percentile. Additionally, while the results of the present study were observed in male children only for Σ DEHP and MEP in the 16-18 weeks sample, mirroring the findings of Olesen et al. (2018), MEP in the pooled sample was associated with lower vocabulary scores on the CDI in all children as well as greater odds of all children having a percentile score $\leq$ 25th percentile. Olesen et al. (2018) had a larger sample size and thus a larger number of children with percentile scores $\leq$ 15th percentile. In contrast, this study with its modest sample size did not have enough children with scores $\leq$ 15th percentile, to explore odds ratios of children having delayed language. Instead, ORs of children having scores $\leq$ 25th percentile were investigated. These estimates had very large confidence intervals and were unstable. However, consistent with the results of Olesen et al. (2018), results of the present study suggest that prenatal exposure to MEP may be associated with lower vocabulary scores on the CDI and increased odds of children having percentile scores at or below levels of concern. This is the first study to date evaluating the relationship of prenatal DEHP exposure and language development, and the results suggest that prenatal exposure to this terephthalate is related to lower vocabulary scores in children at 2 years of age, raising further concern about its increasing use as a replacement for DEHP (Schwedler et al., 2020).

Except for Σ DINP, all phthalates were associated with changes in M3L (Figure 7.6). While MEP and Σ DEHP were both associated with shorter M3L, the association only

approached statistical significance for MEP. Σ AA phthalates and Σ DEHP in the 16-18 weeks sample were actually associated with longer M3L. When M3L scores were converted to percentile scores and dichotomized as greater than or less than the 25th percentile, Σ AA phthalates in the 16-18 weeks sample and Σ DINP in the pooled sample were associated with lower odds of children having a M3L score $\leq$ 25th percentile (Figure 7.8). These results of Σ AA phthalates, Σ DEHP, and Σ DINP are unexpected, although it is interesting that there was some evidence that each of the phthalates with known antiandrogenic effects (Hannas et al., 2011; Howdeshell et al., 2015), had a positive impact on M3L whereas MEP and Σ DEHTP had a negative impact. To our knowledge, this is only the second study to evaluate the relationship between prenatal phthalate exposure and language development outcomes assessed via the CDI, and the first to examine this relationship for M3L. Olesen et al. (2018) used the CDI but did not examine the association of phthalates with the odds of M3L scores being at or below the cutoff for clinical concern of language delay. As for vocabulary, it is concerning that a replacement chemical for DEHP, DEHTP, was the only phthalate associated with significantly reduced M3L further highlighting its negative impact on language development

In analyses of the relationship between prenatal phthalate exposure and sentence complexity as measured via the CDI when the child was 2 years old, MEP in the pooled sample was associated with lower complexity scores, and Σ DEHTP in both the 16-18 weeks sample and the pooled sample was associated with lower complexity scores in all children (Figure 7.7). When complexity scores were converted to percentile scores and dichotomized as > 25 th percentile or ≤ 25 th percentile, only Σ DEHTP in both the 16-18 weeks and pooled samples was associated with significantly greater odds of children having a complexity percentile score ≤ 25 th percentile (Figure 7.8). This is the first study to investigate potential associations of prenatal exposure to Σ DEHTP with language development, and thus there are no results from previous studies to compare to. However, Olesen et al. (2018) also observed associations between

prenatal MEP exposure with lower complexity scores. Like the associations they observed for vocabulary, Olesen et al. (2018) found that MEP was associated with increased odds of male children having a complexity percentile score ≤ 15 th percentile. While results of the present study indicate that prenatal MEP exposure during prenatal development is associated with lower raw complexity scores, unlike Olesen et al. (2018), there was no association of MEP and the odds of children having complexity percentile scores ≤ 25 th percentile. This may be due to the difference in sample size and the number of children in each cohort with low complexity percentile scores, as well as the cutoff used in each study, as Olesen et al. (2018) both had a larger sample size and more children with percentile scores ≤ 15 th percentile compared to the sample included in the present study.

Speech and Language Assessment Scale

In analyses of the relationship between prenatal phthalate scores and SLAS scores at 3 years, there were very few associations that approached statistical significance. Only Σ DEHTP in the 16-18 weeks sample was associated with lower assertiveness and articulation scores (Figure 7.9). Unlike for the CDI at age 2, the results for Σ DEHTP were not consistent between the 16-18 weeks and pooled samples. It is interesting that while there were several significant associations between prenatal phthalate exposure and language outcomes at 2 years, there were only two significant associations at 3 years. This difference could be due to the difference in age or in the two language assessments themselves. As described in Chapter 1, children begin producing language around 12 months of age, followed by a spurt of rapid development at 16-18 months of age, and females tend to begin producing language earlier than males, and children's' vocabularies grow exponentially through about 20-24 months of age, at which point vocabulary growth becomes more linear (Huttenlocher, et al., 1991; Bauer et al., 2002; Fernald et al., 2006; Rose et al., 2009; Eriksson et al., 2012). Thus, it may be that the assessment at 2 years captures effects of prenatal exposure to phthalates on early language development that

resolve as children age. It may also be due to a methodological difference between the CDI, a norm-referenced scale that asks parents to provide objective information about their child's language development, and the SLAS, which asks parents to make a judgement about their child's language skills, that effects of prenatal phthalate exposure on language development are primarily seen at 2 years. Additionally, because the SLAS asks parents to judge their child's language abilities, it may be that the SLAS is not as sensitive of a measure to measure the effects of prenatal phthalate exposure on language development. Future studies using the SLAS and other direct assessments to assess language development in these children at 4 and 7 years of age will help answer this question.

7.4.2. Prenatal acetaminophen exposure and language development

MacArthur-Bates Communicative Development Inventory

In general, there were fewer associations of prenatal acetaminophen exposure with language development outcomes on the CDI at 2 years. Prenatal acetaminophen exposure during the second trimester and cumulative exposure across pregnancy were both associated with lower vocabulary scores, and third trimester exposure was associated with shorter M3L (Figure 7.15). Other studies that have specifically evaluated language development or communication skills more generally have also found associations between prenatal acetaminophen exposure and poorer language outcomes. Using samples of the same cohort, Skovlund et al. (2017) and Brandlistuen et al. (2013) found that prenatal acetaminophen exposure was associated with poorer communication skills at 3 years. Vlenterie et al. (2016) also found that prenatal acetaminophen exposure was associated with poorer communication skills in this cohort, but they observed this association much earlier in children 18 months of age. The findings of Brandlistuen et al. (2013) are particularly of interest here because they found this association specifically with long-term acetaminophen use during pregnancy, which they classified as use for more than 28 days, aligning with the findings of the present study

indicating that higher acetaminophen exposure throughout pregnancy is associated with lower vocabulary scores in children at 2 years of age.

At 2 years, prenatal acetaminophen exposure during the first trimester was associated with female children having vocabulary and complexity scores $\leq$ 25th percentile, and exposure during the second trimester and throughout pregnancy was associated with female children having both vocabulary and M3L scores $\leq$ 25th percentile (Figure 7.16). Third trimester acetaminophen exposure was associated with male children having complexity scores $\leq$ 25th percentile (Figure 7.16). These results align with Bornehag et al. (2018b) who observed that prenatal acetaminophen exposure was associated with increased language delays in female children as assessed via a routine nurse assessment when children were 30 months, suggesting that the association they observed in female children can be detected earlier. However, Bornehag et al. (2018b) did not observe an association of prenatal acetaminophen exposure and language delay in male children. This difference in findings for male children may be related to the timing of exposure as Bornehag et al. (2018b) only looked at acetaminophen exposure during the first trimester, and the association observed here was only found for third trimester exposure.

Speech and Language Assessment Scale

This study is the first to our knowledge to utilize the SLAS to evaluate associations of prenatal exposure to acetaminophen and language development in children. Additionally, while a few previous studies have also investigated the association of prenatal exposure to acetaminophen and language development at or around 3 years of age, this is the first study to examine specific aspects of language development at that age. In this analysis, prenatal acetaminophen exposure was generally associated with lower scores on all SLAS outcomes, as well as total SLAS score at 3 years of age (Figures 7.17, 7.18). For some outcomes (syntax, articulation, and total score), associations were seen only in female children or were more

pronounced in female children. Cumulative exposure across pregnancy was also generally associated with lower SLAS scores. Exposure during the third trimester of prenatal development was associated with lower syntax scores, and lower assertiveness scores in male children only. Interestingly, Bornehag et al. (2018b) found that higher prenatal acetaminophen exposure was associated with greater language delay in female children at 30 months of age as evaluated via a routine nurse and parent assessment. While Bornehag et al. (2018b) classified language delay at 30 months as a child producing fewer than 50 words, and did not investigate specific aspects of language development, their results are generally consistent with these findings.

While prenatal acetaminophen exposure was associated with few outcomes utilizing the CDI raw scores, associations with the odds of scores $\leq$ 25th percentile were more pronounced, as were associations observed using the SLAS at 3 years. As described earlier, this difference may be due to age or the difference in assessments. It may be that the effects of prenatal exposure to acetaminophen only become apparent as children age, although this seems unlikely to be the case as many associations are observed with the odds of children having scores $\leq$ 25th percentile at 2 years. It seems more likely that the difference is due to the method and the aspects of language development each assessment measures. Interestingly, at 2 years and 3 years, there were similarities in patterns of associations with both phthalates and acetaminophen. This is particularly interesting for observations of prenatal Σ DEHP exposure in the 16-18 weeks sample and the associations of prenatal acetaminophen exposure during the second trimester, suggesting that the second trimester may be especially sensitive to effects of prenatal exposure on language development, as hypothesized. The neocortex, the location of both Wernicke's and Broca's areas which are known to be important in language production and comprehension (Hickok, 2009; Friederici, 2011), finishes forming during the second trimester which may be why second trimester exposure appears to have the strongest effects on measures of language development at 2 and 3 years of age (Howdeshell, 2002). While these

similarities in findings between exposures suggest they may share a common mechanism, it seems unlikely that this potential mechanism is antiandrogenic in nature, as prenatal acetaminophen exposure primarily affected female children, and results of associations of antiandrogenic phthalates with language development were inconsistent.

7.4.3. Cumulative effects of exposure to both phthalates and acetaminophen on language development

At both 2 and 3 years of age, there were significant interactions of prenatal acetaminophen exposure and phthalate exposure. At 2 years, interactions of prenatal acetaminophen and phthalate exposure were observed for M3L and for complexity, and depended upon the trimester of acetaminophen exposure, the phthalate metabolite, and the language outcome. There were no significant interactions of exposure on vocabulary at 2 years which was unexpected given that the effects of each exposure alone on the raw scores appeared to be strongest for vocabulary. However, Σ DEHP modified the effects of third trimester acetaminophen exposure on complexity in female children with acetaminophen exposure associated with lower complexity scores in the high Σ DEHP group (Figure 7.19). A similar pattern was seen for Σ DEHP, but for the effects of first trimester acetaminophen exposure on complexity, where acetaminophen exposure was associated with lower complexity scores within the high Σ DEHP group (Figure 7.19). While these results are concerning, as they indicate there may be a cumulative effect of high prenatal exposure to phthalates and acetaminophen, they must be interpreted carefully. First, within the low Σ DEHP and Σ DEHP exposure groups, acetaminophen exposure was associated with higher complexity scores compared to the association of acetaminophen exposure with complexity scores in children in the high Σ DEHP and Σ DEHP groups, suggesting that this may only be a concern when both phthalate and acetaminophen exposure are high. Also, as described earlier in this chapter, not all children had complexity data available, resulting in a smaller sample for these analyses

which was further reduced by categorizing the sample as having high, medium, or low phthalate exposure and stratifying by child sex. Additionally, fewer participants had Σ DEHP exposure data available due to the development of detection methods for Σ DEHP metabolites after the first batch of urine samples were sent for analyses. Although these results must be interpreted with caution, they suggest that there may be a cumulative effect of prenatal acetaminophen exposure and Σ DEHP or Σ DEHP exposure at high phthalate exposure levels, indicating that future studies with larger sample sizes should be conducted to further explore the potential for cumulative effects.

At 3 years, the only phthalate that was associated with any of the language outcomes was Σ DEHP at 16-18 weeks (assertiveness and articulation). However, in interaction models there were no significant differences in prenatal acetaminophen exposure on assertiveness or articulation across the three levels of Σ DEHP exposure groups either overall or when stratified by child sex. Again, due to the small number of children with SLAS data available at 3 years and the even smaller sample with Σ DEHP exposure data available, these results should be interpreted with caution. It is likely that given the increasingly small sample sizes as the sample was categorized and stratified, statistical power was too low to detect modification of effects of acetaminophen exposure by Σ DEHP. However, these results can provide direction for future studies investigating the potential cumulative effects of prenatal exposure to acetaminophen and phthalates.

7.4.4. Summary and conclusions

While there were more significant associations of prenatal phthalate exposure with language development outcomes at 2 years, there were more significant associations of prenatal acetaminophen exposure with language development outcomes at 3 years. However, there was some overlap in associations between exposures. At 2 years, both prenatal acetaminophen exposure and prenatal Σ DEHP and MEP exposure were associated with

reductions in vocabulary size and M3L. Specifically of interest, the association of Σ DEHP was observed for the 16-18 weeks sample, and the association of acetaminophen was observed for second trimester, indicating that the second trimester may be a particularly sensitive period for impacts on language development. At 3 years, prenatal exposure to acetaminophen during the second trimester and Σ DEHP in the 16-18 weeks sample were also associated with lower assertiveness scores, providing additional support for the idea that the second trimester is important. As previously described, 16-18 weeks of gestation, is thought to be the time of peak androgen production in male fetuses (Auyeung et al. 2013), but associations of prenatal phthalate exposure and language development were inconsistent for antiandrogenic phthalates at 2 years, and there were no associations for known antiandrogenic phthalates and language outcomes at 3 years, indicating this may not be the mechanism of action involved. These findings for Σ DEHP are both interesting and concerning as DEHP is increasingly used as a replacement for DEHP in consumer products (Schwedler, et al., 2020). While there were some associations for Σ DEHP and language outcomes at 2 years, the associations for Σ DEHP were stronger and observed at both 2 and 3 years. Additionally, these results add to evidence suggesting that prenatal exposure to acetaminophen impacts language development, strengthening the argument for reevaluation of the safety of acetaminophen use during pregnancy (Cendejas-Hernandez et al., 2020).

7.5. TABLES AND FIGURES

Table 7.1. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal phthalate exposure with language outcomes on the CDI at 2 years.¹

		16-18 weeks gestation sample		
		Stratified by Sex		
Outcome	Exposure	Main Effect	Females	Males
Vocabulary	Σ AA Phthalates	6.088 (17.122, -4.945)	8.152 (19.967, -3.664)	-8.200 (23.407, -39.807)
	Σ DEHP	1.737 (5.248, -1.774)	^a 2.398 (5.946, -1.149)	[#] -16.472 (2.099, -35.044)
	Σ DINP	-0.279 (2.389, -2.948)	-1.42 (1.878, -4.718)	1.850 (6.355, -2.654)
	MEP	-5.540 (1.984, -13.064)	^a -1.969 (6.405, -10.344)	[*] -19.532 (-2.947, -36.116)
	Σ DEHTP	[*] -5.337 (-0.238, -10.437)	-5.27 (1.137, -11.676)	-5.459 (3.148, -14.065)
M3L	Σ AA Phthalates	[*] 0.171 (0.334, 0.007)	0.206 (0.381, 0.032)	-0.091 (0.391, -0.572)
	Σ DEHP	[*] 0.060 (0.112, 0.008)	0.066 (0.119, 0.014)	-0.136 (0.152, -0.424)
	Σ DINP	0.008 (0.048, -0.031)	0.012 (0.061, -0.037)	0.002 (0.068, -0.065)
	MEP	-0.071 (0.041, -0.183)	-0.043 (0.082, -0.168)	-0.181 (0.068, -0.429)
	Σ DEHTP	[*] -0.079 (-0.010, -0.148)	-0.088 (-0.002, -0.173)	-0.063 (0.057, -0.184)
Complexity	Σ AA Phthalates	0.252 (1.053, -0.550)	0.316 (1.173, -0.540)	-0.225 (2.150, -2.600)
	Σ DEHP	0.199 (0.451, -0.053)	0.215 (0.469, -0.039)	-0.531 (1.132, -2.195)
	Σ DINP	-0.051 (0.140, -0.243)	-0.152 (0.083, -0.387)	0.143 (0.468, -0.182)
	MEP	-0.289 (0.252, -0.830)	-0.098 (0.503, -0.699)	-1.056 (0.147, -2.259)
	Σ DEHTP	[#] -0.334 (0.036, -0.704)	-0.270 (0.193, -0.733)	-0.450 (0.176, -1.076)
		Pooled sample		
		Stratified by Sex		
Outcome	Exposure	Main Effect	Females	Males
Vocabulary	Σ AA Phthalates	1.963 (12.911, -8.986)	^a 5.431 (16.842, -5.979)	[#] -31.861 (3.743, -67.466)
	Σ DEHP	0.857 (11.043, -9.329)	^b 6.484 (17.902, -4.934)	[#] -19.889 (2.183, -41.961)
	Σ DINP	2.668 (8.768, -3.431)	^a -0.871 (6.213, -7.955)	[*] 12.495 (24.357, 0.634)
	MEP	^{**} -12.157 (-3.168, -21.146)	-12.960 (-0.490, -25.430)	-11.294 (1.632, -24.219)
	Σ DEHTP	[*] -21.094 (-3.358, -38.830)	-13.837 (9.728, -37.401)	-30.556 (-3.634, -57.478)
M3L	Σ AA Phthalates	0.086 (0.250, -0.079)	0.095 (0.265, -0.075)	-0.043 (0.586, -0.672)
	Σ DEHP	0.108 (0.260, -0.044)	0.166 (0.335, -0.004)	-0.121 (0.217, -0.459)
	Σ DINP	0.075 (0.191, -0.041)	0.085 (0.239, -0.070)	0.062 (0.239, -0.116)
	MEP	[#] -0.118 (0.018, -0.253)	-0.183 (0.004, -0.370)	-0.047 (0.146, -0.241)
	Σ DEHTP	[*] -0.279 (-0.017, -0.541)	-0.325 (-0.012, -0.638)	-0.172 (0.303, -0.648)
Complexity	Σ AA Phthalates	-0.332 (0.457, -1.120)	-0.183 (0.639, -1.006)	-1.879 (0.771, -4.528)
	Σ DEHP	0.032 (0.780, -0.716)	^b 0.365 (1.199, -0.469)	-1.26 (0.388, -2.908)
	Σ DINP	-0.019 (0.423, -0.461)	-0.222 (0.292, -0.735)	0.549 (1.414, -0.317)
	MEP	[*] -0.671 (-0.016, -1.326)	-0.692 (0.206, -1.590)	-0.648 (0.298, -1.594)
	Σ DEHTP	[#] -1.147 (0.160, -2.453)	-1.025 (0.690, -2.74)	-1.314 (0.699, -3.327)

¹All models were adjusted for maternal age, maternal education, household income, child sex, and number of older siblings.

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

[#] $p < 0.10$, ^{*} $p < 0.05$, ^{**} $p < 0.01$

Table 7.2. Odds ratios (odds, 95% CI) of children with prenatal phthalate exposure having a percentile score $\leq$ 25th percentile adjusted for child sex and age.¹

Outcome		16-18 weeks gestation sample	Pooled sample
Vocabulary	Σ AA Phthalates	0.545 (0.206, 1.446)	0.913 (0.456, 1.827)
	Σ DEHP	0.290 (0.011, 7.408)	1.080 (0.100, 11.62)
	Σ DINP	1.129 (0.434, 2.935)	1.770 (0.299, 10.494)
	MEP	1.455 (0.812, 2.607)	* 2.219 (1.043, 4.720)
	Σ DEHTP	1.154 (0.763, 1.746)	1.418 (0.787, 2.552)
M3L	Σ AA Phthalates	# 0.207 (0.040, 1.067)	0.206 (0.030, 1.390)
	Σ DEHP	0.094 (0.002, 5.577)	1.035 (0.040, 26.646)
	Σ DINP	0.014 (0.00006, 3.327)	* 0.002 (0.000004, 0.778)
	MEP	1.634 (0.900, 2.966)	1.615 (0.743, 3.511)
	Σ DEHTP	1.314 (0.829, 2.085)	1.034 (0.409, 2.611)
Complexity	Σ AA Phthalates	0.247 (0.038, 1.610)	0.942 (0.398, 2.225)
	Σ DEHP	0.017 (0.00002, 13.224)	3.168 (0.294, 34.087)
	Σ DINP	0.513 (0.065, 4.066)	0.129 (0.002, 8.183)
	MEP	0.562 (0.132, 2.400)	0.694 (0.193, 2.499)
	Σ DEHTP	* 1.774 (1.114, 2.824)	* 2.014 (1.050, 3.863)

¹All models were adjusted for maternal age, maternal education, household income, child sex, and number of older siblings.

#p < 0.10, *p < 0.05

Table 7.3. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal phthalate exposure with language outcomes on the SLAS at 3 years.¹

Outcome	Exposure	16-18 weeks gestation sample	Pooled sample
		Main Effect	Main Effect
Assertiveness	Σ AA Phthalates	0.069 (0.309, -0.171)	0.137 (0.408, -0.133)
	Σ DEHP	0.021 (0.093, -0.051)	0.007 (0.184, -0.169)
	Σ DINP	-0.013 (0.095, -0.121)	0.110 (0.354, -0.134)
	MEP	-0.199 (0.041, -0.440)	-0.176 (0.073, -0.426)
	Σ DEHTP	# -0.131 (0.013, -0.274)	0.231 (0.764, -0.302)
Responsiveness	Σ AA Phthalates	0.004 (0.160, -0.153)	0.064 (0.241, -0.112)
	Σ DEHP	0.007 (0.054, -0.040)	0.004 (0.118, -0.109)
	Σ DINP	-0.032 (0.038, -0.102)	0.027 (0.186, -0.132)
	MEP	-0.090 (0.066, -0.247)	-0.089 (0.073, -0.252)
	Σ DEHTP	-0.058 (0.039, -0.154)	0.168 (0.524, -0.188)
Semantics	Σ AA Phthalates	0.048 (0.273, -0.177)	0.068 (0.322, -0.186)
	Σ DEHP	0.022 (0.089, -0.046)	0.021 (0.186, -0.144)
	Σ DINP	0.025 (0.126, -0.076)	0.186 (0.413, -0.041)
	MEP	-0.112 (0.114, -0.338)	-0.061 (0.175, -0.296)
	Σ DEHTP	-0.107 (0.028, -0.241)	-0.012 (0.488, -0.511)
Syntax	Σ AA Phthalates	0.062 (0.234, -0.111)	0.058 (0.253, -0.137)
	Σ DEHP	0.025 (0.077, -0.027)	0.042 (0.167, -0.084)
	Σ DINP	0.005 (0.083, -0.072)	0.108 (0.282, -0.067)
	MEP	0.013 (0.187, -0.161)	-0.024 (0.156, -0.204)
	Σ DEHTP	-0.049 (0.055, -0.154)	-0.110 (0.275, -0.496)
Articulation	Σ AA Phthalates	0.075 (0.321, -0.172)	0.153 (0.430, -0.125)
	Σ DEHP	0.022 (0.096, -0.052)	0.027 (0.208, -0.154)
	Σ DINP	0.011 (0.122, -0.099)	0.199 (0.449, -0.050)
	MEP	-0.149 (0.099, -0.396)	-0.072 (0.186, -0.329)
	Σ DEHTP	* -0.165 (-0.024, -0.305)	-0.207 (0.322, -0.736)
Total Score	Σ AA Phthalates	0.313 (1.548, -0.921)	0.580 (1.972, -0.813)
	Σ DEHP	0.128 (0.499, -0.243)	0.077 (0.982, -0.829)
	Σ DINP	-0.041 (0.514, -0.595)	0.861 (2.112, -0.389)
	MEP	-0.652 (0.589, -1.894)	-0.367 (0.923, -1.657)
	Σ DEHTP	-0.590 (0.158, -1.338)	0.419 (3.202, -2.363)

¹All models were adjusted for maternal age, maternal education, maternal parity, delivery type, and child sex.

#p < 0.10, *p < 0.05

Table 7.4. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with language outcomes on the CDI at 2 years.¹

Outcome		Main Effect
Vocabulary	1st Trimester	-3.004 (4.354, -10.362)
	2nd Trimester	* -4.280 (-0.044, -8.515)
	3rd Trimester	-2.231 (0.617, -5.080)
	Full Pregnancy	* -7.427 (-0.507, -14.347)
M3L	1st Trimester	-0.055 (0.053, -0.164)
	2nd Trimester	-0.042 (0.020, -0.105)
	3rd Trimester	# -0.037 (0.005, -0.080)
	Full Pregnancy	-0.075 (0.028, -0.177)
Complexity	1st Trimester	-0.323 (0.237, -0.883)
	2nd Trimester	-0.141 (0.243, -0.525)
	3rd Trimester	-0.115 (0.127, -0.357)
	Full Pregnancy	-0.275 (0.326, -0.876)

¹All models were adjusted for maternal age, maternal education, household income, child sex, and number of older siblings.

#p < 0.10, *p < 0.05

Table 7.5. Odds ratios (odds, 95% CI) of children with prenatal acetaminophen exposure having a percentile score $\leq$ 25th percentile adjusted for child sex and age.¹

Outcome	Exposure	Odds Ratio	Stratified by Sex	
			Females	Males
Vocabulary	1st Trimester	0.999 (1.012, 0.986)	* 0.965 (0.996, 0.936)	1.017 (1.044, 0.990)
	2nd Trimester	0.995 (1.003, 0.987)	* 0.988 (1.000, 0.976)	1.003 (1.015, 0.990)
	3rd Trimester	0.991 (1.011, 0.972)	0.980 (1.007, 0.954)	1.004 (1.041, 0.968)
	Full Pregnancy	0.997 (1.002, 0.992)	* 0.992 (1.000, 0.984)	1.002 (1.009, 0.996)
M3L	1st Trimester	1.005 (1.023, 0.987)	0.992 (1.019, 0.965)	1.020 (1.066, 0.975)
	2nd Trimester	0.996 (1.005, 0.988)	* 0.988 (0.999, 0.978)	1.008 (1.029, 0.987)
	3rd Trimester	0.992 (1.014, 0.971)	0.980 (1.008, 0.953)	1.009 (1.049, 0.970)
	Full Pregnancy	0.998 (1.003, 0.993)	# 0.994 (1.001, 0.988)	1.003 (1.012, 0.994)
Complexity	1st Trimester	0.994 (1.008, 0.980)	# 0.967 (1.003, 0.933)	1.007 (1.030, 0.984)
	2nd Trimester	0.998 (1.009, 0.987)	0.987 (1.004, 0.970)	1.005 (1.028, 0.983)
	3rd Trimester	0.984 (1.007, 0.962)	1.004 (1.046, 0.965)	* 0.966 (0.999, 0.935)
	Full Pregnancy	0.998 (1.003, 0.992)	0.996 (1.007, 0.986)	1.000 (1.009, 0.992)

¹All models were adjusted for maternal age, maternal education, household income, child sex, and number of older siblings.

[#]p < 0.10, *p < 0.05

Table 7.6. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with language outcomes on the SLAS at 2 years.¹

Outcome	Exposure	Main Effect	Stratified by Sex	
			Females	Males
Assertiveness	1st Trimester	-0.115 (0.025, -0.254)	--	--
	2nd Trimester	* -0.130 (-0.009, -0.251)	--	--
	3rd Trimester	-0.045 (0.025, -0.115)	^a -0.003 (0.081, -0.086)	* -0.123 (-0.014, -0.232)
	Full Pregnancy	* -0.176 (-0.015, -0.337)	--	--
Responsiveness	1st Trimester	-0.072 (0.019, -0.163)	--	--
	2nd Trimester	# -0.078 (0.001, -0.157)	--	--
	3rd Trimester	-0.020 (0.026, -0.066)	--	--
	Full Pregnancy	* -0.106 (-0.001, -0.212)	--	--
Semantics	1st Trimester	-0.081 (0.051, -0.213)	--	--
	2nd Trimester	* -0.114 (0.0003, -0.229)	--	--
	3rd Trimester	-0.041 (0.025, -0.107)	--	--
	Full Pregnancy	* -0.157 (-0.005, -0.309)	--	--
Syntax	1st Trimester	-0.080 (0.021, -0.181)	--	--
	2nd Trimester	* -0.113 (-0.026, -0.199)	^b ** -0.188 (-0.078, -0.297)	-0.007 (0.122, -0.136)
	3rd Trimester	# -0.044 (0.007, -0.094)	--	--
	Full Pregnancy	** -0.152 (-0.037, -0.268)	--	--
Articulation	1st Trimester	-0.066 (0.079, -0.210)	--	--
	2nd Trimester	-0.085 (0.040, -0.209)	^a * -0.171 (-0.013, -0.328)	0.036 (0.222, -0.150)
	3rd Trimester	-0.056 (0.017, -0.128)	--	--
	Full Pregnancy	-0.131 (0.035, -0.296)	--	--
Total Score	1st Trimester	-0.556 (0.165, -1.277)	--	--
	2nd Trimester	* -0.642 (-0.019, -1.264)	^a ** -1.076 (-0.287, -1.866)	-0.027 (0.903, -0.957)
	3rd Trimester	-0.249 (0.111, -0.608)	--	--
	Full Pregnancy	* -0.890 (-0.063, -1.717)	--	--

¹All models were adjusted for acetaminophen formulation, maternal age, maternal education, maternal parity, and child sex.

Interaction p-value: ^a p < 0.10, ^b p < 0.05

#p < 0.10, * p < 0.05, ** p < 0.01

Table 7.7. Phthalate exposure cutoffs for acetaminophen and phthalate interaction analyses for the CDI and SLAS outcomes.

Instrument (Age)	Phthalate exposure	Units	25th percentile	75th percentile
CDI (2 years)	ΣAA phthalates	μmol/L	0.181	0.422
	ΣDEHP	μmol/L	0.045	0.106
	ΣDEHTP	μmol/L	0.094	0.513
SLAS (3 years)	ΣDEHTP	μmol/L	0.094	0.513

Table 7.8. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with language development outcomes on the CDI at 2 years at different levels of phthalate exposure.¹

Outcome	Exposure Interaction	Interaction p-value	Stratified by Phthalate Exposure Level		
			Low	Medium	High
M3L	Full Pregnancy x ΣAA phthalates	0.090	** 4.754 (5.818, 4.099)	** 5.403 (5.818, 4.988)	** 4.754 (5.408, 4.099)
Complexity	1st Trimester x ΣDEHTP	0.013	^a ** 5.121 (5.74, 4.503)	^b ** 5.827 (6.285, 5.369)	^{ab} ** 4.221 (4.847, 3.596)

¹All models were adjusted for acetaminophen formulation, maternal age, household income, delivery type, child sex, and number of older siblings.

Post hoc comparison: ^a $p < 0.10$, ^b $p < 0.05$

****** $p < 0.01$

Table 7.9. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with language development outcomes on the CDI at 2 years for stratified by child sex and phthalate exposure level.¹

		Females		
		Stratified by Phthalate Exposure Level		
Outcome	Exposure Interaction	Low	Medium	High
M3L	1st Trimester x Σ DEHP	** 5.513 (6.43, 4.596)	** 6.342 (7.027, 5.658)	** 5.801 (6.673, 4.93)
Complexity	3rd Trimester x Σ DEHP	** 22.305 (26.646, 17.964) ^a	** 23.708 (27.727, 19.688) ^a	** 17.89 (22.406, 13.374)

		Males		
		Stratified by Phthalate Exposure Level		
Outcome	Exposure Interaction	Low	Medium	High
M3L	1st Trimester x Σ DEHP	** 4.514 (5.351, 3.676)	** 4.094 (4.683, 3.505)	** 4.578 (5.351, 3.676)
Complexity	3rd Trimester x Σ DEHP	** 13.43 (19.066, 7.794)	** 12.553 (16.469, 8.638)	** 14.309 (19.647, 8.971)

¹All models were adjusted for acetaminophen formulation, maternal age, household income, delivery type, child sex, and number of older siblings.

Post hoc comparison: ^a $p < 0.10$, ^b $p < 0.05$

** $p < 0.01$

Table 7.10. Multivariable linear regression analyses of the relation (β estimate and 95% confidence interval) of prenatal acetaminophen exposure with attention-related outcomes on the SLAS at 3 years at different levels of phthalate exposure by child sex.¹

		Females		
		Stratified by Phthalate Exposure Level		
Outcome	Exposure Interaction	Low	Medium	High
Assertiveness	Entire Pregnancy x Σ DEHP	** 17.107 (18.524, 15.691)	** 18.01 (19.182, 16.839)	** 17.865 (22.089, 13.641)
Articulation	Entire Pregnancy x Σ DEHP	** 16.558 (17.749, 15.366)	** 16.839 (17.824, 15.854)	** 18.369 (21.922, 14.816)

		Males		
		Stratified by Phthalate Exposure Level		
Outcome	Exposure Interaction	Low	Medium	High
Assertiveness	Entire Pregnancy x Σ DEHP	** 16.84 (18.986, 14.694)	** 15.436 (17.013, 13.859)	** 15.411 (17.516, 13.306)
Articulation	Entire Pregnancy x Σ DEHP	** 16.293 (18.532, 14.054)	** 14.673 (16.318, 13.028)	** 13.612 (15.808, 11.417)

¹All models were adjusted for acetaminophen formulation, maternal age, household income, delivery type, child sex, and number of older siblings.

** p < 0.01

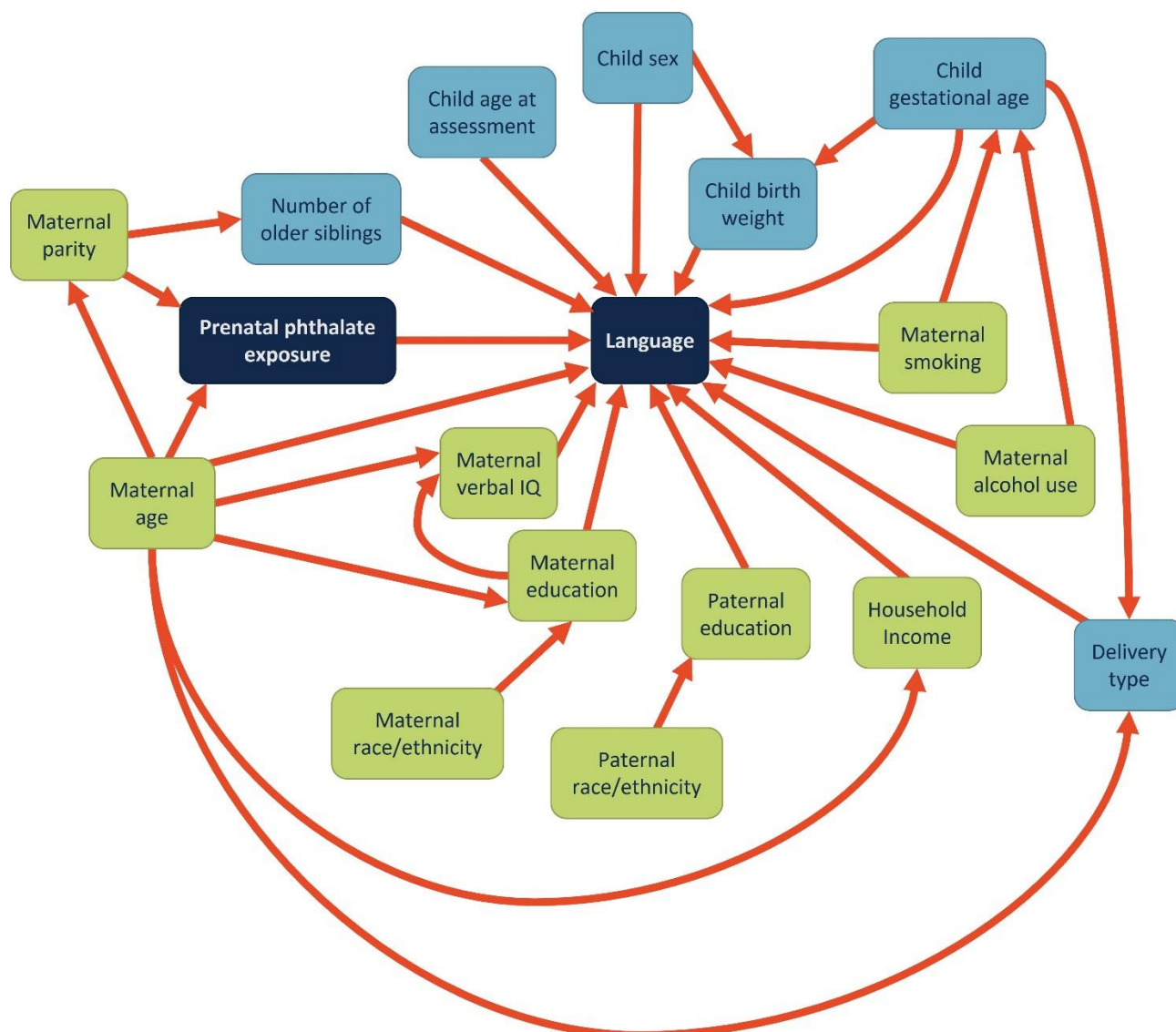


Figure 7.1. Directed acyclic graph (DAG) used to identify potential covariates for inclusion in models examining the association of prenatal phthalate exposure and language outcomes. Green squares indicate parent-related variables, and light blue squares indicate child-related variables

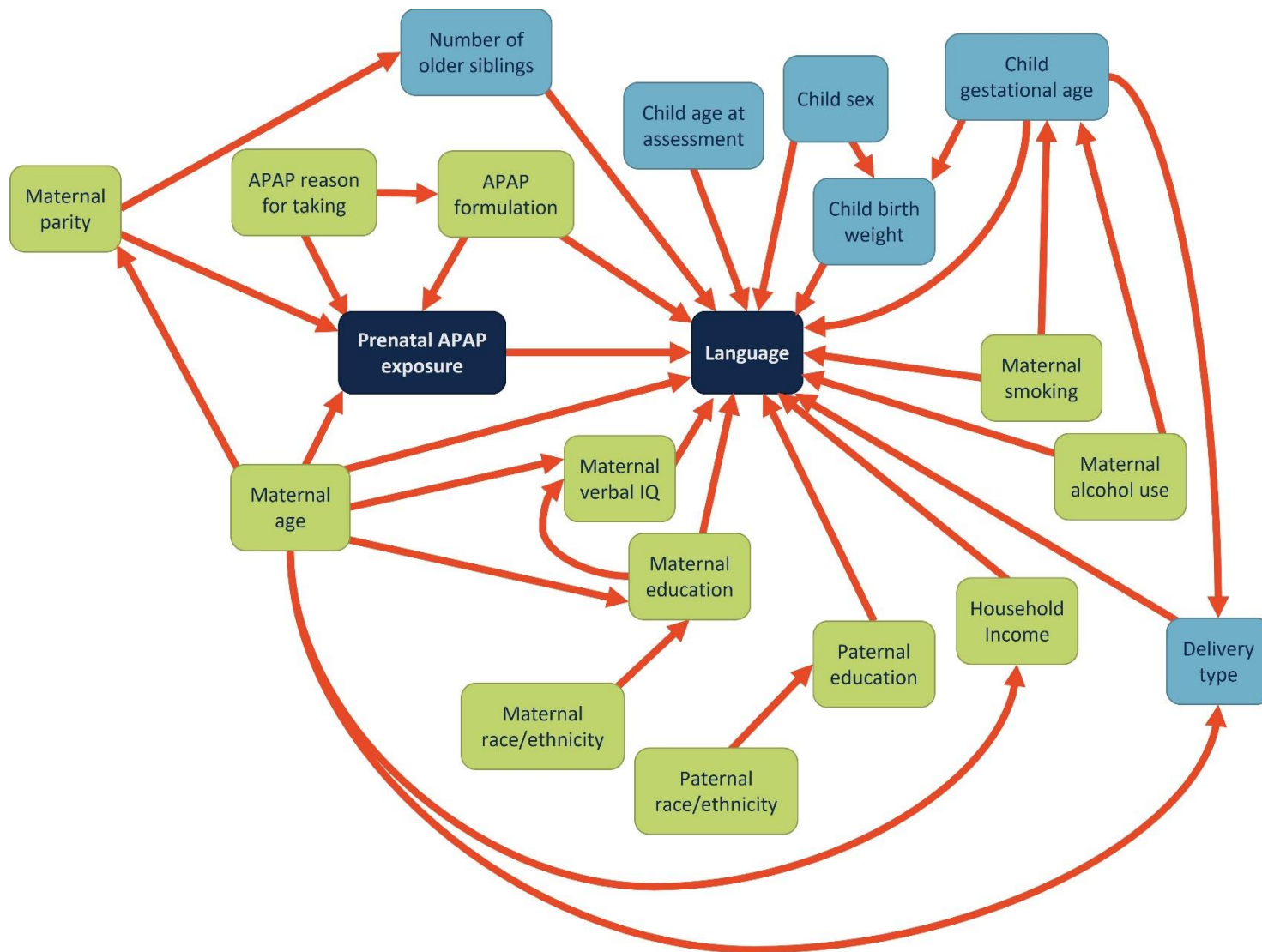


Figure 7.2. Directed acyclic graph (DAG) used to identify potential covariates for inclusion in models examining the association of prenatal acetaminophen exposure and language outcomes. Green squares indicate parent-related variables, and light blue squares indicate child-related variables

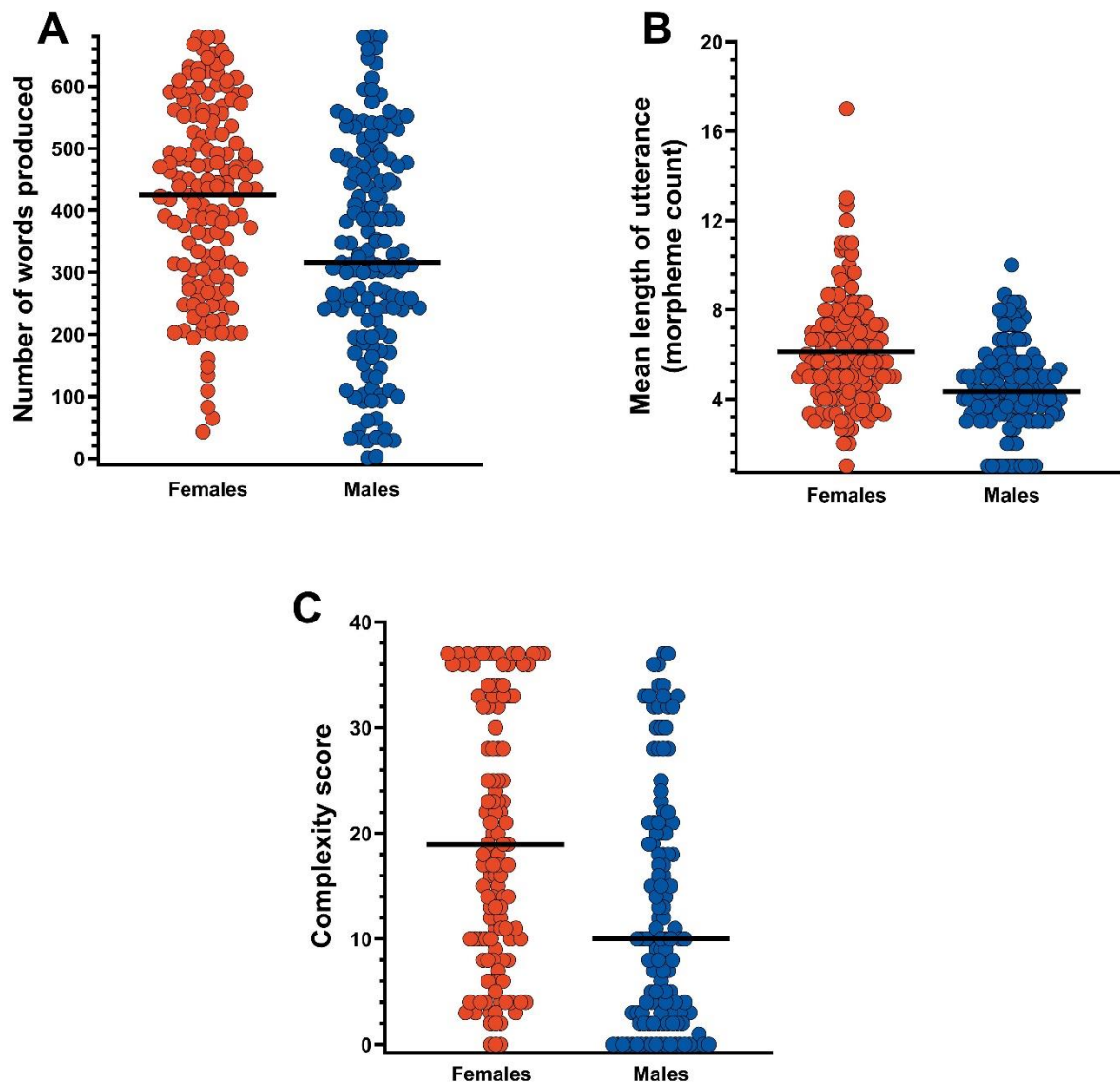


Figure 7.3. Distribution and mean of outcomes on the CDI at 2 years in the IKIDS cohort. Female children had (A) larger vocabularies, (B) higher mean length of utterances, and (C) higher complexity compared to male children.

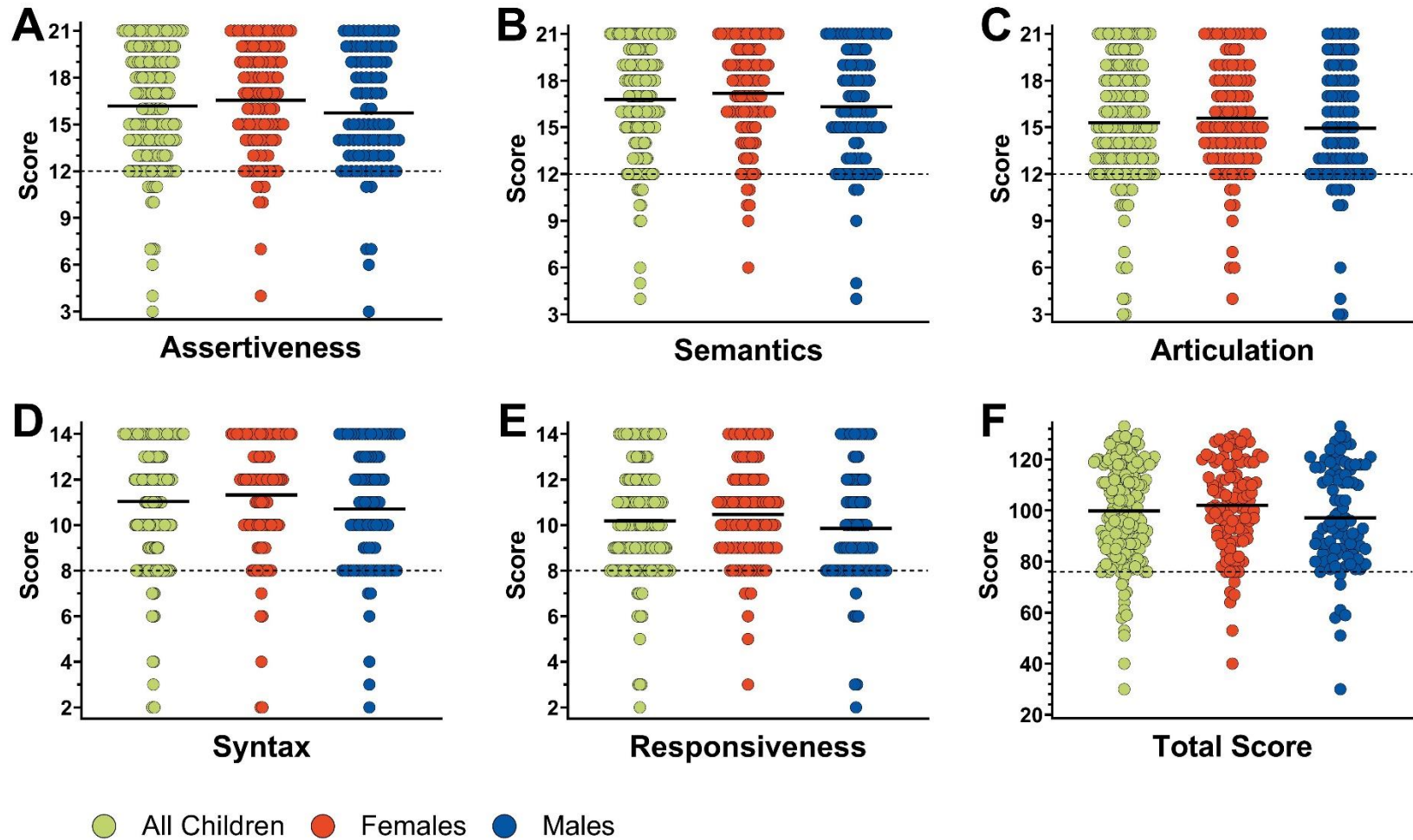


Figure 7.4. Distribution and mean outcomes on the SLAS at 3 years in the IKIDS cohort. Female children had marginally higher (A) assertiveness, (B) semantics, (C) articulation, (D) syntax, (E) responsiveness, and (F) total scores compared to male children. The dashed lines indicate scores which would be considered “normal for age.” Whether males and females were combined or stratified, on average, children had scores above the “normal for age” cutoffs.

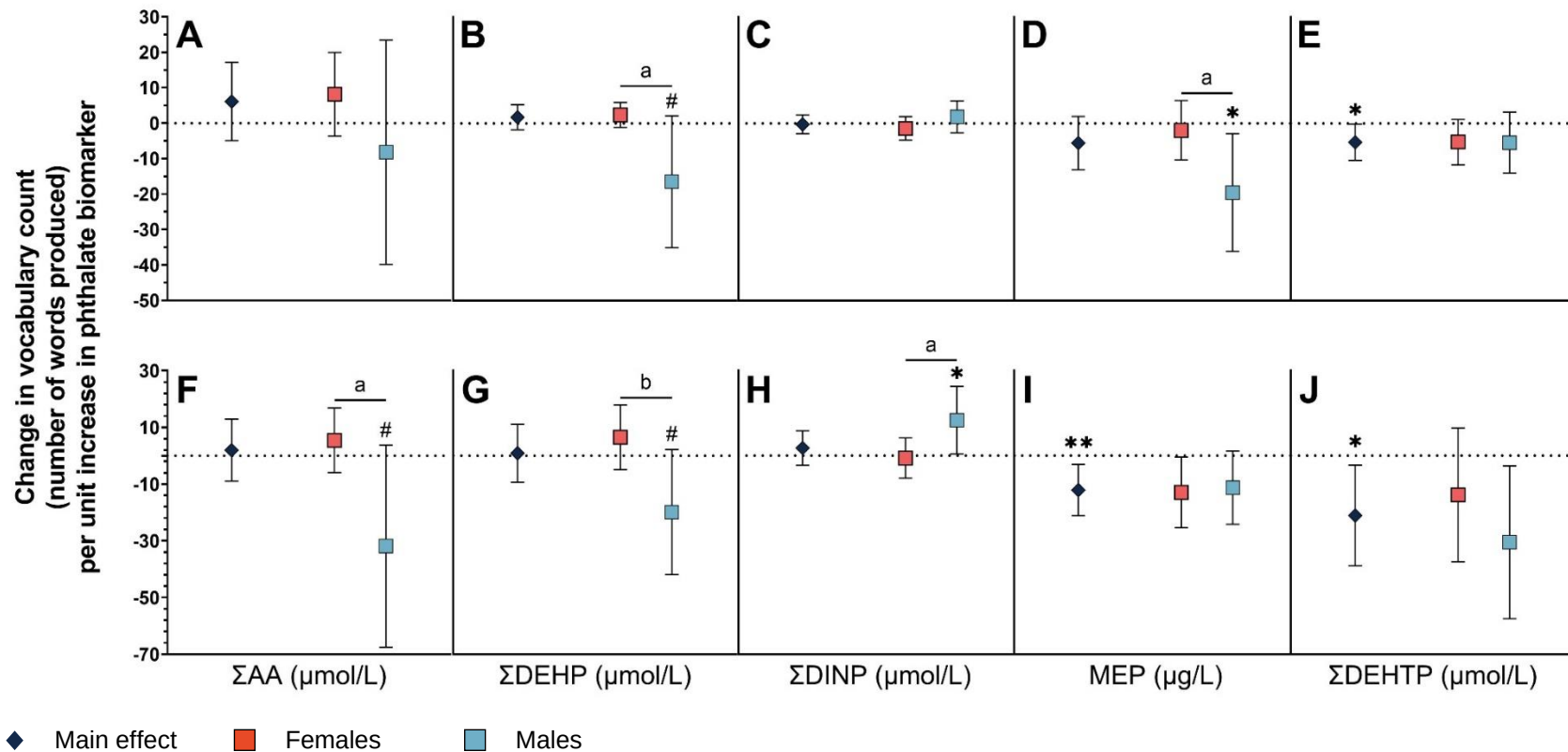


Figure 7.5. Associations of prenatal phthalate exposure with vocabulary size at 2 years of age measured via the CDI. Prenatal exposure to ΣAA phthalates was associated with a decrease in vocabulary size in males only for both the sample at (A) 16-18 weeks and (F) the pooled sample, although the association only approached significance for the pooled sample. ΣDEHP in both the (B) 16-18 weeks sample and (G) the pooled sample was associated with a decrease in vocabulary size in males only as well. ΣDINP in only the pooled sample (H) was associated with an increase in vocabulary size in males only; there was no relationship between ΣDINP in the 16-18 weeks sample and vocabulary size (C). MEP in the 16-18 weeks sample (D) was only associated with a decrease in vocabulary size in male children, but MEP in the pooled sample (I) was associated with a decrease in all children. ΣDEHTP in both the (E) 16-18 weeks sample and (J) the pooled sample was associated with a decrease in vocabulary size in all children. Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$
[#] $p < 0.10$, * $p < 0.05$, ** $p < 0.01$

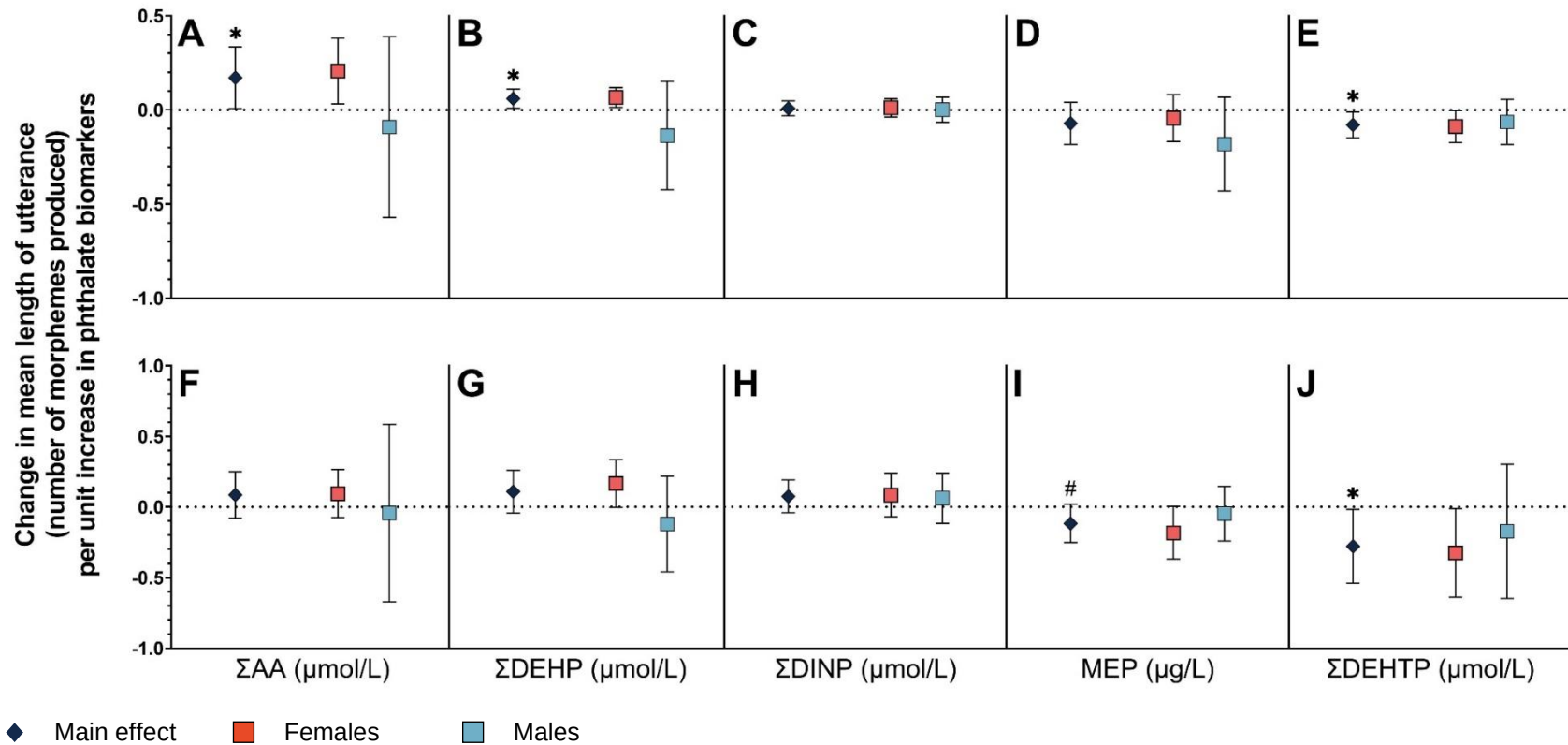


Figure 7.6. Associations of prenatal phthalate exposure with mean length of utterance at 2 years of age measured via the CDI. ΣAA in the 16-18 weeks sample (A) and the pooled sample (F) was associated with an increase in mean length of utterance in all children, but this association was only significant for the 16-18 weeks sample. ΣDEHP in the 16-18 weeks sample (B) and the pooled sample (G) was also associated with an increase in mean length of utterance in all children, but this association was only significant for the 16-18 weeks sample. There was no relationship between ΣDINP in (C) the 16-18 weeks sample or (H) the pooled sample and mean length of utterance. MEP in both (D) the 16-18 weeks and (I) the pooled sample was associated with a decrease in the mean length of utterance, although this was only significant for the pooled sample. ΣDEHTP in both (E) the 16-18 weeks sample and (J) the pooled sample was also associated with a decrease in mean length of utterance.

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$

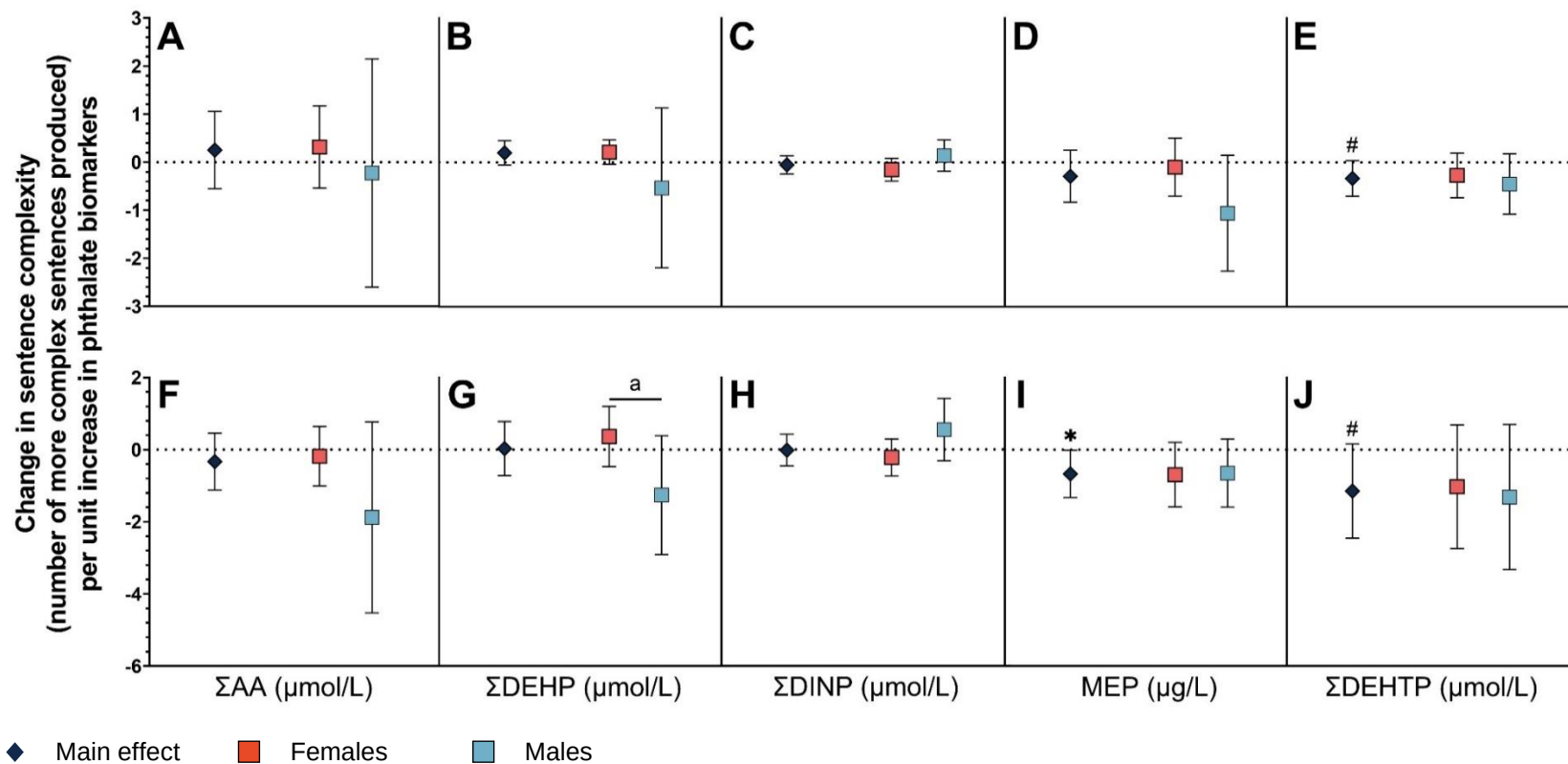


Figure 7.7. Associations of prenatal phthalate exposure with sentence complexity at 2 years of age measured via the CDI. There were no associations of ΣAA phthalates (A, F) ΣDEHP (B,G), or ΣDINP (C, H) in either the 16-18 weeks sample or the pooled sample with complexity. While the sex-by-ΣDEHP interaction was $p < 0.10$ for the pooled sample (G), the associations for males or females were not significant. Both MEP (D, I) and ΣDEHTP (E, J) were associated with lower complexity scores in all children, and while this was significant for both the 16-18 weeks sample and the pooled sample for ΣDEHTP, it was only significant for the pooled sample for MEP.

Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$

$p < 0.10$, * $p < 0.05$, ** $p < 0.01$

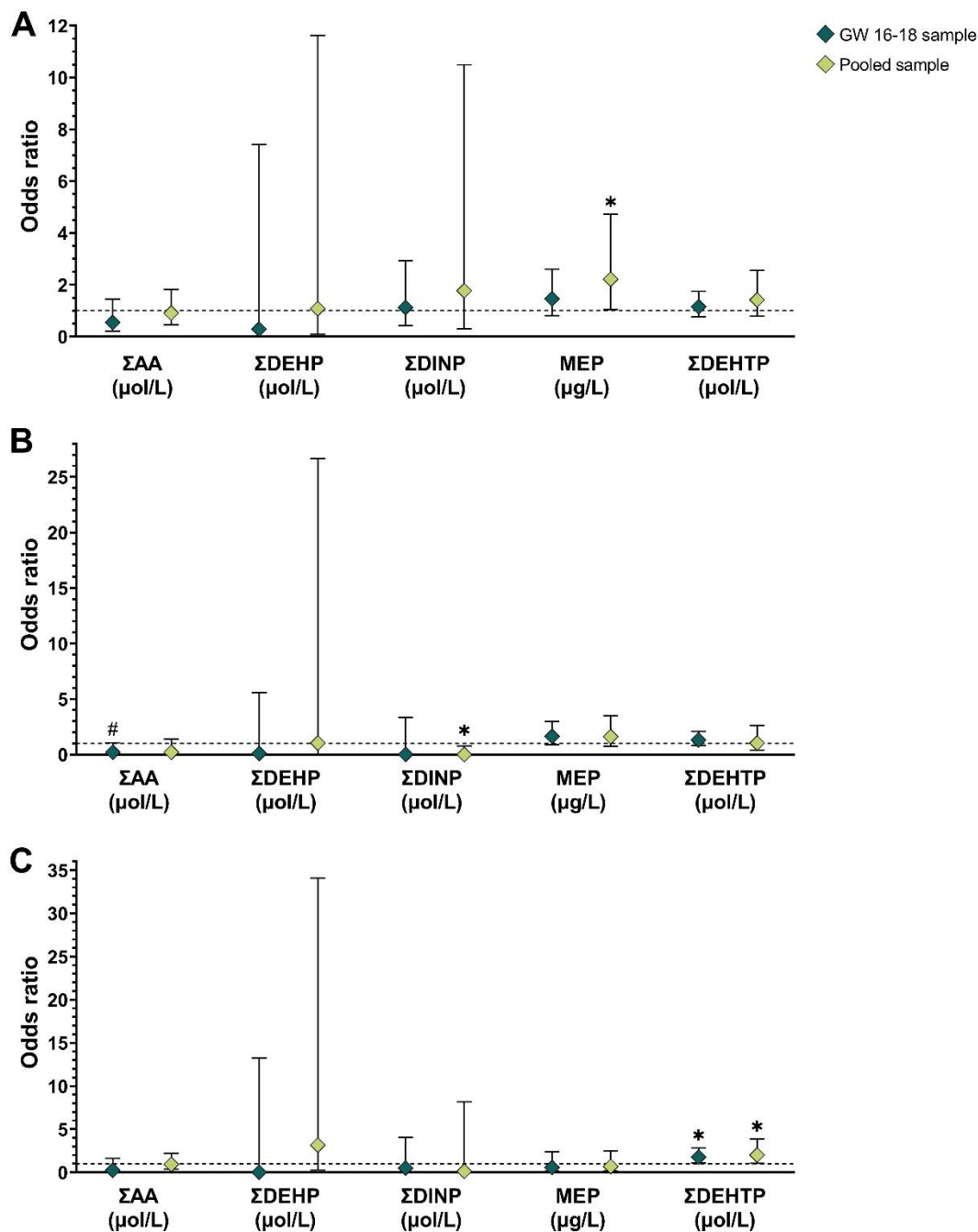


Figure 7.8. Odds ratios and 95% confidence intervals for prenatal phthalate exposure biomarkers and (A) vocabulary count, (B) mean length of utterance, and (C) complexity at or below the 25th percentile. (A) Only MEP in the pooled sample was associated with a significantly increased odds of children having a vocabulary percentile scores at or below the 25th percentile. (B) ΣAA phthalates in the 16-18 weeks sample was associated with a borderline significant decreased odds of children having a mean length of utterance percentile score at or below the 25th percentile. ΣDINP in the pooled sample was associated with a significant decrease in the odds of children having a mean length utterance at or below the 25th percentile. (C) ΣDEHTP in both the 16-18 weeks sample and the pooled sample was associated with an increased odds of children having a percentile score at or below the 25th percentile. #p < 0.10, *p < 0.05

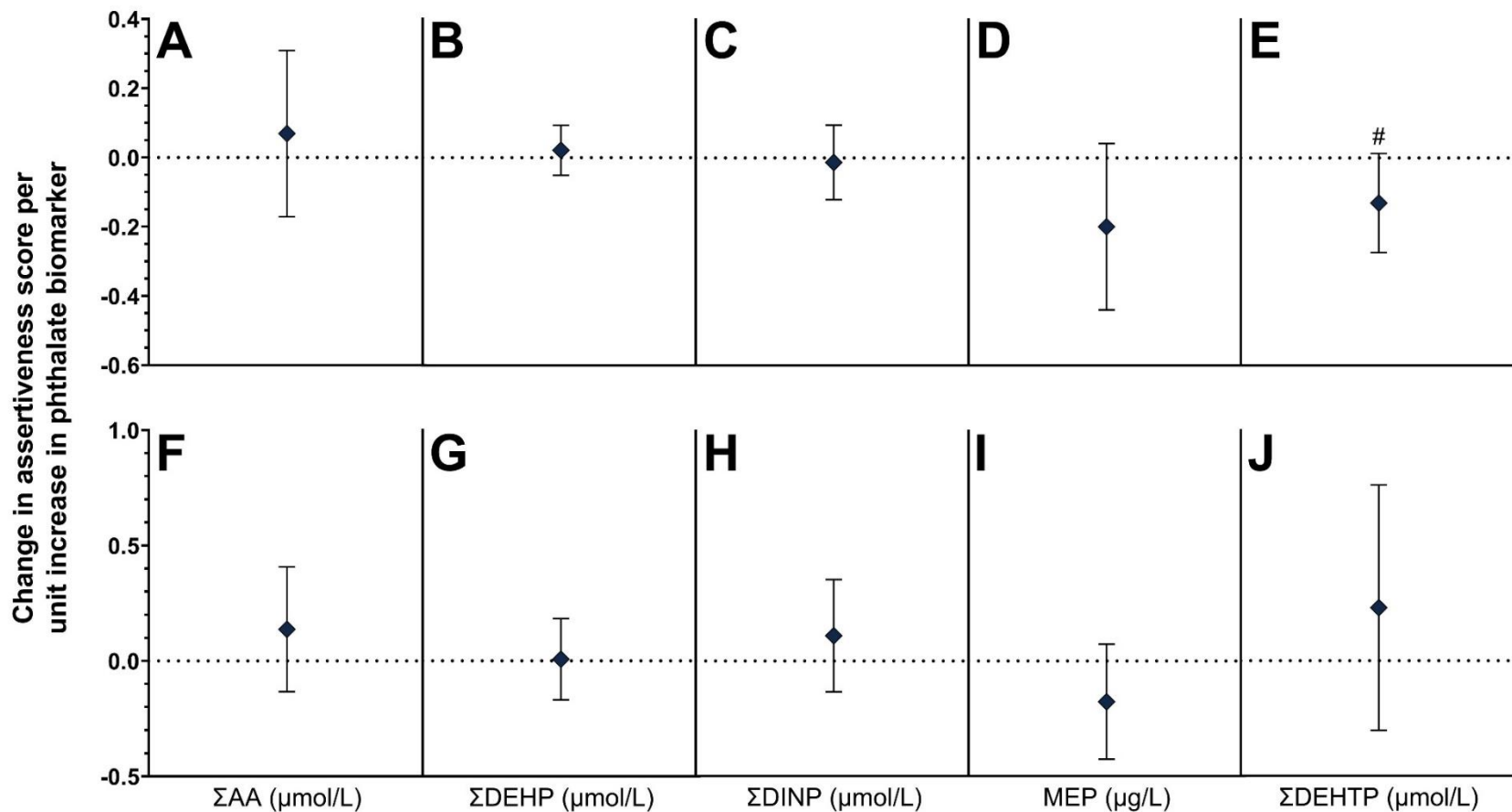


Figure 7.9. The association of prenatal phthalate exposure and conversational assertiveness as measured by the SLAS at 3 years. ΣAA phthalates in both (A) the 16-18 weeks and (F) the pooled samples were associated with a non-significant increase in assertiveness. There was no association of ΣDEHP (B, G) or ΣDINP in the 16-18 weeks sample (C) and assertiveness. MEP in both (D) the 16-18 weeks and (I) the pooled samples was associated with a non-significant decrease in assertiveness. ΣDEHTP in the 16-18 weeks sample was associated with a borderline decrease in assertiveness (E), but ΣDEHTP in the pooled sample was associated with a non-significant increase in assertiveness (J).

#p < 0.10, *p < 0.05, **p < 0.01

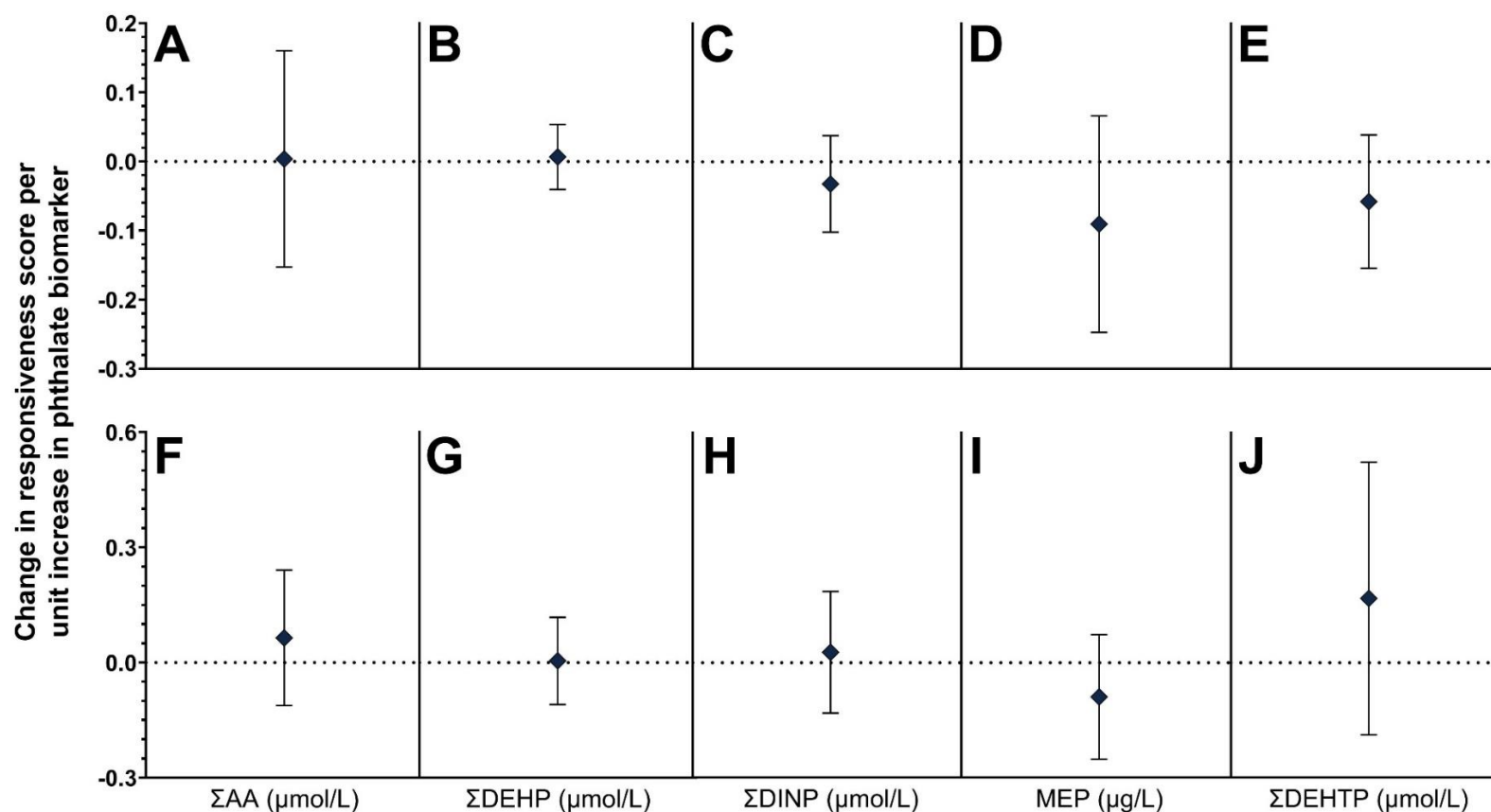


Figure 7.10. The association of prenatal phthalate exposure and conversational responsiveness as measured by the SLAS at 3 years. Σ AA phthalates in the 16-18 weeks sample was not associated with a change in responsiveness (A), but in the pooled sample, Σ AA phthalates was associated with a small non-significant increase in responsiveness (F). Σ DEHP was not associated with a change in responsiveness (B, G). Σ DINP in the 16-18 weeks sample was associated with a very small, non-significant decrease in responsiveness (C) while Σ DINP in the pooled sample was associated with a very small, non-significant increase in responsiveness (H). MEP in both the (D) 16-18 weeks sample and the (I) pooled sample was associated with a decrease in responsiveness. Σ DEHTP in the 16-18 weeks sample was associated with a non-significant decrease in responsiveness (E) while in the pooled sample, Σ DEHTP was associated with an increase in responsiveness (J).

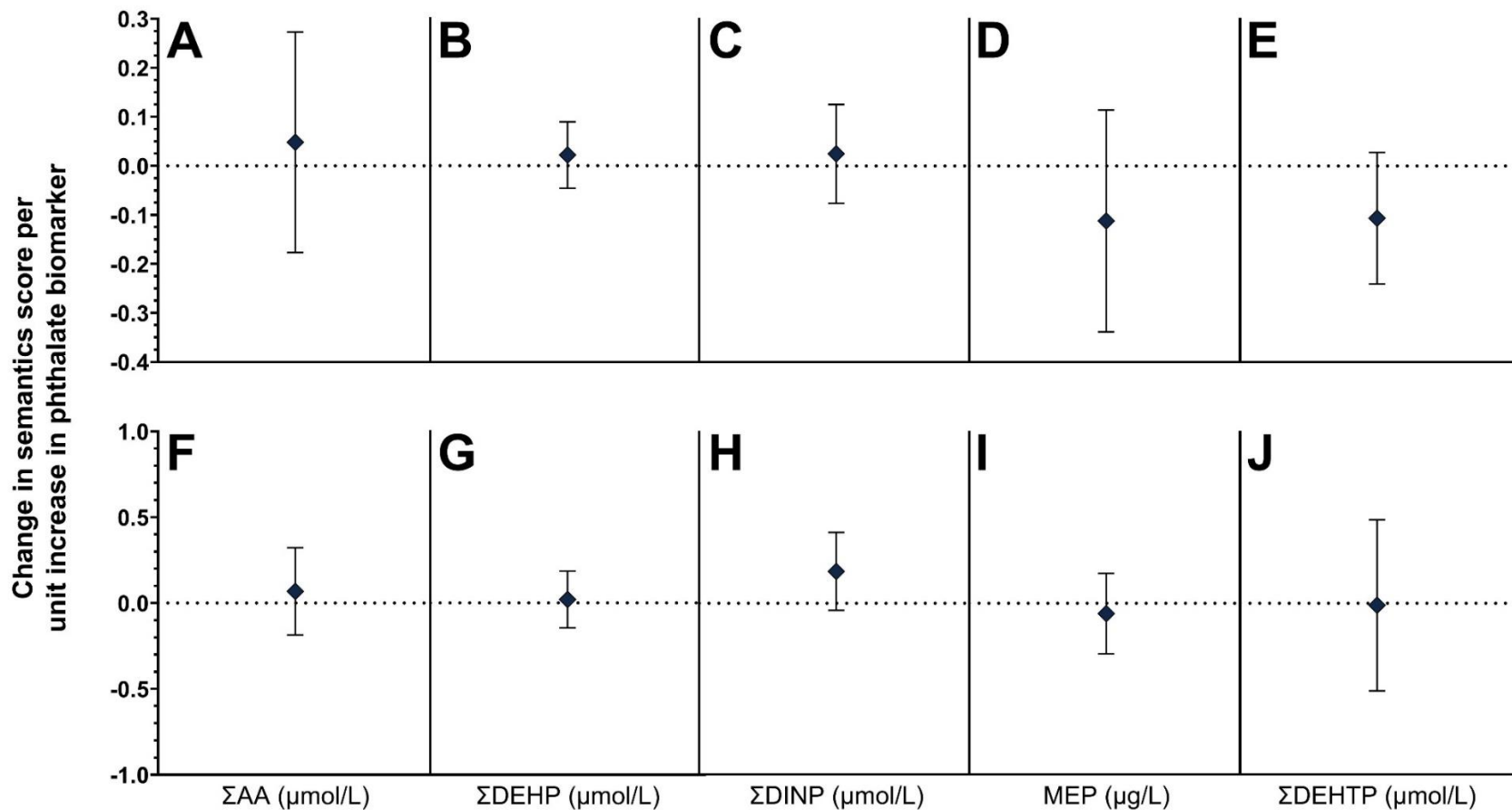


Figure 7.11. The association of prenatal phthalate exposure and semantics as measured by the SLAS at 3 years. Σ AA phthalates in both (A) the 16-18 weeks and (F) the pooled samples was associated with a small, non-significant increase in semantics. There was no association of Σ DEHP and semantics (B, G). Σ DINP in the 16-18 weeks sample was not associated with a change in semantics (C), but in the pooled sample, it was associated with a small, non-significant increase in semantics (H). MEP in the 16-18 weeks sample was associated with a non-significant decrease in semantics (D) while MEP in the pooled sample was not associated with a change in semantics (I). Σ DEHTP in the 16-18 weeks sample was associated with a non-significant decrease in semantics (E), but in the pooled sample, it was not associated with a change in semantics.

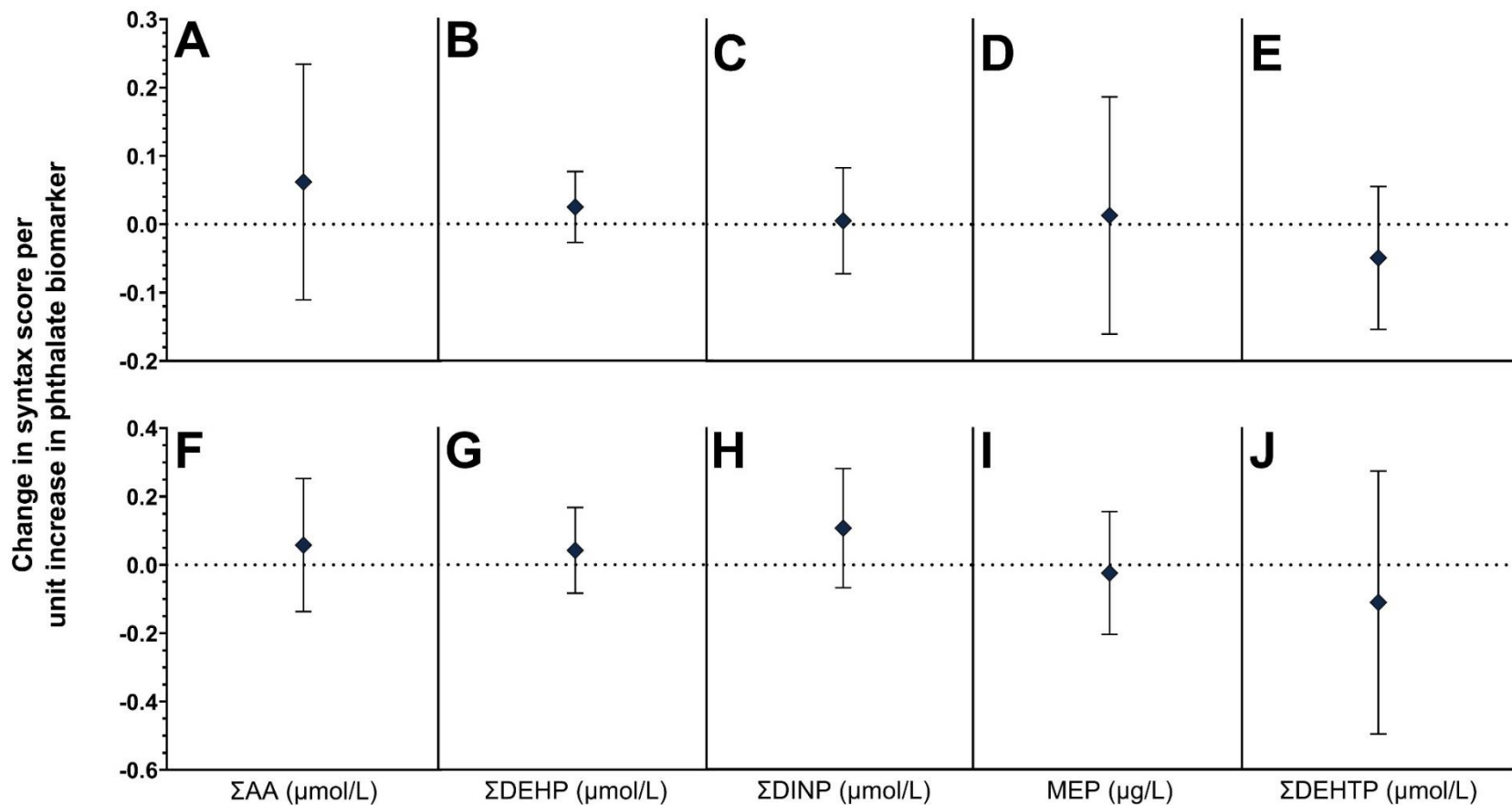


Figure 7.12. The association of prenatal phthalate exposure and syntax as measured by the SLAS at 3 years. ΣAA (A, F) and ΣDEHP (B, G) was associated with a non-significant increase in syntax. ΣDINP in the pooled sample was also associated with a non-significant increase in syntax (C), but it was not associated with a change in syntax in the 16-18 weeks sample (H). MEP was not associated with a change in syntax (D, I), but ΣDEHTP was associated with a small, non-significant decrease in syntax (E, J).

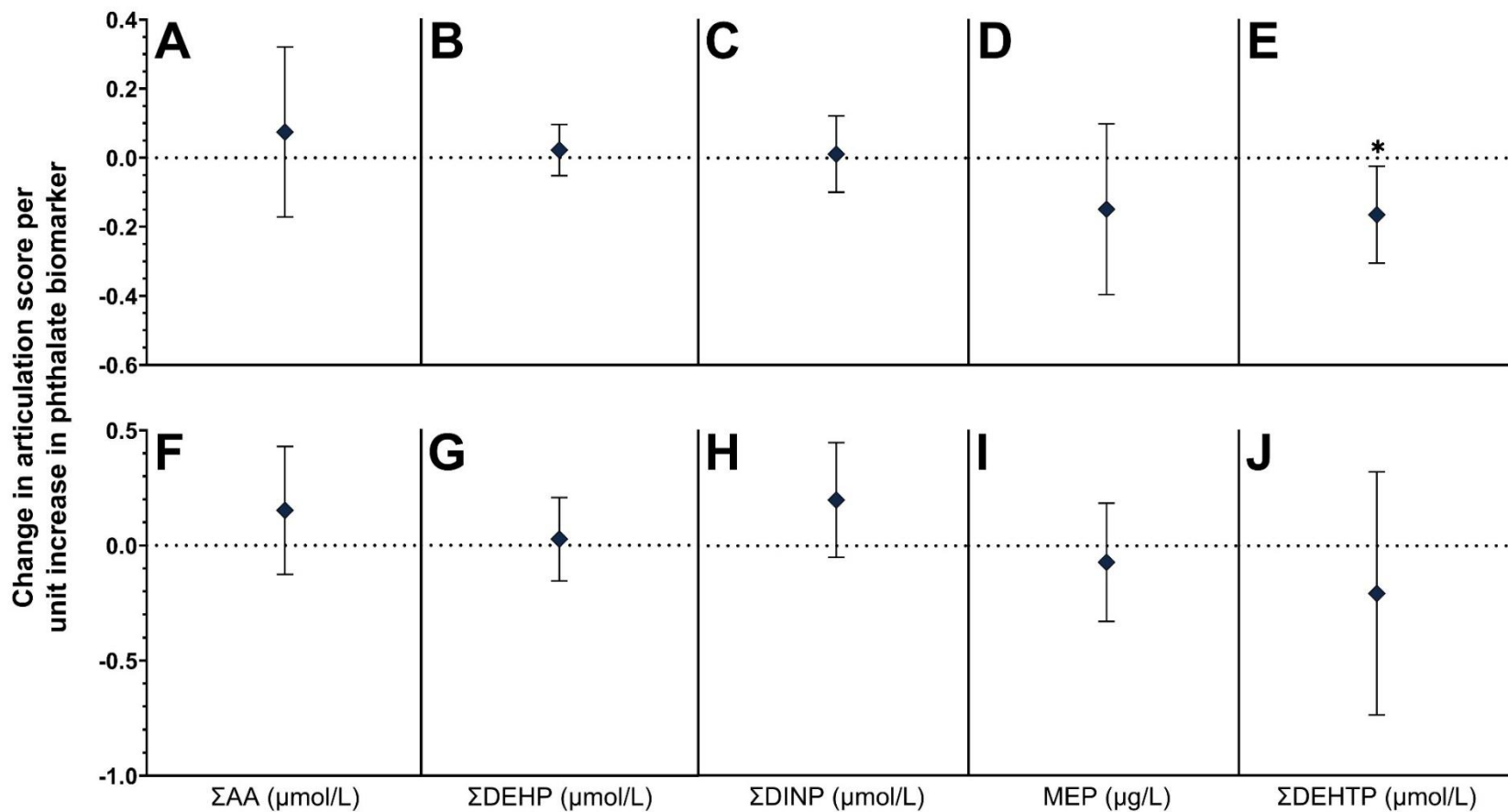


Figure 7.13. The association of prenatal phthalate exposure and articulation as measured by the SLAS at 3 years. ΣAA phthalates were associated with a small, non-significant increase in articulation. ΣDEHP was not associated with articulation (B, G), nor was ΣDINP in the 16-18 weeks sample (C). ΣDINP in the pooled sample was associated with a non-significant increase in articulation (H). MEP (D, I) and ΣDEHTP (E, J) were associated with a decrease in articulation, but this association was only significant for ΣDEHTP in the 16-18 weeks sample.

*p < 0.05

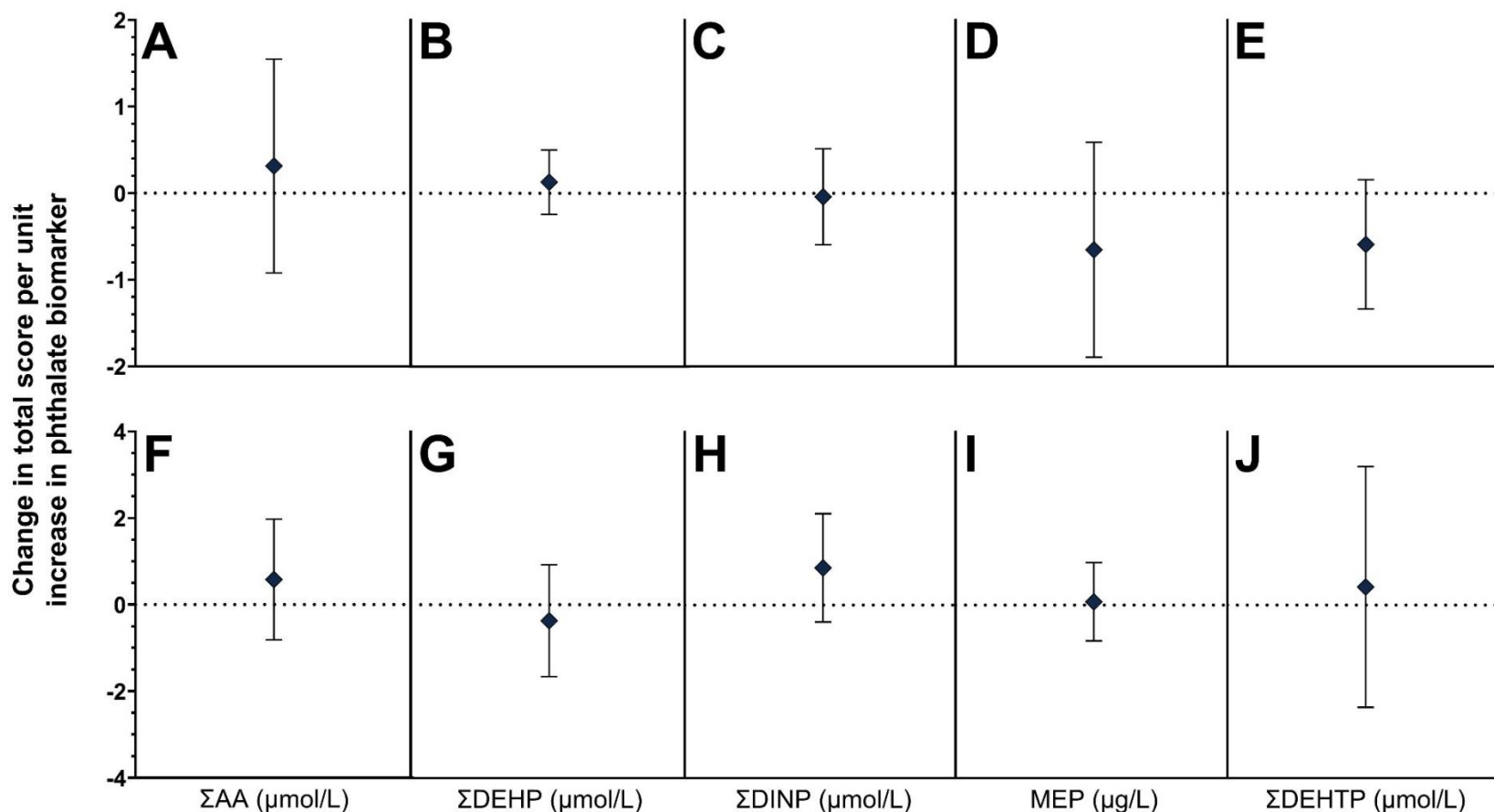


Figure 7.14. The association of prenatal phthalate exposure and the SLAS total score at 3 years. Σ AA phthalates was associated with a small, non-significant increase in total score (A, F). There was no significant association of Σ DEHP and total score (B, G). Σ DINP in the 16-18 weeks sample was not associated with total score (C), but in the pooled sample, Σ DINP was associated with a small, non-significant increase in total score (H). MEP in the 16-18 weeks sample was associated with a non-significant decrease in total score (D), but there was no association of MEP in the pooled sample and total score (I). Σ DEHTP in the 16-18 weeks sample was associated with a non-significant decrease in total score (E) while Σ DEHTP in the pooled sample was not associated with a change in total score (J).

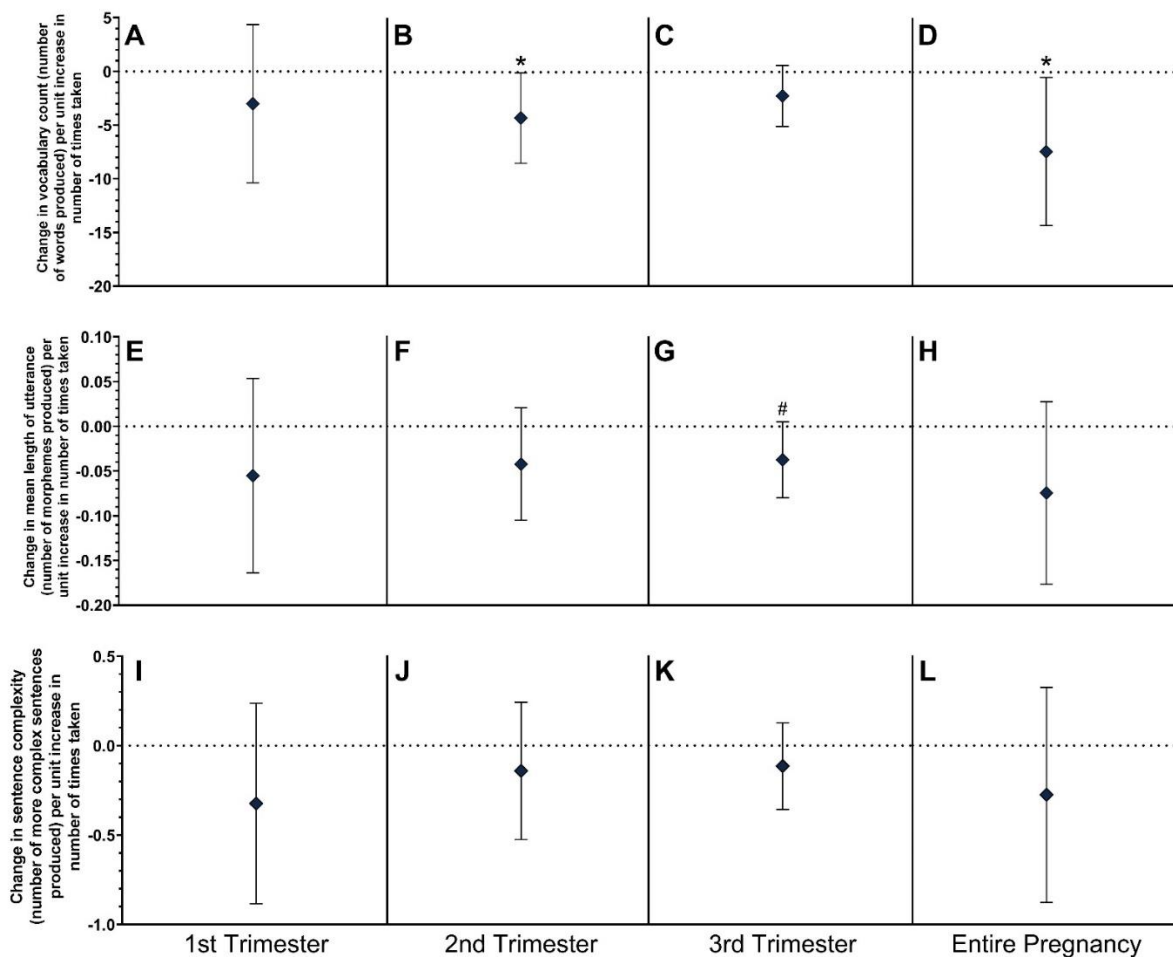


Figure 7.15. Associations of prenatal acetaminophen exposure and vocabulary size (top), mean length of utterance (middle), and complexity (bottom) at 2 years. While prenatal acetaminophen exposure was associated with a decrease in vocabulary size in all infants at all trimesters as well as cumulative exposure throughout pregnancy, only the associations for second trimester exposure (B) and cumulative exposure throughout pregnancy (D) were significant. Exposure during any trimester as well as throughout pregnancy was associated with a decrease in mean length of utterance; however this association only approached significance for third trimester exposure (G). While exposure during any trimester and throughout pregnancy was associated with a decrease in complexity, no associations were significant.

$p < 0.10$, * $p < 0.05$

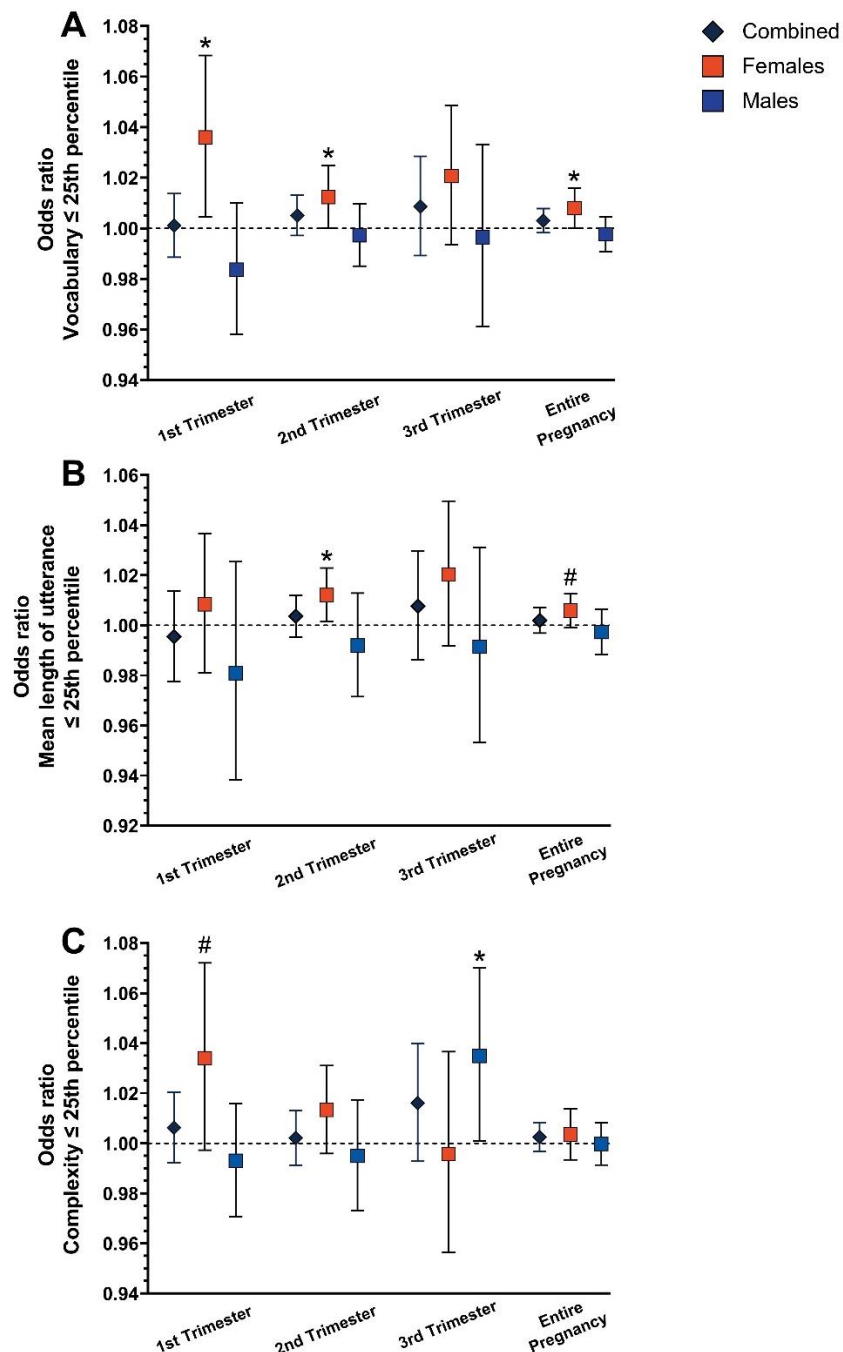


Figure 7.16. Odds ratios and 95% confidence intervals for prenatal acetaminophen exposure and (A) vocabulary size, (B) mean length of utterance, and (C) complexity $\leq$ 25th percentile. (A) Prenatal acetaminophen exposure during the first and second trimester, as well as exposure throughout pregnancy, was associated with a greater odds of female children having a vocabulary percentile score $\leq$ 25th percentile. (B) Acetaminophen exposure during the second trimester and exposure throughout pregnancy was associated with a greater odds of female children having a mean length of utterance score $\leq$ 25th percentile. (C) Acetaminophen exposure during the first trimester was associated with a greater odds of female children having a complexity score $\leq$ 25th percentile while exposure during the third trimester was associated with a greater odds of male children having a complexity score $\leq$ 25th percentile. # $p < 0.10$, * $p < 0.05$

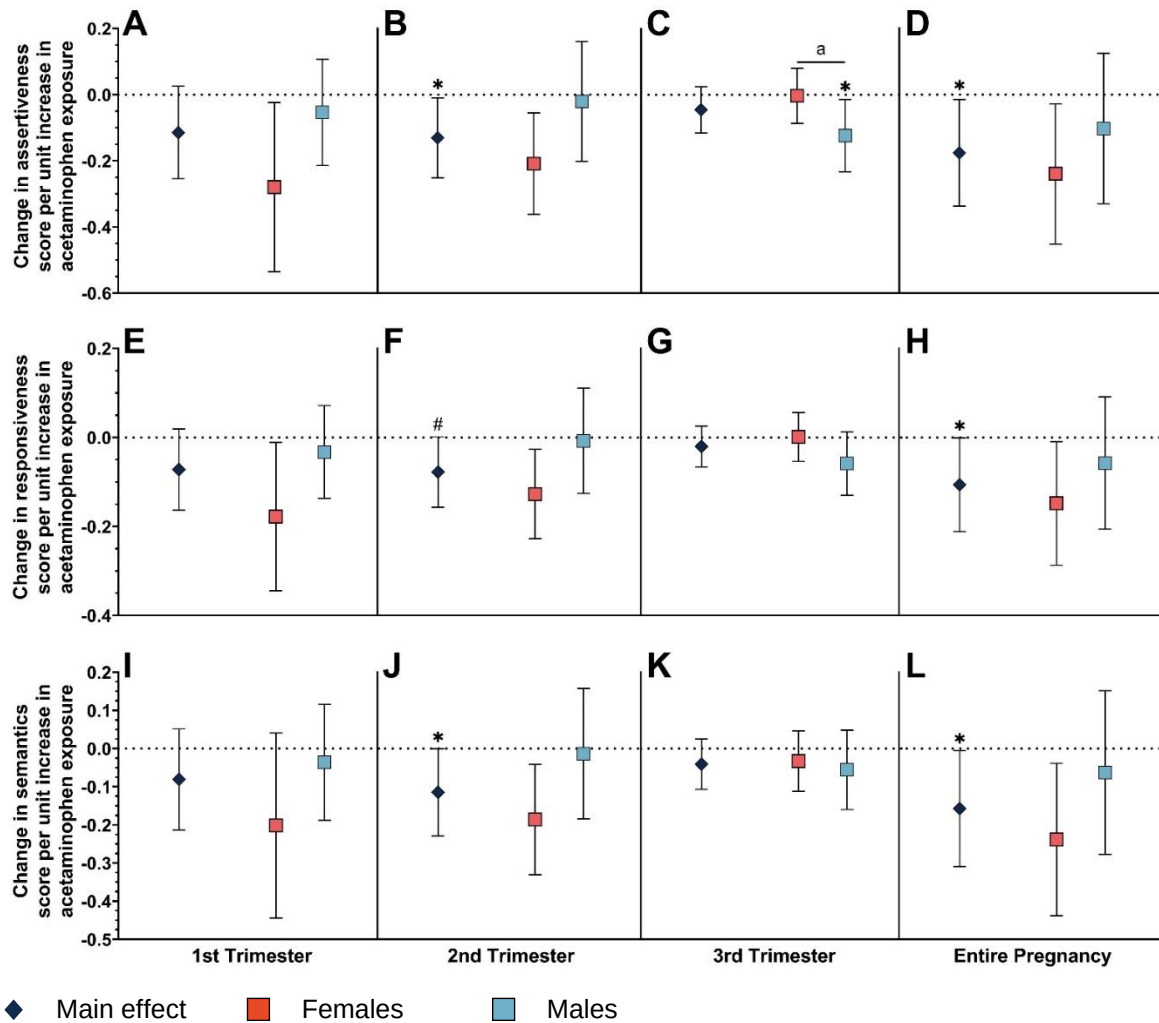


Figure 7.17. Associations of prenatal acetaminophen exposure and conversational assertiveness (top), responsiveness (middle), and semantics (bottom) on the SLAS at 3 years. Acetaminophen exposure during the second trimester and throughout pregnancy was associated with lower (B, D) assertiveness, (F, H) responsiveness, and (J, L) semantics scores. Third trimester exposure (C) was also associated with lower assertiveness scores in male children only.

Interaction p-value: ^a $p < 0.10$

[#] $p < 0.10$, * $p < 0.05$

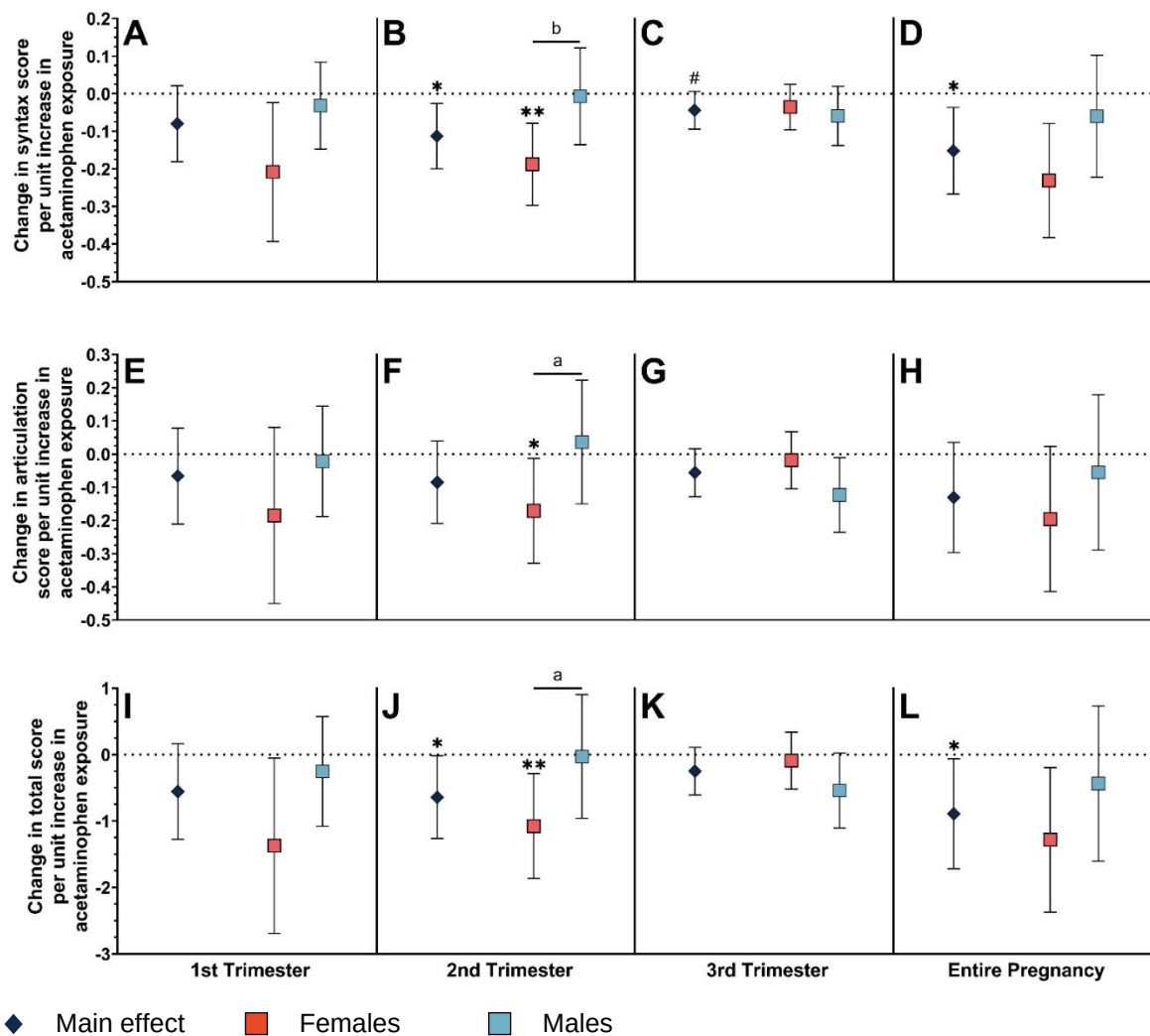


Figure 7.18. Associations of prenatal acetaminophen exposure and syntax (top), articulation (middle), and total score (bottom) on the SLAS at 3 years. (B) Second trimester exposure, (C) third trimester exposure, and (D) exposure throughout pregnancy was associated with lower syntax scores. (B) Third trimester exposure was associated with lower syntax scores in female children specifically as well. (F) Second trimester exposure was associated with lower articulation scores in female children only. (J) Second trimester exposure and (L) exposure throughout pregnancy was associated with lower total SLAS scores. (J) Second trimester exposure was also specifically associated with lower total SLAS scores in female children. Interaction p-value: ^a $p < 0.10$, ^b $p < 0.05$
 * $p < 0.05$, ** $p < 0.01$

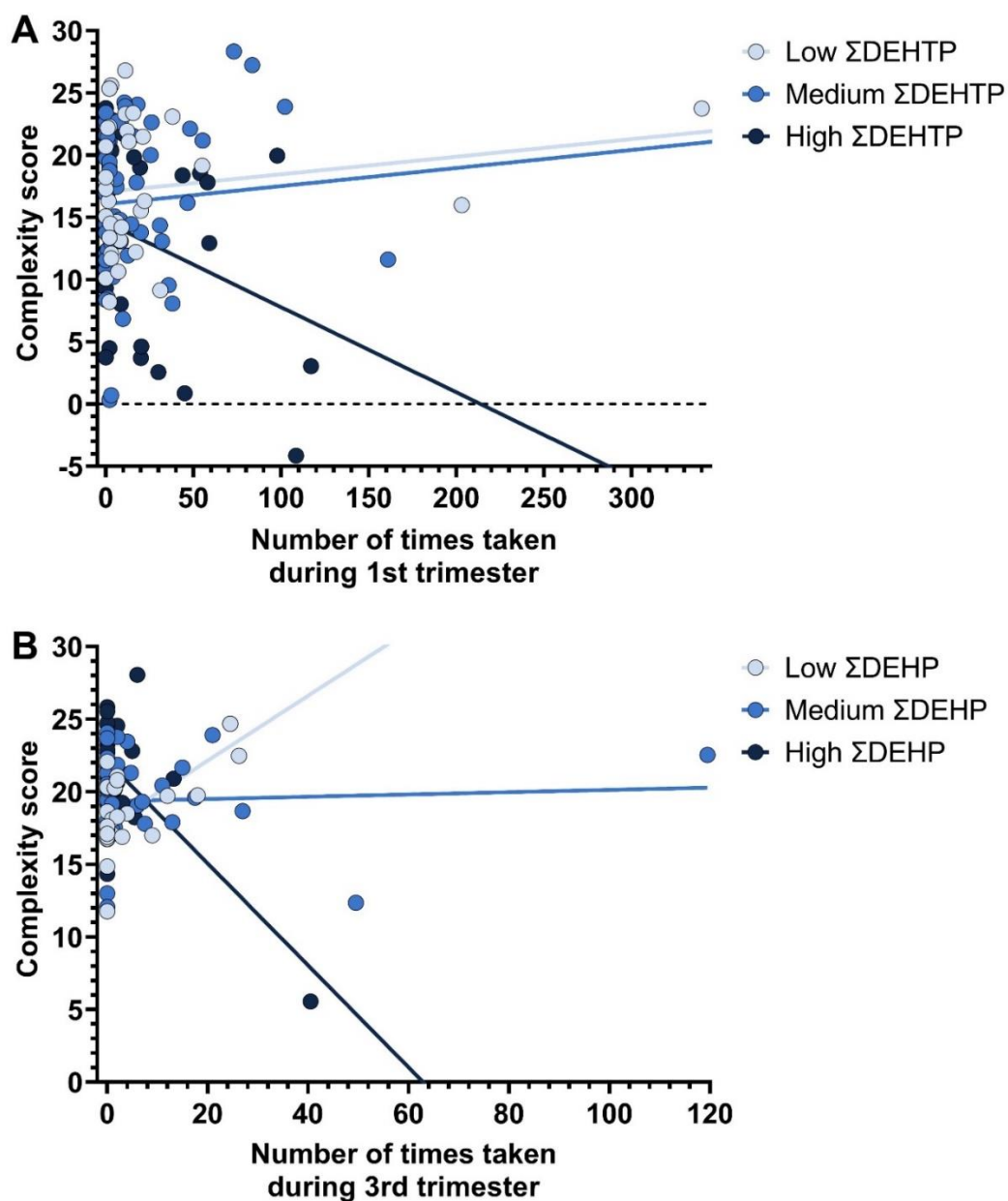


Figure 7.19. The association of acetaminophen exposure during (A) the first trimester and (B) the third trimester and complexity at 2 years of age by Σ DEHP exposure in males and females combined (A) and Σ DEHP exposure in only females (B). (A) Acetaminophen exposure during the first trimester was associated with higher complexity scores in both the low and medium Σ DEHP exposure groups, but they were not significantly different from one another. However, acetaminophen exposure was associated with lower complexity scores in the high Σ DEHP group compared to both the low and medium Σ DEHP groups. (B) In female children only, acetaminophen exposure was associated with higher complexity scores in both the low and medium Σ DEHP exposure groups, although they were not significantly different from one another. Acetaminophen exposure was associated with significantly lower complexity scores in female children in the high Σ DEHP group compared to those in the low and medium Σ DEHP groups.

CHAPTER 8: INTEGRATIVE DISCUSSION

Both acetaminophen (Table 1.1) and phthalate exposure (Table 1.2) during prenatal development have been linked to attention problems and delayed or impaired language development. However, the results of studies examining these associations for phthalates have been inconsistent, and the evaluation of prenatal acetaminophen exposure has been imprecise in studies published to date. Additionally, both phthalates (Parks et al., 2000; Howdeshell et al., 2007; MacLeod et al., 2009; Hannas et al., 2011; Swan et al., 2015) and acetaminophen (Kristensen et al., 2011; Kristensen et al., 2012; Mazaud-Guittot et al., 2013; Holm et al., 2016; Hay-Schmidt et al., 2017; Lind et al., 2017) have been shown to have antiandrogenic action, and therefore may share a common mechanism for disrupting neurodevelopment. Finally, exposure to both is likely during pregnancy (Centers for Disease Control, 2019; Servey and Chang, 2014). Therefore, it was important to assess exposure to phthalates and acetaminophen individually as well as to explore whether co-exposure could have cumulative effects on neurodevelopment. Utilizing data from the Illinois Kids Development Study (IKIDS) prospective birth cohort, this study aimed to evaluate the association of prenatal exposure to acetaminophen collected via maternal report at 6 time points across pregnancy and attention in infancy and early childhood, as well as language development in early childhood. This study also aimed to examine the association of prenatal exposure to several phthalate biomarkers with these outcomes, and, finally, the potential interactive effects of prenatal phthalate and acetaminophen exposure.

8.1. ATTENTION

8.1.1. The relationship of prenatal phthalate exposure and attention

In infancy, time to accrue 20 seconds total looking time at two identical faces or 10 seconds total looking time at two identical shapes (referred to as familiarization) was considered

to be a measure of visual attention, with shorter times indicating better attention. The results were mixed for phthalates, with the direction of the association varying by stimulus set, whether it was the faces or shapes trials, child sex and the specific phthalate biomarker (Figure 4.6). There was also no clear pattern of associations by phthalate type (antiandrogenic or not). At 2 and 3 years of age attention was assessed via maternal report using the Child Behavior Checklist (CBCL), and the results for phthalates were, again, mixed. Most phthalates had no effect or were associated with decreased externalizing behaviors, ADHD behaviors, or attention problems, mainly in boys and mainly at 2 years of age (Figure 6.2). This is similar to findings of Gascon et al. (2015) who found that di-(2-ethylhexyl) phthalate (DEHP) was associated with decreased risk of inattention at 4 and 7 years, and that monoethyl phthalate (MEP) was also associated with a decreased risk of inattention. However, many more previous studies have observed no associations of the phthalates measured in the present study with measures of attention (Whyatt et al., 2012; Kobrosly et al., 2014; Lien et al., Philippat et al., 2017; Kim et al., 2018; Hyland et al., 2019; Jankowska et al., 2019; Colicino et al., 2021). The exception in this study was MEP which was associated with increased ADHD behaviors and attention problems in boys at both 2 and 3 years of age (Figure 6.2O, Y, Z). Engel et al. (2010) also observed an association of increased attention problems with prenatal MEP exposure at 4-5 years; however, the effects they observed were not sex specific. However, several other studies have observed no effect of MEP on attention problems or found it was associated with decreases in attention problems (Table 1.2). These differences could be due to differences in the assessment instruments that were used, as all but Kobrosly et al. (2014) utilized methods other than the CBCL, or the different ages at which children were assessed (Table 1.2). Only Whyatt et al. (2012) used the CBCL to evaluate the association between prenatal phthalate exposure and behavioral outcomes at about 3 years, but they did not report associations with MEP.

8.1.2. The relationship of prenatal acetaminophen exposure and attention

Associations of acetaminophen with measures of attention were more consistent, although in infancy, there was little indication of any association of acetaminophen exposure with visual attention as measured by time to familiarization (Figure 5.2). In contrast, at 2 and 3 years, the results were remarkably consistent with acetaminophen exposure in the second trimester associated with higher (worse) scores on all three measures (externalizing behavior, attention-deficit/hyperactivity disorder [ADHD] behavior and attention problems) at both 2 and 3 years (Figure 6.3), aligning with the results of many previous studies which also found that prenatal acetaminophen exposure was associated with poorer attention, albeit typically at older ages than those examined in the present study (Brandlistuen et al., 2013; Liew et al., 2014; Thompson et al., 2014; Avella-Garcia et al., 2016; Liew et al., 2016a; Ystrom et al., 2017; Ji et al., 2018; Golding et al., 2019; Liew et al., 2019; Inoue et al., 2020; Ji et al., 2020; Trønnnes et al., 2020). Brandlistuen et al. (2013) also utilized the CBCL to evaluate associations of prenatal acetaminophen exposure and behavioral outcomes at 3 years and found that it was associated with increased externalizing behavior at that age as well (Table 1.1).

8.1.3. The relationship of prenatal exposure to phthalates and acetaminophen and attention

In models exploring interactive effects of prenatal acetaminophen and phthalate exposure on attention, there were a couple of interesting interactions for the CBCL at 2 years. Interactions of MEP exposure at 16-18 weeks and second trimester acetaminophen exposure (Figure 6.4A) as well as MEP at 16-18 weeks and acetaminophen exposure throughout pregnancy (Figure 6.4B) showed that the effects of acetaminophen exposure on ADHD Problems may be modified by MEP exposure with increasing MEP and acetaminophen exposure possibly resulting more ADHD problems at 2 years. However, given the small sample size, these analyses were significantly under-powered and must be interpreted cautiously.

8.1.4. Relevance of the present study

ADHD is a prevalent condition, and meta-analyses and systematic reviews have found that individuals with untreated ADHD have poorer academic performance and lower social functioning compared to individuals receiving treatment for their ADHD (Shaw et al., 2012; Harpin et al., 2016; Arnold et al., 2020; Posner et al., 2020). The implications of untreated ADHD strongly suggest that early detection and treatment is important (Shaw et al., 2012). At this time, ADHD cannot be diagnosed in children under age 4, but early detection and intervention at preschool age could dramatically reduce the long-term burden of ADHD (Wolraich et al., 2011; Halperin et al., 2012; Franke et al., 2018; Chang et al., 2020). Therefore, understanding risk factors for ADHD and detecting those risks early is imperative. While prenatal phthalate exposure has been linked to attention problems, ADHD-related behaviors, and ADHD in children, the results, including those of the present study, have been mixed (Table 1.2), indicating need for further study to understand this potential relationship. However, the literature for prenatal acetaminophen exposure has consistently suggested a link between higher exposure and increases in ADHD-like behaviors and attention problems (Table 1.1). The present study both confirms what earlier studies reported and shows that associations of acetaminophen with attention problems can be reliably detected in pre-school aged children. These results coupled with our other finding that prenatal acetaminophen exposure is associated with impaired language development strongly suggest that reevaluation of its safety for use during pregnancy is needed.

8.2. LANGUAGE

8.2.1. The relationship of prenatal phthalate exposure and language development

While associations of prenatal phthalate exposure with language outcomes at 2 and 3 years of age were mixed, most indicated poorer language abilities and males tended to be more adversely affected. At 2 years, both MEP and di-2-ethylhexyl terephthalate (DEHP) were

associated with smaller vocabularies (Figure 7.5), shorter mean length of utterance (M3L; Figure 7.6), and lower complexity scores (Figure 7.7) on the CDI particularly in male children, although only MEP was associated with higher odds of children having vocabulary scores $\leq$ 25th percentile (Figure 7.8A), and only Σ DEHTP was associated with higher odds of children having complexity scores $\leq$ 25th percentile (Figure 7.8C). There were few effects of phthalates on language as assessed by the Speech and Language Assessment Scale (SLAS) at 3 years of age (Figure 7.9-7.12). Only Σ DEHTP was associated with lower assertiveness (Figure 7.9) and articulation scores (Figure 7.13) at 3 years, suggesting that Σ DEHTP may have longer-lasting effects on language development, and that orthophthalates may primarily have effects on early language development which are resolved with age.

8.2.2. The relationship of prenatal acetaminophen exposure and language development

Associations of prenatal acetaminophen exposure with language outcomes were present across ages and assessment instruments. The primary associations with the raw scores on the MacArthur-Bates Communicative Development Inventories (CDI) at 2 years, were a decrease in vocabulary scores associated with both second trimester and cumulative exposure (Figure 7.15). Associations also indicated that second trimester and cumulative acetaminophen exposure was associated with increased odds of female children having vocabulary and M3L scores $\leq$ 25th percentile (Figure 7.16B). At 3 years, more associations of prenatal acetaminophen exposure with language outcomes were observed, possibly indicating that effects of prenatal exposure to acetaminophen on language development may become more apparent with age. Second trimester and cumulative acetaminophen exposure were both associated with lower scores on most of the language outcomes, although this was only for female children for syntax, articulation, and the total score (Figures 7.15, 7.16).

8.2.3. The relationship of prenatal exposure to phthalates and acetaminophen and language development

While there were no indications for cumulative effects of prenatal exposure to acetaminophen and phthalates on language outcomes at 3 years, the interaction of first trimester acetaminophen exposure with Σ DEHP indicated there may be cumulative effects for complexity scores at 2 years of age (Figure 7.19A). The interaction of Σ DEHP with third trimester acetaminophen exposure also suggested there may be a cumulative effect of exposure on complexity scores, but only for female children (Figure 7.19B). However, given the small number of children with Σ DEHP data available, these analyses were significantly under-powered and must be interpreted with great caution.

8.2.4. Relevance of the present study

The results of the present study indicate that prenatal exposure to acetaminophen or phthalates may play a role in delayed language development. Few previous studies have evaluated the association of prenatal phthalate exposure and language development (Bornehag et al., 2018a; Olesen et al., 2018), and none have examined this relationship for DEHP, so these results will be an important contribution to the literature. Interestingly, prenatal exposure to phthalates was associated with lower scores on language development outcomes at 2 years, but these effects largely seem to resolve by 3 years. Previous work examining language development has found that children who begin talking later than typical catch up with their peers by the time they begin school, but these children often continue to show deficits in language as they age (Zubrick et al., 2007; Fernald and Marchman, 2012), aligning with the results observed for prenatal phthalate exposure. Alternatively, as described in Chapter 7, this difference may be due to methodological differences between the two measures.

Additionally, this is the first study to find associations between prenatal acetaminophen exposure and poorer language development using standardized parental-report measures

intended to assess language development specifically, rather than communication in general via measures such as the CBCL or Ages and Stages Questionnaire (ASQ) or language delay as a more general outcome (Brandlistuen et al., 2013; Vlenterie et al., 2016; Skovlund et al., 2017; Bornehag et al., 2018b). These results showed that prenatal acetaminophen was associated with few effects on language development at 2 years, but at 3 years, it was associated with lower scores on several measures of language development. This has important implications because as described in previous chapters, language skills are an important factor in assessing a child's readiness for school, as they are predictive of reading ability and IQ (Hadley and Rice, 1993; Zubrick et al., 2007). This cohort continues to be followed, and assessments at ages 4 and 7 will help to elucidate the persistence of the observed impairments in language development in relation to prenatal phthalate and acetaminophen exposure.

8.3. POTENTIAL MECHANISMS

These results suggest that neither prenatal phthalate exposure nor acetaminophen exposure effect early attention or language development via antiandrogenic actions, as hypothesized. While there were associations of antiandrogenic phthalates with measures of poorer attention and language development, they were often not observed for all antiandrogenic phthalates or were observed for both antiandrogenic phthalates and MEP, or only in MEP, indicating that these effects are not likely to be mediated by antiandrogenic actions of the chemicals. Other possible mechanisms that have been proposed to mediate neurodevelopmental effects of phthalates include disruption of thyroid hormones, and induction of oxidative stress (Minatoya and Kishi, 2021). Regarding associations of prenatal acetaminophen exposure and its associations with attention and language development, this study also does not suggest that its effects are via antiandrogenic actions as most effects did not show a clear sex-specific pattern. As with prenatal phthalate exposure, induction of oxidative stress has been proposed as a potential alternative mechanism through which acetaminophen

may alter neurodevelopment. Another potential mechanism through which acetaminophen may influence neurodevelopment is via the endocannabinoid system (Bauer et al., 2018).

Both animal and human studies have demonstrated a link between phthalate exposure and oxidative stress (Ferguson et al., 2014; Braun, 2017; Minatoya and Kishi, 2021), as well as acetaminophen exposure and oxidative stress (da Silva et al., 2011; Brandlistuen et al., 2013; Parker et al., 2017; Furnary et al., 2021). While the mechanism by which phthalates may induce oxidative stress remains unclear, acetaminophen has been shown to have directly toxic effects on neurons and astroglia and can be metabolized to its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), in the brain, which is thought to be how it induces oxidative stress (Minatoya and Kishi, 2021; Ghanem et al., 2016). Oxidative stress during prenatal development has been shown to impact hippocampal cell proliferation, neuronal differentiation, and microglia development in animal models, as well as depression- and anxiety-like behaviors in animal models (Raeis-Abdollahi et al., 2019; Bauer et al., 2018). In humans, oxidative stress has repeatedly been linked to autism spectrum disorder (ASD; Parker et al., 2017; Ponsonby et al., 2020). More recently in humans, oxidative stress during prenatal development has been associated with increases in externalizing problems, and ADHD-related behaviors, in addition to ASD-related behaviors (Rommel et al., 2020). Thus far, the evidence for phthalates and acetaminophen exerting their effects on neurodevelopment via prenatal oxidative stress seem like the most likely shared mechanism other than their antiandrogenic effects, but further research is needed to determine whether this is the case.

It may also be the case that phthalates and acetaminophen do not share a mechanism. In this study, while both phthalates and acetaminophen had effects on attention and language development, the pattern was not always consistent between the two exposures. For example, in multiple measures of language development, acetaminophen had more pronounced effects on females while phthalates had more pronounced effects in males, and in measures of

attention at 2 and 3 years, acetaminophen had effects that were not sex specific, while phthalates primarily affected males. Several alternative mechanisms of action have been explored for each exposure, including disruption of thyroid hormone by phthalates and disruption of the endocannabinoid system by acetaminophen. Mechanistic studies have demonstrated that phthalates can disrupt the hypothalamic-pituitary-thyroid axis and interfere with the synthesis, transport, and metabolism of thyroid hormone (Minatoya and Kishi, 2021; Howdeshell, 2002). Appropriate levels of maternal thyroid hormone are essential to prenatal brain development, particularly during the first trimester and second trimester before the fetus is able to produce its own thyroid hormone (Howdeshell, 2002). Insufficient levels of thyroid hormone during prenatal development have been shown to affect gliogenesis, neural migration, synaptogenesis, and myelination (Howdeshell, 2002) and have been linked to deficits in attention and learning and memory in animal studies (Moog et al., 2017). In humans, results have been mixed with some studies showing associations between mild to moderate deficiencies in maternal thyroid hormone during pregnancy and lower IQ and language delay and others showing no association (Moog et al., 2017). However, recent studies investigating this relationship with respect to prenatal phthalate exposure have found no link between the effect of prenatal phthalate exposure on thyroid hormone and neurodevelopment in children (Minatoya et al., 2016; Moog et al., 2017; Engel et al., 2018). Thus, to date the results of human studies do not seem to support this mechanism.

Regarding prenatal acetaminophen exposure, there has been growing interest in its actions via the endocannabinoid system, which is how it is thought that acetaminophen exerts its analgesic effects (Bauer et al., 2018). The cannabinoid system has been shown to play an important role in brain development, playing roles in cell differentiation, migration, and synaptogenesis, in addition to immune regulation via microglia (Bauer et al., 2018). In an animal model of prenatal acetaminophen exposure, Philippot et al. (2017) observed similar results to

those they had found in a previous study examining the effects of perinatal tetrahydrocannabinol (THC) exposure on adult animals, specifically, that developmental exposure induced hyperactivity in the adult animals. Other studies have also noted similarities in studies of acetaminophen and cannabinoids on neurodevelopment and behavior in animals (Blecharz-Klin et al., 2013; Milewski et al., 2018). In humans, the endocannabinoid system has been repeatedly implicated in the etiology of ASD (Araujo et al., 2019; Navarrete et al., 2020), and, more recently, has also been linked to ADHD (Navarrete et al., 2020). While the evidence for acetaminophen potentially having effects on neurodevelopment and behavior via disruptions of the endocannabinoid system seems to be mounting, there have been few, if any, studies directly evaluating this association; therefore, further investigation of this potential relationship is needed.

8.4. REGRETTABLE SUBSTITUTION OF PHTHALATES

As described in earlier chapters, phthalates are commonly used as plasticizers in consumer products (Centers for Disease Control and Prevention, 2017). Unfortunately, these chemicals usually are not covalently bound to the products they are found in and can leech into the environment, accumulating in household dust, and food and beverage products, resulting in widespread human exposure (Schwedler et al., 2020). In recent years, concern regarding the effects of exposure on child development has led to calls for the use of phthalates in consumer products to cease (Engel et al., 2021). While the use of DEHP in consumer products has decreased in recent years (Zota et al., 2014; McGrath et al., 2021), it has quietly been replaced by DINP and, increasingly, DEHTP as “safer” alternatives (Schwedler et al., 2020; Engel et al., 2021; Kambia et al., 2021; Moche et al., 2021). However, as shown in this study, this may not be the case. The results of this study indicate that DINP exposure during prenatal development affects information processing in infancy. More concerning, DEHTP had strikingly consistent effects on both attention in infancy and early childhood and language development at both 2

and 3 years. As shown in Table 3.3, measures of this terephthalate are high in the IKIDS cohort, much higher than what has been reported for a nationally represented sample (CDC 2019), and DEHP levels have been increasing world-wide (Schwedler et al., 2020). Many experts, such as Engel et al. (2021) and other members of Project TENDR (Targeting Environmental Neuro-Development Risks), have recently called for industry to cease use of orthophthalates. DEHP, however, belongs to the family of terephthalates, and there is little research regarding the safety of its use in consumer products; thus, additional research on the potential effects of developmental exposure to this compound are critically important.

8.5. ACETAMINOPHEN USE IN PREGNANCY

As previously described, acetaminophen is the most common drug ingredient on the market, and it is one of very few medications currently considered safe for use during pregnancy (Servey and Chang, 2014). Unfortunately, as recently found in a systematic review by Cendejas-Hernandez et al. (2020), the idea that it is safe for use has been taken for granted for many years without any demonstration of its safety regarding neurodevelopment. As shown in Table 1.1, there are a growing number of studies indicating that it may be related to poorer neurodevelopmental outcomes. The results of the present study add to the growing body of literature suggesting that prenatal acetaminophen exposure is related to problems in both attention and language development and demonstrate that these problems can be detected early in life.

8.6. STRENGTHS AND LIMITATIONS

This study has several strengths. First, it utilizes data from the IKIDS cohort, which is an ongoing prospective birth cohort study. Women are recruited early in their pregnancy and are followed through the birth of their child, and their child is evaluated multiple times in infancy and early childhood. As previously mentioned, the children in the IKIDS cohort continue to be followed, and follow-up assessments will be conducted at both 4 and 7 years of age. These

follow-up assessments will allow for future studies to determine whether the problems detected at these younger ages persist. Second, this study utilized data collected multiple times during pregnancy to measure prenatal acetaminophen exposure. Previous studies have relied on self-report measures of acetaminophen collected only once or a few times during pregnancy, leaving ample room for recall bias and under- or over-estimation of exposure. In this study, women were asked about medication use roughly every 4 to 6 weeks during pregnancy beginning at 10-14 weeks and ending at the birth of their child. They reported the medication name, reason for taking, dates of use, and frequency of use, resulting in a more complete and less biased self-report measure of acetaminophen use. This study also utilized pooled samples of urine collected across pregnancy for analysis of phthalate exposure. Many previous studies have relied on only one or two spot urine samples to evaluate prenatal phthalate exposure, but because phthalates are quickly metabolized, these are likely representing exposure during very small windows of time and thus are subject to exposure misclassification (Fisher et al. 2014). For estimation of phthalate exposure throughout pregnancy, pooled urine samples have been shown to provide more reliable estimates of average exposure (Brookmeyer 1999; Liu et al 2003; Saha-Chaudhuri et al. 2013; Sham et al. 2002; Weinberg et al. 1999). The adaptation of state-of-the-art eye tracking for use in infants is another strength of this study. This allows for direct measures of neurodevelopment across several domains of cognition very early in life, and this method has been implemented successfully in several other cohorts across the U.S. in order to replicate these findings in larger and more diverse samples. Another strength is in the use of standardized measures to evaluate development at multiple ages and across multiple domains. Both attention and language data were collected at 2 and 3 years, and this study adds to the limited research on the relationship of prenatal exposures to developmental outcomes in early childhood, providing direction for future and follow-up studies.

While there are multiple strengths, this study also has some limitations. First, many analyses were conducted without correction for multiple comparisons. While it is common practice and often recommended to apply corrections such as a Bonferroni adjustment when multiple comparisons are made to reduce the type I error rate, this method results in an increase in the type II error rate, and more results will not be significant. In the field of environmental epidemiology, it has been argued that the focus should be on looking for trends in results to better-inform future research (Rothman, 1990), which is the approach that was taken for this study. When this approach is used, it is important to consider patterns in the results and biological plausibility of findings, as well as use more care when interpreting findings. Second, this cohort is relatively homogenous, limiting its applicability to other populations. Additionally, the participants who continue to participate in the study after the assessments in infancy tend to be even more biased towards being white, non-Hispanic, high income and highly educated, further limiting the applicability of these findings to the general population. Therefore, it is important that our results be replicated in more diverse samples. Second, while there were multiple times of self-report of medication use during pregnancy, it is likely these reports were not entirely accurate due to recall bias. While a few studies have attempted utilizing biomarkers of acetaminophen exposure in maternal urine, maternal plasma, umbilical cord blood, and meconium, these have their own limitations (Bornehag et al., 2018b; Ji et al., 2018; Ji et al., 2020; Laue et al., 2019; Baker et al., 2020). Like phthalates, acetaminophen is quickly metabolized and excreted, with urine measurements only reflecting use in the previous 36 to 48 hours (Bornehag et al., 2018b), plasma and cord blood samples reflecting recent use (Ji et al., 2018; Ji et al., 2020), and meconium reflecting exposure during the last trimester of prenatal development (Laue et al., 2019; Baker et al., 2020). One previous study has found that acetaminophen use during infancy can be detected and measured in shed molars, but teeth have not yet been investigated as a measure of prenatal exposure (Camann et al., 2012). Future studies should investigate shed teeth as a method of measuring biomarkers of

acetaminophen exposure in addition to collecting self-report data. Finally, due to the small sample size included in these analyses, the aim of examining cumulative effects of prenatal acetaminophen and phthalate exposure was not able to be fully addressed. This was particularly true for interactions of acetaminophen with DEHP as fewer participants had measures of this phthalate available. As previously mentioned, IKIDS is an ongoing prospective birth cohort with participants continuing to be recruited and the intention of following the children through at least 7 years of age. Additionally, IKIDS is part of the Environmental influences on Child Health Outcomes (ECHO) Program combining data from multiple cohorts across the United States. This will allow for future analyses to be conducted with much larger and more diverse samples, allowing for future studies to replicate the findings presented here with greater statistical power as well as investigate the potential cumulative effects of combined prenatal exposure to both acetaminophen and phthalates.

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