UNIVERSITY OF CALIFORNIA SAN DIEGO

Prenatal acetaminophen exposure and neurological development through 48 Months

A thesis submitted in partial satisfaction of the requirements for the Master's degree

in

Public Health

by

Emily S. Reiter

Committee in charge:

Professor Gretchen Bandoli, Chair
Professor Tina Chambers
Professor Steven Edland

2022

The thesis of Emily S. Reiter is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

University of California San Diego

2022

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AD	Anti-depressant
ADHD	Attention-Deficit/Hyperactivity Disorder
AI	Autoimmune
ASD	Autism Spectrum Disorder
ASQ	Ages and Stages Questionnaire
BMI	Body Mass Index
CAST	Childhood Autism Spectrum Test
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
HR	Hazard Ratio
ID	Identification
INTER-NDA	Intergrowth 21st Neurodevelopment Assessment
MCHAT	Modified Checklist for Autism in Toddlers
N/A	Not Applicable
OTC	Over the Counter
OTIS	Organization of Teratology Information Specialists
RR	Risk Ratio
SD	Standard Deviation
WG	Words & Gestures Forms
WRAVMA	Wide Range Assessment of Visual Motor Abilities
WS	Words & Sentences Forms

LIST OF TABLES AND FIGURES

ACKNOWLEDGEMENTS

I would like to take this opportunity to recognize all of the people who made this thesis and the completion of my MPH degree possible. The value of the guidance and support of Dr. Gretchen Bandoli was immeasurable, throughout both my practicum and thesis. I am so thankful for the amount of time she spent problem solving and guiding me through this complicated data set. I would also like to acknowledge my other committee members, Dr. Christina Chambers and Dr. Steven Edland. Thank you for reading multiple drafts, giving valuable feedback, and your continuous support of this project. Lastly, I would like to express my sincerest gratitude to my family. I am so humbled to have had this opportunity to advance my education, and it would not have been possible without your support.

ABSTRACT OF THESIS

Prenatal acetaminophen exposure and neurological development through 48 months

by

Emily S. Reiter

Master's degree in Public Health

University of California San Diego, 2022

Professor Gretchen Bandoli, Chair

Background: Acetaminophen is the most commonly recommended over-the-counter drug during pregnancy yet is an endocrine-disrupting medication. This study's objective was to evaluate the neurological effects of prenatal acetaminophen exposure, up to 48 months of age.

Methods: In this prospective cohort study, 586 women in the UCSD MothertoBaby Study were surveyed throughout pregnancy and 600 children were followed between 12 and 48 months

of age. Exposure was operationalized based on categorical exposure to acetaminophen (none, low, moderate, high). Four neurological scales (ASQ, MCHAT, WG, WS) were used to evaluate neurodevelopment.

Results: ASQ gross motor scores for the low exposure group were significantly lower than those unexposed (β = -2.036, p = 0.0053, 95% CI(-5.30, -0.929)). Adjusted ASQ problem solving scores by quantile showed individuals in both the low (β = -2.471, p = 0.0402, 95% CI(-4.98,-0.113)) and high (β = -2.958, p = 0.0158, 95% CI(-5.67,-0.590)) groups had significantly lower scores than those unexposed. On the WS test, varying levels of acetaminophen exposure was associated with a higher risk of failure to use absent object production (moderate exposure RR = 1.103, 95% CI (1.03, 1.18)), possessive tense (low exposure RR = 1.24, 95% CI (1.02, 1.49)), and combine words proficiently (high exposure RR = 1.13, 95% CI (1.02, 1.26)), compared to those unexposed.

Conclusion: Prenatal acetaminophen exposure was a risk factor for lower ASQ and Words & Sentences scores. Results did not show an association between acetaminophen and neurological outcomes on all scales, indicating the need for additional research.

CHAPTER 1: INTRODUCTION

(NOTE: In this paper, we use the term "women," however we recognize this term is limiting. In this document, we use the term "women" to refer to all pregnancy capable individuals and those with female reproductive organs regardless of gender identity.)

In the last 20 years, the number of children diagnosed with neurological disorders has continued to rise. From 2003 to 2011, attention-deficit/hyperactivity disorder (ADHD) diagnoses in children has increased 42% (Visser, 2014). Childhood bipolar diagnoses have increased 56% from 1996 to 2004 (Blader, 2007). While these are just two examples, this trend can be seen across childhood neurological development (Perrin, 2007). As these diagnoses are on the rise, we can expect to see a growing number of children who struggle to reach developmental and emotional milestones. Neurological deficits in children change the way they learn, behave, handle emotions, and develop social skills – all leading to interference in a child's every-day activities (CDC, 2021).

Acetaminophen is the most commonly used medication during pregnancy and is recommended by physicians to treat both fever and pain (Thiele, 2013). Studies have found that the use of acetaminophen is typically higher during pregnancy than before pregnancy and acetaminophen is used by at least 65% of pregnant women (Werler, 2005).

Despite the widespread use of acetaminophen in pregnancy, studies of the potential adverse neurological effects for the offspring are few and far between. Previous studies have looked specifically at autism spectrum disorder (ASD) (Liew, 2016), hyperkinetic disorders (Liew, 2014), ADHD (Thompson, 2014), and behavioral problems (Stergiakouli, 2016) individually, but few have taken an over-arching analysis of neurological development as a

whole. This study used four different neurological development tests to depict the risk of prenatal acetaminophen exposure.

CHAPTER 2: LITERATURE REVIEW

This review describes studies showing the effects of prenatal acetaminophen exposure on the child's neurological outcomes. There are a variety of neurological outcomes that could come as a result of acetaminophen exposure, most of which have been shown to be on the rise in the last 10 years.

2.1 Methods

A systematic literature review was conducted in November of 2021. The search engines used to conduct this search were PubMed, Google Scholar, and the UC SanDiego Library Database. The key search terms used were acetaminophen and/or paracetamol, AND pregnancy and/or prenatal, AND neurological outcomes. This search resulted in a total of 2,672 results. Studies were limited to those published 2010-2021 and had a full text available. After additionally limiting results to those published in English, 1,349 results were available. Requiring Acetaminophen/paracetamol and pregnancy to occur in the title yielded 39 results. Removal of studies that were not primary sources (removal of meta-analyses and literature reviews) gave 26 results. The abstracts of these 26 studies were reviewed. 15 articles were removed as they did not include neurological outcomes, leaving 11 articles for review.

The following final inclusion criteria were used: (1) the article must have been published between 2010 and 2021; (2) the study must have been published in English; (3) the study must focus on neurological outcomes associated with acetaminophen use in pregnancy. The exclusion criteria used to remove articles for this review were as follows: (1) The article was published before 2010; (2) the study was irrelevant to the study aim; (3) the full article was not available to analyze; (4) the study was a literature review, systematic review, or meta-analysis. Overall,

out of the 39 results from our primary article search, 28 articles were excluded and a total of 11 articles met our criteria for inclusion.

2.2 Results

2.2.1 General Study Characteristics

While the studies were filtered to all evaluate the effects of prenatal acetaminophen exposure on neurological outcomes, there were a variety of outcomes evaluated. Nine of the eleven relevant studies were prospective cohort studies that prospectively recorded acetaminophen use during pregnancy. Two studies did not prospectively record acetaminophen use during pregnancy but did measure acetaminophen metabolites measured in cord plasma samples collected at birth (Ji et al., 2020) and acetaminophen levels in meconium after birth (Baker et al., 2020). This is a strength, as all of the studies included were able to show a temporal relationship between prenatal acetaminophen exposure and the outcome of interest.

Contrary to the consistency in study type and exposure measurement, there were a variety of outcomes reported. The most common outcomes in this review were autism spectrum disorder (ASD) and attention deficit/hyperactivity disorder (ADHD). Additional outcomes included hyperkinetic disorders, general behavioral problems, overall neurodevelopment, and executive function.

Ten of the eleven studies (Liew et al. 2016, Liew et al. 2014, Stergiakouli et al. 2016, Brandlistuen et al. 2013, Thompson et al. 2014, Ji et al. 2020, Liew et al. 2016, Avella-Garcia et al. 2016, Baker et al. 2020, Ystrom et al. 2017) found an association between prenatal acetaminophen exposure and the risk and/or odds of developing the outcome of interest. One study, Bertoldi et al. 2020, found that in one subgroup of their study acetaminophen exposure

was associated with lower Visual Motor Abilities (WRAVMA), but in the other subgroup exposure was associated was not associated with INTER-NDA motor scores and was actually associated with higher INTER-NDA total scores.

Six of the eleven studies (Liew et al. 2014, Brandlistuen et al. 2013, Ji et al. 2020, Avella-Garcia et al. 2016, Baker et al. 2020, Ystrom et al. 2017) examined a dose-response relationship, proxied by frequency, between acetaminophen exposure and the outcome. All five found a statistically significant association when stratified by dose. The 2017 study by Ystrom et al. found that acetaminophen use for < 8 days was negatively associated with ADHD (HR = 0.90, 95% CI 0.81-1.00). However, they found that more than 29 days of maternal acetaminophen use had a HR of 2.20 (95% CI 1.50-3.24) and, even more staggering, acetaminophen use for an indication of fever or infection for 22 to 28 days was HR = 6.15 (95% CI 1.71-22.05).

2.2.2 ADHD, Attention Scores, or Hyperactivity Symptoms

Nine of the eleven studies reviewed had ADHD as an outcome of interest (Liew et al. 2014, Stergiakouli et al. 2016, Brandlistuen et al. 2013, Thompson et al. 2014, Ji et al. 2020, Liew et al. 2016, Avella-Garcia et al. 2016, Baker et al. 2020, Ystrom et al. 2017). All of the studies with ADHD as an outcome found a statistically significant association between prenatal acetaminophen exposure and the child's attention skills. Six of these studies found a dose-response relationship between exposure and outcome (Liew et al. 2014, Brandlistuen et al. 2013, Ji et al. 2020, Avella-Garcia et al. 2016, Baker et al. 2020, Ystrom et al. 2017).

Two studies found that associations were based on gestational time of exposure. Stergiakouli et al. (2016) found that acetaminophen use at 18 weeks gestation was associated with higher odds of hyperactivity symptoms (Risk Ratio (RR)= 1.31, 95% CI 1.16-1.49) but

acetaminophen use at 32 weeks was associated with higher odds of having emotional symptoms (RR = 1.29, 95% CI 1.09-1.53) and total difficulties (RR = 1.46, 95% CI 1.21-1.77).

2.2.3 ASD

Three of the eleven studies reviewed had ASD as an outcome of interest (Liew et al. 2016, Ji et al. 2020, Avella-Garcia et al. 2016). Of these three studies, one found significantly higher odds of ASD diagnosis among women in the second and third tertile of acetaminophen exposure (Ji et al., 2020). The other two studies found associations, within certain conditions. The 2016 study by Liew et al., examining maternal use of acetaminophen during pregnancy and risk of ASD in childhood, reported an increased risk of ASD when accompanied by hyperkinetic symptoms but not with other ASD cases. The study completed by Avella-Garcia et al. in 2016 showed an association between acetaminophen use in pregnancy and Childhood Autism Spectrum Test (CAST) scores in males only.

2.2.4 Overall Neurodevelopment

Out of the eleven studies included in this review, only two examined the effects of prenatal acetaminophen exposure on the child's overall neurodevelopment. Brandlistuen et al. (2013) examined the effects of acetaminophen on psychomotor development (communication, fine and gross motor), externalizing and internalizing behavior problems, and temperament (emotionality, activity, sociability and shyness). This study found that paracetamol exposure for more than 28 days resulted in poorer gross motor development, communication, externalizing behavior, internalizing behavior, and higher activity levels.

The study conducted by Bertoldi et al. in 2020 examined neurodevelopment using the Picture Vocabulary Test, Wide Range Assessment of Visual Motor Abilities (WRAVMA), and INTER-NDA. Their study was conducted among two different cohorts. In the first, they found

that exposure to acetaminophen in the first and second trimesters was associated with lower WRAVMA drawing scores. In the second, they found that there was no association between first and second trimester acetaminophen exposure and motor scores. Instead, exposure in this cohort was associated with higher INTER-NDA total scores.

2.3 Discussion

The impact of prenatal acetaminophen exposure on the neurodevelopment of children is an issue that is complexed and nuanced. As was seen in this review, there are many possibilities for measuring acetaminophen exposure as well as depicting the total neurologic effects. This review showed that there are large gaps in the research for an issue that has important individual, familial and public health implications.

Many of the articles looked at a specific diagnosis as an outcome but few used overall neurodevelopment as their outcome of interest. There are many different tests for measuring neurodevelopment in children so an overarching goal for this research should be to expand the methods used in order to determine the overall association between acetaminophen exposure and neurological development.

Additionally, only half of the studies examined whether there was a dose-response relationship between acetaminophen use and their outcome of interest. This is imperative to understanding the total impact of acetaminophen, as it is unlikely that taking acetaminophen once has the same burden as taking it regularly. This information is imperative for guiding medical recommendations during pregnancy. Examining the outcomes as divided by trimester of acetaminophen use would be another avenue for determining the safety of consumption during pregnancy.

CHAPTER 3: METHODS

3.1 Study Design

The MotherToBaby California Study is a prospective cohort study evaluating adverse birth and developmental outcomes for a variety of prenatal exposures for women residing in California. This study used a sub-population of the MothertoBaby California Study to assess the relationship between prenatal acetaminophen exposure and the neurological development of the child. Between 2010 and 2020, participants completed up to four telephone surveys, while pregnant, assessing maternal risk factors, medical history, pregnancy complications, exposures, and demographic factors. A postnatal interview was completed to assess late gestation information and pregnancy outcomes.

Ages and Stages Questionnaire (ASQ), Modified Checklist for Autism in Toddlers (MCHAT), Words and Gestures (WG), and Words and Sentences (WS) scores were determined through parents completing a home evaluation and reporting scores via phone interview. All participants provided written informed consent prior to the start of monitoring. The MotherToBaby pregnancy study was approved by the University of California, San Diego Institutional Review Board.

3.2 Setting & Sample

Participants were recruited between January 2010 and January 2020 through advertising, Facebook, callers of the Organization of Teratology Information Specialists (OTIS) and physician's offices. Women who were currently pregnant and living in California, USA were eligible for participation in the study. Exclusion criteria for this study included women whose pregnancies did not result in a live birth, and those who had not completed at least one of the

developmental outcomes for evaluation (ASQ, MCHAT, WS, WG). Multiples (n = 29) were not excluded to maintain power.

3.3 Measures

3.3.1 Study Exposure:

All medications, including OTC medications, used were self-reported at each interview. Mothers were asked to report start and stop dates, frequency, dosage, and reason for use for each medication reported. Acetaminophen exposure was defined as any prenatal acetaminophen use during gestation. The average daily dose of acetaminophen and acetaminophen containing products was calculated from the reported frequency and dosage each week, corresponding with gestational week. In the instance where multiple acetaminophen products were used in the same day, the dosages were summed. The cumulative dose of acetaminophen use through delivery was summed for overall exposure.

3.3.2 Study Outcomes:

Women in the MotherToBaby study included as participants completed at least one of the neurological development outcome tests. All tests were completed by the mothers, with guidance, before 48 months of age. The Ages and Stages Questionnaire (ASQ) is a parent-completed questionnaire, recommended by the American Academy of Neurology, First Signs, and The Child Neurology Society (Brookes, 2014). The ASQ-2 and ASQ-3 provide separate tests for each age group (every 2 months, from 2-60 months) with five categories and six questions in each category, designed to screen young children for developmental delays (Squires, 2009). ASQ was scored by child development professionals and reported as normal, borderline, or abnormal for communication, gross motor skills, fine motor skills, problem solving, and

personal/social skills, based on the child's age-adjusted score. The Modified Checklist for Autism in Toddlers (MCHAT) is a screening completed by parents that asks 20 questions about the child's typical behavioral tendencies (Robins, 2009). Recent studies have found that the MCHAT has the highest positive predictive value for identifying children at risk of a developmental concern (Weitlauf, 2016). MCHAT was reported as a count of critical items and other items, with 2 or more critical items failed = critical and 3 or more *any* items failed = critical.

The Words and Gestures Forms (WG) are completed by parents between 8 and 18 months of age. The child's understanding of common vocabulary as well as their use of communicative and symbolic gestures are recorded (Fenson, 2007). The WG test was marked as a yes or no for responds to name, responds to no, responds to mom/dad, imitation, and labeling. The number of phrases understood, vocabulary understood, vocabulary produced, early gestures, later gestures, and total gestures were recorded as total numbers and the number per hour of study time. The Words and Sentences Forms (WS) are completed by parents between 16 and 30 months. The skills assessed are the child's understanding of word forms, complexity of multi-word utterances, the early phases of grammar, and the child's production and use of words (Fenson, 2007). The WS test was marked as a yes or no for a child that uses future tense, past tense, absent object production, absent object comprehension, absent owner, possessive, plural, progressive, past tense, and combining words. The number of vocabulary produced, irregular words, over-regularized words, and complexity were recorded as total numbers and the number per hour of study time. The three longest sentences produced were recorded and then averaged.

3.3.3 Covariates

The potential covariates for this study included self-reported autoimmune disorders, including inflammatory bowel disease, psoriasis, rheumatoid arthritis or other (multiple sclerosis, Type I diabetes, antiphospholipid syndrome, celiac disease, Raynaud's syndrome, and Sjogren's syndrome), as well as other pre-existing conditions such as hypertension, depression, fibromyalgia, anxiety, and other mental health disorders. All chronic conditions were reported as yes/no. Demographic and behavioral information included race (White, Black, Asian, Native American, or Other), ethnicity (Hispanic/Non-Hispanic), mother's age, mother's pre-pregnancy body mass index, prenatal tobacco use (yes/no), and ADHD medication use (yes/no). The indication for acetaminophen use could also be considered a potential covariate. Testing instruments for this study were scored with adjustment for gestational age at delivery, eliminating prematurity as a potential confounding factor.

3.4 Statistical Analysis

Data was coded by participant ID (mother) and outcome ID (child) along with exposure status (yes/no) and cumulative use throughout pregnancy (cumulative avg. daily dose/week) stratified into quartiles of none (no acetaminophen exposure), low (cumulative dose throughout pregnancy < 1300mg), moderate (cumulative dose throughout pregnancy of >1300.0 ≤ 6500.0mg), and high (cumulative dose throughout pregnancy of >6500.0mg).

For continuous outcomes, equal variances were tested through the quotient and reciprocal of standard deviations. For outcomes with equal variances, two sample t-tests were used to determine significance of the association. When analyzed without equal variances, a Welch two-sample t test was used. Covariates were selected based on the hypothesized causal mechanism.

Multivariable linear regressions with 95% confidence intervals, adjusting for maternal depression, asthma, and any autoimmune condition, were used to determine adjusted beta estimates for continuous outcomes. For each categorical outcome (Overall ASQ normal/abnormal, MCHAT normal/abnormal, WG + WS yes/no responses), adjusted risk ratios were used to estimate effect. Significance was found for p values < 0.05. For all statistical analyses, the absence of prenatal acetaminophen exposure was the reference group. RStudio version 1.3.1093 was used for all analyses.

CHAPTER 4: RESULTS

The demographic characteristics of the study population are shown in table 1, stratified by prenatal acetaminophen exposure. Of the 600 individuals included in the study, 389 (64.8%) were prenatally exposed to acetaminophen. The mean age of the pregnant person was 33.25 years in the unexposed group and 32.92 years in the exposed group. Both groups – those who used acetaminophen and those who did not – were largely White (82.5% and 70.6%, respectively) and Non-Hispanic/Latina (71.5% and 59.7%, respectively).

There was no difference in gravidity (average 2.1 pregnancies vs. 2.02 pregnancies) or parity (average 0.57 births vs. 0.53 births) between groups. The average gestational age at delivery – for both the exposed and unexposed groups – was full term (39.05 and 39.14 weeks, respectively). It was found that 72.41% of pregnancies carrying multiples were exposed to acetaminophen as compared to 64.4% of singleton pregnancies. All baseline characteristics were considered when creating a hypothesized causal mechanism for selecting covariates.

Table 2 shows the prevalence of maternal chronic disease, as well as anti-depressant (AD) and ADHD medication use, in this cohort. The most common chronic disease in this study was depression, with 138 participants (23%) reporting a diagnosis of depression. Other common chronic diseases in this population included asthma (n=115, 19.17%) and any autoimmune disease (n=93, 15.5%). Additionally, 108 participants (18%) reported AD medication use.

Indications for use by exposure group are presented in table 3. Of the participants who reported an indication for acetaminophen use, there were a variety of indications reported. The most commonly reported indications were headache, backache, fever, overall pain (including AI pain, abdominal pain, cramps, and muscle pain), cold/flu, dental pain, and acute injury/illness (including surgery). Overall, the most frequently reported indication was headache, with 64.15%

of individuals who used acetaminophen it as an indication for use. Following this, 33.24% of those who used acetaminophen reported overall pain as an indication for use.

4.1 Ages and Stages Questionnaire

The crude ASQ score averages by category were found to be generally higher among those who were not exposed to Acetaminophen prenatally. Those differences were found to be statistically significant for both the gross motor scores ($t = 2.817$, $p = 0.00504$, 95% CI(0.705, 3.95)) and the average score for each individual ($t = 2.745$, $p = 0.00629$, 95% CI(0.521, 3.15)).

When grouped into quantiles of exposure and adjusting for potential confounders (maternal depression, maternal autoimmune disease, and maternal asthma), the ASQ gross motor scores for those in the low exposure group were significantly poorer than those who were not exposed ($\beta = -2.036$, $p = 0.0053$, 95% CI(-5.30, -0.929)). The adjusted ASQ problem solving scores by quantile showed that individuals in both the low ($\beta = -2.471$, $p = 0.0402$, 95% CI(-4.98,-0.113)) and high ($\beta = -2.958$, $p = 0.0158$, 95% CI(-5.67,-0.590)) exposure groups had significantly poorer scores than those who had not been exposed to any acetaminophen prenatally. The adjusted average ASQ score by quantile reflected this and showed similar results, with the low ($\beta = -2.111$, $p = 0.0180$, 95% CI(-3.86,-0.363)) and high ($\beta = -1.876$, $p = 0.0441$, 95% CI(-3.70,-0.0495)) exposure groups having significantly lower scores than those who were not exposed. Figure 1 shows the distribution of overall ASQ scores, categorized into an abnormal or normal result. Being in the high, moderate, or low exposure groups is associated with a 1.37 (95% CI 0.877, 2.14), 1.41 (95% CI 0.988, 2.19), and 1.48 (95% CI 0.958, 2.28) –fold adjusted increased risk (respectively) of an abnormal ASQ result compared to the unexposed group.

4.2 Modified Checklist for Autism in Toddlers

Figure 2 shows the overall distribution of MCHAT results, categorized as normal or critical, between exposure groups. Being in the high, moderate, or low exposure groups was associated with a 1.17 (95% CI 0.764, 1.78), 1.11 (95% CI 0.715, 1.72), and 0.925 (95% CI 0.925, 1.47) -fold increased risk (respectively) of a critical MCHAT result compared to the unexposed group. After adjusting for potential confounders, the difference in MCHAT total item counts for the low (β = -0.07385, p = 0.770, 95% CI(-0.569, 0.422)), moderate (β = 0.15756, p = 0.540, 95% CI(-0.347, 0.662)), and high (β = 0.115, p = 0.648, 95% CI(-0.379, 0.609)) groups were not significant compared to the group with no acetaminophen exposure at 95% confidence.

4.3 Words and Gestures & Words and Sentences

After analyzing each category of the Words & Gestures test, it was determined that there was no statistically significant difference between the scores of those who were not exposed to acetaminophen and those who were exposed to low, moderate or high levels of acetaminophen prenatally. It was, however, found that the presence of any maternal autoimmune disease was significantly associated with the number of later gestures percentile score (β = -8.918, p = 0.0115, 95% CI(-15.82, -2.01)) and the total number of later gestures (β = -2.996, p = 0.00695, 95% CI(-5.16, -0.825)). Any maternal autoimmune disease was also found to be significantly associated with the number of vocabulary understood percentile (β = -7.977, p = 0.0328, 95% CI(-15.29, -0.656)).

The Words & Sentences test showed that individuals exposed to moderate levels of acetaminophen prenatally were at a 1.103 times increased (adjusted) risk for being unable to complete absent object production, compared to those with no exposure (RR = 1.103, 95% CI

(1.03, 1.18)). Additionally, those who were exposed to low levels of acetaminophen prenatally were at a 1.24 times increased (adjusted) risk for being unable to show proficiency of possessive word forms, compared to those who were unexposed (RR = 1.24, 95% CI (1.02, 1.49)). Lastly, it was found that those in the high exposure group were at a 1.13 times increased (adjusted) risk for being unable to combine words proficiently, compared to those who were unexposed (RR = 1.13, 95% CI (1.02, 1.26)).

CHAPTER 5: DISCUSSION

In this large cohort study with prospective data, the objective was to evaluate the potential association between prenatal acetaminophen exposure and general neurodevelopmental outcomes, using four different neurological scales. Due to the high rate of acetaminophen use among pregnant women and the potential it poses for adverse neurological outcomes of the offspring (Bauer et al., 2021), it was imperative to use a variety of scales to estimate the true effects of acetaminophen on neurodevelopment.

Our findings indicate that there is an association between acetaminophen use and adverse neurological outcomes as categorized by the ASQ test, as well as some categories of the WS test, but there was no evidence of a significant association for the MCHAT or WG tests. It was found that acetaminophen exposure was significantly associated with lower gross motor scores, lower problem solving scores, and lower average ASQ scores. However, there was no significant difference in scores across the other categories (communication, fine motor or personal/social). On the Words & Sentences test, varying levels of acetaminophen exposure was associated with a higher risk of failure to use absent object production, possessive tense, and combine words proficiently.

It is well-known that maternal hormones play an important role in regulating fetal development, even more specifically in regulating fetal brain development (Rubinow, 1996). Given that acetaminophen can not only cross the placenta but also has endocrine-disrupting properties (Jensen, 2010), it is plausible that prenatal acetaminophen use could disrupt fetal brain development.

The Ages and Stages Questionnaire (ASQ) screens for developmental delays in children between birth and 6 years of age. Given that these scores were significantly associated with

acetaminophen use, it is safe to say that children who are exposed to acetaminophen prenatally should be monitored for developmental delays throughout childhood.

Our tests were not used for the purpose of a neurodevelopmental diagnosis but other, similar studies, found significant associations between acetaminophen use and diagnosis of common childhood neurodevelopmental disorders. Studies within the Danish National Birth Cohort showed that maternal acetaminophen use was associated with increased risk of autism spectrum disorders accompanied by hyperkinetic symptoms (Liew, 2016). An additional study using this same cohort found that prenatal acetaminophen exposure resulted in a higher risk for use of ADHD medications, or having ADHD-like symptoms at age 7 (Liew, 2014).

Other studies have used cord blood to measure the amount of acetaminophen the infant was exposed to and found that cord biomarkers of fetal exposure to acetaminophen were associated with a dose-response increased risk of ADHD and ASD (Ji, 2020). The one difference between this study and others that have been completed is the broad spectrum of neurological scores. This fills the gap in the literature about the overarching neurological development of the child associated with acetaminophen exposure, as opposed to a specific neurologic outcome.

Our findings should be considered with regard to the limitations of our study. The most obvious limitation in a study where the exposure is self-reported is recall bias and a lack of accurately information. Most of the women in this study were White and non-Hispanic/Latino. This may limit the generalizability of these results to the general population. It is known that maternal fever during pregnancy may interfere with fetal brain development and has been linked to some neurodevelopmental disorders, including ASD (Dreier, 2017). This study did not record ailments for the unexposed population, resulting in an inability to adjust for indication of acetaminophen use. Additionally, our neurological development scores only followed children

through 48 months of age. Of the most common developmental disorders, ADHD can only be diagnosed after age 4 (American Psychiatric Association, 2013) and the average age for an ASD diagnosis is 4.33 years of age (CDC, 2021). From birth to age five, a child's brain develops more rapidly than at any other time in life (Arizona PBS, 2020). This indicates that if children were studied further into their development, their outcomes have the potential to change.

Future research should focus on continued monitoring of neurological development through childhood to determine the long term effects of prenatal acetaminophen exposure. More research needs to be done to determine the relationship between acetaminophen use by trimester and neurological development, as well as the overall neurological effects and their relationship to specific neurological disorders. It was found that autoimmune disease was significantly associated with some outcomes of the Words & Gestures test. After adjusting for this association though, it was found that acetaminophen exposure was still not associated with test outcomes. Future research should examine the association between neurodevelopment and maternal autoimmune disease. If the results of this study the other existing literature on the subject are corroborated in the future, they have significant implications for the recommendations of acetaminophen use in pregnancy.

APPENDIX

Table 1: Maternal Characteristics by Acetaminophen Use (N = 600)

	Acetaminophen Use Group			
	None	**Low**	**Moderate**	**High**
Maternal Characteristics	n = 228	n = 127	n =121	n = 124
Age (yrs)				
Mean (SD)	33.5 (5.23)	32.93 (5.66)	32.64 (4.83)	32.66 (4.77)
Pre-Pregnancy BMI				
Mean (SD)	23.45 (4.34)	24.55 (5.13)	24.42 (4.23)	24.30 (6.66)
Race – n (%)				
1 White	162 (71.05%)	106 (83.46%)	102 (84.29%)	101 (81.45%)
2 Black	4 (1.75%)	4 (3.15%)	6 (4.96%)	4 (3.22%)
4 Asian	39 (17.11%)	5 (3.94%)	8 (6.61%)	6 (4.84%)
5 Native American	0 (0%)	1 (0.787%)	1 (0.826%)	5 (4.03%)
6 Other	13 (5.70%)	6 (4.72%)	2 (1.65%)	4 (3.23%)
8 Did Not Answer	6 (2.63%)	1 (0.787%)	1 (0.826%)	2 (1.61%)
9 Pacific Islander	1 (0.439%)	0 (0%)	0 (0%)	0 (0%)
11 Unknown	3 (1.32%)	4 (3.15%)	1 (0.826%)	2 (1.61%)
Ethnicity – n (%)				
Hispanic/Latina	48 (23.52%)	26 (20.97%)	20 (16.53%)	22 (17.88%)
Non-Hispanic/Latina	156 (76.47%)	98 (79.03%)	101 (83.47%)	101 (82.11%)
Gravidity				
Mean (SD)	2.12 (1.31)	2.06 (1.37)	1.92 (1.08)	2.03 (1.27)
Parity				
Mean (SD)	0.571 (0.872)	0.543 (0.732)	0.521 (0.786)	0.516 (0.738)
Tobacco Use – n (%)				
Yes	10 (5.03%)	8 (6.30%)	8 (6.61%)	12 (9.68%)
Infant Characteristics				
Infant Sex – n (%)				
Female	118 (51.75%)	57 (44.88%)	63 (52.07%)	52 (41.94%)
Male	110 (48.25%)	70 (55.12%)	58 (47.93%)	72 (58.06%)
Gestational Age at Delivery (wks)				
Mean (SD)	39.14 (1.95)	38.87 (2.03)	39.22 (1.62)	39.08 (1.70)
Multiples – n (%)				
Singleton	218 (95.61%)	112 (96.06%)	113 (93.39%)	118 (95.16%)
Multiples	10 (4.39%)	5 (3.94%)	8 (6.61%)	6 (4.84%)
Birth Weight Percentile				
Mean (SD)	46.78 (26.89)	44.33 (27.11)	42.29 (26.35)	46.58 (24.68)

Table 2: Maternal Chronic Disease by Acetaminophen Use (N = 600)

	Acetaminophen Use Group			
	None	**Low**	**Moderate**	**High**
Maternal Chronic Disease	n = 228	n = 127	n =121	n = 124
Asthma – n (%)				
Yes	32 (14.04%)	20 (15.78%)	28 (23.14%)	35 (28.23%)
IBD – n (%)				
Yes	7 (3.07%)	3 (2.36%)	7 (5.79%)	4 (3.23%)
PSO – n (%)				
Yes	6 (2.63%)	3 (2.36%)	7 (5.79%)	5 (4.03%)
Rheumatoid Arthritis – n (%)				
Yes	4 (1.75%)	6 (4.72%)	7 (5.79%)	16 (12.90%)
Other AI – n (%)				
Yes	6 (2.63%)	6 (4.72%)	8 (6.61%)	13 (10.48%)
Any AI – n (%)				
Yes	19 (8.33%)	16 (12.60%)	25 (20.66%)	33 (26.61%)
Depression – n (%)				
Yes	33 (14.47%)	37 (29.13%)	25 (20.66%)	43 (34.68%)
Hypertension – n (%)				
Yes	10 (4.39%)	11 (8.66%)	6 (4.96%)	14 (11.29%)
Other Mental Health – n (%)				
Yes	13 (5.70%)	9 (7.09%)	13 (10.74%)	22 (17.74%)
Anxiety – n (%)				
Yes	11 (4.82%)	9 (7.09%)	11 (9.09%)	11 (8.87%)
Diabetes – n (%)				
Yes	7 (3.07%)	3 (2.36%)	2 (1.65%)	6 (4.84%)
Fibromyalgia – n (%)				
Yes	1 (0.439%)	0	1 (0.826%)	0
AD medication use – n (%)				
Yes	16 (7.02%)	31 (24.41%)	25 (20.66%)	36 (29.03%)
ADHD medication use – n (%)				
Yes	2 (0.877%)	0	3 (2.48%)	2 (1.61%)

Table 3: Indications for Use

	Acetaminophen Use Group			
	Low	**Moderate**	**High**	**Any Use**
Patient Reported Indication	n = 127	n = 121	n = 124	n = 389
Headache – n (%)				
Yes	69 (54.33%)	86 (71.07%)	75 (60.48%)	238 (64.15%)
Backache – n (%)				
Yes	6 (4.72%)	11 (9.09%)	26 (20.97%)	42 (11.32%)
Fever – n (%)				
Yes	7 (5.51%)	11 (9.09%)	17 (13.71%)	36 (9.72%)
Pain – n (%)				
Yes	41 (32.28%)	32 (26.44%)	47 (37.90%)	123 (33.24%)
Cold/Flu – n (%)				
Yes	13 (10.24%)	25 (20.66%)	28 (22.58%)	69 (18.64%)
Dental Pain – n (%)				
Yes	0	2 (1.65%)	2 (1.61%)	4 (1.08%)
Insomnia – n (%)				
Yes	3 (2.36%)	3 (2.48%)	3 (2.42%)	9 (2.43%)
Acute Illness or Injury – n (%)				
Yes	4 (3.15%)	7 (5.79%)	10 (8.06%)	21 (5.68%)
Other – n (%)				
Yes	3 (2.36%)	4 (3.31%)	5 (4.03%)	12 (3.25%)

Table 4: Testing Statistics

	ASQ	MCHAT	W&G	W&S
Test Characteristics	n = 595	n = 524	n = 444	n = 393
Age Administered (months)				
Range	3 – 48	N/A	11 – 18.6	17.6 – 42.3
Mean (SD)	18.22 (8.08)	N/A	13.44 (1.38)	25.10 (2.29)
Sex Distribution – n (%)				
Male	308 (51.76%)	276 (52.67%)	227 (51.13%)	212 (53.94%)
Female	287 (48.24%)	248 (47.33%)	217 (48.87%)	181 (46.06%)
Exam Version – n (%)				
2	579 (97.31%)	306 (58.40%)	439 (98.87%)	2 (0.51%)
3	16 (2.69%)	182 (34.73%)	N/A	352 (89.57%)
4	N/A	36 (6.87%)	5 (1.13%)	39 (9.92%)

Table 5: Summary of Adjusted ASQ Results

	Acetaminophen Use Group		
	Low	**Moderate**	**High**
ASQ Results	n = 127	n =120	n = 123
Communication	β = -1.271 p = 0.370 CI (-4.05, 1.51)	β = -0.886 p = 0.541 CI (-3.73, 1.96)	β = -1.264 p = 0.393 CI (-4.17, 1.64)
Gross Motor	β = -2.936 ◊ p = 0.0093 CI (-5.14, -0.726)	β = -1.839 p = 0.109 CI (-4.09, 0.415)	β = -1.656 p = 0.159 CI (-3.96, 0.653)
Fine Motor	β = -1.628 p = 0.139 CI (-3.79, 0.530)	β = -1.221 p = 0.279 CI (-3.44, 0.993)	β = -2.192 p = 0.0567 CI (-4.45, 0.062)
Problem Solving	β = -2.548◊ p = 0.0402 CI (-4.98, -0.114)	β = -1.147 p = 0.368 CI (-3.64, 1.35)	β = -3.132◊ p = 0.0158 CI (-5.67, -0.590)
Personal/Social	β = -2.248 p = 0.071 CI (-4.69, 0.193)	β = -1.258 p = 0.323 CI (-3.76, 1.24)	β = -0.925 p = 0.476 CI (-3.47, 1.62)
Reported Parental Concern	RR = 0.960 CI (0.851, 1.08)	RR = 1.019 CI (0.909, 1.14)	RR = 1.045 CI (0.938, 1.16)
Result (Score)	β = -2.111◊ p = 0.0180 CI (-3.86, -0.363)	β = -1.759 p = 0.0531 CI (-3.54, 0.023)	β = -1.876◊ p = 0.0441 CI (-3.70, -0.049)
Result (Normal/Abnormal)	RR = 1.476 CI (0.957, 2.28)	RR = 1.406 CI (0.899, 2.19)	RR = 1.372 CI (0.876, 2.15)

* these results are after adjusting for the potential confounders as determined by the hypothesized causal mechanism: maternal depression, maternal autoimmune disease, and maternal asthma

** all results are referencing the "none" group as the comparison

◊ indicates statistically significant results

Table 6: Summary of Adjusted MCHAT Results

	Acetaminophen Use Group		
	Low	**Moderate**	**High**
MCHAT Results	n = 113	n = 107	n = 114
Total Item Count	β = -0.0656 p = 0.796 CI (-0.565, 0.434)	β = -0.100 p = 0.700 CI (-0.410, 0.610)	β = -0.0564 p = 0.829 CI (-0.456, 0.569)
Result (Normal/Critical)	RR = 0.925 CI (0.581, 1.47)	RR = 1.109 CI (0.715, 1.72)	RR = 1.167 CI (0.764, 1.78)

* these results are after adjusting for the potential confounders as determined by the hypothesized causal mechanism: maternal depression, maternal autoimmune disease, and maternal asthma

** all results are referencing the "none" group as the comparison

Table 7: Summary of Adjusted Words & Gestures Results

	Acetaminophen Use Group		
	Low	**Moderate**	**High**
Words & Gestures	n = 99	n = 88	n = 94
Responds to Name	RR = 1.021 CI (0.987, 1.06)	RR = 1.019 CI (0.985, 1.06)	RR = 1.01 CI (0.969, 1.05)
Responds to No	RR = 1.003 CI (0.952, 1.06)	RR = 1.01 CI (0.959, 1.06)	RR = 0.989 CI (0.934, 1.05)
Responds to Parent	RR = 1.04 CI (1.00, 1.08)	RR = 1.03 CI (0.980, 1.07)	RR = 0.995 CI (0.937, 1.06)
Phrases Understood	β = 0.404 p = 0.669 CI (-1.45, 2.26)	β = -0.189 p = 0.847 CI (-2.12, 1.74)	β = -0.893 p = 0.3672 CI (-2.84, 1.05)
Imitation	RR = 1.069 CI (0.917, 1.24)	RR = 1.085 CI (0.928, 1.27)	RR = 1.09 CI (0.942, 1.27)
Labeling	RR = 1.041 CI (1.00, 1.08)	RR = 1.027 CI (0.980, 1.07)	RR = 0.995 CI (0l.937, 1.06)
Vocab. Understood	β = 2.304 p = 0.539 CI (-5.07, 9.67)	β = 1.522 p = 0.696 CI (-6.13, 9.17)	β = -0.8191 p = 0.835 CI (-8.54, 6.91)
Vocab. Produced	β = 0.5454 p = 0.879 CI (-6.52, 7.61)	β = 2.526 p = 0.499 CI (-4.81, 9.86)	β = -1.849 p = 0.624 CI (-9.25, 5.56)
Early Gesture	β = 0.4555 p = 0.254 CI (-0.328, 1.24)	β = 0.2768 p = 0.504 CI (-0.536, 1.09)	β = -0.07358 p = 0.860 CI (-0.895, 0.748)
Later Gesture	β = 1.154 p = 0.301 CI (-1.04, 3.34)	β = 2.059 p = 0.0757 CI (-0.214, 4.33)	β = -0.7781 p = 0.506 CI (-3.07, 1.52)
Total Gesture	β = 0.5686 p = 0.683 CI (-2.17, 3.31)	β = 2.514 p = 0.0833 CI (-0.333, 5.36)	β = 0.3255 p = 0.824 CI (-2.54, 3.19)

* these results are after adjusting for the potential confounders as determined by the hypothesized causal mechanism: maternal depression, maternal autoimmune disease, and maternal asthma

** all results are referencing the “none” group as the comparison

Table 8: Summary of Adjusted Words & Sentences Results

	Acetaminophen Use Group		
	Low	**Moderate**	**High**
Words & Sentences	n = 80	n = 78	n = 90
Vocab. Produced	β = 12.945 p = 0.607 CI (-36.46, 62.35)	β = 4.928 p = 0.848 CI (-45.65, 55.50)	β = 28.782 p = 0.250 CI (-20.34, 77.91)
Future	RR = 1.024 CI (0.895, 1.17)	RR = 1.002 CI (0.871, 1.15)	RR = 0.981 CI (0.854, 1.13)
Past	RR = 1.071 CI (0.931, 1.23)	RR = 0.997 CI (0.853, 1.17)	RR = 0.952 CI (0.813, 1.11)
Absent Obj. Production	RR = 1.062 CI (0.978, 1.15)	RR = 1.103◊ CI (1.03, 1.18)	RR = 1.03 CI (0.946, 1.127)
Absent Obj. Comprehension	RR = 1.010 CI (0.964, 1.06)	RR = 1.022 CI (0.983, 1.06)	RR = 0.978 CI (0.922, 1.04)
Absent Owner	RR = 1.005 CI (0.943, 1.07)	RR = 1.004 CI (0.941, 1.07)	RR = 0.976 CI (0.909, 1.05)
Possessive	RR = 1.128 CI (0.953, 1.33)	RR = 0.979 CI (0.805, 1.19)	RR = 0.969 CI (0.800, 1.17)
Plural	RR = 1.11 CI (0.947, 1.29)	RR = 0.929 CI (0.768, 1.13)	RR = 0.936 CI (0.780, 1.12)
Progressive	RR = 1.24◊ CI (1.02, 1.49)	RR = 0.935 CI (0.735, 1.19)	RR = 1.12 CI (0.912, 1.37)
Past Tense	RR = 1.22 CI (0.943, 1.58)	RR = 0.949 CI (0.699, 1.29)	RR = 0.913 CI (0.676, 1.23)
Irregular Words	β = 0.184 p = 0.814 CI (-1.36, 1.72)	β = 0.0354 p = 0.965 CI (-1.55, 1.62)	β = 0.756 p = 0.339 CI (-0.796, 2.31)
Over-Regularized Words	β = 0.456 p = 0.340 CI (-0.483, 1.39)	β = 0.328 p = 0.506 CI (-0.639, 1.29)	β = 0.505 p = 0.295 CI (-0.442, 1.45)
Combining Words	RR = 1.04 CI (0.909, 1.18)	RR = 1.09 CI (0.973, 1.23)	RR = 1.13◊ CI (1.02, 1.26)
Longest Sentence Mean	β = 0.327 p = 0.347 CI (-0.356, 1.01)	β = 0.195 p = 0.588 CI (-0.512, 0.901)	β = 0.469 p = 0.176 CI (-0.212, 1.15)
Complexity	β = 1.34 p = 0.347 CI (-1.46, 4.13)	β = -0.0690 p = 0.962 CI (-2.93, 2.79)	β = 0.704 p = 0.618 CI (-2.07, 3.48)

* these results are after adjusting for the potential confounders as determined by the hypothesized causal mechanism: maternal depression, maternal autoimmune disease, and maternal asthma

** all results are referencing the "none" group as the comparison

◊ indicates statistically significant results

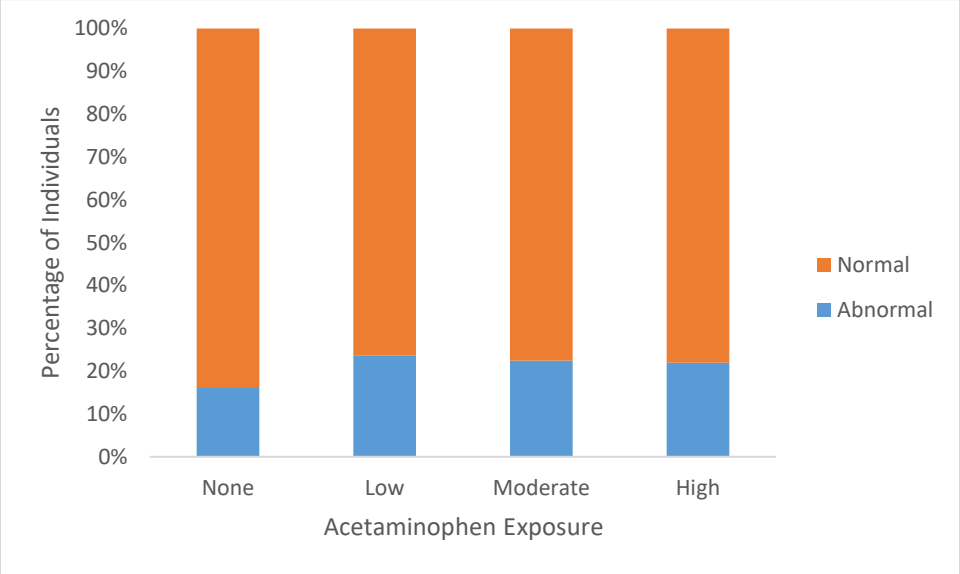

Figure 1: ASQ Result by Acetaminophen Exposure (N = 600)

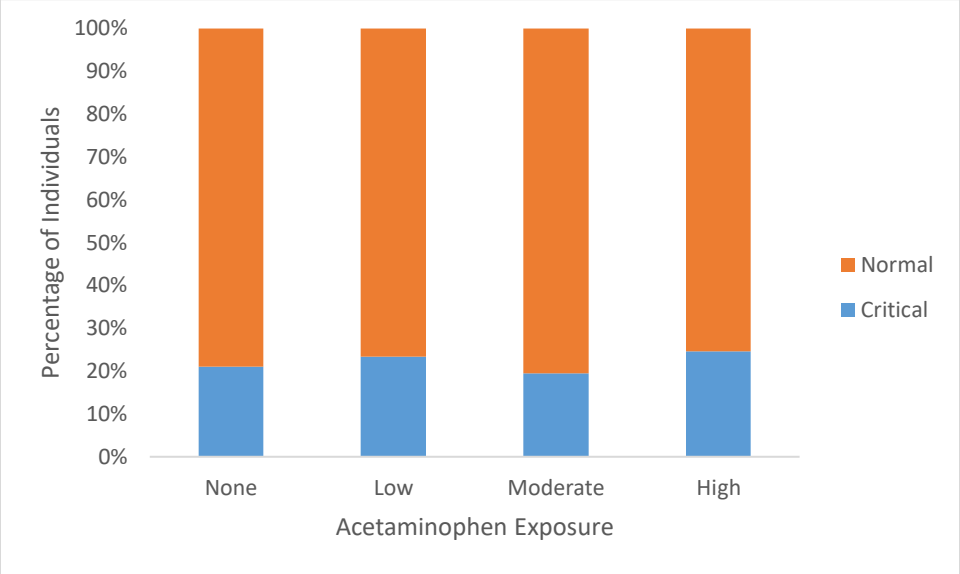

Figure 2: MCHAT Result by Acetaminophen Exposure (N = 600)

REFERENCES

American Psychiatric Association. 2013. *Diagnostic and statistical manual of mental disorders.* (5th ed.). doi: 10.1176/appi.books.9780890425596

Arizona PBS. 2020. *Early Childhood Brain Development Has Lifelong Impact*. https://azpbs.org/2017/11/early-childhood-brain-development-lifelong-impact/\

Avella-Garcia, C., Julvez, J., Fortuny, J., Rebordosa, C., Esteban, R., Galan, I., Tardon, A., Bernal, C., Iniguez, C., Andiarena, A., Santa-Marina, L., Sunyer, J. 2016. Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms. *International Journal of Epidemiology. 45*(6):1987-1996. doi: 10.1093/ije/dyw115.

Baker, B., Lugo-Candelas, C., Wu, H., Laue, H., Boivin, A., Gillet, V., Aw, N., Rahman, T., Lepage, J., Wittingstall, C., Bellenger, J., Posner, J., Takser, L., Baccarelli, A. 2020. *JAMA Pediatrics. 174*(11):1073-1081. doi: 10.1001/jamapediatrics.2020.3080.

Bauer, A., Swan, S., Kriebel, D., Liew, Z., Taylor, H., Bornehag, C. Andrade, A., Olsen, J., Jensen, R., Mitchell, R., Skakkebaek, N., Jégou, B., Kristensen, D. 2021. Paracetamol use during pregnancy – a call for precautionary action. *Nature Reviews Endocrinology*. 17: 757-766. doi: 10.1038/s41574-021-00553-7.

Bertoldi, A., Shiman, S., Boing, A., Pizzol, T., Miranda, V., Silveira, M., Silveira, M., Domingues, M., Santos, I., Bassani, D., Rodrigues, L., Oken, E. 2020. Associations of acetaminophen use during prengnacy and the first year of life with neurodevelopment in early childhood. *Paediatric and Perinatal Epidemiology. 34*(3):267-277. doi: 10.1111/ppe.12632.

Blader JC, Carlson GA. 2007. Increased rates of bipolar disorder diagnoses among U.S. child, adolescent, and adult inpatients, 1996-2004. *Biol Psychiatry*. 62(2):107-114. doi:10.1016/j.biopsych.2006.11.006.

Brandlistuen, R., Ystrom, E., Nulman, I., Koren, G., Nordeng, H. 2013. Prenatal paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study. *International Journal of Epidemiology. 42*(6): 1702-1713. doi: 10.1093/ije/dyt183.

Brookes Publishing. 2014. *Screening & Assessments: ASQ-3*. https://brookespublishing.com/product/asq-3/

Centers for Disease Control and Prevention. 2021. *Delay Between First Concern to Accessing Services.* https://www.cdc.gov/ncbddd/autism/addm-community-report/delay-to-accessing-services.html

Centers for Disease Control and Prevention. 2021. *Children's Mental Health*. https://www.cdc.gov/childrensmentalhealth/basics.html

Dreier, J. 2017. *Fever and infections in Pregnancy and neurodevelopmental impairment in the child.* University of Southern Denmark.

Fenson, L., Marchman, V., Thal, D., Dale, P., Reznick, S., Bates, E. 2007. *MacArthur-Bates Communicative Development Inventories (CDI) Words and Gestures, NCS Scannable, English.* Paul H. Brookes Publishing Co.

Jensen MS, Rebordosa C, Thulstrup AM, et al. 2010. Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. *Epidemiology*. 21(6):779-785.

Ji, Y., Azuine, R., Zhang, Y., Hou, W., Hong, X., Wang, G., et al. 2020. Association of cord plasma biomarkers of in utero acetaminophen exposure with risk of Attention-Deficit/Hyperactivity Disorder and Autism Spectrum Disorder in childhood. *JAMA Psychiatry. 77*(2):180-189. Doi: 10.1001/jamapsychiatry.2019.3259.

Liew, Z. Bach, C., Asarnow, R., Ritz, B., Olsen, J. 2016. Paracetamol use during pregnancy and attention and executive function in offspring at age 5 years. *International Journal of Epidemiology. 45*(6):2009-2017. doi: 10.1093/ije/dyw296.

Liew, Z. Ritz, B., Virk, J., Olsen, J. 2016. Maternal use of acetaminophen during pregnancy and risk of autism spectrum disorders in childhood: A Danish national birth cohort study. *Autism Research: official journal of the International Society for Autism Research*. *9*(9):951-8. doi: 10.1002/aur.1591.

Liew, Z., Ritz, B., Rebordosa, C., Lee, P.C., Olsen, J. 2014. Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatr. 168*(4):313-20. Doi: 10.1001/jamapediatrics.2013.4914.

Perrin JM, Bloom SR, Gortmaker SL. 2007. The increase of childhood chronic conditions in the United States. *JAMA.* 297(24):2755-2759. doi:10.1001/jama.297.24.2755.

Robins, D., Fein, D., Barton, M. 2009. Instructions for Taking and Scoring the M-CHAT-R Autism Test. *Autism Speaks.* https://www.autismspeaks.org/screen-your-child

Rubinow DR, Schmidt PJ. Androgens, brain, and behavior. 1996. *Am J Psychiatry*. 153(8):974-984.

Squires, J., Bricker, D. 2009. *Ages & Stages Questionnaires: A Parent-Completed Child Monitoring System, Third Edition.* Paul H. Brookes Publishing Co.

Stergiakouli, E., Thapar, A., Smith, G. 2016. Association of acetaminophen use during prengnacy with behavioral problems in childhood: evidence against confounding. *JAMA Pediatrics. 170*(10):964-970. doi: 10.1001/jamapediatrics.2016.1775.

Thiele, K., Kessler, T., Arck, P., Erhardt, A., Tiegs, G. 2013. Acetaminophen and pregnancy: short- and long-term consequences for mother and child. *Journal of Reproductive Immunology*. *97*:1 (128-139). Doi: https://doi.org/10.1016/j.jri.2012.10.014

Thompson, J., Waldie, K., Wall, C., Murphy, R., Mitchell, E. ABC study group. 2014. Associations between acetaminophen use during pregnancy and ADHD symptoms measured at ages 7 and 11 years. *PLoS One. 9*(9): e108210. doi: 10.1371/journal.pone.0108210.

Visser SN, Danielson ML, Bitsko RH, et al. 2014. Trends in the parent-report of health care provider diagnosed and medicated ADHD: United States, 2003-2011. *J Am Acad Child Adolesc Psychiatry*. 53(1):34-46.e2. doi:10.1016/j.jaac.2013.09.001.

Weitlauf, A., Vehorn, A., Stone, W., Fein, D., Warren, Z. 2016. Using the M-CHAT-R/F to Identify Developmental Concerns in a High-Risk 18-Month-Old Sibling Sample. *J Dev Behav Pediatr*. *36*(7): 497-502. Doi: 10.1097/DBP.0000000000000194

Werler, M., Mitchell, A., Hernandez-Dias, S., Honein, M. 2005. Use of over-the-counter medications during pregnancy. *Am J Obstet Gynecol. 193*(3 Pt 1):771-7. Doi: 10.1016/j.ajog.2005.02.100.

Ystrom, E., Gustavson, K., Brandlistuen, R., Knudsen, G., Magnus, P., Susser, E., Smith, G., Stoltenberg, C., Suren, P., Haberg, S., Hornig, M., Lipkin, W., Nordeng, H., Kjennerud, T. 2017. Prenatal exposure to acetaminophen and risk of ADHD. *Pediatrics. 140*(5): e20163840. doi: 10.1542/peds.2016-3840.