August 2024

THE IMPACT OF PRENATAL EXPOSURE TO ENDOCRINE DISRUPTING CHEMICALS ON LANGUAGE DEVELOPMENT TRAJECTORIES

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by

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A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

in Environmental Health Sciences

at

The University of Wisconsin-Milwaukee

August 2024

ABSTRACT

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Under the Supervision of Professor Amy Kalkbrenner

Environmental contaminants that can impact and disrupt the endocrine system, known as endocrine disrupting chemicals (EDCs), are of growing concern, found in water, air, and common household goods. The disruption to the endocrine system can have severe impacts on the development of the fetus, particularly with regards to neurodevelopment. Understanding and elucidating the effects of prenatal exposure to EDCs on neurodevelopment will help us develop policies and interventions that minimize exposure and risk. In this research, we examined the effects of prenatal exposure to EDCs and their impact on language development trajectories using two enhanced risk autism cohorts. Chapter 1 introduces the concepts of neurodevelopment, endocrine disruption, the EDCs we intend to examine, and the statistical methods we intend to use in this research. Chapter 2 examines the effect that autism diagnostic status has on language development trajectories, with the hypothesis that those with a diagnosis of autism or that present with sub-clinical symptoms are at greater risk of abnormal development. Our results indicated that children with a diagnosis of autism, or who present with sub-clinical symptoms are at a greater risk of falling into an abnormal

language trajectory. Chapter 3 examines the effects of prenatal exposure to phthalates on language development trajectories, with the hypothesis that increased exposure results in greater risk of abnormal development. Our results indicated that low molecular weight phthalates tended to increase risk, while high molecular weight phthalate metabolites tended to decrease risk, though only a few reached statistical significance. Chapter 4 examines the effects of prenatal exposure to air toxics on language development trajectories, with the hypothesis that increased exposure will lead to greater risk of abnormal development. Our results indicated that nearly all air toxics did not have significant effect on risk of abnormal language development, with only acetaldehyde showing a decreased risk. Chapter 5 discusses the previous chapters and the implications of our findings. This research highlights the need for further research in a larger and more representative population, and that the effects of EDCs need to be more thoroughly explored to better elucidate their effects on neurodevelopment.

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LIST OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactive Disorder
ADOS	Autism Diagnostic Observation Schedule
AIC	Akaike Information Criteria
ASD	Autism Spectrum Disorder
BIC	Bayesian Information Criteria
BKMR	Bayesian Kernel Machine Regression
BzBP	Butyl benzyl phthalate
CDC	Centers for Disease Control and Prevention
DAG	Directed Acyclic Graph
DBP	Di-n-butyl phthalate
DEP	Di-ethyl phthalate
DEHP	Di(2-ehtylhexyl) phthalate
DiBP	Di-isobutyl phthalate
DiDP	Di-isodecyl phthalate
DiNP	Di-isononyl phthalate
DOP	Dioctyl phthalate, Di-n-octyl phthalate
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EARLI	Early Autism Risk Longitudinal Investigation
EDC	Endocrine Disrupting Chemical/Compound
EPA	Environmental Protection Agency
ICL	Integrated Complete Likelihood
LCGA	Latent Class Growth Analysis
LI	Language Impairment
MARBLES	Markers of Autism Risk in Babies – Learning Early Signs
mBP2	Mono-n-butyl phthalate
mBzP2	Monobenzyl phthalate
mCNP	Mono-carboxyisononyl phthalate
mCOP	Mono-carboxyisooctyl phthalate
mCPP	Mono(3-carboxypropyl) phthalate
mEP2	Monoethyl phthalate
mEHP	Mono(2-ethylhexyl) phthalate
mECPP	Mono(2-ethyl-5-carboxypentyl) phthalate
mEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
mEOHP	Mono(2-ethyl-5-oxohexyl) phthalate
MHBP	Monohydroxy-n-butyl phthalate
MHiBP	Monohydroxy-isobutyl phthalate
miBP	Mono-isobutyl phthalate
mNP2	Mono-isononyl phthalate
MSEL	Mullen Scales of Early Learning
NATA	National Air Toxics Assessment
Non-TD	Non-Typically Developing
RRR	Relative Risk Ratio
SABIC	Sample Size Adjusted Bayesian Information Criteria
TD	Typically Developing

ACKNOWLEDGEMENTS

I would like to thank several individuals who have provided advice, support, and mentorship throughout the duration of my degree. Thank you to my advisor Dr. Amy Kalkbrenner and my committee members Dr. Bonita Klein-Tasman, Dr. Kurt Svoboda, Dr. Cheng Zheng, and Dr. Heather Volk for their advice, support, and guidance throughout the duration of this dissertation. Without your help and support, I do not think I'd be able to get to this point in my academic career.

Thank you to the members of Kalkbrenner Labs, Tara Jensen, Victor Garcia Florez, and Mozhgan Nagi. The discussions we had during our weekly lab sessions, along with your feedback and support have proven invaluable to the writing of this dissertation.

Thank you to the staff and faculty of Zilber, who ignited a passion for teaching and public health within me. Thank you for all the administrative and technical support throughout the years, without which I'd likely still be lost in reams of paperwork and confusion.

Thank you to my friends, family, and loved ones, especially to Nathan, Samantha, and Cobie. You kept my spirits high during my lowest moments, and provided support and care when I needed it most. The nights spent together in laughter and fun kept my motivation and morale high, and I certainly wouldn't have made it this far with you all.

Chapter 1 – General Introduction

I. Introduction

The establishment of language is an important milestone in early childhood development, as it plays a role in almost every aspect of daily life. The effects of delays and impairments in language development may continue into adulthood, and include atypical social and emotional development, poor academic performance, and increased risk for diagnosis with neurodevelopmental disorders (1–7). There are many factors that can influence language development, including exposure to certain environmental pollutants. It is well known and documented that exposure to environmental chemicals during pregnancy can lead to abnormal neurodevelopment. Understanding the relationship between language development, its overall trajectories, and prenatal exposure to environmental chemicals, particularly within high-risk populations, may provide additional insight in identifying specific environmental hazards that may negatively impact language development. Once identified, targeted interventions can be implemented by policy makers in order to better mitigate the impact of these environmental exposures.

This research focuses on prenatal exposure to chemicals that act as endocrine disruptors (EDCs), which may play a role in fetal neurodevelopment. Exposure to these chemicals may negatively affect fetal neurodevelopment, which may result in abnormal language development. Rather than focusing on a single time point outcome, this research aims to examine language development over a longitudinal time period. By developing and using these language development trajectories as our outcome of interest, we are able to perform a more nuanced examination on the effects of EDCs on language development and gain several advantages over more traditional

epidemiological methods that examine single time point outcomes. By using multiple measures of language to develop these trajectories, we are able to improve our measurement of language development, reducing the chance of outcome misclassification. In addition, statistical analysis of trajectory groups allows for the examination of both within and between group variation, allowing researchers to identify risk factors unique to a specific trajectory class. This, in turn, may allow for more targeted interventions for those who fall within that trajectory class.

II. Language Development

The establishment of language is an important milestone in early childhood development. Delays or impairment of language development may lead to adverse effects later in life. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) lists the following as criteria for diagnosis of language disorder: persistent difficulties in the acquisition and use of language across modalities due to deficits in comprehension or production, language abilities that are substantially and quantifiably below those expected for age, onset during an early developmental period, and the difficulties are not attributable to hearing or other sensory impairment, motor dysfunction, or another medical or neurological condition and are not better explained by intellectual disability or global developmental delay (8). Language impairment (LI) has also been linked with and often co-occurs with several neurodevelopmental disorders, including attention deficit hyperactive disorder (ADHD) and autism (9,10,11, p.). As a result, understanding how, when, and why language impairment occur are important in order to prevent detrimental outcomes in the future.

Due to the multi-faceted aspects of language development, the suspected causes of LI, and the impacts of those causes, are varied; causes that result in a smaller vocabulary may not be the same causes that cause delays in verbal communications, for example. From a biological perspective, there have been several studies that have indicated that the basal ganglia may play an important role in language development (12,13). In addition, a region of the brain known as Broca's area or Broca's region has widely been regarded as an important structure for language development (14–16). Disruption of the development of these areas may result in language impairment. Thus, anything that may impact neurodevelopment has a possibility of directly impacting language development.

Although the study of environmental pollutants and LI is currently limited, some environmental pollutants have been linked to increased risk of LI including polychlorinated biphenyls (17), certain heavy metals (18), air pollutants (19,20), and polycyclic aromatic hydrocarbons (21,22). Many other environmental pollutants (e.g. air toxics, pesticides, and perfluoroalkyl substances) are likely candidates for study because they have been linked to neurodevelopmental disorders generally, such as autism (23–25), but have not yet been explicitly explored for links with language development. Beyond biological risk factors, consistently supported demographic and behavioral risk factors include: lower maternal education, having a family history of LI, higher birth order, male sex, preterm birth, lower 5 minute Apgar score, maternal smoking, and maternal alcohol use (26–30).

Human language development is not static but unfolds over time, following different trajectories that vary from person to person. Even among children without

diagnoses of developmental disorders or language impairment, subtle differences in language development trajectories can be detected and measured. These differences result in several trajectories of language development (31–33). However, impaired language development follows distinct and different trajectories than typical development. Studies of individuals with language impairment have found several different trajectories (34,35, p.,36,37). In addition, a large portion of existing research that examines language development trajectories has been in the context of autism spectrum disorder (ASD). For example, Landa et al. identified four groups using latent class growth analysis based on scores from the Mullen Scales of Early Learning on 204 siblings of children with autism (38). For the classes identified in these studies, they can be generalized into two broad categories: typical development and delayed or slowed improvement. Typical development (TD) is the trajectory defined by control individuals; that is, individuals with no clinical diagnosis of autism or other neurodevelopmental disorder, including LI. However, the definition of TD varies by study due to the nature of their cohorts, along with the method used to measure language. Those with delayed or slowed improvement may show improvement but either at a slower pace or begin at a less developed point than TD individuals, though they may still develop at an equal pace. When compared to children without a diagnosis of autism, children with autism tend to score lower on psychometric tests and have trajectories that are significantly different (39,40), though the general trend of the trajectory may be similar. However, both studies did not examine a third group: individuals who show “sub-clinical” symptoms. A portion of this research dissertation includes this third group, which we call

non-typically developing (non-TD), for trajectory comparison, which may provide more insight into possible trajectory differences between these diagnostic groups.

III. The Endocrine System

The endocrine system refers to the system of organs within the body that produce hormones, chemical messengers that regulate bodily functions, and the effect these hormones have on the body. These organs include the thyroid, gonads, and pituitary glands, which produce hormones such as sex hormones (e.g. estrogen), thyroid hormones (e.g. T3, T4), and peptide hormones (e.g. insulin, oxytocin). Because hormones are responsible for cell-to-cell communication during development, disruption of the endocrine system during critical developmental windows can lead to severe detriments in development. Pollutants that interfere with proper endocrine functioning are called endocrine disrupting compounds (EDCs), and are commonly linked to many different developmental outcomes, including neurodevelopmental deficits. For example, thyroid hormones play important roles during fetal neurodevelopment, acting on processes such as cell migration and differentiation, myelination, and synaptogenesis (41,42). As a result, prenatal thyroid disruption can lead to severe neurodevelopmental defects, including cretinism, lower IQ, and an increased risk of developing neurodevelopmental disorders (43–47).

More than 1800 EDCs have been identified by the US Food and Drug Administration (FDA) that disrupt at least one endocrine pathway (48). Some examples of common EDCs include bisphenol A, organophosphate pesticides, phthalates, polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and per/polyfluoroalkyl substances (PFAS).

Disruption of the endocrine system can occur through several different methods, including disrupting hormone metabolism by antagonizing or activating hormone receptors, modulating receptor coactivators, and influencing DNA methylation (49). EDCs can be found in various mediums, including groundwater, air pollution, and household products, and have been linked with many different health effects, including obesity, diabetes, reproductive health, certain types of cancer, and neurodevelopment disorders (50–53). Because of the large number of EDCs, combined with the multiple number of possible exposure routes, there has been a large emphasis on identifying exposure-outcome relationships and promoting the removal of EDCs with known health effects from general use.

As mentioned previously, hormones play a key role in infant development, particularly with neurodevelopment. Many EDCs have documented associations with neurodevelopmental disorders such as autism and ADHD. Polychlorinated biphenyls (PCBs) have been associated with ADHD (54), along with more general neurodevelopmental impairments, such as lower IQ, mental development, and psychomotor development (17,55). Polybrominated diphenyl ethers (PBDEs) have consistently shown a connection with neurodevelopment and neurotoxicity (56–58), along with associations with certain neurodevelopmental detriments in children related to attention, fine motor coordination, and cognition (59,60). Polycyclic aromatic hydrocarbons (PAHs) have been associated with adverse neurodevelopmental effects, such as cognitive delays and autism (21,61–63). Certain pesticides have been linked to lower IQ, lower motor speed, lower motor coordination, lower visuospatial performance, worse visual memory, and an increased risk for ADHD (64–71).

While research into the role of hormones on language development has been sparser, there is still evidence that both thyroid and sex hormones may play a role in language development. Postnatal treatment of thyroid deficiencies led to improvements in language deficits in children with hypothyroidism (72). Quast et al. found that sex hormone levels were linked with infant babbling, an important marker of articulatory skills and vocal development (73). Given the important role of hormones in neurodevelopment, it is reasonable to suspect that some EDCs that impact neurodevelopment may also interfere with language development. Indeed, some studies have found that certain EDCs have been associated with delays in language development. Bornehag et al. found that certain phthalates were associated with language delays, and both carbamate and organochlorine pesticides have been found to decrease language abilities in children (74–76).

IV. Phthalates

Phthalates, or phthalate esters, are a class of chemicals that are primarily used as plasticizers. They are used in a wide variety of products, including cosmetics, food packaging, cleaning materials, and pharmaceuticals. Phthalates have been shown to be endocrine disruptors through both animal and human studies, affecting estrogen and thyroid hormone pathways (77–79). These effects have been linked to several health effects, including neurotoxicity (80–82), hepatotoxicity (82,83), metabolic syndromes (84,85), and reproductive effects (83,86). Phthalates impair neurodevelopment via several pathophysiologies, which are varied, due to the wide range and types of phthalates and the possibility of different biological effects from different phthalate compounds. Several of the mechanisms of action of phthalates that have been

observed in animal studies include cell apoptosis (80,87), affecting neurotransmitter pathways (80,88), changing gene expression (87,89), and altering hormone levels (89,90). These effects have been connected to potential neurodevelopmental issues, which indicates that there may be more than one mechanism of action taking place that is resulting in an association with neurodevelopmental disorders and delays.

Currently, there is a significant amount of literature that indicates that phthalates have a negative impact on neurodevelopment. Phthalates have been found to be associated with neurodevelopmental disorders such as autism and ADHD (78,91,92) along with deficits in other neurological domains such as cognition (93,94), motor effects (95), and behavioral outcomes (96–98). Disruption of functionality and plasticity in the hippocampus, alterations in gene regulation and DNA damage, and disruption of several hormonal pathways are possible explanations for the neurodevelopmental effects of phthalates (99–102). There have been several studies that examined prenatal phthalate exposure and its effects on language development, four finding associations with delays in language development (74,103–105), and four finding no association (97,106–108). These studies generally excelled in prospective designs where phthalates were primarily measured via biomarkers. They used psychometric tests to measure language development, but only examined a single time point during development. Although the reasons for their differing results are not yet well-established, possible reasons for the contradicting findings include exposure misclassification, precision or sampling error (particularly for those studies with smaller sample sizes), and confounding. In addition, there were little to no examinations on multi-phthalate models, which are important to consider given that exposure to

phthalates often occurs in a mixture setting. One weakness that has been noted in prior studies involve the possibility of exposure misclassification, perhaps due to single measurements of phthalate exposures (97,107,108), which may result in no association being observed between phthalate exposure and delayed language development.

It is also important to note that when discussing phthalates and their possible association with language development, individual phthalates may have differing results, which may help to explain some of the inconsistencies. When we reviewed multiple studies that covered several phthalates and their metabolites, we found that results were inconsistent across phthalates, with some studies showing some phthalates with an increased risk for abnormal language development with others showing protective or null effects (74,97,103–108). Part of this difference may be in part due to the chemical composition of each phthalate and its metabolites, since it is thought that phthalate toxicity may be due in part to their overall molecular weight. Phthalates are classified into low and high molecular weight classes. The difference in weight may account for the differences between each individual phthalate ester. Each ester has different solubilities, with lower weight phthalates being more volatile in their pure state, but have low volatility in aqueous solutions, while high weight esters are hydrophobic in comparison, whose solubility is dependent on the length of the aryl chains of the phthalate (109).

Currently, evidence points to a possible sex difference in phthalates' various effects (110–112); this is to be expected as many endocrine disruptors impact female health differently than male health. This may be due to phthalate's impact on different hormonal pathways, some of which may be active during different periods of

development between the different sexes. Prior studies have found that sex differences vary based on phthalates, with two studies showing greater risk for boys (74,105) and one showing greater risk for girls (113). This may also be further compounded by the effect sex may have on certain neurodevelopmental outcomes. A literature review performed by Etchell et al., for instance, found inconsistent evidence for sex differences in brain function and structure with respect to language development (114). There is also a sex difference in prevalence in some neurodevelopmental disorders, such as autism and ADHD, with males being diagnosed at a higher rate than females. This may be due to interactions between sex and other factors, such as genetics, hormones, and environment (115).

V. Air Toxics

Air toxics, as defined by the United States Environmental Protection Agency (EPA), are toxic or hazardous air pollutants that cause or may cause serious health effects such as cancer, reproductive effects, and/or adverse environmental and ecological effects (116,117). Because of this broad definition, there are several classes of chemicals that are considered to be air toxics; the Clean Air Act identifies a total of 187 air toxics the EPA is required to control, though there are many more hazardous air pollutant (117). Pesticides, polychlorinated biphenyls (PCBs), particulate matter (e.g. PM_{10} and $PM_{2.5}$), polycyclic aromatic hydrocarbons (PAHs), various volatile organic compounds (VOCs, e.g. benzene, formaldehyde), and heavy metals (e.g. lead, mercury) are just a few examples. With the wide variety of chemicals, air toxics have been linked to a vast array of health effects, including neurodevelopmental disorders (54,57,118–120), cognitive decline (121), and cancer (122).

Of the numerous air toxics, PCBs, phthalates, PAHs, polybrominated diphenyl ethers (PBDEs), and certain heavy metals are a few examples with known endocrine disrupting effects (61,123–142). The mechanisms by which these air toxics cause these effects are varied, with some mimicking sex hormones (123,143–145), while others disrupt thyroid hormone pathways (123,124,146–149). Air toxics that act as EDCs have been linked to several health outcomes, including insulin resistance, cancers, fetal development, and reproductive issues (150,151, p.2,152–156). The routes of exposure to these air toxics vary, depending on the specific EDC. For instance, exposure to PAHs is due to combustion of organic materials, while exposure to phthalates is due to exposure to plastics or plasticizers (157). Exposure to these toxics also varies depending on environment, with some toxics being more prevalent within indoor environments compared to outdoor environments. In addition to individual effects, it is also important to note that exposure to these chemicals often do not occur in isolation; that is, an individual is often exposed to more than one class of chemicals at a time. Exposure to such mixtures may result in a compounding effect on the body, resulting in a stronger effect than if an individual were exposed to an air toxic individually.

A large number of studies focusing on the relationship between chemicals considered to be air toxics and neurodevelopmental disorders examined all routes of exposure, rather than focusing on solely airborne exposure. Of the studies that focused on airborne exposure, several air toxics have been linked with an increased risk for autism (24,119,158–160), and airborne exposure to PAHs have been linked to an increased risk of ADHD (161), as well as cognitive delays and autism (21,61,62). Furthermore, there have been few studies done on these chemicals and language

development. A total of 5 studies have examined exposure to these chemicals and language development, with all but one finding associations between exposure and abnormal language development (162–166). However, these studies did not exclusively examine airborne exposure to these air toxics; all but one examined all routes of exposure and one focused on dietary exposure. Other issues include the fact that all of these studies have examined single time point outcomes, rather than a trajectory outcome, and that they did not adjust for possible co-pollutant confounding due to limited exposure assessment.

Perhaps more importantly than their individual effects, exposure to air toxics often means all individuals are exposed to multiple chemicals. This arises because air toxics share common sources, creating similar geographic gradients after originating from vehicle exhaust, power plants, and industrial activity. Within a mixture of chemicals, health effects may be magnified, resulting in a more potent effect than exposure to a single chemical alone (e.g. pollutant-pollutant interactions). Furthermore, due to these exposures, there is a high risk of confounding due to the high correlation between the various pollutants and the correlation of the pollutants with the outcome of interest, along with a greater risk of Type I and Type II errors (167). While there are several statistical methods that can deal with pollutant-pollutant confounding, there is no single accepted method for examining pollutant-pollutant confounding. Rather, each method has its own strengths and weaknesses, along with purpose of use, so determining which statistical approach is most appropriate relies on the question being asked and what information is desired (168).

VI. Trajectory Analysis

When evaluating developmental outcomes, there are two common approaches researchers can use: a single time point approach or a longitudinal approach. In the former, researchers focus on a developmental outcome measurement at a single point in time, often resulting in a cross-sectional view of the outcome. In the latter, researchers use multiple outcome measurements over a period of time, resulting in an analysis that takes into consideration an individual's growth over time. While there are many forms of longitudinal analysis, this research focuses on trajectory analysis, which aims to take multiple outcome measurements in order to build and compare growth patterns between individuals.

Trajectory analysis can be performed using several different statistical techniques. These include growth mixture modeling, group based trajectory modelling, latent class analysis, and latent transition analysis (169). However, despite the different methods one can employ, there are multiple strengths of trajectory analysis. Perhaps the clearest strength of studying language trajectories is that trajectories are more representative of development than examining diagnostic outcomes at a single time point (170), given that development by its nature unfolds over time, and is not well-represented by a single-time snapshot. This benefit can be framed as improved measurement of the outcome of interest; that is, reduced outcome misclassification. In addition, by creating trajectory groups, it allows researchers to examine between-person variation within each group, along with between-group variation as well. This may permit researchers to identify unique risk factors within each group, allowing for more targeted interventions to aid those specific groups in preventing detrimental health outcomes.

Chapter 2 - Language Trajectories in Siblings of Children with Autism

Abstract

Background: Language development is a critical part of human development that unfolds over time. We aimed to examine and characterize the trajectories of language development within children with an older sibling with autism, as these children are more likely to have neurodevelopmental delays, thus allowing more detailed exploration of suboptimal language acquisition trajectories.

Methods: Participants were drawn from the Early Autism Risk Longitudinal Investigation (EARLI) (n=251) and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) (n=393) cohorts that recruited pregnant mothers who previously had a child with autism (ASD). Expressive and receptive language development was measured using the Mullen Scales of Early Learning (MSEL) at ages 6,12, 24, and 36 months of age. Each child was classified into one of 3 neurodevelopmental classifications: Autism Spectrum Disorder (ASD) (n=93), non-typically developing with no ASD (non-TD) (n=79), or typically developing (TD) (n=250). We used latent class growth analysis (LCGA) to determine unique language trajectories based on MSEL receptive or expressive language raw scores, the child's study-derived neurodevelopmental classification, child sex, maternal education, and maternal race.

Results: We determined four language trajectories for expressive (High Growth, Moderate Growth, Low Growth, and Tracking) and two trajectories for receptive language (High Growth and Late Growth). Overall, we observed that suboptimal language trajectories were most common for children classified as ASD and intermediate for non-TD children versus TD. Non-TD and ASD children were more likely to belong to the Low Growth, Late Growth, and Tracking trajectories.

Conclusion: While previous studies have shown that children with ASD are more likely to have sub-optimal language development, we newly showed that children with non-typical development not meeting ASD criteria also exhibit higher proportions with suboptimal trajectories, even when symptoms associated with neurodevelopmental delays are sub-clinical and do not meet diagnostic criteria for ASD.

Keywords: language development, language trajectory, autism, autism spectrum disorder, latent class growth analysis

Abbreviations: autism spectrum disorder (ASD), latent class growth analysis (LCGA), typically developing (TD), non-typically developing (non-TD), Mullen Scales of Early Learning (MSEL), neurodevelopmental delays (NDDs)

Introduction

Language abilities play a role in almost every aspect of daily life and early childhood is a time of rapid language acquisition. Language development delay or impairment can contribute to atypical social and emotional development, poor academic performance, and increased risk for diagnosis with neurodevelopmental disorders (1–7). Understanding the developmental trajectory of language in populations enriched for likelihood of neurodevelopmental disorders may aid the early identification of those conditions, as well as better target language focused interventions among at risk groups.

Human language development unfolds over time, following trajectories that vary from person to person, but that also cluster in classes. In addition to an optimal language acquisition trajectory defined by the most growth without observed degradation, prior research has identified between 4 to 7 different atypical trajectories or patterns in children from grade school to adolescence (34,35, p.,36,37). Law et al., for example, found evidence for three atypical trajectories: one where individuals start at a lower point but track with normally developing individuals ("tracking"), one where individuals develop at a similar rate but plateau at a certain time point ("plateau"), and one where individuals start at a similar point to normally developing individuals but with slower growth rates ("deterioration") (34). Pickles et al. labeled some of their trajectories as "delay" or "catch-up", both of which appear to follow the proposed "tracking" trajectory from Law et al. Tambyraja et al. likewise found several trajectories, two of which following the proposed "tracking" as described above and one that followed

"deterioration". The patterns identified will vary by the sample size, age of children, and characteristics of the sample, including the prevalence of developmental disabilities.

A substantive portion of existing research that examines language development trajectories has been in the context of autism spectrum disorder (ASD). Individuals diagnosed with ASD, compared to those without, tend to score lower on language tests and have trajectories with a decrease or a slower increase in language abilities (39,40). Although the presence of language impairment is common in individuals with ASD, language development among those with ASD is highly heterogenous (171,172).

Less studied are children who have "sub-clinical" symptoms of ASD or who may otherwise be identified as neuroatypical among other cognitive and behavioral dimensions. Studies including sufficient numbers of these children may be beneficial in more fully elucidating the relationship between neurodevelopment and language impairment trajectories, because the study will have better statistical ability to resolve language patterns in this group. An example of one such study that was enhanced for atypical development and sub-threshold autism-like traits was Landa et al.'s investigation of 204 siblings of children with ASD. This study identified four trajectory groups using latent class growth analysis based on scores from the Mullen Scales of Early Learning (38).

To add to the understanding of language development trajectories among children, especially those with atypical development, we conducted a study of similar design based on data collected from two ASD sibling cohorts, using latent class growth analysis (LCGA). We evaluated how language development varied across children in

these cohorts meeting diagnostic criteria for ASD, showing evidence of atypical development, and appearing to be typically developing.

Methods

Population

We included individuals in the Early Autism Risk Longitudinal Investigation (EARLI) and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) studies (173,174). Both studies recruited pregnant women who already had a child with a diagnosis of ASD, or where the biological father had a child with ASD and followed both the mother and the expected child longitudinally. EARLI recruited from several sites, spanning across northeast Maryland, southeast Pennsylvania, and northern California, while MARBLES recruited primarily from northern California. Children in EARLI were born between 2009 and 2013, while children in MARBLES were born between 2006 and 2023. Demographic information was obtained via in-person interviews and questionnaires (Table 2.1).

From 621 children in both cohorts, we excluded persons with 1 or no recorded language scores or who had no study-based classification as ASD, non-TD, or TD, because these were key study variables and to be consistent with prior literature, yielding a sample size of 493 individuals (Table 2.3).

Language Development and Neurodevelopmental Classification

Trained staff administered the Mullen Scales of Early Learning (MSEL, 20) to assess cognitive development (including language), in person, at 6, 12, 24, and 36 months of age. The MSEL is a standardized psychometric test that is used to measure

cognitive development in children ages 3 to 60 months (175). The MSEL generates five subscores (gross motor, fine motor, expressive language, receptive language, and visual reception). We used expressive language and receptive language raw subscores to generate our language development trajectories. Both expressive and receptive language involve the ability to process visual, auditory, and written language, though in different ways. Expressive language involves the ability to communicate ideas and thoughts using words and gestures to convey messages accurately and appropriately to others. Receptive language involves the ability to understand and process the meaning of language directed towards an individual.

All study children were assigned a research-based neurodevelopmental classification. When study children were 3 years old, licensed clinical psychologists evaluated them using the Autism Diagnostic Observation Schedules (ADOS, 19). These ADOS scores, together with MSEL composite standardized scores and clinical best estimate of an ASD diagnosis, were used to categorize study children as meeting criteria for autism (ASD), non-typical development (non-TD), and typical development (TD) based on a previously reported algorithm (177). Children classified as ASD had scores over the ADOS cutoff and met the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria for ASD. Non-TD children had ADOS scores within three points of the diagnostic cutoff or had either one MSEL subscore 2.0 standard deviations below average or two MSEL age adjusted subscores 1.5 standard deviations below average. Thus, non-TD children may have autism-related symptoms subthreshold of diagnostic criteria and/or poorer performance on any 1-2 of 5 MSEL subscales. Of the 96 children who were classified as non-TD, 71 of them were categorized as such due to low scores

on receptive or expressive language. Children who did not meet these criteria were classified as TD.

Statistical Analysis

The most frequently missing variables were expressive and receptive language scores at the middle time point: 24 months: 29% missing (Table 2.4). This may be explained due to a funding cut in EARLI, where this collection point was then dropped. A smaller number were missing other covariates, including expressive and receptive MSEL scores at other time points (6% for 6 months, 2% for 12 months, and 7% for 36 months), maternal educational attainment (1%), homeownership (3%), and maternal race (1%). To maximize our statistical precision and avoid a potential bias by excluding children missing data, we used multiple imputation by chained equations using the R package *mice* to impute missing values for variables listed above, including MSEL scores (178). Imputed distribution of these variables can be seen in Table 2.4.

We used latent class growth analysis (LCGA) to characterize language development trajectories, separately modeling expressive vs. receptive language. LCGA is a method to identify unmeasured (or latent) class membership using observed variables, with the goal of creating classes (or trajectories) so that individuals within a class are more similar than individuals between classes (179,180). We used a "one-step" approach where the same LCGA model was used to find the trajectories that best fit the data and estimate associations between neurodevelopmental classification (ASD, non-TD, TD) with trajectories. In addition to MSEL scores and neurodevelopmental classification, we also included the following a priori potential predictors of language for more accurate prediction for language trajectory assignments: child sex, race/ethnicity,

and maternal education (as a proxy for socioeconomic status) (27–30). Parameters from the LCGA models were used to generate relative risk ratios and 95% confidence intervals, and we performed an overall chi-squared test of the association between neurodevelopmental classification and language trajectories.

We ran LCGA models with 2-7 classes, assessing each model for goodness of fit, as indicated by lower Bayesian information criteria (BIC), sample size adjusted BIC (SABIC), and integrated complete likelihood (ICL), and higher entropy values. These fit measures do not always agree with each other, often pointing to different models as the best fitting model. Because our models generally had low entropy (>0.8), we selected the best-fitting model (number of classes) using the BIC, based on recommendations in Diallo et al. (181), along with favoring fewer over more classes for interpretability, and avoiding models with classes with <5% of the study population. While the complete models use probabilities of language trajectory membership (where a child has probabilities for each trajectory), we assigned each child to the trajectory class for which they had the highest probability for figures.

We selected language trajectory class names based on the overall shape of the class in the plot and guided by designations used in prior literature. We examined the patterns of expressive versus receptive language, examining both MSEL raw score correlations at each time point and concordance between our assigned language trajectories.

All analyses were conducted using Rstudio version 2022.02.1 Build 461 with R Version 4.1.3 “One Push-Up”. The LCGA was conducted using the *lcmm* package (182).

Results

Of the included children from EARLI and MARBLES, 114 were designated as ASD, 96 as non-TD, and 283 as TD. Children classified as having ASD were more likely to be male and from families with lower-SES (e.g., lower educational attainment) and of non-white race compared to the other neurodevelopmental classifications (Table 2.1). Children classified as TD were from families with higher SES (higher maternal education and more homeownership).

Mean expressive and receptive MSEL language raw scores increased across age (Figure 2.5). While the mean language scores in infancy (6 and 12 months) were similar across ASD, non-TD, and TD, the scores began to diverge at 24 and 36 months. During those later time points, the mean expressive and receptive MSEL scores were lowest for ASD, intermediate for non-TD, and highest for children classified as TD (Table 2.1). Expressive and receptive language raw scores were correlated at all ages, with higher correlations with later developmental periods, with no individuals exhibiting highly discordant scores (e.g., high expressive with low receptive) (Figure 2.6).

For expressive language, 4 trajectory classes were optimal (Table 2.5). The 4 assigned trajectory classes started at a similar level of language ability at 6 months, diverged slightly at 12 months, and then increasingly diverged at 24 and 36 months of age, resulting in classes that we named High Growth, Tracking, Moderate Growth, and Low Growth (Figure 2.1). The Tracking class mostly followed the High Growth class, albeit with lower scores at 24 months, along with a noticeable increase at 36 months. The majority of children, overall and for all neurodevelopmental classifications, were assigned to the intermediate Tracking trajectory for expressive language (273 of 493,

Figure 2.3). Moderate growth had consistent growth, but mean scores were lower than both Tracking and High Growth classes. The Low Growth class had almost no change in scores between 12 and 24 months, with a slight increase in scores at 36 months.

For receptive language, both the BIC and ICL indicated that the 4-class model was the best fit (Table 2.6). However, the 3 and 4-class models all had at least one class that had less than 5 percent of the population, and so we selected the next best fitting, 2-class model, labeling them as High Growth and Late Growth. Here, the Late Growth trajectory saw slight changes in raw scores between 12 and 24 months, but scores at 36 months jumped considerably (Figure 2.2). A majority of children, overall and for all neurodevelopmental classifications, were assigned to the High Growth trajectory for receptive language (373 of 493, Figure 2.4).

For both receptive and expressive language, we observed that our results had low entropy (entropy > 0.8), which indicated that the class separation was good for our LCGA. There were no individuals who had clearly discordant language trajectory assignments between expressive vs. receptive language (e.g., being assigned to High Growth for expressive but Late Growth for receptive). All individuals were either in the same class or in the adjacent class (Table 2.9).

For both expressive and receptive language, individuals in the Low or Late Growth trajectory were more likely to be male, have mothers who were less educated, and were less likely to identify as non-Hispanic white than individuals who fell in the High Growth trajectory (Tables 2.7 and 2.8). Individuals in the Tracking or Moderate Growth trajectory were less likely to identify as non-Hispanic white and more likely to have mothers who were less educated compared to the High Growth trajectory.

Both expressive and receptive language trajectories were highly associated with a child's neurodevelopmental classification, as shown by very low p-values from our overall chi square tests (Table 2.2 and Figures 2.3 and 2.4).

For expressive language, children with ASD at 36 months were substantially more likely to be in the Low Growth trajectory and were more likely to fall into the Tracking trajectory when compared to children classified as TD (Table 2.2 and Figures 2.3 and 2.4). Language trajectories for children classified as non-TD showed intermediate patterns of suboptimal language – between the more severely impacted ASD distribution which was largely of Moderate and Low Growth language trajectories, and the TD distribution which was largely made up of children with High Growth language trajectories. Non-TD children compared to TD children were more likely to be classified in the Low Growth and Tracking language trajectories, though the confidence bands include the null (Table 2.2). No TD children were classified as Moderate Growth, leading to an inability to calculate relative risk ratios (RRR) for these comparisons.

For receptive language, children with ASD were more likely to fall into the Late Growth trajectory when compared to both TD and non-TD children (Table 2.2 and Figures 2.3 and 2.4). When compared to TD children, non-TD children were more likely to fall into the Late Growth category.

Discussion

The goal of this study was to characterize the trajectory of language development in two cohorts of children with enhanced risk for neurodevelopmental disorders because they had a sibling with ASD, including the distribution of trajectories across

neurodevelopmental classification. Our results supported the hypothesis that a child's neurodevelopmental outcome classification was highly related to their language development trajectory. More specifically, children who were classified as ASD were more likely to belong trajectories that indicate a slower rate of language development and children with non-TD (but without ASD) were generally intermediate in trajectories of language acquisition compared to children classified as having typical development (TD).

Children classified as having ASD were more likely to belong to the Low Growth and Late Growth trajectories for expressive and receptive language, respectively, when compared to both non-TD and TD classified children. The Low Growth trajectory is distinct from other trajectories by having lower overall scores at each time point. Each of the other expressive language trajectories showed a marked increase in mean score at 24 months, which was not reflected in the Low Growth trajectory. Notably, for the Late Growth trajectory, we observed a large jump in score in receptive language at 36 months, an indication that there may be some "catch up" to the High Growth trajectory scores with certain developmental trajectories, though our analysis was not able to observe these other hypothetical trajectories in receptive language. Children classified as non-TD were also more likely to belong to both the Low Growth and Tracking trajectory classes for expressive and the Late Growth class for receptive language when compared to TD children. These results are consistent with prior literature, which shows that children with ASD generally score lower on language tests than those who do not have ASD, although there is a high amount of heterogeneity with language growth (37,39,40). While Landa and Pickles were in comparable cohorts (Landa included

children with a sibling with ASD while Pickles used referrals for possible autism), Brignell pulled from a broader community-based population.

Similar to the “catch up” effect observed in receptive language for the Late Growth trajectory, a smaller jump in score was seen in the Low Growth class for expressive language. Prior literature has indicated that such trajectory shapes are possible to observe in language development (37), and as such, it cannot be discounted that the Late Growth trajectory may be closer to a “tracking” trajectory, or that the Low Growth trajectory may be closer to a “late growth” trajectory. However, the LCGA algorithm did not determine that this was the case, though models that have greater number of classes may have been able to resolve more distinct and nuanced trajectory patterns. These trends highlight that the selection of the number of classes has a significant impact on the findings of all LCGA studies, including ours.

The use of the MSEL is important to note, as the MSEL is one of several inputs used in determining child neurodevelopmental classification as having non-TD, in addition to being the primary determinant of language trajectories. The distinction between a non-TD and TD classification involves criteria across the 5 MSEL subscores, of which expressive and receptive language are 2 of the 5. From our sample, a total of 82 of 96 individuals did not meet these language subscore thresholds. As a result, there is some level of correlation between our neurodevelopmental classifications and independent variables (language trajectories), at least for individuals with a non-TD classification. However, the neurodevelopmental classification algorithm does not only rely solely on the expressive and receptive language subscores of the MSEL, but also uses the fine motor and visual receptive subscores, so there may still be some level of

independence there. Further, the algorithm utilizes the standard score, which reflects distance from the normative mean, while our analysis uses the raw score, which reflects absolute level of language, as our dependent variable.

Our findings are in concert with previous studies and extend the understanding of language trajectories among children with suboptimal neurodevelopment. Landa et al. placed 235 children into three groups: Early-ASD (diagnostic impression of ASD at 14 months), Late-ASD (did not receive a diagnostic impression of ASD at 14 months), and non-ASD (40). They used generalized estimating equations (GEE) to determine differences in mean language scores using the MSEL between the three groups, finding significant differences for receptive and expressive language between the Early-ASD group and the Non-ASD group at all ages, whereas separation between the Late-ASD group and the Non-ASD group occurred only at later developmental periods: 24 and 36 months. Our approach in contrast to that of Landa et al. was able to shed light on a group that had non-typical development but did not meet ASD study criteria. Furthermore, it is only with our LCGA/trajectory approach that we could go describe the shape of language acquisition trajectories (such as we found with our Late Growth group), in contrast to only determining mean differences with a GEE approach. Tek et al. examined 35 children using Brown's 14 grammatical morphemes, dividing them into ASD and TD groups, using individual growth curve analysis to examine the differences (183). They found two distinct language development profiles, which they labeled as ASD-high verbal and ASD-low verbal. Our larger sample size (493) allowed us to detect a greater number of language trajectories (such as the four trajectories we resolved for expressive language) and their links with neurodevelopmental classification. Visser et

al. examined 203 children using LCGA to generate five different trajectory groups based on their Autism Diagnostic Observation Schedule (ADOS) scores, including the ADOS language score, along with other domains such as IQ (184). While Visser et al. was able to describe ASD phenotypic subgroups, the subgroups were more holistic and included other behavioral and cognitive domains beyond just language. Furthermore, all children were referred for ASD, thus preventing the evaluation of language in relation to more typical developmental classifications.

Strengths of our study include the use of language development trajectories, rather than single time point outcomes, which allowed for a more detailed and accurate examination of language acquisition across development. In addition, we had 4 repeated, robust measures of both expressive and receptive language, reducing the possibility of incorrectly assigning an individual to a wrong trajectory class. Second, the use of cohorts which prospectively follow siblings of children with autism allowed for a greater ability to detect abnormal language development trajectories that may be less common in unselected cohorts. Our included cohorts have a heightened prevalence for ASD compared to the general population and have increased prevalence of other neurodevelopmental delays. Third, the use of a continuous neurodevelopmental outcome measurement (i.e., MSEL raw scores), rather than a dichotomous neurodevelopmental outcome, allowed for the examination of individuals who present with “sub-clinical” symptoms (non-TD). Few studies have included these individuals as a separate group, with some including this group as part of the controls or not including them in the analysis altogether. The inclusion of this non-TD group allows a more

complete picture of language development in early childhood across a range of neurodevelopmental outcomes.

Our measurement of language using the MSEL was robust in that it is considered to be a suitable psychometric test for measuring expressive and receptive language (185) and has been shown to have good construct validity (186). Yet the MSEL is limited to expressive and receptive language domains and may miss other aspects of language - a complex phenotype. Future research should explore other facets of language development, such as vocabulary, grammatical development, or semantics. Our analysis was limited to the first 36 months of age, so we are unable to extrapolate language trajectories after this period. Studies that have examined language development patterns in children with ASD past the age of 36 months have largely found that, while children with ASD generally start with lower scores, their growth patterns are similar to those without ASD, with some instances of children with ASD scoring near identically to those without ASD (37,39). Due to the nature of LCGA analysis, our results are sensitive to the number of classes selected and our total sample size. While our sample size is sufficiently large enough to utilize LCGA, selecting for a different number of classes or changing the sample size may alter the results. However, we are confident that the overall direction of effect would remain consistent between various class choices.

In conclusion, we identified unique language trajectories for expressive and receptive language development in a population of children more likely to develop ASD or a developmental delay. Children with an ASD exhibited the most impairment; while children with non-typical development were intermediate, with both of these groups

more likely to belong to the Low or Late Growth category as opposed to the High Growth category when compared to children with typical development. Further exploration of these results should be conducted in larger and more diverse populations and/or with more in-depth characterization of language development measures.

Tables and Figures

Table 2.1: Characteristics of Participants in Early Autism Risk Longitudinal Investigation (EARLI) and Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) by Study Neurodevelopmental Classification

	ASD (N=114)	Non-TD (N=96)	TD (N=283)	Overall (N=493)
Child Gender				
Female	28 (24.6%)	43 (44.8%)	145 (51.2%)	216 (43.8%)
Male	86 (75.4%)	53 (55.2%)	138 (48.8%)	277 (56.2%)
Maternal Educational Attainment				
Less than high school	5 (4.5%)	3 (3.1%)	6 (2.1%)	14 (2.9%)
High school diploma/GED	8 (7.1%)	9 (9.4%)	12 (4.3%)	29 (5.9%)
Some college	49 (43.8%)	41 (42.7%)	92 (32.6%)	182 (37.1%)
Bachelor's degree	25 (22.3%)	25 (26.0%)	101 (35.8%)	151 (30.8%)
Graduate or professional degree	25 (22.3%)	18 (18.8%)	71 (25.2%)	114 (23.3%)
Missing	2	0	1	3
Homeownership				
Rent	52 (50.5%)	40 (43.0%)	95 (34.2%)	187 (39.5%)
Own	51 (49.5%)	53 (57.0%)	183 (65.8%)	287 (60.5%)
Missing	11	3	5	19
Maternal Race/Ethnicity				
Non-Hispanic White	56 (50.5%)	43 (45.7%)	165 (58.3%)	264 (54.1%)

	ASD (N=114)	Non-TD (N=96)	TD (N=283)	Overall (N=493)
Black/African American	11 (9.9%)	13 (13.9%)	7 (2.5%)	31 (6.4%)
Hispanic	24 (21.6%)	19 (20.2%)	57 (20.1%)	100 (20.5%)
Other/Multiracial	20 (18.0%)	19 (20.2%)	54 (19.1%)	93 (19.0%)
Missing	3	2	0	5
Expressive language raw score (6 months)				
Mean (SD)	6.49 (1.29)	6.11 (1.12)	6.33 (1.27)	6.32 (1.25)
Median [Min, Max]	6.00 [4.00, 11.0]	6.00 [3.00, 10.0]	6.00 [3.00, 12.0]	6.00 [3.00, 12.0]
Missing	18	12	37	67
Receptive language raw score (6 months)				
Mean (SD)	7.85 (1.51)	7.73 (1.56)	8.04 (1.48)	7.94 (1.51)
Median [Min, Max]	8.00 [4.00, 12.0]	8.00 [2.00, 12.0]	8.00 [3.00, 13.0]	8.00 [2.00, 13.0]
Missing	18	12	37	67
Expressive language raw score (12 months)				
Mean (SD)	11.0 (2.70)	11.4 (2.46)	12.5 (2.45)	12.0 (2.60)
Median [Min, Max]	11.0 [5.00, 16.0]	12.0 [6.00, 16.0]	13.0 [5.00, 19.0]	12.0 [5.00, 19.0]
Missing	9	10	10	29

	ASD (N=114)	Non-TD (N=96)	TD (N=283)	Overall (N=493)
Receptive language raw score (12 months)				
Mean (SD)	11.2 (2.14)	12.2 (1.84)	13.0 (1.83)	12.5 (2.04)
Median [Min, Max]	11.0 [5.00, 17.0]	12.0 [7.00, 16.0]	13.0 [9.00, 26.0]	13.0 [5.00, 26.0]
Missing	9	10	10	29
Expressive language raw score (24 months)				
Mean (SD)	16.3 (5.04)	19.4 (3.65)	22.4 (3.84)	20.6 (4.83)
Median [Min, Max]	16.0 [6.00, 28.0]	20.0 [9.00, 26.0]	22.0 [12.0, 33.0]	21.0 [6.00, 33.0]
Missing	42	57	83	182
Receptive language raw score (24 months)				
Mean (SD)	17.3 (5.98)	21.7 (4.62)	25.8 (3.24)	23.3 (5.47)
Median [Min, Max]	15.0 [2.00, 28.0]	22.0 [13.0, 30.0]	26.0 [14.0, 33.0]	25.0 [2.00, 33.0]
Missing	43	57	83	183
Expressive language raw score (36 months)				
Mean (SD)	25.2 (7.18)	29.7 (5.98)	33.8 (3.77)	31.0 (6.27)
Median [Min, Max]	26.0 [6.00, 41.0]	31.0 [15.0, 42.0]	34.0 [23.0, 45.0]	32.0 [6.00, 45.0]

	ASD (N=114)	Non-TD (N=96)	TD (N=283)	Overall (N=493)
Missing	14	5	29	48
Receptive language raw score (36 months)				
Mean (SD)	25.6 (6.89)	30.1 (4.28)	32.6 (3.81)	30.5 (5.54)
Median [Min, Max]	28.0 [2.00, 41.0]	30.0 [22.0, 42.0]	32.0 [24.0, 46.0]	31.0 [2.00, 46.0]
Missing	13	5	31	49

Footnotes for Table 2.1: TD = Typically Developing, Non-TD = Non-typically Developing; Percentages were calculated after removing missing individuals from the sample; We present maternal education here as a categorical variable, but because it is coded as ordinal, we elected to use it as a continuous variable in our analysis to avoid problems with model convergence

Table 2.2: Relative Risk Ratios of Language Development Trajectory by Neurodevelopmental Classification, Participants in Early Autism Risk Longitudinal Investigation (EARLI) and Markers of Autism Risk in Babies – Learning Early Signs (MARBLES)

		Relative Risk Ratios and 95% Confidence Intervals		
	Total (ASD/Non-TD/TD)	**ASD vs TD**	**ASD vs Non-TD**	**Non-TD vs TD**
		Expressive Language (overall chi-square p value < 2.2e-16)		
High Growth (n=106)	106 (5/13/88)	Referent	Referent	Referent
Tracking (n=273)	273 (40/56/177)	3.7 (1.1, 12.3)	1.5 (0.4, 5.9)	2.4 (0.6, 10.6)
Moderate Growth (n=34)	34 (29/5/0)	*	12.3 (1.9, 81.3)	*
Low Growth (n=80)	80 (40/22/18)	32.1 (7.7, 133.0)	3.5 (0.9, 13.5)	9.2 (0.7, 115.8)
		Receptive Language (overall chi-square p value < 2.2e-16)		
High Growth (n=373)	373 (43/63/267)	Referent	Referent	Referent
Late Growth (n=120)	120 (71/33/16)	25.9 (12.8, 52.4)	2.8 (1.4, 5.5)	9.0 (2.5, 32.1)

Footnote for Table 2.2: TD = Typically Developing, Non-TD = Non-typically Developing; n=114 for ASD, n=96 for non-TD, n=283 for TD; Multinomial logistic regression models with neurodevelopmental classification status as the main predictor and the trajectory classes as the outcome; All models were adjusted for child sex, maternal race (categorical), and maternal education (continuous, higher is more educated); * = because there were no TD individuals with that fell into the Moderate Growth trajectory class, those RRR were unable to be calculated

Table 2.3: Characteristics of Participants Included and Excluded from Analyses

	Included (N=493)	Excluded (N=128)	Overall (N=621)
Child Gender			
Female	216 (43.8%)	64 (54.7%)	280 (45.9%)
Male	277 (56.2%)	53 (45.3%)	330 (54.1%)
Missing	0	11	11
Maternal Educational Attainment			
Less than high school	14 (2.9%)	4 (5.8%)	18 (3.2%)
High school diploma/GED	29 (5.9%)	7 (10.2%)	36 (6.4%)
Some college	182 (37.1%)	19 (27.5%)	201 (36.0%)
Bachelor's degree	151 (30.8%)	20 (29.0%)	171 (30.6%)
Graduate or professional degree	114 (23.3%)	19 (27.5%)	133 (23.8%)
Missing	3	59	62
Homeownership			
Rent	187 (39.5%)	31 (44.3%)	218 (40.1%)
Own	287 (60.5%)	39 (55.7%)	326 (59.9%)
Missing	19	58	77

	Included (N=493)	Excluded (N=128)	Overall (N=621)
Neurodevelopmental Classification Status			
Typically Developing	283 (59.3%)	3 (33.3%)	286 (57.0%)
Non-typically Developing	96 (18.7%)	3 (33.4%)	99 (19.7%)
ASD	114 (22.0%)	3 (33.3%)	117 (23.3%)
Missing	0	119	119
Maternal Race/Ethnicity			
Non-Hispanic White	264 (54.1%)	39 (52.7%)	303 (53.9%)
Black/African American	31 (6.4%)	8 (10.8%)	39 (6.9%)
Hispanic	100 (20.5%)	12 (16.2%)	112 (19.9%)
Other/Multiracial	93 (19.0%)	15 (20.3%)	108 (19.3%)
Missing	5	54	59
Expressive language raw score (6 months)			
Mean (SD)	6.32 (1.25)	6.33 (1.54)	6.32 (1.28)
Median [Min, Max]	6.00 [3.00, 12.0]	6.00 [3.00, 11.0]	6.00 [3.00, 12.0]
Missing	67	88	155
Receptive language raw score (6 months)			

	Included (N=493)	Excluded (N=128)	Overall (N=621)
Mean (SD)	7.94 (1.51)	8.03 (1.93)	7.95 (1.55)
Median [Min, Max]	8.00 [2.00, 13.0]	8.00 [1.00, 11.0]	8.00 [1.00, 13.0]
Missing	67	88	155
Expressive language raw score (12 months)			
Mean (SD)	12.0 (2.60)	12.0 (2.91)	12.0 (2.62)
Median [Min, Max]	12.0 [5.00, 19.0]	12.0 [6.00, 17.0]	12.0 [5.00, 19.0]
Missing	29	91	120
Receptive language raw score (12 months)			
Mean (SD)	12.5 (2.04)	12.4 (2.49)	12.5 (2.07)
Median [Min, Max]	13.0 [5.00, 26.0]	13.0 [3.00, 16.0]	13.0 [3.00, 26.0]
Missing	29	91	120
Expressive language raw score (24 months)			
Mean (SD)	20.6 (4.83)	20.1 (5.45)	20.6 (4.85)
Median [Min, Max]	21.0 [6.00, 33.0]	20.0 [11.0, 28.0]	21.0 [6.00, 33.0]
Missing	182	116	298

	Included (N=493)	Excluded (N=128)	Overall (N=621)
Receptive language raw score (24 months)			
Mean (SD)	23.3 (5.47)	19.6 (5.62)	23.2 (5.51)
Median [Min, Max]	25.0 [2.00, 33.0]	18.0 [13.0, 29.0]	25.0 [2.00, 33.0]
Missing	183	116	299
Expressive language raw score (36 months)			
Mean (SD)	31.0 (6.27)	26.8 (9.82)	31.0 (6.36)
Median [Min, Max]	32.0 [6.00, 45.0]	25.0 [12.0, 39.0]	32.0 [6.00, 45.0]
Missing	48	120	168
Receptive language raw score (36 months)			
Mean (SD)	30.5 (5.54)	26.5 (9.80)	30.4 (5.65)
Median [Min, Max]	31.0 [2.00, 46.0]	28.0 [5.00, 38.0]	31.0 [2.00, 46.0]
Missing	49	120	169

Footnote for Table 2.3: To be removed from the analysis, an individual needed to be missing 3 or more MSEL scores for either expressive or receptive language or be missing a study classification (ASD, non-TD, or TD)

Table 2.4: Characteristics of Included Study Sample Before and After Imputing Missing Data

	Original Data (N=493)	Imputed (N=493)
Child Gender		
Female	216 (43.8%)	216 (43.8%)
Male	277 (56.2%)	277 (56.2%)
Maternal Educational Attainment		
Less than high school	14 (2.9%)	14 (2.8%)
High school diploma/GED	29 (5.9%)	29 (5.9%)
Some college	182 (37.1%)	184 (37.3%)
Bachelor's degree	151 (30.8%)	151 (30.6%)
Graduate or professional degree	114 (23.3%)	115 (23.3%)
Missing	3	
Homeownership		
Rent	187 (39.5%)	194 (39.4%)
Own	287 (60.5%)	299 (60.6%)
Missing	19	
Maternal Race/Ethnicity		

	Original Data (N=493)	Imputed (N=493)
Non-Hispanic White	264 (54.1%)	265 (53.8%)
Black/African American	31 (6.4%)	31 (6.3%)
Hispanic	100 (20.5%)	100 (20.3%)
Other/Multiracial	93 (19.0%)	97 (19.7%)
Missing	5	
Expressive language raw score (6 months)		
Mean (SD)	6.32 (1.25)	6.27 (1.25)
Median [Min, Max]	6.00 [3.00, 12.0]	6.00 [3.00, 12.0]
Missing	67	
Receptive language raw score (6 months)		
Mean (SD)	7.94 (1.51)	7.86 (1.54)
Median [Min, Max]	8.00 [2.00, 13.0]	8.00 [2.00, 13.0]
Missing	67	
Expressive language raw score (12 months)		
Mean (SD)	12.0 (2.60)	12.0 (2.61)

	Original Data (N=493)	Imputed (N=493)
Median [Min, Max]	12.0 [5.00, 19.0]	12.0 [5.00, 19.0]
Missing	29	
Receptive language raw score (12 months)		
Mean (SD)	12.5 (2.04)	12.5 (2.00)
Median [Min, Max]	13.0 [5.00, 26.0]	13.0 [5.00, 26.0]
Missing	29	
Expressive language raw score (24 months)		
Mean (SD)	20.6 (4.83)	20.4 (4.93)
Median [Min, Max]	21.0 [6.00, 33.0]	20.0 [6.00, 33.0]
Missing	182	
Receptive language raw score (24 months)		
Mean (SD)	23.3 (5.47)	22.9 (5.62)
Median [Min, Max]	25.0 [2.00, 33.0]	25.0 [2.00, 33.0]
Missing	183	

	Original Data (N=493)	Imputed (N=493)
Expressive language raw score (36 months)		
Mean (SD)	31.0 (6.27)	31.1 (6.12)
Median [Min, Max]	32.0 [6.00, 45.0]	32.0 [6.00, 45.0]
Missing	48	
Receptive language raw score (36 months)		
Mean (SD)	30.5 (5.54)	30.6 (5.43)
Median [Min, Max]	31.0 [2.00, 46.0]	31.0 [2.00, 46.0]
Missing	49	

Footnote for Table 2.4: To be removed from the analysis, an individual needed to be missing 3 or more MSEL scores for either expressive or receptive language or be missing a neurodevelopmental classification; Percentages were calculated after removing missing individuals from the sample; We present maternal education here as a categorical variable, but because it is coded as ordinal, we elected to use it as a continuous variable in our analysis to avoid problems with model convergence

Table 2.5: Measures of Model Fit for Expressive Language Latent Class Growth Analysis

Number of classes	Log-likelihood	SABIC	BIC	Entropy	ICL
1	-5440.39	10917.1	10955.19	1	10955.19
2	-5127.54	10336.8	10422.5	0.915236*	10451.47
3	-4979.2	10085.51	10218.82	0.844211	10303.2
4	-4908.17	9988.841	10169.76*	0.851522	10271.24*
5	-4887.5	9992.901	10221.43	0.788803	10389
6	-4843.89*	9951.077*	10227.22	0.87692	10335.94
7	-1.00E+09	2E+09	2E+09	1	2E+09

Footnote for Table 2.5: Higher log-likelihood, lower SABIC (sample adjusted BIC), lower BIC (Bayesian Inclusion Criterion), higher entropy, and lower ICL (integrated classification likelihood) indicates an overall better fit; * indicates the best model based on the above criteria (single class will always have an entropy of 1 so we select the next highest entropy instead); we prioritized BIC and ICL based on the findings in Diallo et al., 2016

Table 2.6: Measures of Model Fit for Receptive Language Latent Class Growth Analysis

Number of classes	Log-likelihood	SABIC	BIC	Entropy	ICL
1	-5346.83	10729.97	10768.06	1	10768.06
2	-5003.98	10089.68	10175.38	0.900346	10209.44
3	-4860.16	9847.437	9980.746	0.95275*	10006.34
4	-4658.26	9489.037	9669.955*	0.920754	9724.116*
5	-1.00E+09	2E+09	2E+09	1	2E+09
6	-4576.24*	9415.777*	9691.915	0.83438	9838.213
7	-4581.78	9472.269	9796.017	0.911532	9880.888

Footnote for Table 2.6: Higher log-likelihood, lower SABIC (sample adjusted BIC), lower BIC (Bayesian Inclusion Criterion), higher entropy, and lower ICL (integrated classification likelihood) indicates an overall better fit; * indicates the best model based on the above criteria (single class will always have an entropy of 1 so we select the next highest entropy instead); 5 and 6 class models failed to converge; we prioritized BIC and ICL based on the findings in Diallo et al., 2016; classes had <5% of the population in the 3 and 4 class models, so we selected the next best fit model instead

Table 2.7: Characteristics of Study Population Stratified by Assigned Expressive Language Trajectory Classes

	High Growth (N=106)	Tracking (N=273)	Moderate Growth (N=34)	Low Growth (N=80)	Overall (N=493)
Child Gender					
Female	51 (48.1%)	128 (46.9%)	7 (20.6%)	30 (37.5%)	216 (43.8%)
Male	55 (51.9%)	145 (53.1%)	27 (79.4%)	50 (62.5%)	277 (56.2%)
Maternal Educational Attainment					
Less than high school	44 (41.5%)	82 (30.0%)	5 (14.7%)	20 (25.0%)	151 (30.6%)
High school diploma/GED	30 (28.3%)	65 (23.8%)	8 (23.5%)	12 (15.0%)	115 (23.3%)
Some college	3 (2.8%)	17 (6.2%)	3 (8.8%)	6 (7.5%)	29 (5.9%)
Bachelor's degree	2 (1.9%)	5 (1.8%)	3 (8.8%)	4 (5.0%)	14 (2.8%)
Graduate or professional degree	27 (25.5%)	104 (38.1%)	15 (44.1%)	38 (47.5%)	184 (37.3%)
Homeownership					
Rent	35 (33.0%)	105 (38.5%)	19 (55.9%)	35 (43.8%)	194 (39.4%)
Own	71 (67.0%)	168 (61.5%)	15 (44.1%)	45 (56.3%)	299 (60.6%)
Maternal Race/Ethnicity					
Non-Hispanic White	60 (56.6%)	155 (56.8%)	14 (41.2%)	36 (45.0%)	265 (53.8%)

	High Growth (N=106)	Tracking (N=273)	Moderate Growth (N=34)	Low Growth (N=80)	Overall (N=493)
Black/African American	6 (5.7%)	12 (4.4%)	4 (11.8%)	9 (11.3%)	31 (6.3%)
Hispanic	14 (13.2%)	56 (20.5%)	10 (29.4%)	20 (25.0%)	100 (20.3%)
Other/Multiracial	26 (24.5%)	50 (18.3%)	6 (17.6%)	15 (18.8%)	97 (19.7%)
Expressive language raw score (6 months)					
Mean (SD)	6.33 (1.34)	6.25 (1.14)	6.62 (1.71)	6.14 (1.25)	6.27 (1.25)
Median [Min, Max]	6.00 [3.00, 10.0]	6.00 [4.00, 12.0]	6.00 [3.00, 11.0]	6.00 [3.00, 11.0]	6.00 [3.00, 12.0]
Expressive language raw score (12 months)					
Mean (SD)	13.0 (2.58)	12.0 (2.45)	11.3 (2.41)	11.0 (2.79)	12.0 (2.61)
Median [Min, Max]	13.0 [5.00, 18.0]	12.0 [5.00, 19.0]	12.0 [6.00, 15.0]	11.5 [6.00, 15.0]	12.0 [5.00, 19.0]
Expressive language raw score (24 months)					
Mean (SD)	27.0 (2.74)	19.8 (2.80)	11.9 (3.47)	17.5 (3.17)	20.4 (4.93)
Median [Min, Max]	26.0 [21.0, 33.0]	20.0 [6.00, 26.0]	12.0 [6.00, 19.0]	18.0 [11.0, 27.0]	20.0 [6.00, 33.0]
Expressive language raw score (36 months)					
Mean (SD)	36.8 (2.76)	32.6 (2.49)	16.7 (4.50)	24.3 (2.48)	31.1 (6.12)

	High Growth (N=106)	Tracking (N=273)	Moderate Growth (N=34)	Low Growth (N=80)	Overall (N=493)
Median [Min, Max]	37.0 [31.0, 45.0]	32.0 [26.0, 42.0]	18.0 [6.00, 23.0]	25.0 [18.0, 29.0]	32.0 [6.00, 45.0]

Table 2.8: Characteristics of Study Population Stratified by Assigned Receptive Language Trajectory Classes

	High Growth (N=373)	Late Growth (N=120)	Overall (N=493)
Child Gender			
Female	179 (48.0%)	37 (30.8%)	216 (43.8%)
Male	194 (52.0%)	83 (69.2%)	277 (56.2%)
Maternal Educational Attainment			
Less than high school	10 (2.7%)	4 (3.3%)	14 (2.8%)
High School/GED	18 (4.8%)	11 (9.2%)	29 (5.9%)
Some college	121 (32.4%)	63 (52.5%)	184 (37.3%)
Bachelor's degree	127 (34.0%)	24 (20.0%)	151 (30.6%)
Graduate or Professional degree	97 (26.0%)	18 (15.0%)	115 (23.3%)
Homeownership			
Rent	127 (34.0%)	67 (55.8%)	194 (39.4%)
Own	246 (66.0%)	53 (44.2%)	299 (60.6%)
Maternal Race/Ethnicity			
Non-Hispanic White	212 (56.8%)	53 (44.2%)	265 (53.8%)

	High Growth (N=373)	Late Growth (N=120)	Overall (N=493)
Black/African American	18 (4.8%)	13 (10.8%)	31 (6.3%)
Hispanic	67 (18.0%)	33 (27.5%)	100 (20.3%)
Other/Multiracial	76 (20.4%)	21 (17.5%)	97 (19.7%)
Receptive language raw score (6 months)			
Mean (SD)	7.95 (1.52)	7.59 (1.56)	7.86 (1.54)
Median [Min, Max]	8.00 [2.00, 13.0]	8.00 [3.00, 11.0]	8.00 [2.00, 13.0]
Receptive language raw score (12 months)			
Mean (SD)	12.8 (2.00)	11.6 (1.75)	12.5 (2.00)
Median [Min, Max]	13.0 [5.00, 26.0]	12.0 [7.00, 15.0]	13.0 [5.00, 26.0]
Receptive language raw score (24 months)			
Mean (SD)	25.6 (2.69)	14.4 (3.42)	22.9 (5.62)
Median [Min, Max]	26.0 [16.0, 33.0]	14.0 [2.00, 21.0]	25.0 [2.00, 33.0]
Receptive language raw score (36 months)			
Mean (SD)	32.4 (3.83)	25.1 (6.02)	30.6 (5.43)
Median [Min, Max]	32.0 [24.0, 46.0]	27.0 [2.00, 35.0]	31.0 [2.00, 46.0]

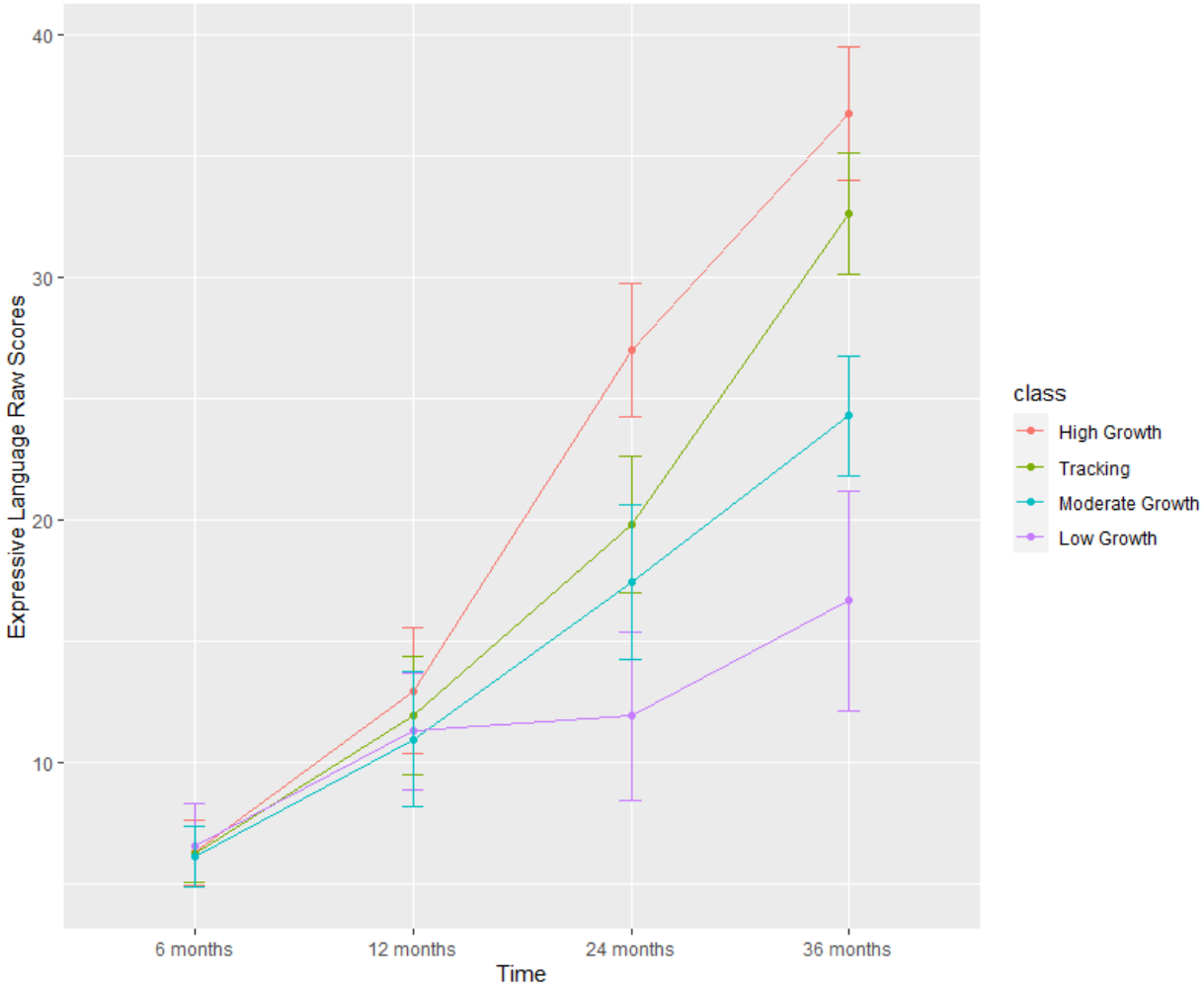
Table 2.9: Cross-classification of study population by assigned language trajectories

Expressive Language Trajectories	Receptive Language Trajectories	
	High Growth	Late Growth
High Growth	105	1
Tracking	232	41
Moderate Growth	3	31
Low Growth	33	47

Table 2.10: Total number of children falling into trajectories based on Neurodevelopmental Classification

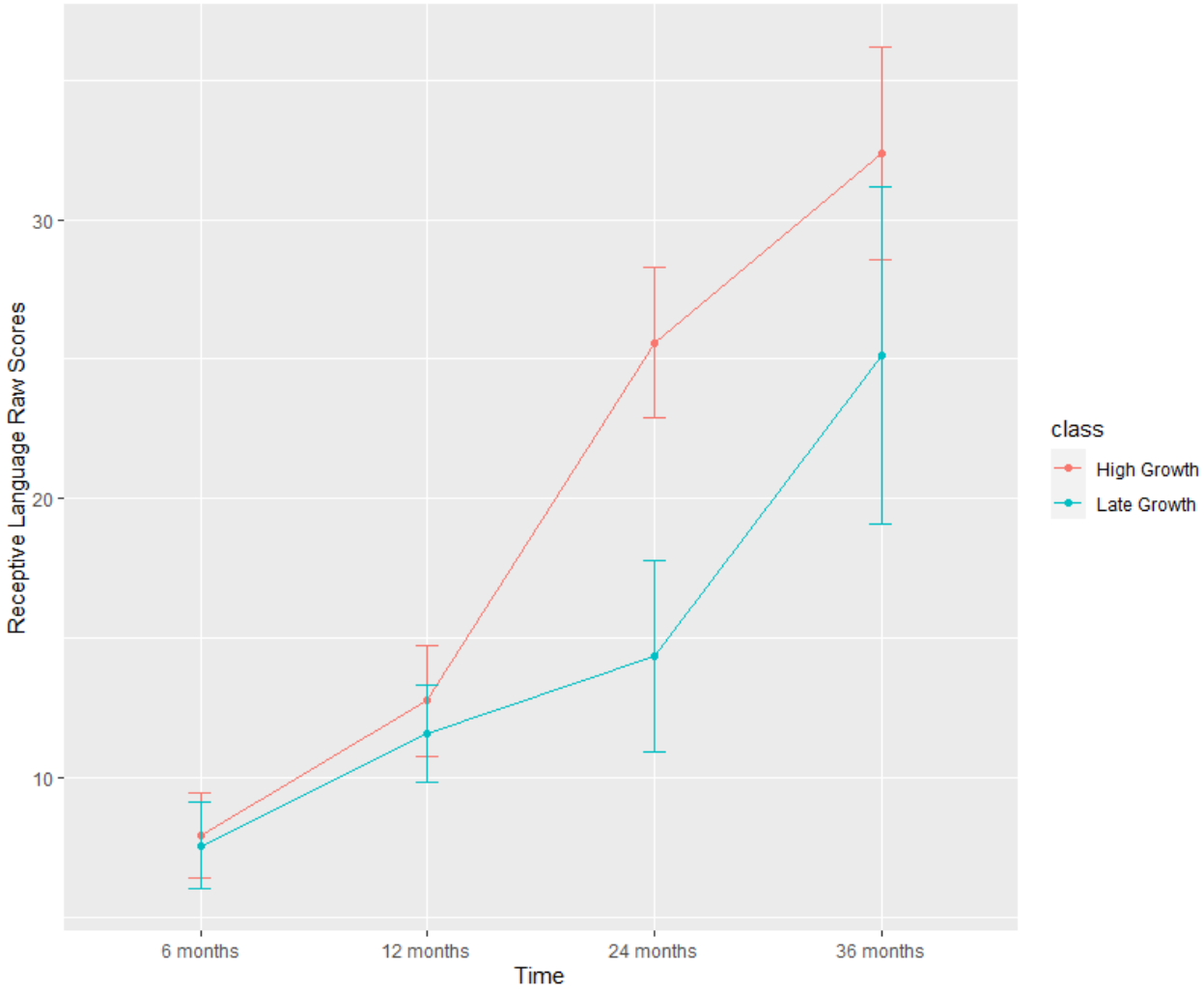
	Neurodevelopmental Classification		
Expressive Language Trajectories	ASD	Non-TD	TD
High Growth	5	13	88
Tracking	40	56	177
Moderate Growth	29	5	0
Low Growth	40	22	18
Receptive Language Trajectories			
High Growth	43	63	267
Late Growth	71	33	16

Figure 2.1: Expressive Language Trajectories



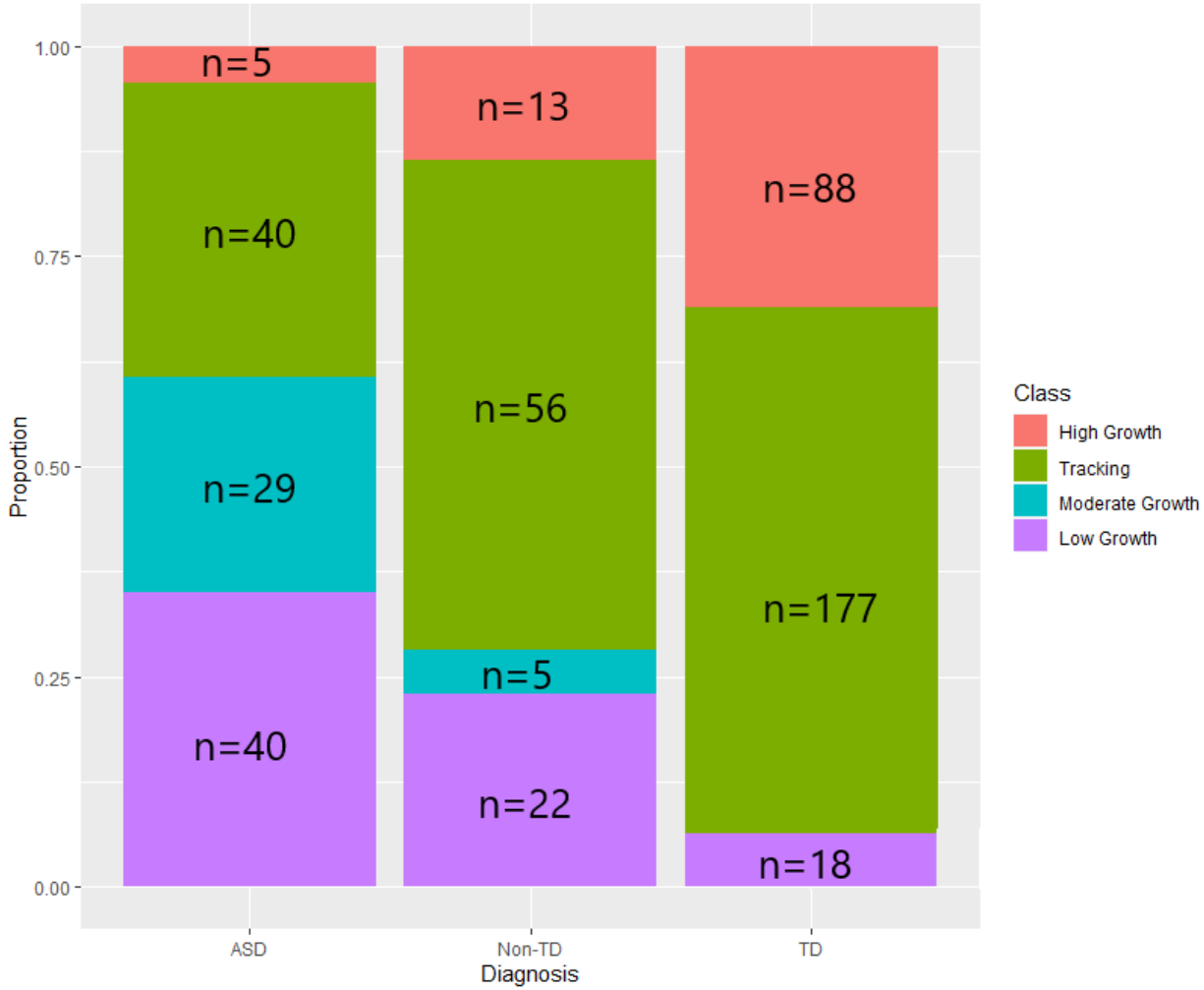
Footnotes for Figure 2.1: Bars indicate 95 percent confidence interval centered around the mean at each time point

Figure 2.2: Receptive Language Trajectories



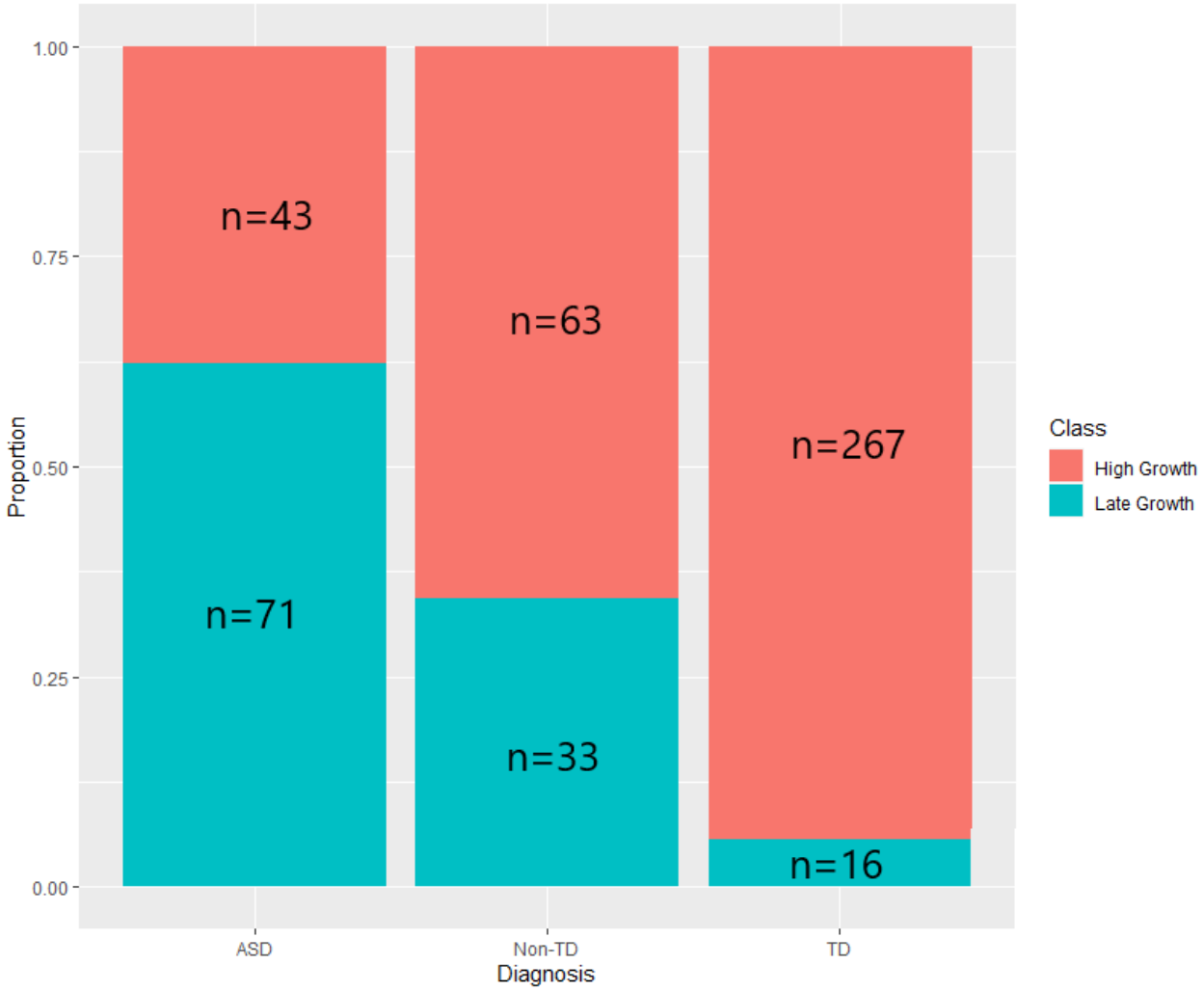
Footnotes for Figure 2.2: Bars indicate 95 percent confidence interval centered around the mean at each time point

Figure 2.3: Proportions of trajectory class assignment for expressive language



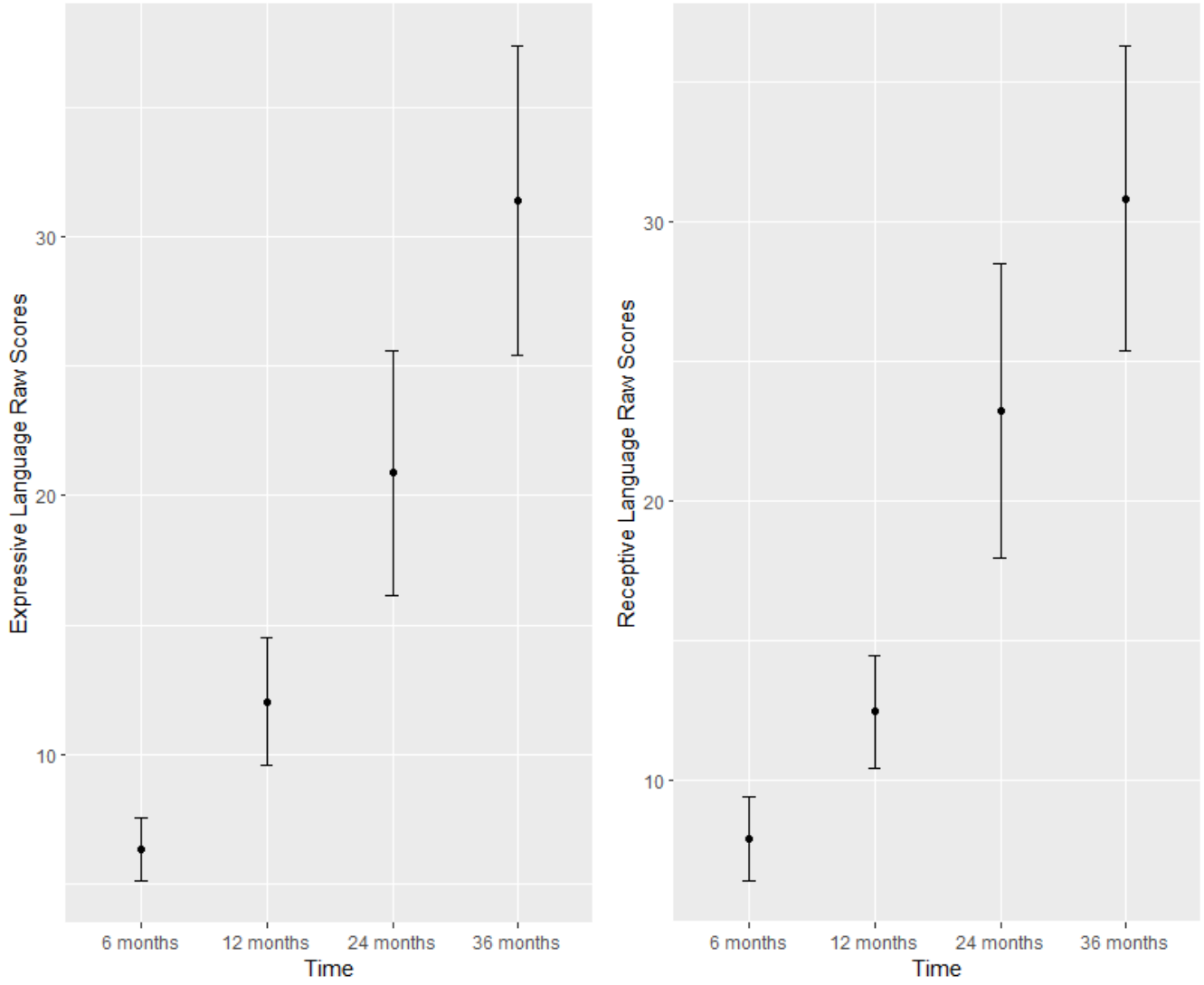
Footnotes for Figure 2.3: Trend shows that those with a diagnosis of ASD or Non-TD are more likely to fall into Low and Moderate Growth compared to Tracking and High Growth

Figure 2.4: Proportions of trajectory class assignment for receptive language



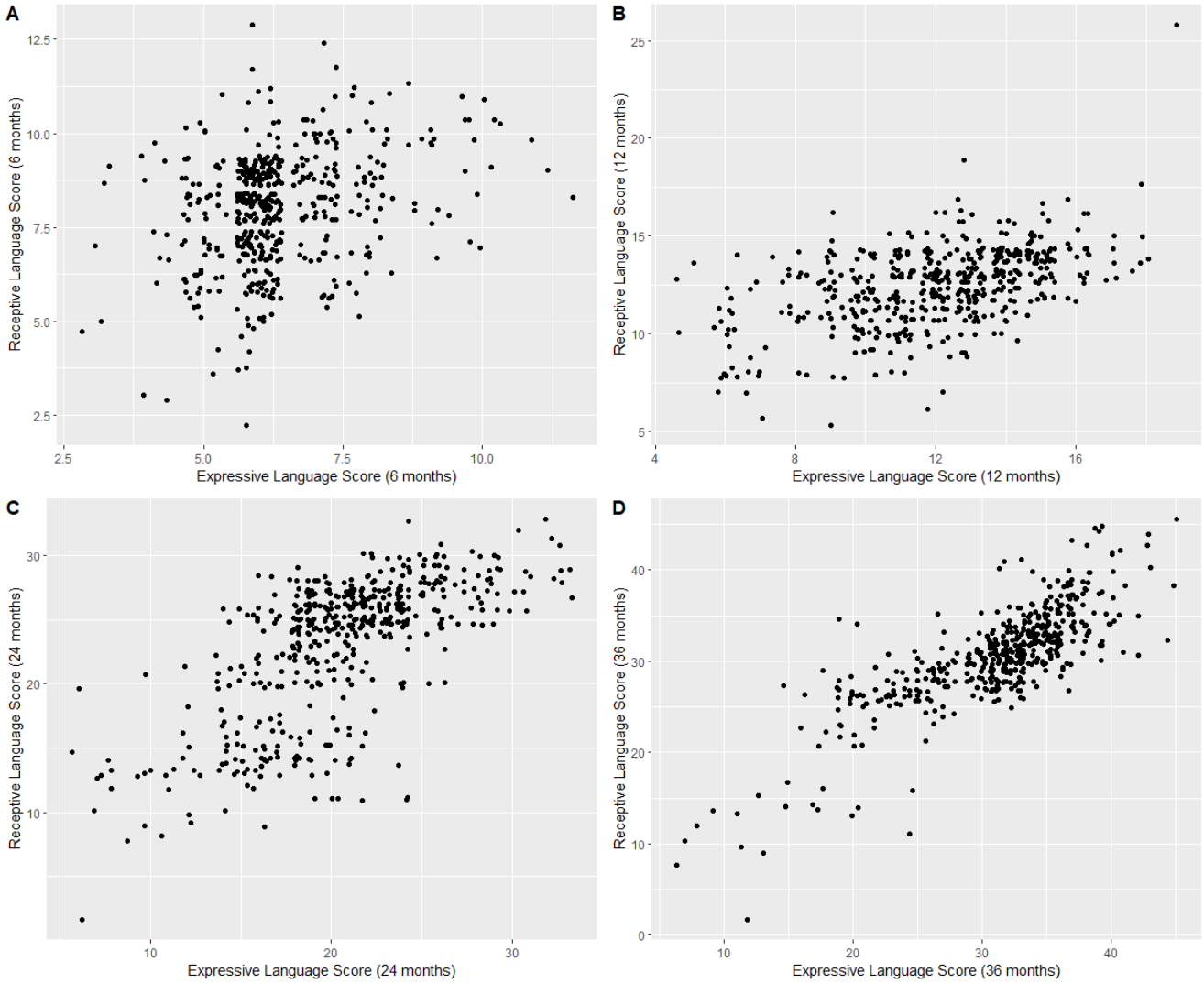
Footnotes for Figure 2.4: Trend shows that those with a diagnosis of ASD or Non-TD are more likely to fall into Late Growth

Figure 2.5: Distributions of MSEL Expressive and Receptive Language Raw Scores at 6, 12, 24, and 36 Months



Footnotes for Figure 2.5: Bars indicate 95 percent confidence intervals centered on mean

Figure 2.6: Scatterplots of MSEL Expressive vs Receptive Language Raw Scores at Each Time of Data Collection



Footnote for Figure 2.6: Comparison of expressive and receptive language scores; disjoint scores would be present in the upper left and lower right corners; we observe a mostly linear relationship between the two scores

Chapter 3 - The Impact of Prenatal Phthalate Exposure on Language Development Trajectories in Siblings of Children with Autism

Abstract

Background: Language development is a critical part of human development that unfolds across time. We aimed to examine how prenatal phthalate exposure affects early childhood language development, utilizing a robust longitudinal analysis methodology.

Methods: Participants were drawn from the Early Autism Risk Longitudinal Investigation (EARLI) (n=251) and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) (n=393) cohorts that recruited pregnant mothers who previously had a child with autism (ASD). Expressive and receptive language development was measured using the Mullen Scales of Early Learning (MSEL) at ages 6,12, 24, and 36 months of age. A total of 14 phthalate metabolites were assessed using multiple first morning voids at each trimester of pregnancy. We used latent class growth analysis (LCGA) to determine language trajectories based on MSEL receptive or expressive language raw scores, prenatal phthalate exposure, cohort, child sex, maternal age, maternal race/ethnicity, homeownership, and maternal educational attainment.

Results: We found 3 trajectories for both expressive and receptive language using both a one-step and two-step LCGA approach. The general direction of risk did not change between the one and two step approaches. Most phthalates were not statistically significant. Certain metabolites of di(2-ethylhexyl) phthalate decreased risk of falling into an abnormal trajectory for receptive language, and metabolites of di-isodecyl phthalate decreased risk of falling into the Tracking trajectory for expressive language using the one-step approach. The metabolites of di-n-butyl phthalate

increased risk for falling into the Low Growth trajectory for expressive language using the one-step approach.

Conclusion: Most of our phthalates were not statistically significant, though some trends were observed among low and high molecular weight phthalates. These trends were largely consistent with prior literature.

Introduction

The establishment of language is an important milestone in early childhood development, as it plays a role in almost every aspect of daily life. Language development delay or impairment may continue into adulthood, and can contribute to atypical social and emotional development, poor academic performance, and increased risk for diagnosis with neurodevelopmental disorders (Beitchman & Brownlie, 2012; Howlin & Udwin, 2002; Irwin et al., 2002; Johnson et al., 1999; Roulstone et al., 2011; Snowling et al., 2006; Whitehouse et al., 2009). The causes of language delay are multifaceted and varied, with some common social factors including family history, child sex, maternal education, and home environment (28,187,188). There are also varied environmental chemical exposures that are suspected to be linked with language delays, including certain heavy metals (e.g. lead, mercury), polychlorinated biphenyls, polybrominated diphenyl ethers, phthalates, and organophosphate insecticides (74,189–192), although, for some of these exposures, both the direction and magnitude of causality with language have proven to be inconsistent.

Certain biological systems are critical to early neurodevelopment and language development, such as the endocrine system. Environmental pollutants that disrupt this system, known as endocrine disrupting chemicals (EDCs) are therefore of vital interest, having a higher *a priori* suspicion of causing language impairment. As such, understanding environmental causes that may act as EDCs will not only provide a better understanding behind the etiology of language disorders, but also provide stakeholders with vital knowledge to make impactful changes to reduce overall risk.

Phthalates are a class of environmental exposures with known impacts on the endocrine system and neurodevelopment. They are plasticizers used in a wide variety of products, including cosmetics, food packaging, cleaning materials, and pharmaceuticals. Phthalates may impair neurodevelopment via several proposed pathophysiologies, including cell apoptosis (80,87), affecting neurotransmitter pathways (80,88), and altering gene expression and hormone levels (87,89). These effects are thought to be linked to potential neurodevelopmental issues, including disorders such as autism and ADHD (78,91,92) along with deficits in other neurological domains such as cognition (93,94), motor effects (95), and behavioral outcomes (96–98).

Of the multiple dozens of phthalates used in products, they are grouped broadly by low molecular weight (LMW) and high molecular weight (HMW) (193). HMW phthalates, such as di-(2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DiNP), and di-isodecyl phthalate (DiDP) are typically used to add flexibility to polyvinyl chloride plastic (PVC) and are used in plastic tubing and food packaging. LMW phthalates, such as diethyl phthalate (DEP), dimethyl phthalate (DMP) and dibutyl phthalate (DBP) tend to be used as solvents, and are more present in household products such as adhesives and cosmetics (193,194). The difference in metabolism of the different types of phthalates are thought to result in differing biological pathways. HMW phthalates tend to form oxidized metabolites, while LMW phthalates tend to form monoesters. These monoesters are theorized to be more relevant to androgen insufficiency (193). However, toxicity profiles can vary between various phthalates, and not all phthalates have been thoroughly investigated for their mechanisms of action.

Phthalates have been studied in relation to language impairment, including both HMW and LMW phthalate groups. Results of impacts on language have been inconsistent between molecular weight classes and individual phthalates.

Findings of LMW phthalates (as a class) and language vary widely to include increased and decreased risk (protective associations). One study found increased risk of language impairment for all LMW phthalates examined (106), three studies found increased or decreased risk depending on the individual phthalate compound examined (74,107,108), and one study found protective associations for all LMW phthalates examined (97). Olesen et al. found various results depending on the method of language measurement, with increased risk for complexity scores on the MacArthur-Bates Communicative Development Inventories for all LMW phthalates, but individual LMW phthalates had increased or decreased risk for vocabulary scores on the MacArthur-Bates Communicative Development Inventories. In addition, Olesen found sex differences, where males generally exhibited risk associations between LMW phthalates in that study, while females largely exhibited associations that leaned more protective, though none reached statistical significance, for both measures of language (105).

For HMW phthalates, the direction of association has been more consistent with increased risk across several studies, suggesting that HMW phthalates may be more harmful to the developing nervous system, although results were still dependent on sex and the language measurement tool. For instance, Bornehag et al., Hyland et al, and Polanska et al., all found increased risk for all HMW phthalates observed (74,106,108). Olesen et al. found increased risk in males for both vocabulary and complexity with

HMW phthalates, but females had a decreased risk, except for the metabolite mEHP which had an increased risk (105). Likewise, Huang et al. found decreased risk for all HMW phthalates when examining verbal IQ, but an increased risk when examining verbal comprehension index on the Wechsler Intelligence Scale for Children-IV (103).

Possible reasons for the high variability in findings may reflect true nuance where impairment only follows from exposure to a particular phthalate compound, pertains to only a certain subtle attribute of language that is only detectable by certain psychometric tests, or is only evident in one sex. Alternately, heterogenous findings may be influenced by errors such as exposure misclassification, precision or sampling error (particularly for those studies with smaller sample sizes), and confounding. These studies of phthalates and language generally excelled in prospective designs where phthalates were primarily measured via biomarkers and used psychometric tests to measure language development, but only examined a single time point during development. One weakness that has been noted in prior studies involve the possibility of exposure misclassification, perhaps due to measurements of phthalate exposures at a single point during development (97,107,108), which may result in no association being observed between phthalate exposure and delayed language development.

To clarify the possibility that certain phthalates, and phthalate molecular classes, may exert deleterious impacts on language, we conducted a study based on data collected from two autism sibling cohorts. Phthalates were measured using urinary biomarkers including more than one point of collection during pregnancy. We utilized latent class growth analysis (LCGA) to capture different trajectories of language acquisition measured 4 times in early childhood. We examined the association of 15

prenatal phthalate exposures and their potential impact on language development trajectories and examined whether results differed by child sex.

Methods

Population

We included individuals in the Early Autism Risk Longitudinal Investigation (EARLI) and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) studies (173,174). Both studies recruited pregnant women who already had a child with a diagnosis of ASD or whose fathers who had a previous biological child with ASD. Both the mother and the expected child were followed longitudinally. EARLI recruited from several sites, spanning across northeast Maryland, southeast Pennsylvania, and northern California, while MARBLES recruited primarily from northern California. Children in EARLI were born between 2009 and 2013, while children in MARBLES were born between 2006 and 2023. Demographic information was obtained via in-person interviews and questionnaires.

Language Development and Neurodevelopment Measurement

The Mullen Scales of Early Learning (MSEL, 20) was used to assess language development at 6, 12, 24, and 36 months of age. The MSEL is a standardized psychometric test that is used to measure cognitive development in children ages 3 to 60 months (175). Trained staff administered the MSEL in person at each of the time points above for both cohorts, having the child complete MSEL tasks and grading them based on tasks completed. The MSEL generates five subscores (gross motor, fine motor, expressive language, receptive language, and visual reception). We used

expressive language and receptive language raw subscores to generate our language development trajectories. Both expressive and receptive language involve the ability to process visual, auditory, and written language, though in different ways. Expressive language involves the ability to communicate ideas and thoughts through the use of words and gestures to convey messages accurately and appropriately to others. Receptive language involves the ability to understand and process the meaning of language directed towards an individual. At the 36 month visit, children were assigned a neurodevelopmental classification from licensed clinical psychologists using the Autism Diagnostic Observation Schedules (ADOS, 19). The ADOS, together with MSEL composite standardized scores and clinical best estimate of an ASD diagnosis, were used to categorize study children as meeting criteria for autism (ASD), non-typical development (non-TD), and typical development (TD) based on a previously reported algorithm (177).

Phthalate Measurements

Mothers in EARLI were instructed to provide up to two first morning void (FMV) urine samples during their 1st, 2nd, or 3rd trimester. Further details on the collection and analysis for the EARLI phthalate metabolites are detailed in a previous paper (195). For MARBLES participants, mothers were instructed to collect three FMVs and one 24-hour urine sample in each trimester. Further details on the collection and analysis for the MARBLES phthalate metabolites are detailed in a previous paper (196). A total of 14 metabolites were quantified in both studies: monoethyl phthalate (mEP2), mono-isobutyl phthalate (miBP), monohydroxy-isobutyl phthalate (MHiBP), mono-n-butyl phthalate (mBP2), monohydroxy-n-butyl phthalate (MHBP), monobenzyl phthalate (mBzP2),

mono(2-ethylhexyl) phthalate (mEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (mECPP), mono(3-carboxypropyl) phthalate (mCPP), mono-isononyl phthalate (mNP2), mono-carboxyisooctyl phthalate (mCOP), and mono-carboxyisononyl phthalate (mCNP). We also constructed ΣDEHP due to the high correlation between the metabolites of DEHP (mEHP, mEHHP, mEOHP, and mECPP) by using the molar sum of those metabolites: we divided their concentrations by their molecular weight and then summed the results.

To control for urinary dilution, we used two measurements. For EARLI, creatinine concentration was collected, and for MARBLES, specific gravity was collected. For each measurement, a site-specific Z-score was created and included in all models along with the raw unadjusted reported phthalate concentrations. Creatinine concentrations were log-base2 transformed prior to the calculations of Z-scores. This is consistent with methods described in Chiu et al. (197).

For phthalate concentrations below the limit of detection (LOD), we used machine-observed concentrations without substitution or transformation. Because some women had multiple phthalate measurements, we created an average exposure for each individual for each phthalate metabolite by summing all phthalate measurements taken, then dividing by the total number of samples taken during the prenatal period. Because MARBLES also had pooled samples that used multiple samples for a single phthalate concentration, we generated a weighted average metabolite concentration for that mother using the following formula: $C_{avg} = \frac{C_i + C_{pool} * N_{pool}}{N_{pool} + N_i}$, where C_i is the

concentration for any individual sample (i.e. non-pooled samples), C_{pool} is the concentration of the pooled sample, N_{pool} is the number of samples used in the pool, and N_i is the number of individual samples. For individuals that did not have pooled samples, we averaged the individual samples together to obtain the average metabolite concentration. To best model phthalates numerically in relation to language scores, we examined the AIC for simple models using phthalates and the expressive and receptive language scores at 36 months (Supplemental Table 2). We found that transformations of phthalates were not necessary. However, we winsorized phthalate metabolite measures at the 99th percentile to reduce the effects of extreme measurements. We divided all phthalates by their interquartile range for all statistical analyses.

Statistical Analysis

A total of 621 individuals were in the initial study populations. We excluded persons missing the study-derived diagnostic classification (n=119) or for whom we weren't confident in their language trajectories (those with 1 or no recorded language scores out of the 4 data collections points, n=98), or who were missing all phthalate measurements (n=209), yielding a sample size of 362 individuals (Table 3.2). The most frequently missing variables were expressive and receptive language scores at 24 months: 39.8% and 40.1% missing, respectively (Table 3.3). A total of 209 persons were missing phthalate values, and when one was missing, the entire suite of phthalate metabolites was missing. A smaller number were missing other covariates, including expressive and receptive Mullen scores at other time points (15.7% for 6 months, 6.1% for 12 months, and 0.8 and 1.1% for 36 months respectively), maternal educational attainment (0.3%), homeownership (3.5%), and maternal race (1.0%). To include

persons missing covariates or 1 or 2 Mullen scores, we used multiple imputation by chained equations using the R package *mice* to impute missing values for variables listed above, including Mullen scores (178). Imputed distribution of these variables can be seen in Table 3.3.

We used a latent class growth analysis (LCGA) approach to analyze the relationship between individual phthalates and language development trajectories, separately modeling expressive and receptive language. We used LCGA to both create language development trajectories that best fit the data and to assign children to the trajectory most appropriate to their language development. LCGA is a method to identify unmeasured (or latent) class membership using observed variables, with the goal of creating classes (or trajectories) so that individuals within a class are more similar than individuals between classes (179,180). We performed two types of LCGA analysis: a two-step and a one-step process.

Our primary analyses consisted of a two-step LCGA approach, where we first assigned language trajectories, and then in a separate model regressed these on phthalate exposures. We ran several LCGA models with different numbers of classes, ranging from 2 classes to 7 classes, using only Mullen scores as our inputs. We selected the best number of classes using the following measures of fitness: Bayesian information criteria (BIC), sample size adjusted BIC (SABIC), integrated complete likelihood (ICL), and entropy, selecting the model that had the best measures of fit (i.e. low BIC, ICL, and SABIC with low entropy [>0.8]). We prioritized using the BIC based on recommendations observed in Diallo et al. (181), along with two additional considerations: we favored models with fewer classes to aid in interpretability, and we

avoided models with low number of individuals in a class (<10% of the study population). After individuals were assigned, a multinomial logistic regression was performed using the assigned classes as the outcome and phthalate exposures as our main predictors, along with site-specific Z-score for urine dilution. We adjusted all models for cohort (EARLI vs. MARBLES) and also accounted for the following a priori potential predictors of language: child sex, maternal age, maternal race/ethnicity, maternal education, and home-ownership (as proxies for socioeconomic status) (27–30).

In a sensitivity analysis, we used a one-step LCGA, where the language trajectories were established in one model simultaneously with the same covariates as above and phthalate exposures. The inclusion of these predictors in the LCGA algorithm holds several advantages: misclassification of individuals into the wrong trajectory class is irrelevant (198), measurement errors of trajectory membership are incorporated into the analysis (198), and the inclusion of covariates can improve class separation and reduces standard errors (199). We ran several LCGA models with different numbers of classes, ranging from 2 classes to 5 classes, using the same method described above to select the optimum number of trajectory classes.

For both the two-step and one-step processes, we assessed the coefficients and confidence intervals for each phthalate metabolite as a predictor of language trajectory class. Following each process, each person was assigned a probability of belonging to each trajectory class (separately for two and one step), and we assigned each person to their highest probability class for tables and figures. We selected class names based on the overall shape of the class in the plot, guided by designations used in prior literature.

All analyses were conducted using Rstudio version 2022.02.1 Build 461 with R Version 4.1.3 “One Push-Up”. The LCGA was conducted using the *lcmm* package (182).

Results

Males had slightly lower MSEL raw scores than females at 36 months of age (Table 3.1). Children whose mothers were 30 or younger or who did not receive at least a college degree had lower MSEL raw scores than children who had mothers older than 30 or who received at least a college degree. Children of African American or Hispanic mothers also had lower MSEL raw scores than children from non-Hispanic white or multiracial mothers. Children whose parents rented had lower MSEL scores than children of homeowners. There was no appreciable difference in MSEL scores between birth seasons.

There was a tendency for many of the phthalate metabolites to be higher among females (Supplemental Table 1). Many of the HMW phthalates were increasingly higher for older maternal ages and higher levels of maternal education. Most phthalates did not seem consistently patterned by race, except for mEP2 and mCOP where concentrations were several-fold higher among mothers who identified as African American.

With our two-step LCGA approach, for expressive language, 3 trajectory classes were optimal for capturing patterns in language development. These trajectory classes were named High Growth, Tracking, and Low Growth (Figure 3.1). None of the phthalates were statistically significant for associations with expressive language trajectories (Table 3.4). In general, we observed that most metabolites of LMW

phthalates saw an increase in the likelihood for falling into the Tracking and Low Growth versus the more optimal High Growth trajectory categories. DBP was the LMW phthalate with the strongest risk ratios with the suboptimal trajectories. Higher levels of the HMW butyl benzyl phthalate (BzBP) exhibited increased likelihood of being in the suboptimal expressive language trajectories. Other HMW phthalates saw a decrease in likelihood of falling into Tracking and Low Growth categories (e.g. protective direction), and this pattern was most notable for metabolites of di(2-ethylhexyl) phthalate.

With our two-step LCGA approach, for receptive language, 3 trajectory classes were optimal for capturing patterns in language development, which we named High Growth, Tracking, and Late Growth (Figure 3.2). Metabolites of LMW phthalates generally had near-null associations, except for those of DEP, which exhibited somewhat elevated associations with the Tracking and Low Growth trajectories (but without being statistically significant). Associations with HMW phthalate metabolites were also largely near-null, except for BzBP, which exhibited elevated (risk) associations, and DEHP, for which several metabolites exhibited statistically significant protective associations for both Tracking and Late Growth trajectories.

We examined whether sex was a modifier by including a sex by phthalate metabolite interaction term. For expressive language, based on likelihood ratio testing, sex did not appear to be a modifier for any phthalate metabolites (Table 3.11). For receptive language, sex was suggestive as a possible effect modifier for the metabolites of DiNP (mCOP and mNP2) and was most notable and consistent for metabolites of DEHP (Table 3.12). Especially for the Late Growth Trajectory, the association with these metabolites exerted a stronger protective effect among females versus males.

In sensitivity analyses, we also conducted a one-step LCGA. Like our two-step approach, we used goodness-of-fit measures to determine the total number of classes from between 1 and 5 total classes (Tables 3.9 and 3.10). While goodness-of-fit measures sometimes indicated more classes, an examination of the distribution of population showed that at least one class would have fewer than 10 percent of the study population. In general, the one-step and two-step class distributions were broadly overlapping, with only a few individuals being classified as a different class (Table 3.9 and 3.12).

With our one-step LCGA approach, for expressive language, 3 trajectory classes were found to be optimal for all phthalates, which we named High Growth, Tracking, and Low Growth. The shape of the trajectories generally followed those observed in our two-step approach (Figures 3.3 to 3.16). In general, trends observed in the two-step process were observed in the one-step approach. However, two metabolites were statistically significant where they were not in the two-step process.

With our one-step LCGA approach, for receptive language, 3 trajectory classes were found to be optimal for all metabolites, which we labeled as High Growth, Tracking, and Late Growth. The shape of the trajectories generally followed those observed in our two-step approach (Figures 3.17 to 3.31). In general, trends observed in the two-step process were observed in the one-step approach.

Discussion

The goal of this study was to characterize language development trajectories in two cohorts of children with enhanced familial risk for autism and evaluate if prenatal

phthalate exposure was associated with delayed or abnormal language development. Results were mixed depending on the individual phthalate. Most phthalates, whether HMW or LMW, were not associated with impaired language trajectories. The LMW phthalates DBP and DEP exhibited a trend toward impaired expressive language and DEP with impaired receptive language. Among the HMW phthalates, BzBP exhibited a trend with suboptimal language development. Metabolites of the HMW DiDP, DiNP, DEHP, and the metabolite mCPP consistently exhibited a trend towards protective effects on both expressive and receptive language.

While most phthalate exposures were consistent with the direction of their risk between the various trajectory classes, there were a handful of instances where a metabolite was protective for one class but raised risk or had a null effect for another class. For example, in our two-step approach for expressive language, miBP and mCNP saw a decrease in risk for belonging to the Low Growth class but a null effect for belonging to the Tracking class. This may indicate a true difference between the risk of belonging to those two trajectories. It may also be possible that in this sample which is enhanced for genetic liability for neurodevelopmental disorders generally and autism, the action of phthalates differs from an unselected population. We also observed that certain phthalates were heightened in specific populations (e.g., mEP2 in African Americans were significantly higher compared to other races). This may be due to the relatively small sample size of the categories, or it may indicate a true difference in phthalate distribution based on those categories.

Multiple testing is of particular concern in our study, due to the multiple tests that needed to be performed; we examined a total of 15 metabolites across two different

facets of language using two separate methods. In addition, some of the groups had a small number of individuals, despite our efforts to choose classes that had greater than 10 percent of the total study population. Because of these two factors, it is possible that we found false negatives in our analysis; that is, the fact that we did not find many statistically significant associations does not mean that phthalates are not without risk. It is possible that any given phthalate (or other endocrine disrupting exposure) could truly support a higher uptake of language skills and decrease the risk of falling into a less optimal language development trajectory. Yet this hypothesis is not consistent with prior literature, which primarily shows that phthalate exposure leads to language development impairments and other deleterious aspects of neurodevelopment, and so these results should not be taken to indicate that some phthalates support neurodevelopment.

A strength of our approach over most previous publications was the use of biomarkers collected at more than one time period, allowing us to obtain a more accurate representation of phthalate exposure during the entire pregnancy. The areas of the brain that are most likely related to language appear to develop during the second and third trimesters (200,201), and we collected exposure data during those time periods. However, no postnatal samples were collected, which reduces our ability to determine whether phthalate exposure that occurred following birth may have impacted language development. Because some phthalate exposures arise from behavioral patterns that are relatively stable over time, prenatal exposure may, for some phthalates, serve as a proxy of phthalate exposure following birth, but this is imperfect and with unknown degrees of error. In addition, phthalate metabolites tend to have short

half-lives, in the span of hours, which may limit our ability to determine if the levels of metabolites measured truly reflect actual phthalate exposure (202,203).

We compared phthalate measurements in our study to the National Health and Nutrition Examination Survey (NHANES) laboratory data, combining the reported data from the 2011 to 2012 and 2013 to 2014 reports (n= 5171). We used a two-sided Wilcox Rank Sum test to assess whether the phthalate measures significantly differed from each other. As seen in Table 3.13, we found that levels of nearly all phthalates in our study were higher than levels found in NHANES. There may be several explanations for these discrepancies. The population in our study is not nationally representative, but rather concentrated around a handful of states. While we did not examine statewide averages, it may be possible that the states our participants resided in have generally higher phthalate levels than the national average. Furthermore, because our population had higher concentrations of phthalates than a nationally representative sample, there may be a possibility that the effects observed in this study may not be as pronounced in a more generalizable population.

Di-n-butyl phthalate, or DBP, is one of the more well-studied phthalates. A LMW phthalate, it's commonly used as a plasticizer and can be found in shower curtains, raincoats, food wraps, bowls, car interiors, vinyl fabrics, floor tiles, and other products (204). We found that its metabolites tended to increase the risk of falling into a suboptimal language trajectory for expressive language and decrease the risk of falling into a suboptimal language trajectory for receptive language. This is consistent with prior studies, which show both an increase and decrease in risk depending on the specific measure of language used. Bornehag and Hyland both found that a DBP

metabolite resulted in increased risk at language delays, while Olesen found that it largely increased risk, though it decreased risk for girls (74,105,106). Gascon, on the other hand, found that it improved language development (97). However, results from these studies did not meet statistical significance, much like our own.

Di-ethyl phthalate, or DEP, is a LMW phthalate commonly found in plastics, personal care products, industrial materials (e.g. dyes, rocket propellant, sealants, and lubricants), and medical products (83). We found that its metabolite was linked with higher risk of falling into an abnormal language trajectory for both expressive and receptive language. This is consistent with some prior studies that have examined DEP. Olesen, Miodovnik, and Ramos all found that a DEP metabolite increased risk for various measures of language ability (105,205,206). However, some studies have found a protective effect, such as Bornehag, Gascon, and Polanska (74,97,108). However, just like our results, none of these studies achieved statistical significance.

Butyl benzyl phthalate, or BzBP, is a HMW phthalate that is most commonly found in toys, bag, gloves, and plastic tubing in order to improve flexibility and make such products soft and malleable (207). We found that its metabolite increased the risk of belonging to an abnormal language trajectory for expressive language. For receptive language, it increased the risk of falling into the Late Growth trajectory. Our results are fairly consistent with prior literature. Bornehag, Hyland, and Polanska all similarly found increased risk for various measures of language (74,106,108). However, Olesen found increased risk for males, but decreased risk for females, and Gascon found decreased risk (97,105). None of the above studies reached statistical significance, however.

Di(2-ethylhexyl) phthalate, or DEHP, is also one of the more well-studied phthalates. A HMW phthalate, it's commonly found in medical devices such as medical tubing and catheters, along with soft plastic products like toys and infant products (82). We found that its metabolites tended to decrease the risk of falling into a suboptimal language trajectory for both expressive and receptive language. This is inconsistent with most other studies that have examined this phthalate and its metabolites. Bornehag, Hyland, and Polanska all found increased risk for language delays (74,106,108). Olesen and Huang both found increased risk in some instances (Olesen for males only, Huang for Verbal Comprehension on the Wechsler Intelligence Scale for Children-IV), with decreased risk in others (Olesen for females only, Huang for Wechsler Preschool and Primary Scale of Intelligence-Revised Verbal IQ) (103,105). Most of the studies above did not meet statistical significance, save for mEOHP in Huang's study for increased risk for lower verbal comprehension.

Most phthalates in prior studies did not reach statistical significance, much like our study. Of the studies that did find significance, Huang found that miBP, a metabolite of di-isobutyl phthalate (DiBP), and mEOHP, a metabolite of DEHP, were statistically significant, significantly lowering scores on the Wechsler Intelligence Scale for Children-IV Verbal Comprehension Index (103). However, it should be noted that they did not examine prenatal exposure, but instead children who were exposed to products that were tainted by DEHP between 3 and 12 years of age. Olesen found several statistically significant results, all of which increased risk in males for either the MacArthur-Bates Communicative Development Inventories Vocabulary Scores or Complexity Scores (105). These included the metabolites of DEHP, metabolites of DEP, and metabolites of

BzBP. Bornehag et al. found that metabolites of DBP and BzBP were associated with higher risk of language delay, though other phthalates examined returned null results (74). However, they only examined a single time point and used a method of measuring language development that is noticeably unique, examining number of words known rather than utilizing a psychometric test. Finally, Ramos found some statistically significant results in their path analysis, with organophosphates being associated with lower communication and expressive language in both parent and teacher reported models (206).

Loftus et al. used a pregnancy cohort to examine the effects of phthalate exposure on different neurodevelopmental outcomes. Uniquely, they utilized weighted quantile sum (WQS) regression as their statistical method. While this allowed for examination of phthalate mixtures, there are some limitations to WQS regression. Loftus et al. noted that the WQS regression can't account for "relative toxicity of mixture components"; in other words, they were unable to determine individual risk for each of the phthalates used in the mixture. In addition, WQS regression may have lower accuracy with highly correlated exposures; depending on the specific metabolites analyzed, correlation may be high enough that diminished accuracy may be of concern (208).

We explored the possibility of sex modification via the inclusion of an interaction term of phthalate metabolite and child sex, due to the possibility that these EDCs may be affecting each sex differently. A prior review found that there was inconsistent evidence of sex modification between prenatal phthalate exposure and child language development (209). We found mixed results for different phthalate metabolites. While

we found no evidence of sex modification for expressive language for any metabolite, for receptive language, sex was a suggestive effect modifier for the metabolites mCOP, mNP2, mEHHP, and mEHP, and a significant effect modifier for the metabolite mECPP (Tables 3.13 and 3.14). More specifically, when examining the risk ratios, we find that males generally have a higher risk of belonging in the Tracking trajectory compared to females when exposed to higher concentrations of metabolites of DEHP (i.e. mEHHP, mEHP, and mECPP) but have a lower risk of belonging in the Tracking trajectory compared to females for the metabolites of DiNP (i.e. mCOP, mNP2). Our results with DEHP are consistent with the results found in Olesen et al., where males had increased risk, some being statistically significant, when compared to females (105). However, there have been no prior studies that have examined sex differences for DiNP metabolites. These results suggests that certain phthalates and their metabolites may have a differential impact based on sex, although the specific pathophysiology is unclear. It may be possible that the increased risk in males may be due to phthalates disrupting the development of androgen-dependent structures by affecting testosterone synthesis (210). There is also evidence that suggests that certain phthalates act as anti-estrogens and anti-androgens, with DEP, DEHP, DiBP, and DBP acting as anti-androgen, and DBP and DEHP acting as anti-estrogens (211). These same phthalates can also act as xenobiotics, activating estrogen and androgen receptors, with DEP, DEHP, DiBP, DiNP, and DBP activating estrogen receptors, and DBP and DEHP activating androgen receptors (211). This behavior may help to explain why DiNP results the increase in risk we observed in females compared to males; the activation of estrogen receptors may be influencing neurodevelopment, resulting in an increased risk

of language impairment. Further studies should focus on examining these sex differences and potentially explore further routes or biological pathways that may explain these differences.

Our results examining sex differences may intersect with known sex differences in language within children with autism. Some studies have indicated a sex difference, with some indicating that girls have worse language skills than boys (212–214), while others indicate the reverse (215,216). Our results indicated that prenatal exposure to certain phthalates may decrease the risk of females of falling into an abnormal language trajectory compared to males, while exposure to another may increase that risk. This may help to explain the disparate results from prior literature, namely that the differences observed may be caused by differences in androgen or estrogen levels during development.

Strengths of our study include the use of language development trajectories, rather than single time point outcomes, which allowed for a more detailed and accurate examination of how neurodevelopmental outcome classification affects language development. In addition, the use of repeated measurements reduced the possibility of outcome misclassification, as there are multiple points in time where the outcome is assessed, reducing the possibility of incorrectly assigning an individual to a wrong trajectory class. We used a total of four time points, giving us a robust amount of data to use to construct our trajectories classes. Second, the use of cohorts which prospectively follow siblings of children with autism allowed for a greater ability to detect abnormal language development trajectories that may be less common in unselected cohorts.

These cohorts have a heightened prevalence for neurodevelopmental delays compared to the general population.

Because all children in our study have an older sibling with autism, our results may not be applicable to the general population. In addition, while the MSEL is considered to be a suitable psychometric test for measuring expressive and receptive language (185) and has been shown to have good construct validity (186), language is a complex phenotype that develops throughout a child's early development period. Our analysis is limited to the facets of language development that the MSEL measures: namely expressive and receptive language. Future research should explore other facets of language development, such as vocabulary, grammatical development, or semantics. Our analysis was limited to the first 36 months of age, so we are unable to extrapolate language trajectories after this period. Due to the nature of LCGA analysis, our results are sensitive to the number of classes selected and our total sample size. While our sample size is sufficiently large enough to utilize LCGA, selecting for a different number of classes or changing the sample size may alter the results. However, we are confident that the overall direction of effect would remain consistent between various class choices.

In conclusion, we explored how prenatal exposure to phthalates influences language development trajectories and found that most low molecular weight phthalates increased risk and most high molecular weight phthalates decreased risk for belonging to abnormal language trajectories, though most were not statistically significant. In addition, we found evidence that sex may be acting as a modifier for receptive language. While our study may not be generalizable due to the nature of the cohorts

used, our use of multiple exposure and outcome measures, along with the use of LCGA to explore trajectory growth, are considerable advantages to our study. Future studies should continue to explore potential associations, with an emphasis on exploring potential sex modification and mixture effects.

Tables and Figures

Table 3.1: Mean and Standard Deviation of 36 Month Mullen Scores for Included Population by Child Characteristics

	Expressive language raw score (36 months)	Receptive language raw score (36 months)
Cohort		
MARBLES (N=203)	30.9 (6.31)	30.3 (5.63)
EARLI (N=159)	31.5 (5.86)	31.0 (4.80)
Child Sex		
Female (N=156)	32.2 (4.99)	31.6 (4.19)
Male (N=206)	30.3 (6.76)	30.0 (5.89)
Maternal age		
<30 years (N=70)	29.8 (6.00)	29.7 (4.56)
31 to 35 years (N=142)	31.5 (6.15)	30.7 (5.77)
36 to 40 years (N=104)	31.1 (5.82)	31.1 (4.85)

>40 years (N=49)	32.1 (6.72)	31.1 (5.65)
Maternal Race/Ethnicity		
Non-Hispanic White (N=203)	31.7 (6.02)	31.1 (5.34)
Black/African American (N=24)	30.4 (6.09)	29.9 (4.09)
Hispanic (N=68)	30.0 (5.95)	28.9 (5.39)
Other/Multiracial (N=67)	31.0 (6.56)	31.5 (5.05)
Maternal Educational Attainment		
High School/GED or Less (N=32)	29.0 (6.42)	28.3 (4.74)
Some college (N=128)	30.2 (6.03)	29.7 (5.25)
Bachelor's degree (N=123)	32.2 (5.55)	31.5 (5.11)
Graduate or Professional degree (N=79)	31.8 (6.65)	32.0 (5.27)

Homeownership		
Rent (N=131)	30.3 (6.76)	29.8 (5.83)
Own (N=231)	31.6 (5.70)	31.2 (4.89)
Birth Season		
Spring (n=71)	31.3 (5.36)	30.4 (4.69)
Summer (n=111)	30.6 (6.64)	29.9 (5.78)
Fall (n=94)	31.8 (6.42)	31.6 (5.32)
Winter (n=86)	30.9 (5.69)	30.8 (4.95)
Overall (N=362)	31.1 (6.12)	30.7 (5.28)

Footnotes for Table 3.1: Mean and standard deviations were calculated after removing missing values

Table 3.2: Comparison of characteristics of included versus excluded individuals

	Included (N=378)	Excluded (N=259)	Overall (N=621)
Child Sex			
Female	156 (43.1%)	124 (50.0%)	280 (45.9%)
Male	206 (56.9%)	124 (50.0%)	330 (54.1%)
Missing	0	11	11
Maternal Age			
20 to 30 years	70 (19.3%)	56 (22.3%)	102 (16.6%)
31 to 35 years	142 (39.2%)	102 (40.6%)	244 (39.8%)
36 to 40 years	101 (27.9%)	76 (30.3%)	177 (28.9%)
40 to 49 years	49 (13.5%)	17 (6.8%)	66 (10.8%)
Missing	0	8	8
Maternal Race/Ethnicity			
Non-Hispanic White	203 (56.5%)	100 (49.2%)	303 (53.9%)
Black/African American	24 (6.7%)	15 (7.4%)	39 (6.9%)
Hispanic	66 (18.4%)	46 (22.7%)	112 (19.9%)
Other/Multiracial	66 (18.4%)	42 (20.7%)	108 (19.3%)
Missing	3	56	59

	Included (N=378)	Excluded (N=259)	Overall (N=621)
Maternal Educational Attainment			
High School/GED or less	32 (8.9%)	22 (11.1%)	36 (6.4%)
Some college	128 (35.4%)	73 (36.9%)	201 (36%)
Bachelor's degree	122 (33.8%)	49 (24.7%)	171 (30.6%)
Graduate or Professional degree	79 (21.9%)	54 (27.3%)	133 (23.8%)
Missing	1	61	62
Homeownership			
Rent	128 (36.7%)	90 (46.2%)	218 (40.1%)
Own	221 (63.3%)	105 (53.8%)	326 (59.9%)
Missing	13	64	77
Expressive language raw score (6 months)			
Mean (SD)	6.14 (1.08)	6.66 (1.53)	6.32 (1.28)
Median [IQR]	6.00 [0]	6.00 [1.00]	6.00 [1.00]
Missing	57	98	155
Receptive language raw score (6 months)			
Mean (SD)	7.84 (1.52)	8.14 (1.58)	7.95 (1.55)

	Included (N=378)	Excluded (N=259)	Overall (N=621)
Median [IQR]	8.00 [2.00]	8.00 [2.00]	8.00 [2.00]
Missing	57	98	155
Expressive language raw score (12 months)			
Mean (SD)	11.8 (2.66)	12.4 (2.48)	12.0 (2.62)
Median [IQR]	12.0 [4.00]	13.0 [3.00]	12.0 [4.00]
Missing	22	98	120
Receptive language raw score (12 months)			
Mean (SD)	12.5 (2.03)	12.5 (2.17)	12.5 (2.07)
Median [IQR]	13.0 [3.00]	13.0 [3.00]	13.0 [3.00]
Missing	22	98	120
Expressive language raw score (24 months)			
Mean (SD)	20.4 (4.67)	21.0 (5.20)	20.6 (4.85)
Median [IQR]	21.0 [6.00]	21.0 [6.00]	21.0 [6.00]
Missing	144	154	298
Receptive language raw score (24 months)			

	Included (N=378)	Excluded (N=259)	Overall (N=621)
Mean (SD)	23.2 (5.46)	23.1 (5.65)	23.2 (5.51)
Median [IQR]	25.0 [7.00]	25.0 [7.00]	25.0 [7.00]
Missing	145	154	299
Expressive language raw score (36 months)			
Mean (SD)	31.2 (6.12)	30.2 (7.22)	31.0 (6.36)
Median [IQR]	32.0 [7.00]	32.0 [8.00]	32.0 [7.00]
Missing	3	165	168
Receptive language raw score (36 months)			
Mean (SD)	30.6 (5.29)	29.7 (6.83)	30.4 (5.65)
Median [IQR]	31.0 [5.75]	30.0 [5.75]	31.0 [5.00]
Missing	4	165	169

Footnote for Table 3.2: Percentages were calculated after removing missing individuals from the sample

Table 3.3: Comparison of Imputed versus Non-Imputed Covariates

	Non-imputed (N=362)	Imputed (N=362)
Child Sex		
Female	156 (43.1%)	156 (43.1%)
Male	206 (56.9%)	206 (56.9%)
Maternal Age		
20 to 30 years	70 (19.3%)	70 (19.3%)
31 to 35 years	142 (39.2%)	142 (39.2%)
36 to 40 years	101 (27.9%)	101 (27.9%)
40 to 49 years	49 (13.5%)	49 (13.5%)
Maternal Race/Ethnicity		
Non-Hispanic White	203 (56.5%)	203 (56.1%)
Black/African American	24 (6.7%)	24 (6.6%)
Hispanic	66 (18.4%)	68 (18.8%)
Other/Multiracial	66 (18.4%)	67 (18.5%)
Missing	3	
Maternal Educational Attainment		
High School/GED or less	32 (8.9%)	32 (8.8%)

	Non-imputed (N=362)	Imputed (N=362)
Some college	128 (35.4%)	128 (35.4%)
Bachelor's degree	122 (33.8%)	123 (34.0%)
Graduate or Professional degree	79 (21.9%)	79 (21.8%)
Missing	1	
Homeownership		
Rent	128 (36.7%)	131 (36.2%)
Own	221 (63.3%)	231 (63.8%)
Missing	13	
Expressive language raw score (6 months)		
Mean (SD)	6.14 (1.08)	6.09 (1.11)
Median [IQR]	6.00 [0]	6.00 [0]
Missing	57	
Receptive language raw score (6 months)		
Mean (SD)	7.84 (1.52)	7.90 (1.51)
Median [IQR]	8.00 [2.00]	8.00 [2.00]
Missing	57	

	Non-imputed (N=362)	Imputed (N=362)
Expressive language raw score (12 months)		
Mean (SD)	11.8 (2.66)	11.7 (2.68)
Median [IQR]	12.0 [4.00]	12.0 [4.00]
Missing	22	
Receptive language raw score (12 months)		
Mean (SD)	12.5 (2.03)	12.4 (2.01)
Median [IQR]	13.0 [3.00]	13.0 [3.00]
Missing	22	
Expressive language raw score (24 months)		
Mean (SD)	20.4 (4.67)	20.3 (4.84)
Median [IQR]	21.0 [6.00]	20.0 [5.75]
Missing	144	
Receptive language raw score (24 months)		
Mean (SD)	23.2 (5.46)	23.1 (5.43)
Median [IQR]	25.0 [7.00]	25.0 [7.00]

	Non-imputed (N=362)	Imputed (N=362)
Missing	145	
Expressive language raw score (36 months)		
Mean (SD)	31.2 (6.12)	31.1 (6.12)
Median [IQR]	32.0 [7.00]	32.0 [7.00]
Missing	3	
Receptive language raw score (36 months)		
Mean (SD)	30.6 (5.29)	30.7 (5.28)
Median [IQR]	31.0 [5.75]	31.0 [5.75]
Missing	4	

Footnote for Table 3.3: Percentages were calculated after removing missing individuals from the sample

Table 3.4: Relative Risk Ratios and 95% Confidence Intervals for Phthalate Metabolites and Expressive and Receptive Language Trajectories

Molecular Weight	Parent Compound	Metabolite	Expressive Language				Receptive Language			
			Tracking		Low Growth		Tracking		Late Growth	
			One Step	Two Step	One Step	Two Step	One Step	Two Step	One Step	Two Step
Low Molecular Weight	DBP	mBP	1.10 (0.83, 1.46)	1.08 (0.85, 1.37)	1.46 (1.07, 2.00)	1.24 (0.93, 1.65)	0.89 (0.65, 1.22)	0.85 (0.65, 1.11)	0.91 (0.64, 1.28)	0.92 (0.67, 1.26)
		MHBP	1.08 (0.84, 1.40)	1.13 (0.90, 1.42)	1.32 (0.99, 1.77)	1.26 (0.94, 1.67)	0.99 (0.71, 1.36)	0.90 (0.69, 1.18)	1.03 (0.73, 1.46)	1.05 (0.78, 1.43)
	DEP	mEP2	1.01 (0.92, 1.12)	1.02 (0.93, 1.13)	1.03 (0.92, 1.15)	1.01 (0.90, 1.14)	1.27 (0.92, 1.74)	1.23 (0.93, 1.62)	1.20 (0.87, 1.67)	1.24 (0.93, 1.64)
	DiBP	MHiBP	0.96 (0.71, 1.28)	0.99 (0.78, 1.25)	0.90 (0.59, 1.38)	0.81 (0.57, 1.15)	0.93 (0.66, 1.30)	0.93 (0.68, 1.27)	1.00 (0.70, 1.42)	0.95 (0.66, 1.36)
		miBP	1.01 (0.74, 1.37)	1.00 (0.79, 1.27)	0.98 (0.69, 1.39)	0.85 (0.60, 1.20)	0.98 (0.71, 1.37)	0.96 (0.70, 1.31)	0.99 (0.70, 1.41)	0.90 (0.63, 1.30)
High Molecular Weight	BzBP	mBzP2	1.17 (0.90, 1.51)	1.14 (0.91, 1.43)	1.21 (0.89, 1.64)	1.20 (0.89, 1.62)	0.95 (0.67, 1.35)	0.93 (0.70, 1.22)	1.16 (0.82, 1.65)	1.14 (0.84, 1.55)
	DiDP	mCNP	0.88 (0.79, 0.98)	1.00 (0.96, 1.04)	0.91 (0.80, 1.03)	0.98 (0.91, 1.05)	0.98 (0.94, 1.02)	0.98 (0.95, 1.02)	0.92 (0.82, 1.03)	0.95 (0.88, 1.03)
	DiNP	mCOP	0.94 (0.82, 1.08)	0.95 (0.83, 1.09)	0.92 (0.74, 1.13)	0.91 (0.74, 1.11)	0.91 (0.77, 1.07)	0.94 (0.80, 1.11)	0.85 (0.68, 1.05)	0.85 (0.68, 1.06)

	mNP2	0.95 (0.85, 1.06)	0.97 (0.87, 1.08)	0.99 (0.86, 1.14)	0.97 (0.83, 1.12)	0.93 (0.82, 1.05)	0.95 (0.85, 1.08)	0.86 (0.72, 1.02)	0.86 (0.72, 1.04)
DOP, DBP, other HMW phthalates	mCPP	0.98 (0.91, 1.06)	0.98 (0.92, 1.05)	0.93 (0.82, 1.05)	0.96 (0.88, 1.06)	0.94 (0.87, 1.02)	0.98 (0.91, 1.05)	0.95 (0.86, 1.04)	0.94 (0.84, 1.04)
DEHP	mECPP	0.92 (0.82, 1.04)	0.94 (0.86, 1.02)	0.80 (0.62, 1.02)	0.81 (0.65, 1.00)	0.87 (0.78, 0.97)	0.87 (0.78, 0.96)	0.80 (0.66, 0.96)	0.80 (0.67, 0.96)
	mEHHP	0.96 (0.88, 1.04)	0.96 (0.90, 1.02)	0.82 (0.65, 1.03)	0.81 (0.64, 1.01)	0.92 (0.86, 1.00)	0.92 (0.86, 0.99)	0.87 (0.76, 1.00)	0.87 (0.76, 0.99)
	mEHP	0.97 (0.88, 1.06)	0.98 (0.91, 1.05)	0.81 (0.62, 1.06)	0.80 (0.61, 1.04)	0.94 (0.87, 1.02)	0.93 (0.86, 1.01)	0.87 (0.74, 1.03)	0.87 (0.74, 1.01)
	mEOHP	0.97 (0.89, 1.05)	0.97 (0.92, 1.04)	0.83 (0.66, 1.06)	0.82 (0.65, 1.03)	0.94 (0.87, 1.01)	0.93 (0.88, 1.00)	0.88 (0.77, 1.02)	0.89 (0.78, 1.01)
	ΣDEHP	0.95 (0.87, 1.04)	0.96 (0.89, 1.03)	0.82 (0.64, 1.03)	0.81 (0.65, 1.01)	0.91 (0.84, 0.99)	0.91 (0.84, 0.98)	0.85 (0.73, 0.99)	0.85 (0.74, 0.99)

Footnotes for Table 3.4: High Growth was used as the referent group; All phthalates were winsorized to the 99th percentile; all models were adjusted for site specific Z-score for urinary dilution measure (specific gravity or creatinine), cohort, child sex, maternal age, maternal race/ethnicity, home-ownership, and maternal educational attainment

Table 3.5: Measures of Model Fit for Expressive Language Latent Class Growth Analysis, Two-Step Model

Number of classes	Log-likelihood	SABIC	BIC	Entropy	ICL
1	-4302.09	8617.99	8633.85	1	8633.85
2	-4008.34	8044.31	8076.04	0.90*	8101.04
3	-3905.41	7852.24	7899.84	0.82	7973.67
4	-3857.71	7770.66	7834.11	0.82	7928.66*
5	-3839.51	7748.08	7827.4*	0.79	7957.89
6	-3829.19	7741.24	7836.42	0.81	7967.04
7	-3821.89*	7740.46*	7851.51	0.79	8002.91

Footnote for Table 3.5: Higher log-likelihood, lower SABIC (sample adjusted BIC), lower BIC (Bayesian Inclusion Criterion), higher entropy, and lower ICL (integrated classification likelihood) indicates an overall better fit; * indicates the best model based on the above criteria (single class will always have an entropy of 1 so we select the next highest entropy instead); we prioritized BIC and ICL based on the findings in Diallo et al., 2016; classes had <5% of the population in the 6 class model, so we selected the next best fit model instead

Table 3.6: Measures of Model Fit for Receptive Language Latent Class Growth Analysis, Two-Step Model

Number of classes	Log-likelihood	SABIC	BIC	Entropy	ICL
1	-4247.94	8509.70	8525.56	1	8525.56
2	-3947.36	7922.34	7954.07	0.929*	7972.757
3	-3893.73	7828.89	7876.48	0.844	7941.112
4	-3883.52	7822.29	7885.75	0.805	7987.721
5	-3880.92	7830.89	7910.21	0.777	8045.602
6	-3670.24*	7423.35*	7518.53*	0.8475	7621.971
7	-3880.92	7858.51	7969.56	0.515	8326.024

Footnote for Table 3.6: Higher log-likelihood, lower SABIC (sample adjusted BIC), lower BIC (Bayesian Inclusion Criterion), higher entropy, and lower ICL (integrated classification likelihood) indicates an overall better fit; * indicates the best model based on the above criteria (single class will always have an entropy of 1 so we select the next highest entropy instead); 6 class model failed to converge; we prioritized BIC and ICL based on the findings in Diallo et al., 2016; classes had <5% of the population in the 5 and 7 class models, so we selected the next best fit model instead

Table 3.7: Measure of Model Fit for Expressive Language Latent Class Growth Analysis, One-Step Model

Metabolite	Classes	Log-likelihood	SABIC	BIC	Entropy	ICL
mBP	1	-4081.62	8204.03	8251.62	1	8251.62
	2	-3800.66	7685.60	7783.96	0.92	7803.86
	3	-3700.11	7528.01	7677.12	0.82	7747.43
	4	-3674.03	7519.36	7719.23	0.86	7787.20
	5	-3610.86	7436.53	7687.16	0.88	7759.95
mBzP2	1	-4083.89	8208.57	8256.16	1	8256.16
	2	-3802.72	7689.73	7788.08	0.92	7807.69
	3	-3700.86	7529.52	7678.63	0.82	7749.45
	4	-3677.13	7525.56	7725.43	0.77	7841.44
	5	-3620.79	7456.40	7707.03	0.80	7823.00
mCNP	1	-4085.83	8212.44	8260.03	1	8260.03
	2	-3797.79	7679.87	7778.21	0.92	7798.55
	3	-3694.10	7516.00	7665.11	0.83	7731.22
	4	-3643.75	7458.80	7658.67	0.85	7735.95
	5	-3628.33	7471.47	7722.10	0.82	7825.52
mCOP	1	-4084.49	8209.77	8257.36	1	8257.36
	2	-3803.51	7691.32	7789.67	0.92	7809.49
	3	-3702.61	7533.01	7682.12	0.82	7752.31
	4	-3649.82	7470.94	7670.81	0.85	7748.30
	5	-3632.31	7479.44	7730.07	0.79	7850.62
mCPP	1	-4085.59	8211.96	8259.55	1	8259.55
	2	-3804.88	7694.04	7792.39	0.92	7812.57
	3	-3703.66	7535.12	7684.23	0.83	7753.75

	4	-3651.33	7473.97	7673.84	0.84	7755.78
	5	-3631.55	7477.92	7728.55	0.81	7841.41
mECPP	1	-4082.87	8206.52	8254.11	1	8254.11
	2	-3802.31	7688.92	7787.27	0.92	7807.17
	3	-3702.01	7531.81	7680.92	0.82	7750.60
	4	-3684.74	7540.78	7740.65	0.77	7857.42
	5	-3628.84	7472.49	7723.13	0.78	7851.98
mEHHP	1	-4083.14	8207.07	8254.66	1	8254.66
	2	-3802.02	7688.34	7786.68	0.92	7806.29
	3	-3701.97	7531.74	7680.85	0.82	7750.91
	4	-3648.54	7468.38	7668.25	0.84	7749.67
	5	-3626.75	7468.30	7718.93	0.85	7804.45
mEHP	1	-4083.49	8207.77	8255.36	1	8255.36
	2	-3802.06	7688.41	7786.76	0.92	7806.42
	3	-3702.08	7531.96	7681.07	0.82	7751.62
	4	-3647.90	7467.10	7666.97	0.84	7748.16
	5	-3618.38	7451.57	7702.20	0.87	7776.32
mEOHP	1	-4084.01	8208.82	8256.41	1	8256.41
	2	-3802.70	7689.70	7788.05	0.92	7807.56
	3	-3702.59	7532.97	7682.08	0.82	7752.40
	4	-3648.50	7468.30	7668.17	0.85	7743.74
	5	-3617.72	7450.25	7700.88	0.88	7772.26
mEP2	1	-4085.72	8212.22	8259.81	1	8259.81
	2	-3804.08	7692.46	7790.81	0.92	7810.40
	3	-3704.36	7536.52	7685.63	0.82	7756.11
	4	-3648.32	7467.94	7667.81	0.86	7740.47

	5	-3615.25	7445.30	7695.93	0.88	7767.53
MHBP	1	-4079.16	8199.11	8246.70	1	8246.70
	2	-3799.51	7683.32	7781.67	0.92	7801.69
	3	-3698.55	7524.89	7674.00	0.83	7742.94
	4	-3644.31	7459.92	7659.79	0.84	7739.74
	5	-3607.75	7430.30	7680.93	0.87	7758.55
MHiBP	1	-4085.69	8212.16	8259.75	1	8259.75
	2	-3804.96	7694.21	7792.55	0.92	7812.68
	3	-3702.47	7532.73	7681.84	0.83	7751.28
	4	-3645.90	7463.10	7662.97	0.84	7742.12
	5	-3625.01	7464.83	7715.46	0.80	7830.30
miBP	1	-4085.74	8212.28	8259.86	1	8259.86
	2	-3804.74	7693.78	7792.13	0.92	7811.43
	3	-3702.46	7532.72	7681.83	0.82	7752.15
	4	-3646.12	7463.54	7663.41	0.84	7744.42
	5	-3625.25	7465.31	7715.94	0.81	7829.36
mNP2	1	-4085.22	8211.22	8258.81	1	8258.81
	2	-3804.08	7692.44	7790.79	0.92	7810.44
	3	-3702.07	7531.94	7681.05	0.82	7750.81
	4	-3651.48	7474.27	7674.14	0.84	7756.12
	5	-3634.27	7483.36	7734.00	0.77	7870.71
DEHP	1	-4083.24	8207.27	8254.86	1	8254.86
	2	-3802.29	7688.88	7787.23	0.92	7806.96
	3	-3702.17	7532.14	7681.25	0.82	7751.29
	4	-3648.07	7467.45	7667.32	0.85	7742.69
	5	-3617.92	7450.65	7701.28	0.85	7786.97

Footnotes for Table 3.7: Higher log-likelihood, lower SABIC (sample adjusted BIC), lower BIC (Bayesian Inclusion Criterion), higher entropy, and lower ICL (integrated classification likelihood) indicates an overall better fit; All phthalates were winsorized to the 99th percentile; all models were adjusted for site specific Z-score for urinary dilution measure (specific gravity or creatinine), cohort, child sex, maternal age, maternal race/ethnicity, home-ownership, and maternal educational attainment; 4 and 5 class models had <10% of population in at least one group, which resulted in those models being dropped from consideration, even if they had better fit measures

Table 3.8: Measure of Model Fit for Receptive Language Latent Class Growth Analysis, One-Step Model

Metabolite	Classes	Log-likelihood	SABIC	BIC	Entropy	ICL
mBP	1	-4016.66	8074.12	8121.70	1	8121.70
	2	-3701.74	7487.78	7586.13	0.94	7600.18
	3	-3635.61	7399.01	7548.12	0.89	7590.25
	4	-3465.42	7102.15	7302.02	0.91	7347.13
	5	-3426.35	7067.50	7318.14	0.85	7407.87
mBzP2	1	-4012.83	8066.44	8114.03	1	8114.03
	2	-3702.46	7489.20	7587.55	0.94	7602.32
	3	-3638.71	7405.22	7554.33	0.86	7609.58
	4	-3463.08	7097.46	7297.33	0.91	7344.58
	5	-3425.24	7065.28	7315.91	0.87	7390.53
mCNP	1	-4017.48	8075.75	8123.34	1	8123.34
	2	-3705.69	7495.68	7594.03	0.94	7608.81
	3	-3643.77	7415.33	7564.44	0.86	7621.13
	4	-3466.70	7104.70	7304.57	0.90	7353.36
	5	-3427.69	7070.18	7320.81	0.87	7398.07
mCOP	1	-4016.47	8073.73	8121.31	1	8121.31
	2	-3703.74	7491.77	7590.12	0.94	7605.42
	3	-3640.59	7408.97	7558.08	0.85	7616.31
	4	-3466.41	7104.12	7303.99	0.90	7354.36
	5	-3434.72	7084.24	7334.87	0.85	7421.26
mCPP	1	-4016.53	8073.86	8121.44	1	8121.44
	2	-3701.96	7488.20	7586.55	0.94	7602.29
	3	-3636.49	7400.78	7549.89	0.87	7603.27

	4	-3617.65	7406.61	7606.48	0.79	7713.76
	5	-3601.80	7418.42	7669.05	0.84	7764.51
mECPP	1	-4014.64	8070.07	8117.66	1	8117.66
	2	-3702.93	7490.16	7588.51	0.94	7603.32
	3	-3639.56	7406.92	7556.03	0.86	7610.78
	4	-3468.61	7108.52	7308.39	0.90	7356.53
	5	-3426.34	7067.48	7318.12	0.88	7389.98
mEHHP	1	-4015.60	8071.98	8119.56	1	8119.56
	2	-3704.07	7492.44	7590.79	0.94	7605.67
	3	-3640.88	7409.55	7558.66	0.86	7614.38
	4	-3468.91	7109.13	7309.00	0.90	7357.76
	5	-3442.49	7099.79	7350.42	0.85	7439.93
mEHP	1	-4016.42	8073.63	8121.22	1	8121.22
	2	-3704.76	7493.81	7592.16	0.94	7607.1
	3	-3641.67	7411.14	7560.25	0.86	7617.68
	4	-3464.32	7099.94	7299.81	0.90	7348.76
	5	-3601.12	7417.05	7667.68	0.76	7806.25
mEOHP	1	-4016.47	8073.72	8121.31	1	8121.31
	2	-3704.97	7494.24	7592.58	0.94	7607.48
	3	-3641.31	7410.42	7559.53	0.86	7615.51
	4	-3484.59	7140.48	7340.35	0.90	7388.39
	5	-3447.10	7109.00	7359.63	0.83	7458.90
mEP2	1	-4017.53	8075.85	8123.44	1	8123.44
	2	-3705.67	7495.63	7593.98	0.95	7607.75
	3	-3642.22	7412.24	7561.35	0.85	7620.57
	4	-3470.66	7112.62	7312.49	0.90	7363.41

	5	-3437.22	7089.26	7339.89	0.85	7427.09
MHBP	1	-4015.22	8071.22	8118.81	1	8118.81
	2	-3703.01	7490.31	7588.66	0.94	7602.65
	3	-3638.42	7404.65	7553.76	0.89	7597.47
	4	-3466.86	7105.03	7304.90	0.90	7352.79
	5	-3427.86	7070.53	7321.16	0.85	7406.98
MHiBP	1	-4017.28	8075.34	8122.93	1	8122.93
	2	-3705.24	7494.77	7593.12	0.94	7607.53
	3	-3642.25	7412.29	7561.40	0.86	7618.52
	4	-3469.67	7110.64	7310.51	0.90	7360.60
	5	-3606.55	7427.90	7678.54	0.78	7806.04
miBP	1	-4017.49	8075.76	8123.35	1	8123.35
	2	-3704.63	7493.56	7591.91	0.94	7606.03
	3	-3641.38	7410.56	7559.67	0.86	7615.58
	4	-3622.53	7416.36	7616.23	0.85	7690.92
	5	-3432.45	7079.70	7330.33	0.86	7411.89
mNP2	1	-4016.53	8073.84	8121.43	1	8121.43
	2	-3703.54	7491.36	7589.71	0.94	7604.87
	3	-3640.28	7408.36	7557.47	0.85	7616.24
	4	-3466.74	7104.78	7304.65	0.90	7356.12
	5	-3429.68	7074.18	7324.81	0.85	7409.87
DEHP	1	-4015.55	8071.89	8119.48	1	8119.48
	2	-3704.00	7492.29	7590.63	0.94	7605.49
	3	-3640.63	7409.07	7558.18	0.86	7613.79
	4	-3628.18	7427.66	7627.53	0.80	7729.59
	5	-3438.38	7091.56	7342.19	0.87	7420.80

Footnotes for Table 3.8: Higher log-likelihood, lower SABIC (sample adjusted BIC), lower BIC (Bayesian Inclusion Criterion), higher entropy, and lower ICL (integrated classification likelihood) indicates an overall better fit; All phthalates were winsorized to the 99th percentile; all models were adjusted for site specific Z-score for urinary dilution measure (specific gravity or creatinine), cohort, child sex, maternal age, maternal race/ethnicity, home-ownership, and maternal educational attainment; 4 and 5 class models had <10% of population in at least one group, which resulted in those models being dropped from consideration, even if they had better fit measures

Table 3.9: Comparison of Class Assignment between One-Step and Two-Step Models for Expressive Language

Metabolite	Two-Step Categorizations	One-Step Categorizations		
mBP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	8	179	3
	Low Growth	0	6	58
mBzP2		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	9	180	1
	Low Growth	0	7	57
mCNP		High Growth	Tracking	Low Growth
	High Growth	99	9	0
	Tracking	12	174	4
	Low Growth	0	4	60
mCOP		High Growth	Tracking	Low Growth
	High Growth	99	9	0
	Tracking	8	181	4
	Low Growth	0	5	60
mCPP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	9	180	1

	Low Growth	0	5	59

mECPP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	10	179	1
	Low Growth	0	5	59

mEHHP		High Growth	Tracking	Low Growth
	High Growth	99	9	0
	Tracking	9	180	1
	Low Growth	0	5	59

mEHP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	10	179	1
	Low Growth	0	6	58

mEOHP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	9	180	1
	Low Growth	0	5	59

mEP2		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	8	181	1
	Low Growth	0	6	58

MHBP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	8	181	1
	Low Growth	0	6	58

MHiBP		High Growth	Tracking	Low Growth
	High Growth	99	8	0
	Tracking	11	181	1
	Low Growth	0	7	57

miBP		High Growth	Tracking	Low Growth
	High Growth	100	8	0
	Tracking	11	178	1
	Low Growth	0	5	59

mNP2		High Growth	Tracking	Low Growth
	High Growth	99	9	0
	Tracking	9	180	1
	Low Growth	0	7	57

DEHP		High Growth	Tracking	Low Growth
	High Growth	99	9	0
	Tracking	9	180	1
	Low Growth	0	5	59

Table 3.10: Comparison of Class Assignment between One-Step and Two-Step Models for Receptive Language

Metabolite	Two-Step Categorizations	One-Step Categorizations		
mBP		High Growth	Tracking	Late Growth
	High Growth	35	18	0
	Tracking	1	223	3
	Late Growth	1	2	79
mBzP2		High Growth	Tracking	Late Growth
	High Growth	44	9	0
	Tracking	2	223	2
	Late Growth	0	2	80
mCNP		High Growth	Tracking	Late Growth
	High Growth	44	9	0
	Tracking	2	223	2
	Late Growth	0	3	79
mCOP		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	223	2
	Late Growth	0	2	80
mCPP		High Growth	Tracking	Late Growth
	High Growth	44	9	0
	Tracking	1	223	3

	Late Growth	0	2	80

mECPP		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	224	1
	Late Growth	0	2	80

mEHHP		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	224	1
	Late Growth	0	2	80

mEHP		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	224	1
	Late Growth	0	2	80

mEOHP		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	224	1
	Late Growth	0	2	80

mEP2		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	224	1
	Late Growth	0	3	79

MHBP		High Growth	Tracking	Late Growth
	High Growth	38	15	0
	Tracking	1	223	3
	Late Growth	1	2	79

MHiBP		High Growth	Tracking	Late Growth
	High Growth	44	9	0
	Tracking	2	222	3
	Late Growth	0	2	80

miBP		High Growth	Tracking	Late Growth
	High Growth	44	9	0
	Tracking	2	222	3
	Late Growth	0	2	80

mNP2		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	223	2
	Late Growth	0	2	80

DEHP		High Growth	Tracking	Late Growth
	High Growth	45	8	0
	Tracking	2	224	1
	Late Growth	0	2	80

Footnotes for Table 3.10:

Table 3.11: Sex Modification for Expressive Language

Molecular Weight	Parent Compound	Metabolite	LRT P-value	Tracking (female) (N=81)	Tracking (male) (N=109)	Low Growth (female) (N=16)	Low Growth (male) (N=48)
Low Molecular Weight	DBP	mBP	0.39	1.20 (0.89, 1.63)	0.90 (0.61, 1.31)	1.20 (0.78, 1.85)	1.17 (0.78, 1.76)
		MHBP	0.23	1.30 (0.95, 1.77)	0.89 (0.61, 1.30)	1.22 (0.77, 1.93)	1.16 (0.78, 1.73)
	DEP	mEP2	0.12	1.03 (0.87, 1.21)	1.03 (0.91, 1.16)	0.36 (0.12, 1.11)	1.05 (0.91, 1.20)
	DiBP	MHiBP	0.55	1.03 (0.87, 1.21)	1.03 (0.91, 1.16)	0.36 (0.12, 1.11)	1.05 (0.91, 1.20)
		miBP	0.80	1.06 (0.77, 1.46)	0.93 (0.66, 1.31)	0.90 (0.55, 1.47)	0.79 (0.49, 1.29)
High Molecular Weight	BzBP	mBzP2	0.69	1.16 (0.87, 1.54)	1.12 (0.77, 1.63)	1.09 (0.68, 1.74)	1.26 (0.83, 1.92)
	DiDP	mCNP	0.29	0.98 (0.94, 1.03)	1.19 (0.87, 1.62)	0.86 (0.62, 1.21)	1.18 (0.86, 1.61)
	DiNP	mCOP	0.53	1.03 (0.86, 1.23)	0.85 (0.69, 1.04)	0.91 (0.62, 1.34)	0.86 (0.68, 1.10)
		mNP2	0.53	1.03 (0.90, 1.18)	0.87, 0.73, 1.03)	1.05 (0.85, 1.29)	0.88 (0.72, 1.07)
	DOP, DBP, other HMW phthalates	mCPP	0.67	0.98 (0.89, 1.08)	0.98 (0.89, 1.08)	0.79 (0.50, 1.24)	0.99 (0.89, 1.09)
	DEHP	mECPP	0.17	0.93 (0.83, 1.03)	0.95 (0.82, 1.11)	0.78 (0.51, 1.20)	0.83 (0.63, 1.08)
		mEHHP	0.11	0.95 (0.88, 1.03)	0.98 (0.89, 1.09)	0.82 (0.56, 1.21)	0.81 (0.61, 1.06)
		mEHP	0.18	0.97 (0.89, 1.07)	0.98 (0.88, 1.09)	0.88 (0.66, 1.19)	0.74 (0.51, 1.07)

		mEOHP	0.22	0.97 (0.90, 1.05)	0.98 (0.89 1.07)	0.84 (0.56, 1.25)	0.81 (0.61, 1.08)
		ΣDEHP	0.16	0.95 (0.87, 1.04)	0.97 (0.87, 1.09)	0.82 (0.56, 1.20)	0.82 (0.63, 1.07)

Footnotes for Table 3.11: Female and High Growth were defaulted as the referent group; All phthalates were winsorized to the 99th percentile; models were adjusted for site specific Z-score for urinary dilution measure (specific gravity or creatinine), cohort, child sex, maternal age, maternal race/ethnicity, home-ownership, and maternal educational attainment

Table 3.12: Sex Modification for Receptive Language

Molecular Weight	Parent Compound	Metabolite	LRT P-value	Tracking (Female) (N=106)	Tracking (Male) (N=121)	Late Growth (Female) (N=24)	Late Growth (Male) (N=58)
Low Molecular Weight	DBP	mBP	0.18	1.07 (0.72, 1.60)	0.63 (0.42, 0.94)	1.26 (0.79, 2.00)	0.64 (0.41, 1.01)
		MHBP	0.25	1.09 (0.73, 1.63)	0.69 (0.46, 1.04)	1.34 (0.86, 2.10)	0.78 (0.50, 1.21)
	DEP	mEP2	0.16	1.19 (0.84, 1.68)	1.33 (0.84, 2.10)	0.95 (0.59, 1.53)	1.37 (0.86, 2.17)
	DiBP	MHiBP	0.24	1.19 (0.84, 1.68)	1.33 (0.84, 2.10)	0.95 (0.59, 1.53)	1.37 (0.86, 2.17)
		miBP	0.32	1.55 (0.81, 2.96)	0.77 (0.53, 1.11)	1.53 (0.76, 3.09)	0.69 (0.43, 1.12)
High Molecular Weight	BzBP	mBzP2	0.21	1.11 (0.73, 1.69)	0.75 (0.51, 1.11)	1.44 (0.90, 2. 28)	0.89 (0.59, 1.35)
	DiDP	mCNP	0.36	0.99 (0.95, 1.04)	0.96 (0.90, 1.03)	0.85 (0.62, 1.15)	0.97 (0.89, 1.05)
	DiNP	mCOP	0.06	1.14 (0.87, 1.50)	0.77 (0.62, 0.97)	0.79 (0.44, 1.40)	0.78 (0.61, 1.00)
		mNP2	0.05	1.08 (0.90, 1.23)	0.80 (0.67, 0.97)	0.82 (0.55, 1.21)	0.81 (0.66, 0.99)
	DOP, DBP, other HMW phthalates	mCPP	0.11	1.06 (0.92, 1.23)	0.93 (0.85, 1.02)	0.74 (0.44, 1.23)	0.94 (0.84, 1.04)
	DEHP	mECPP	0.01	0.84 (0.74, 0.96)	0.90 (0.77, 1.06)	0.63 (0.36, 1.10)	0.87 (0.71, 1.08)
		mEHHP	0.05	0.90 (0.82, 0.99)	0.95 (0.85, 1.06)	0.74 (0.48, 1.12)	0.92 (0.79, 1.07)
		mEHP	0.08	0.91 (0.82, 1.02)	0.95 (0.85, 1.07)	0.58 (0.30, 1.12)	0.92 (0.79, 1.08)

		mEOHP	0.17	0.93 (0.85, 1.01)	0.95 (0.86, 1.05)	0.76 (0.49, 1.16)	0.92 (0.80, 1.07)
		ΣDEHP	0.04	0.89 (0.80, 0.99)	0.94 (0.83, 1.06)	0.69 (0.42, 1.12)	0.91 (0.77, 1.07)

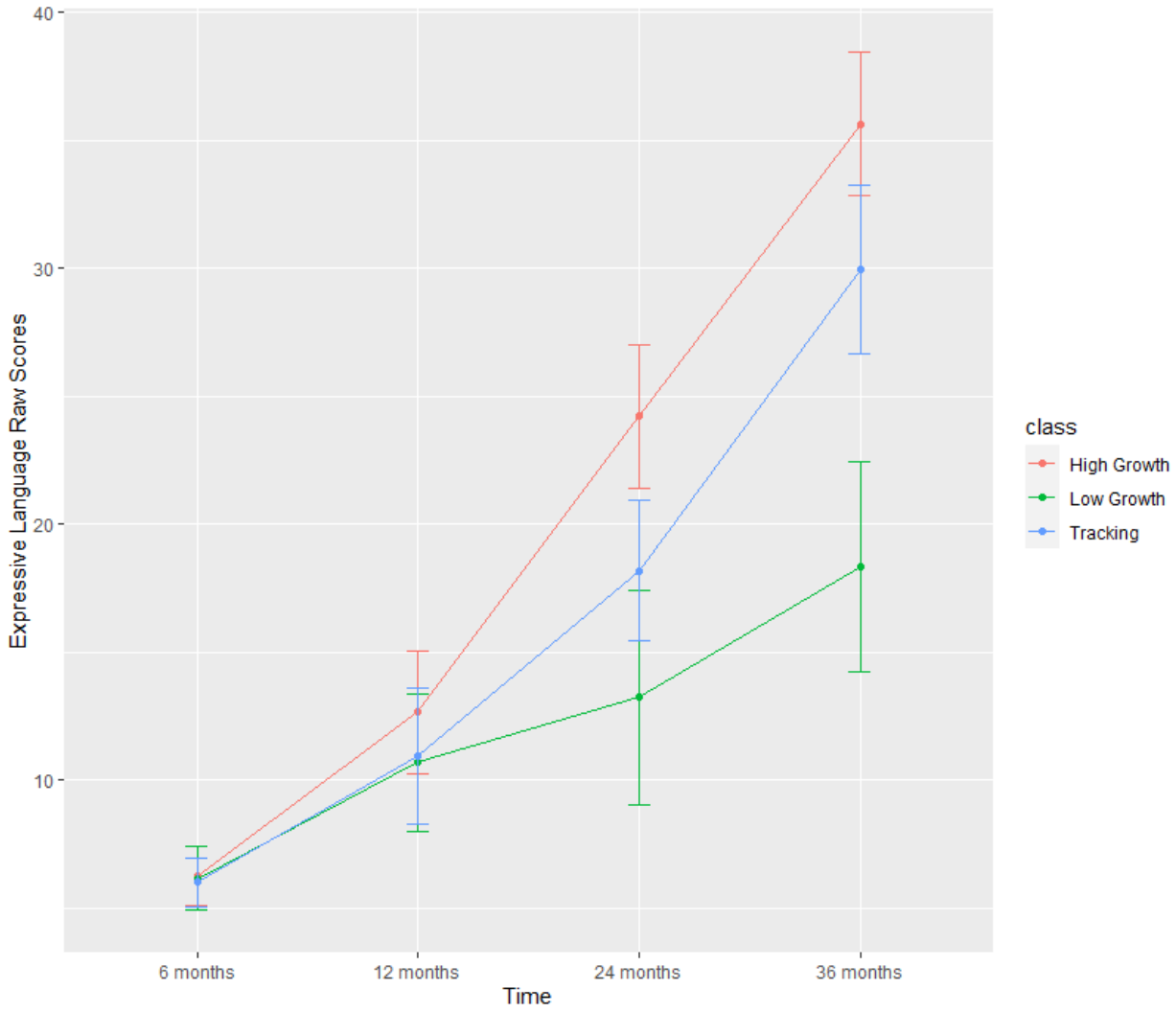
Footnotes for Table 3.12: Female and High Growth were defaulted as the referent group; All phthalates were winsorized to the 99th percentile; models were adjusted for site specific Z-score for urinary dilution measure (specific gravity or creatinine), cohort, child sex, maternal age, maternal race/ethnicity, home-ownership, and maternal educational attainment

Table 3.13: Comparison of Urinary Phthalate Concentrations between NHANES and Study Population

Phthalate metabolite	**Study Population** (N=362)	**Combined NHANES data** (N=5171)	**Wilcox Rank Sum P-value**
mBP	12.9 [14.5]	11.2 [12.8]	<< 0.001
mEP2	28.4 [42.5]	38.4 [83.0]	<< 0.001
miBP	8.46 [11.0]	8.25 [9.05]	0.75
mBzP2	6.68 [10.9]	5.29 [8.02]	<< 0.001
mCNP	3.74 [4.09]	2.64 [3.19]	<< 0.001
mCOP	23.3 [47.7]	19.4 [38.6]	0.01
mNP2	1.41 [2.53]	1.09 [1.87]	<< 0.001
mECPP	20.2 [27.5]	13.7 [15.2]	<< 0.001
mEHHP	12.7 [16.5]	8.33 [9.81]	<< 0.001
mEHP	2.92 [4.86]	1.53 [1.98]	<< 0.001
mEOHP	10.2 [12.8]	5.46 [6.06]	<< 0.001

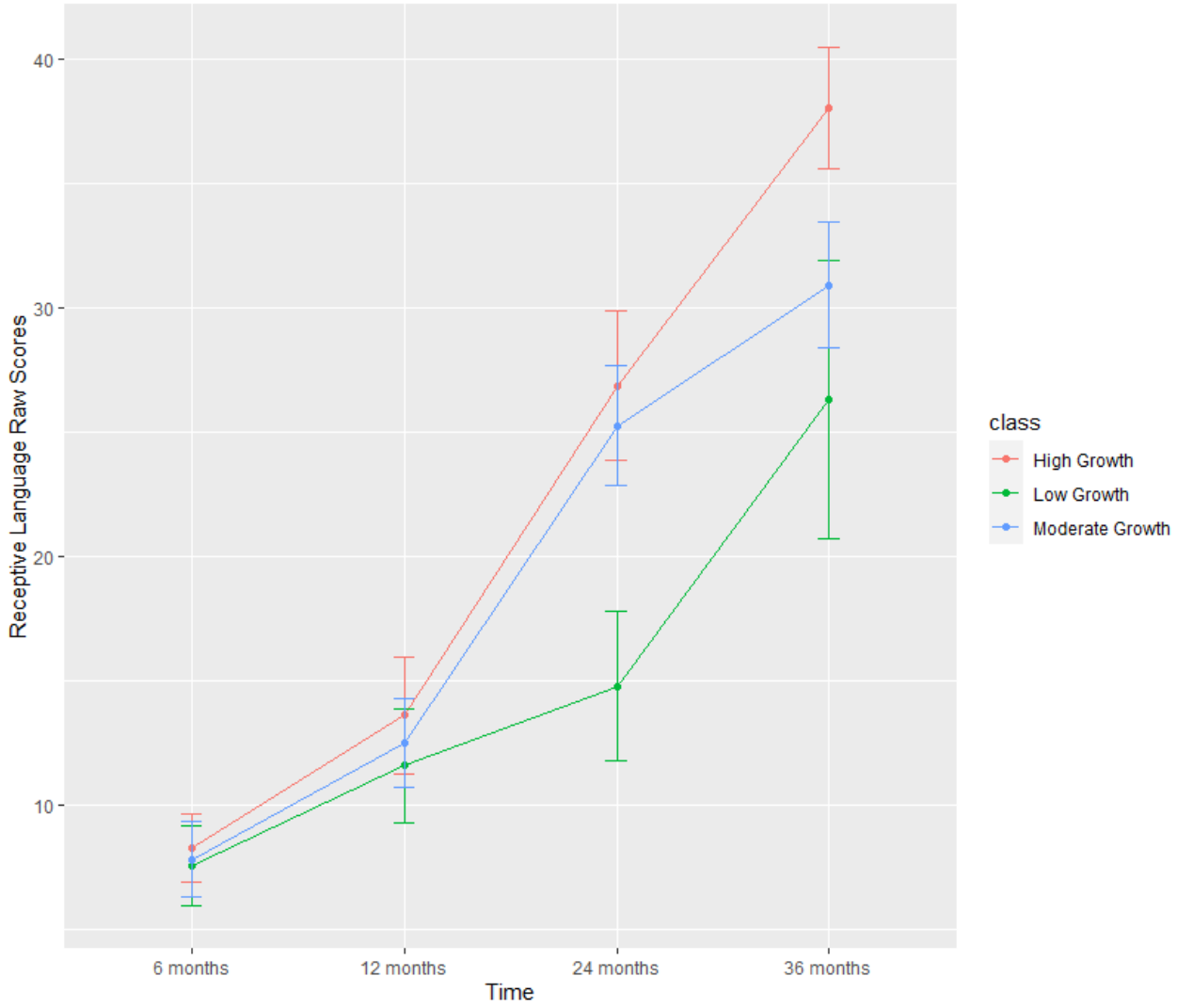
Footnotes for Table 3.13: Due to skewness of data, medians and IQRs were calculated after removing any missing individuals; two-sided Wilcox Rank Sum test was performed using unpaired test; all values were adjusted for creatinine (NHANES and EARLI) or specific gravity (MARBLES)

Figure 3.1: Two-Step Latent Class Growth Analysis Plot for Expressive Language



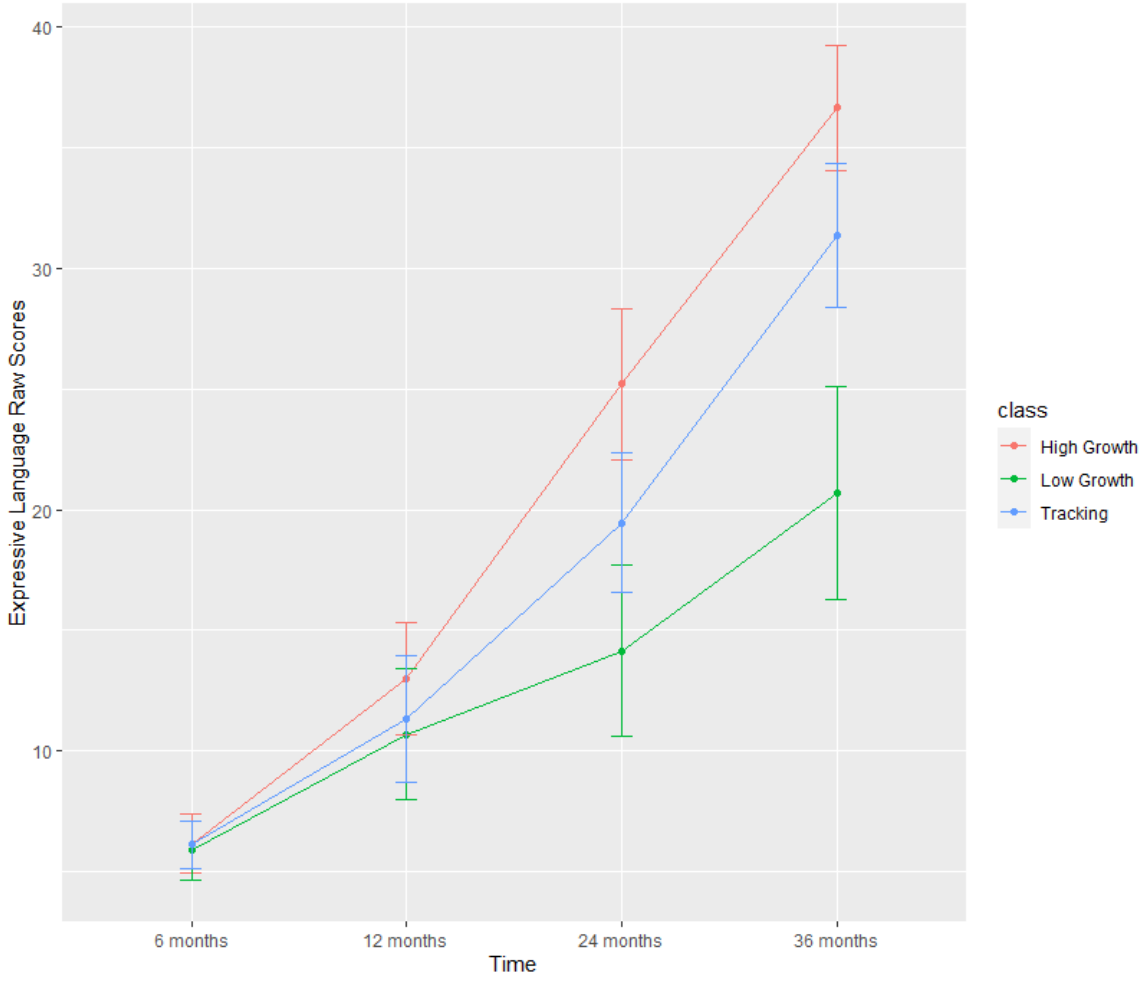
Footnotes for Figure 3.1: Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.2: Two-Step Latent Class Growth Analysis Plot for Receptive Language



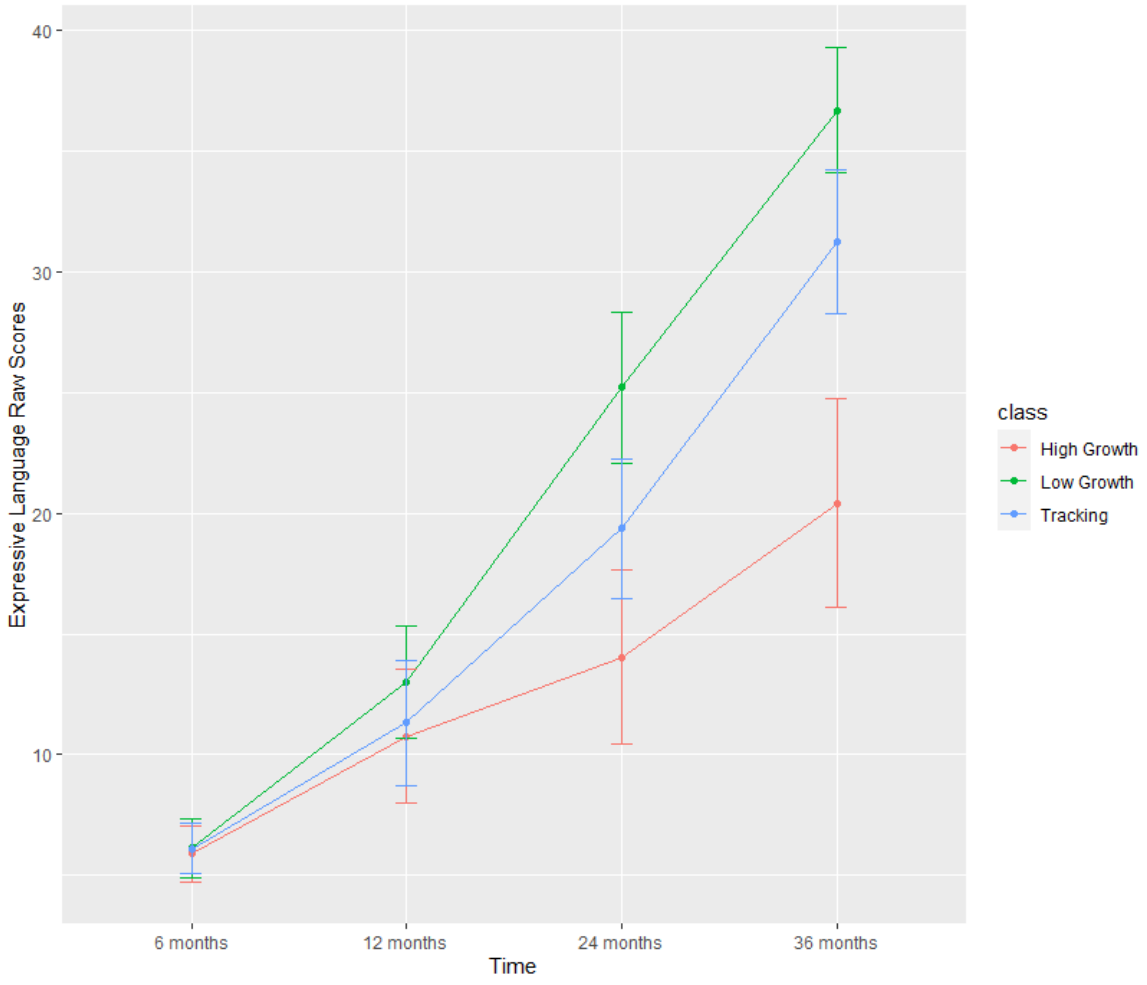
Footnotes for Figure 3.2: Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.3: One-Step Latent Class Growth Analysis Plot for Expressive Language for mBP



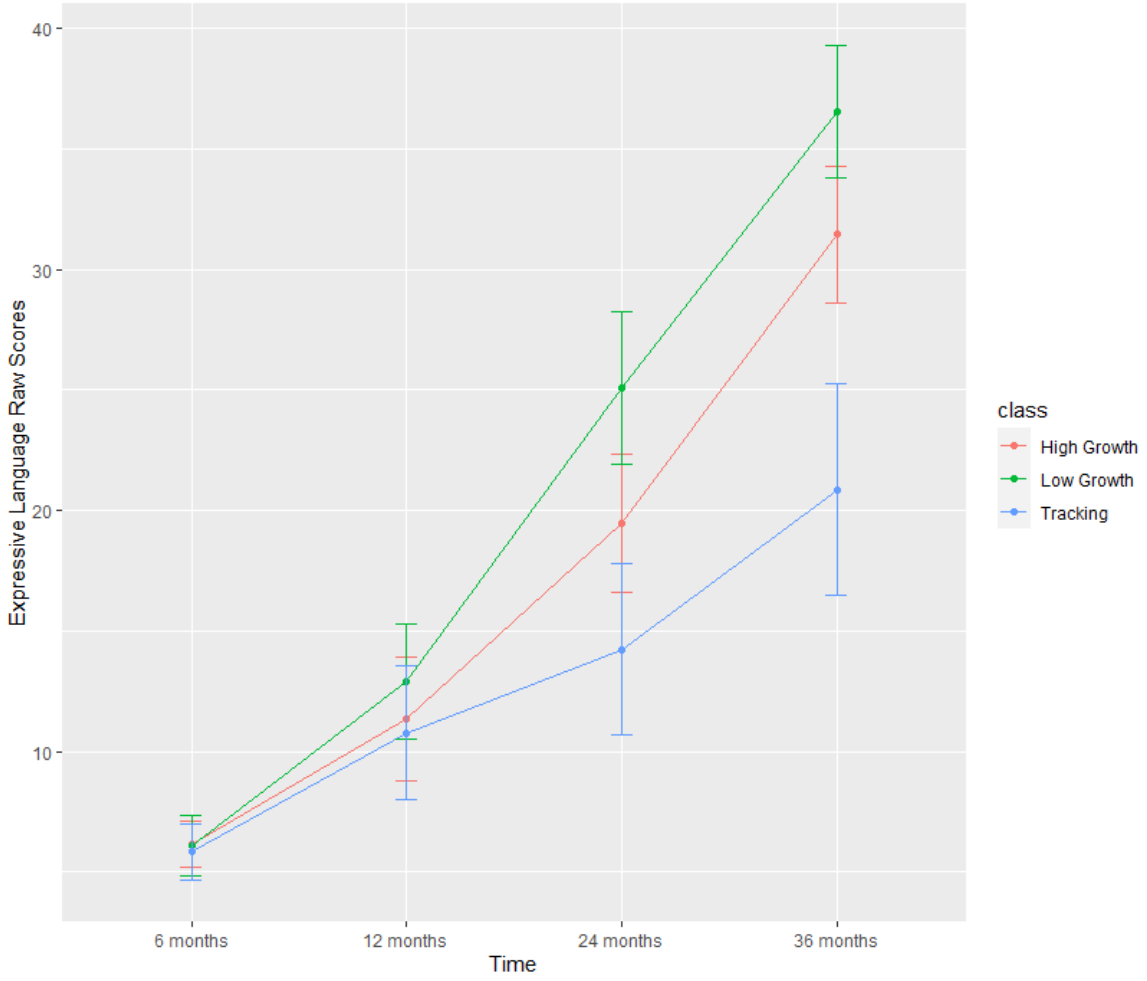
Footnotes for Figure 3.3: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.4: One-Step Latent Class Growth Analysis Plot for Expressive Language for mBzP2



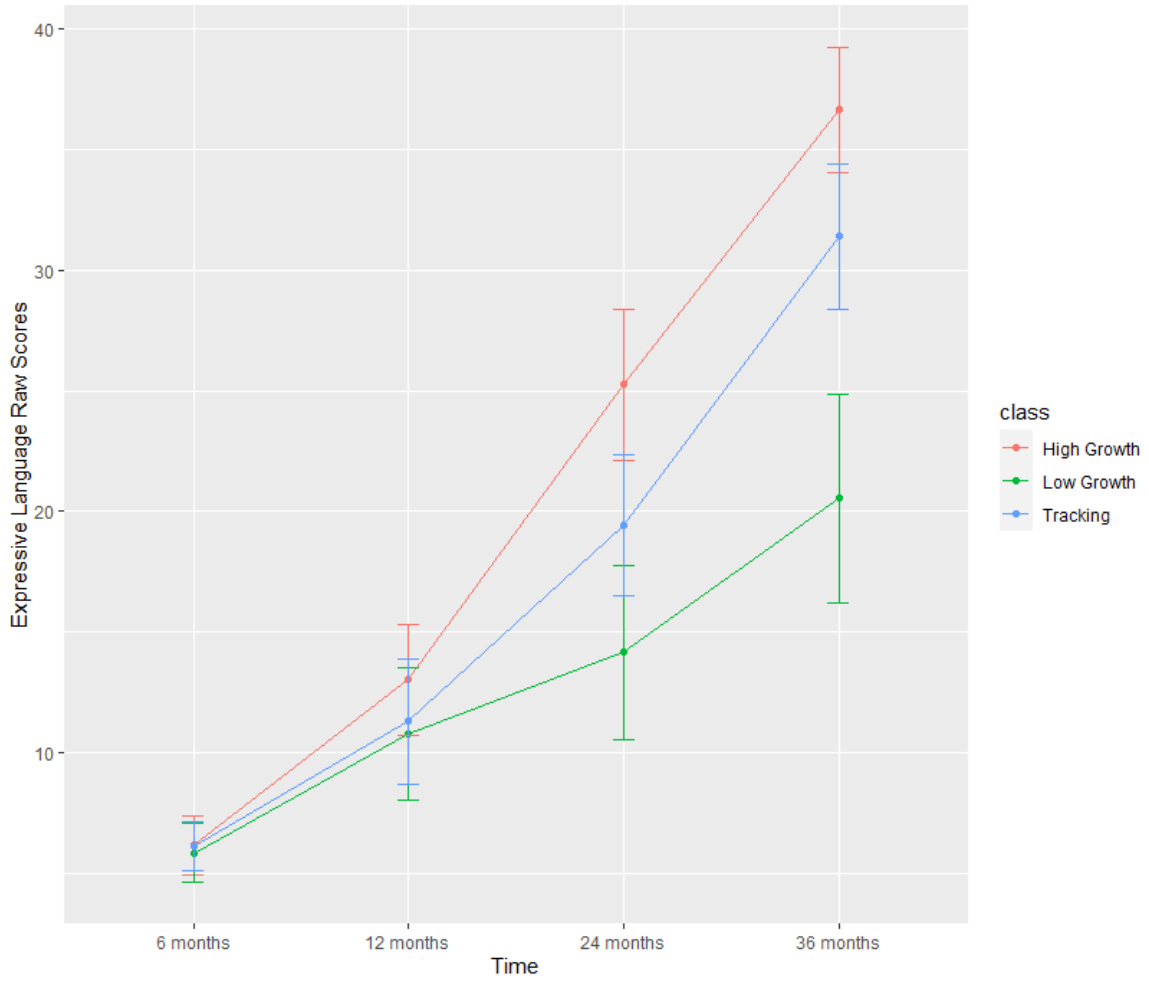
Footnotes for Figure 3.4: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.5: One-Step Latent Class Growth Analysis Plot for Expressive Language for mCNP



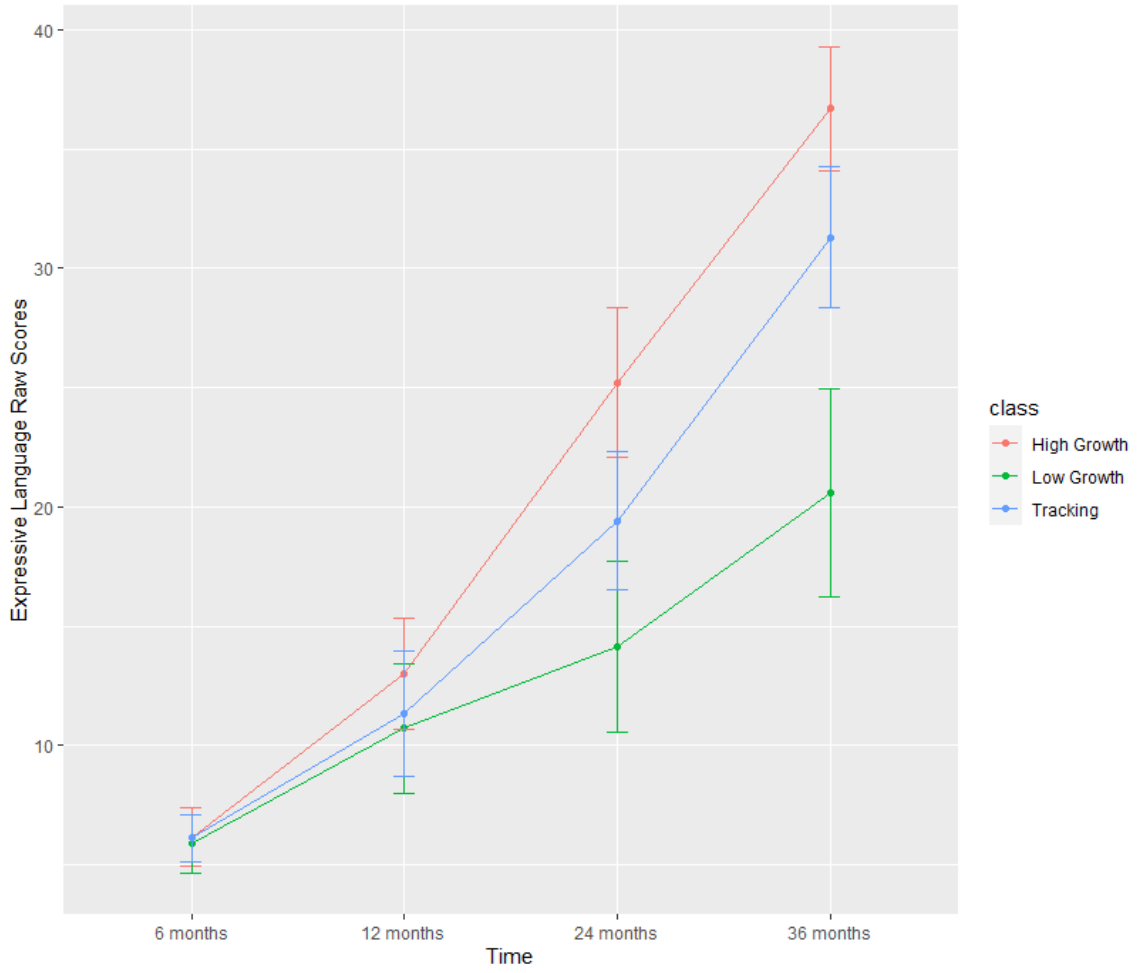
Footnotes for Figure 3.5: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.6: One-Step Latent Class Growth Analysis Plot for Expressive Language for mCOP



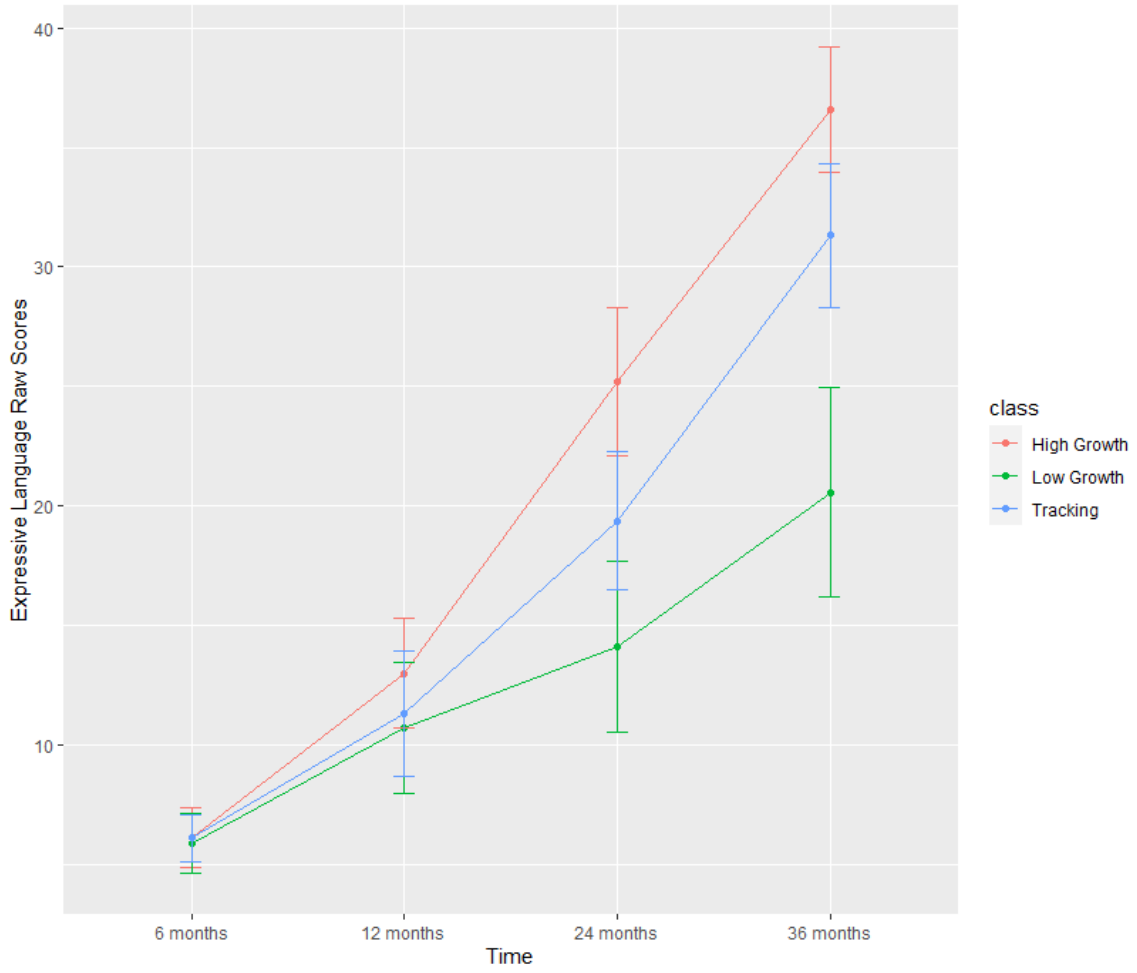
Footnotes for Figure 3.6: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.7: One-Step Latent Class Growth Analysis Plot for Expressive Language for mCPP



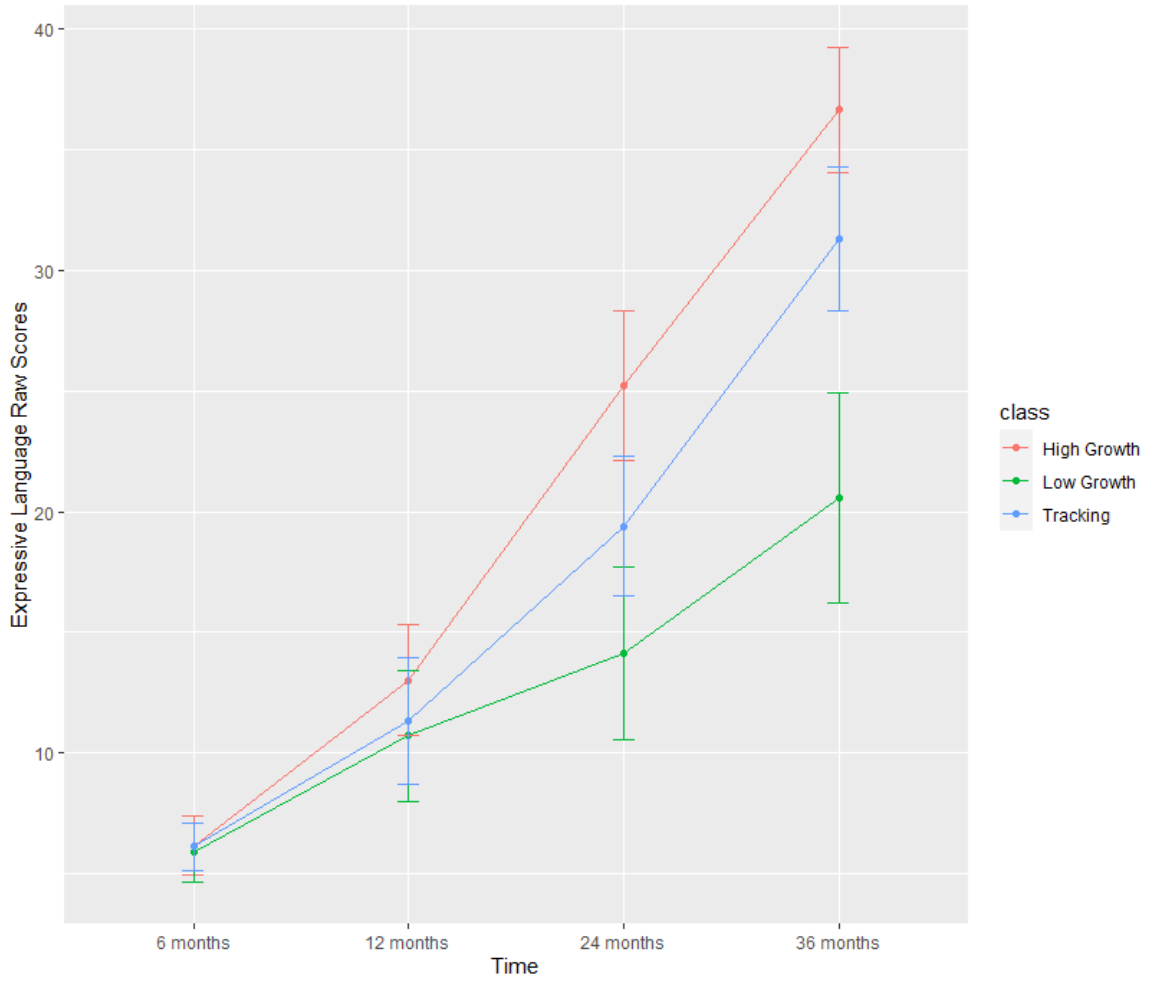
Footnotes for Figure 3.7: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.8: One-Step Latent Class Growth Analysis Plot for Expressive Language for mECPP



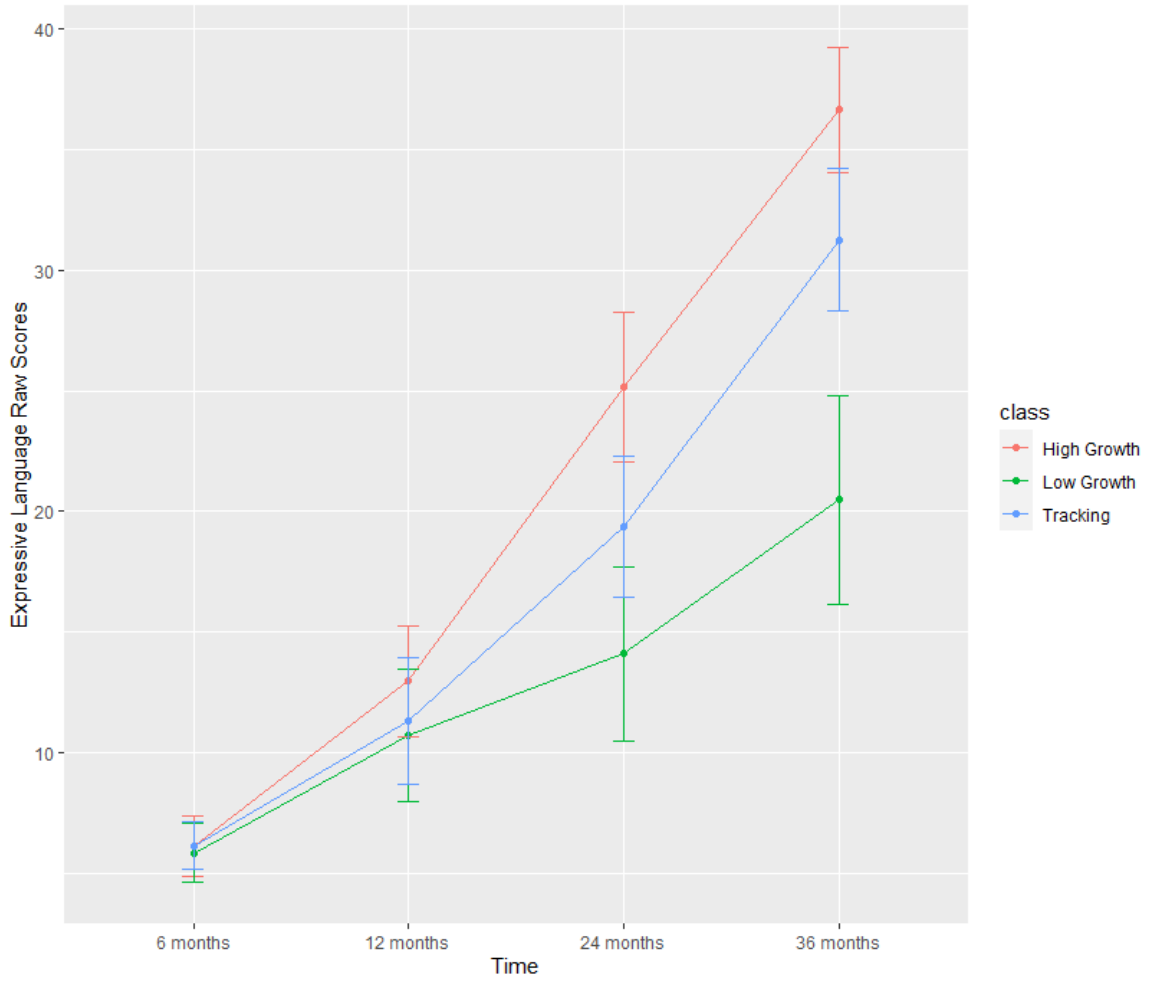
Footnotes for Figure 3.8: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.9: One-Step Latent Class Growth Analysis Plot for Expressive Language for mEHHP



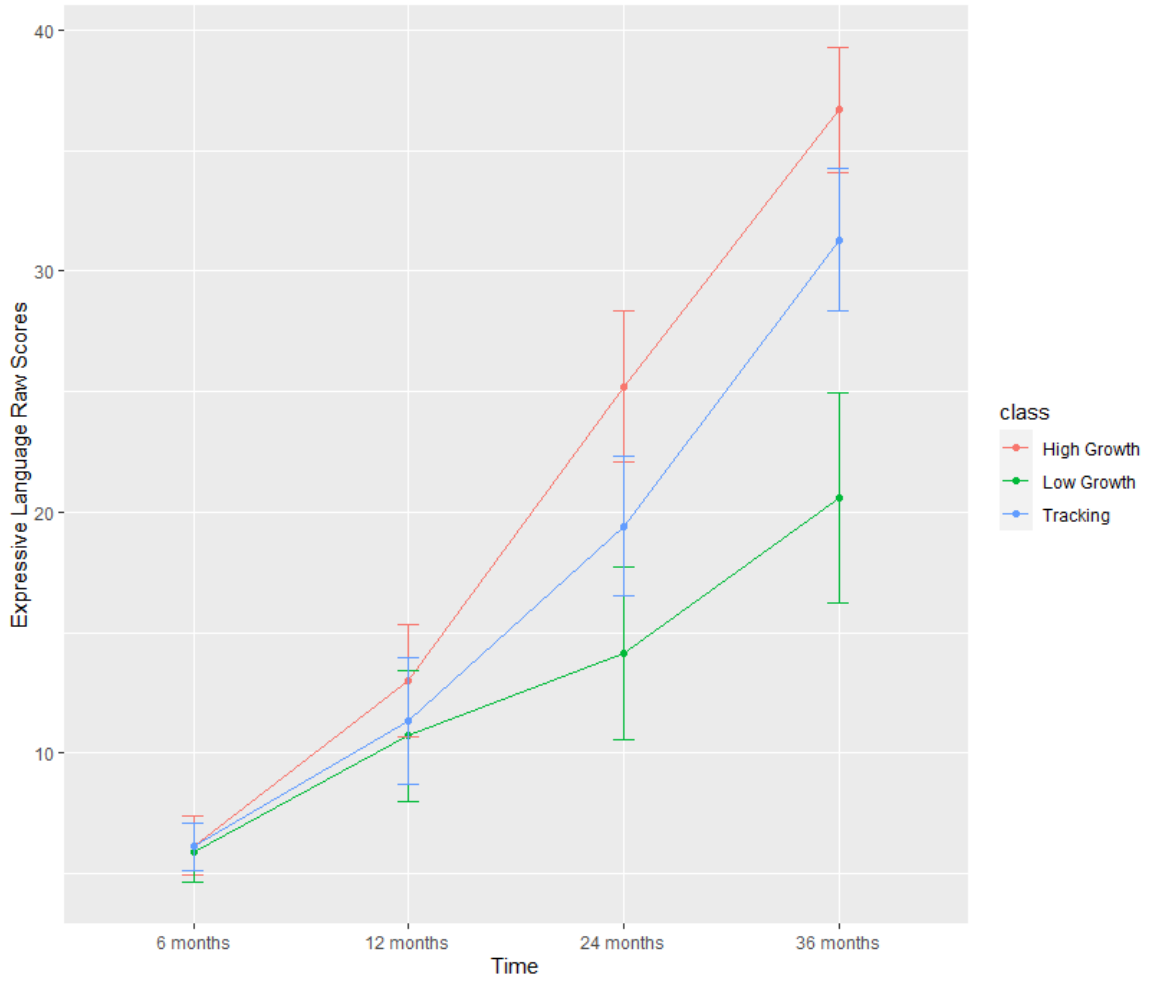
Footnotes for Figure 3.9: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.10: One-Step Latent Class Growth Analysis Plot for Expressive Language for mEHP



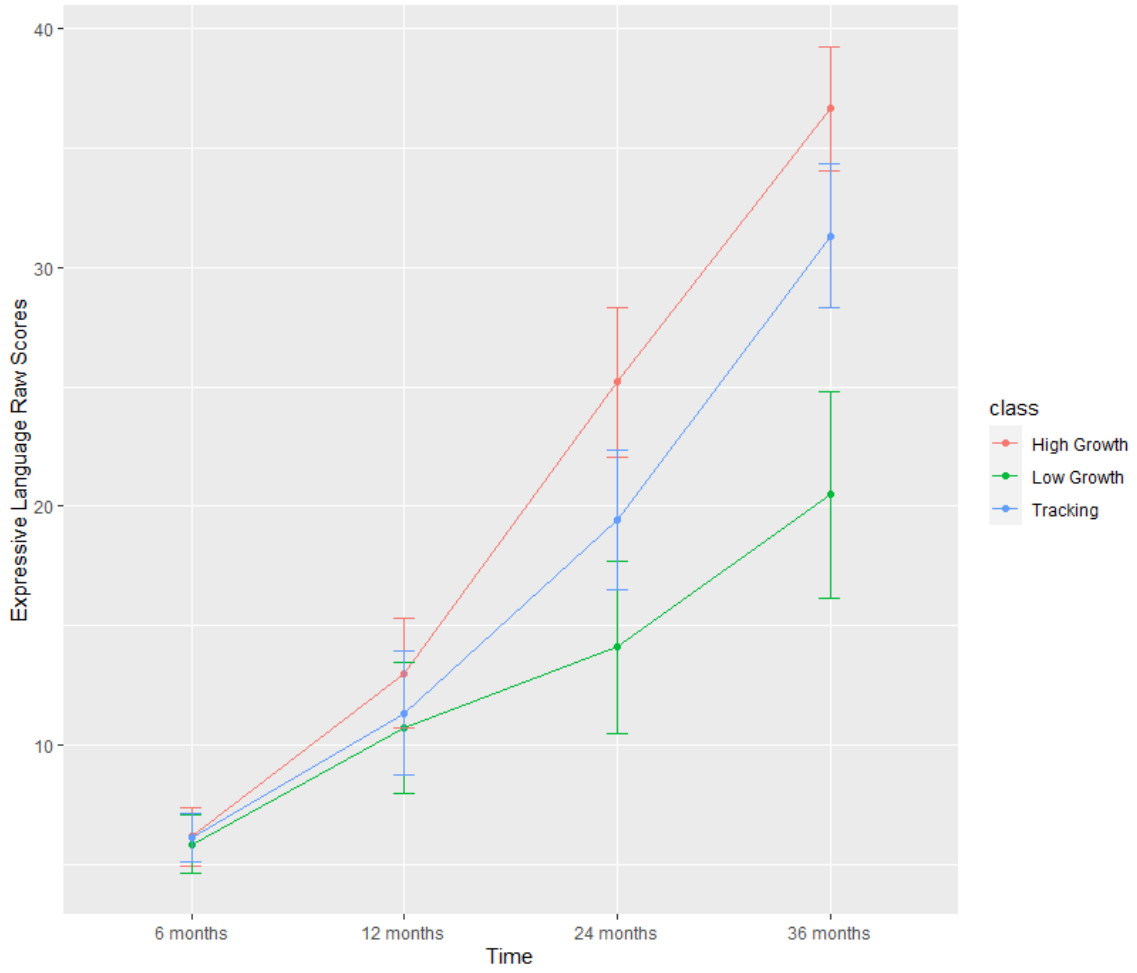
Footnotes for Figure 3.10: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.11: One-Step Latent Class Growth Analysis Plot for Expressive Language for mEOHP



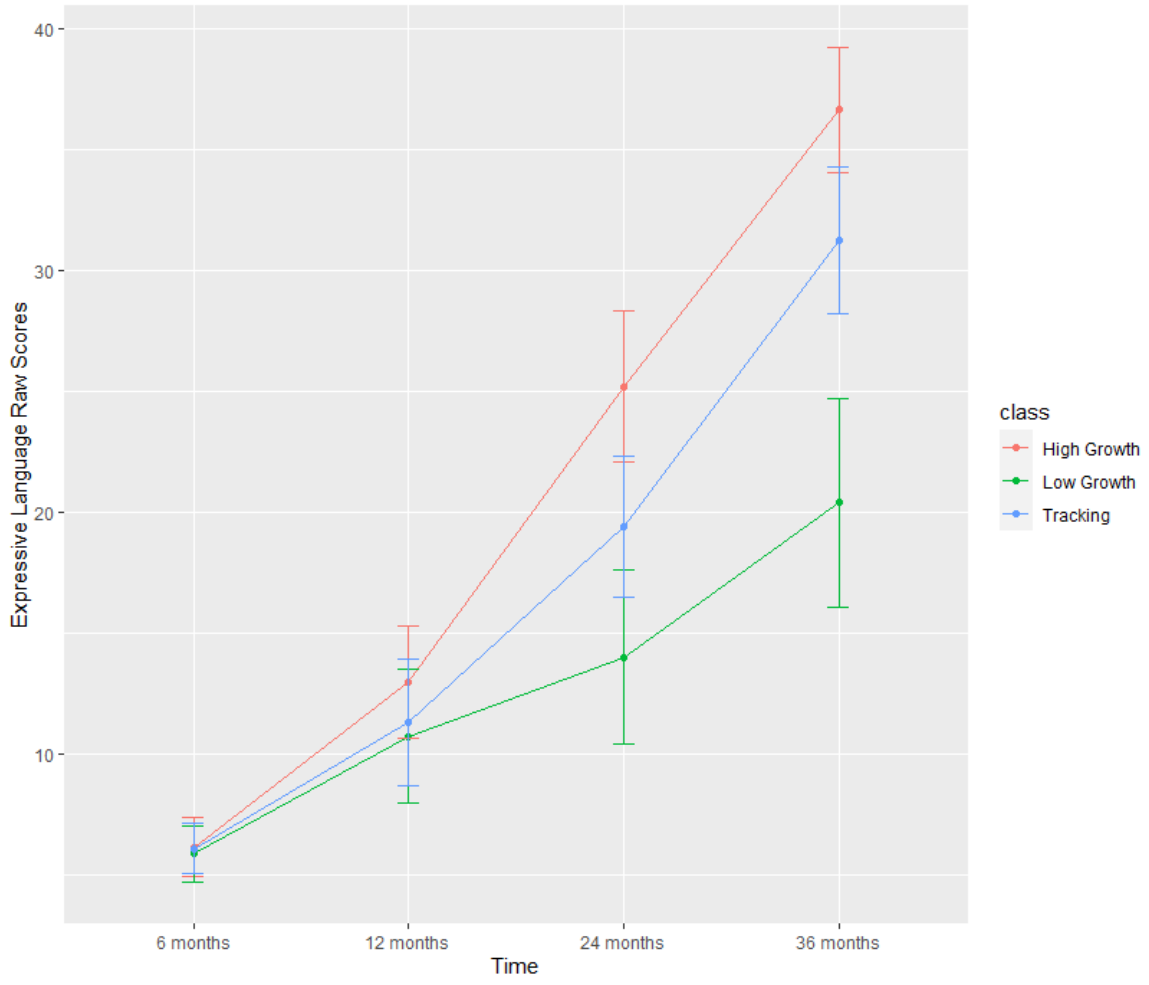
Footnotes for Figure 3.11: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.12: One-Step Latent Class Growth Analysis Plot for Expressive Language for mEP2



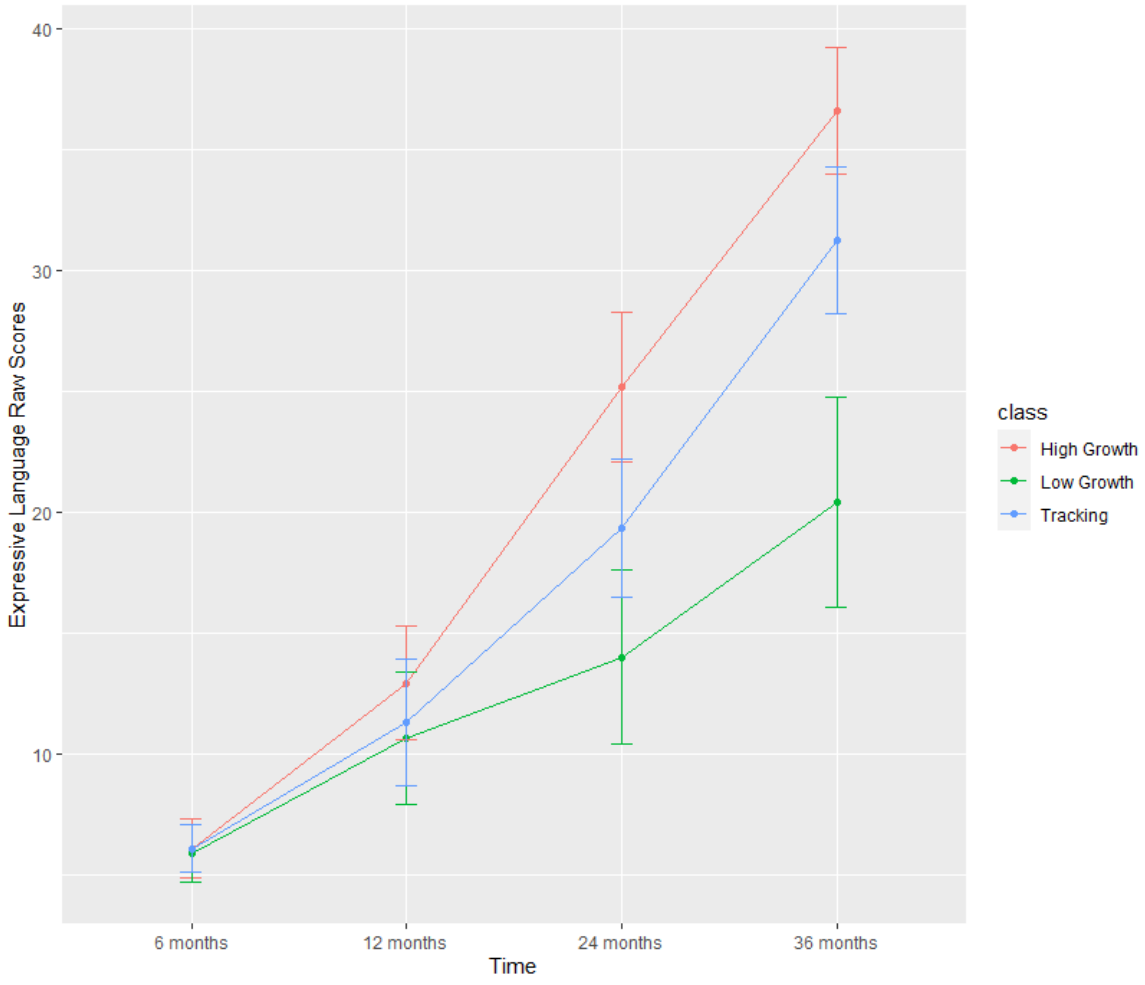
Footnotes for Figure 3.12: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.13: One-Step Latent Class Growth Analysis Plot for Expressive Language for MHBP



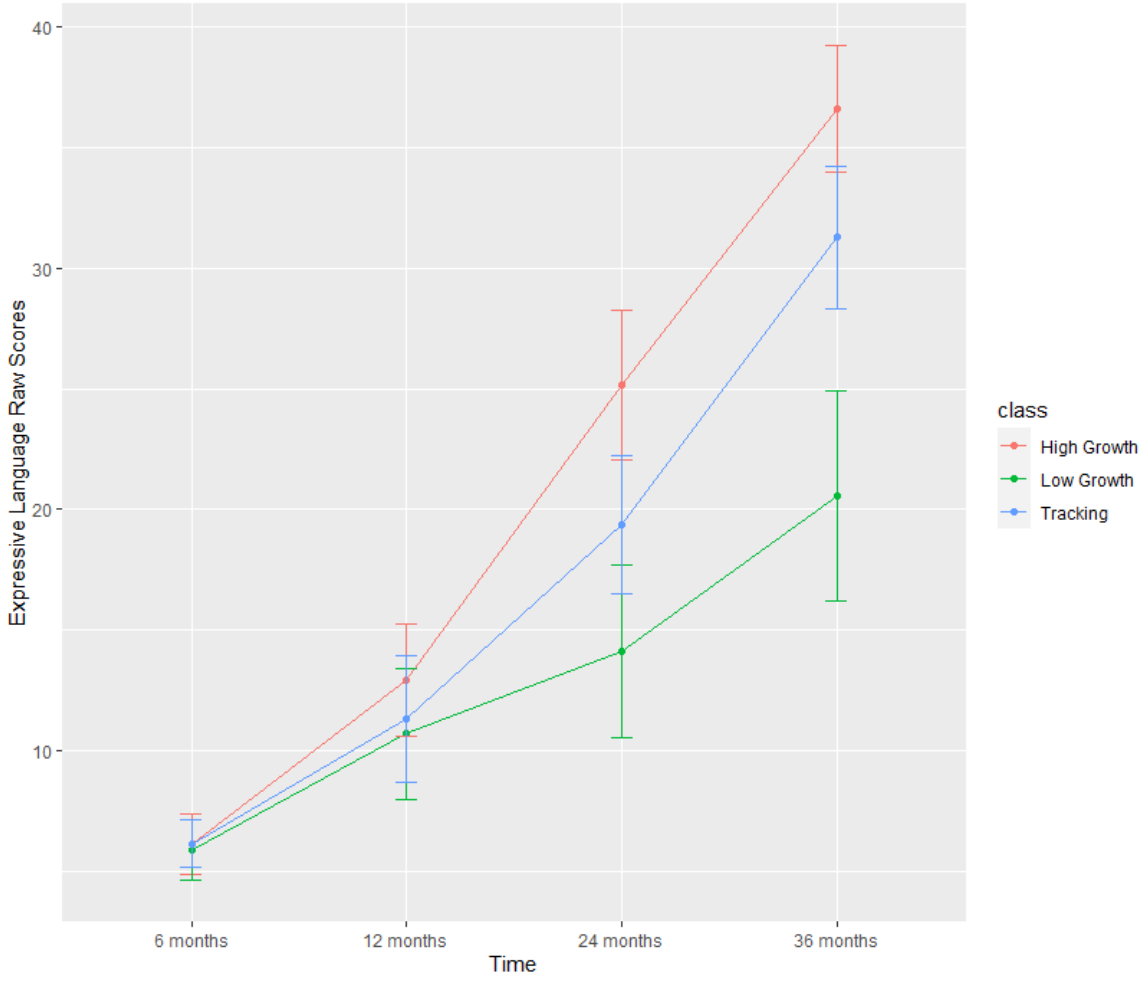
Footnotes for Figure 3.13: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.14: One-Step Latent Class Growth Analysis Plot for Expressive Language for MHiBP



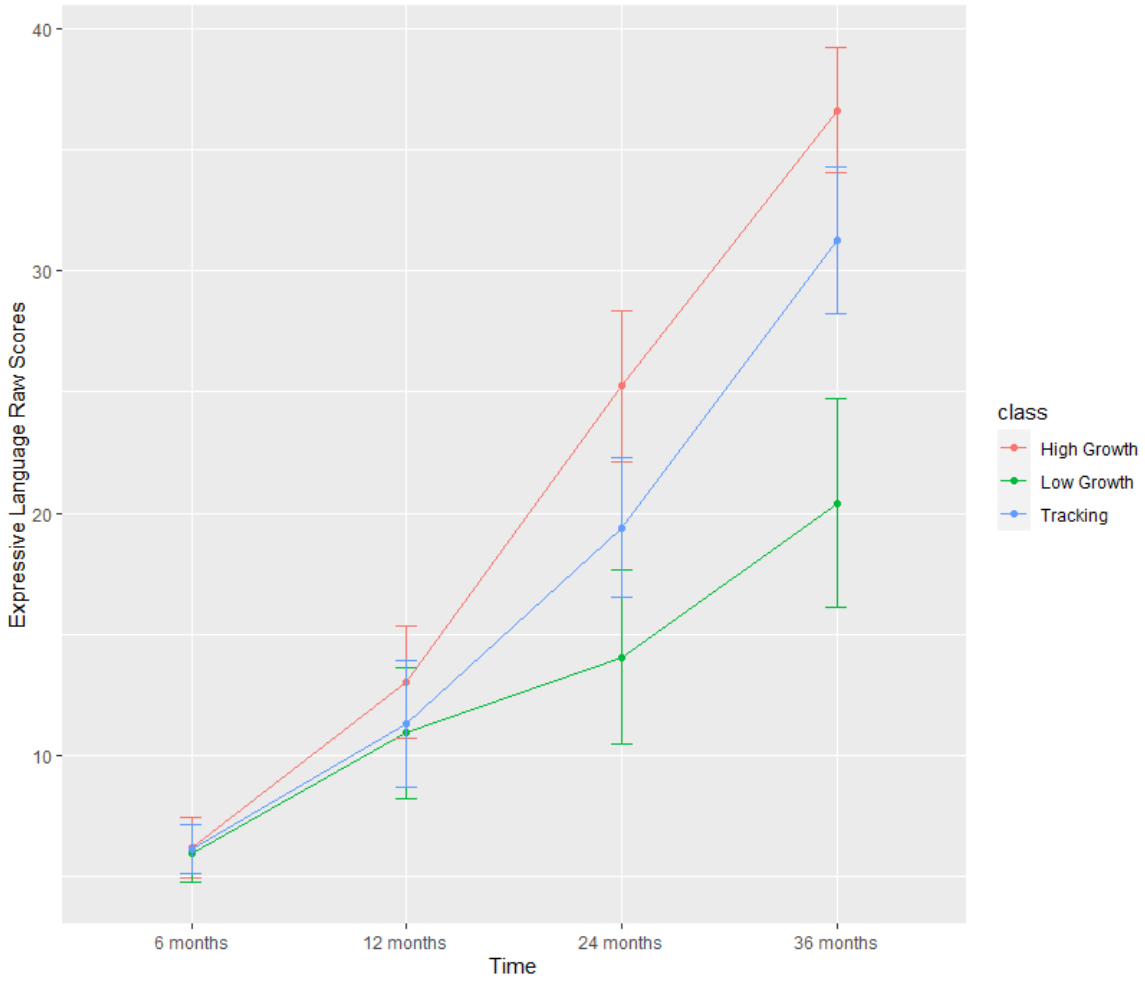
Footnotes for Figure 3.14: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.15: One-Step Latent Class Growth Analysis Plot for Expressive Language for miBP



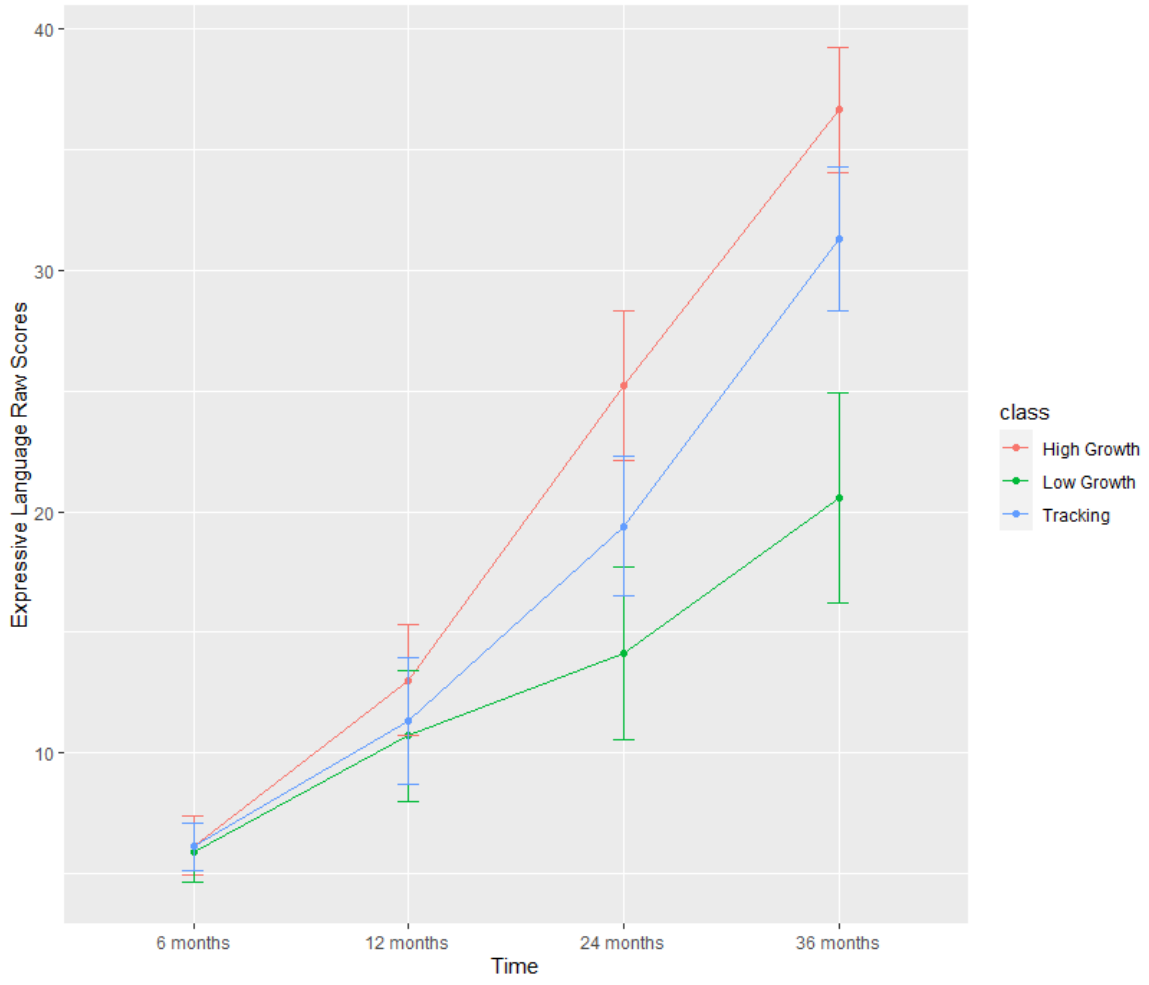
Footnotes for Figure 3.15: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.16: One-Step Latent Class Growth Analysis Plot for Expressive Language for mNP2



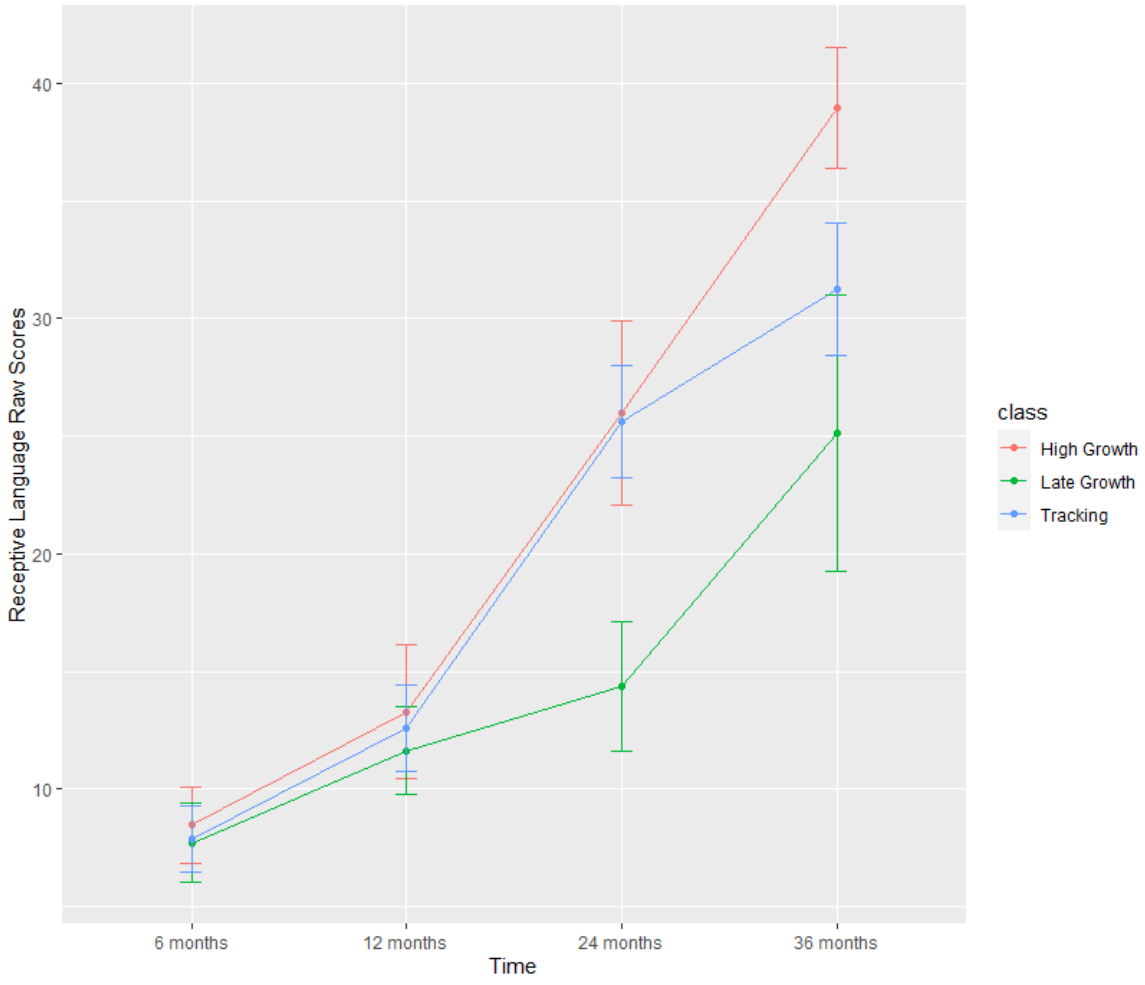
Footnotes for Figure 3.16: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.17: One-Step Latent Class Growth Analysis Plot for Expressive Language for DEHP



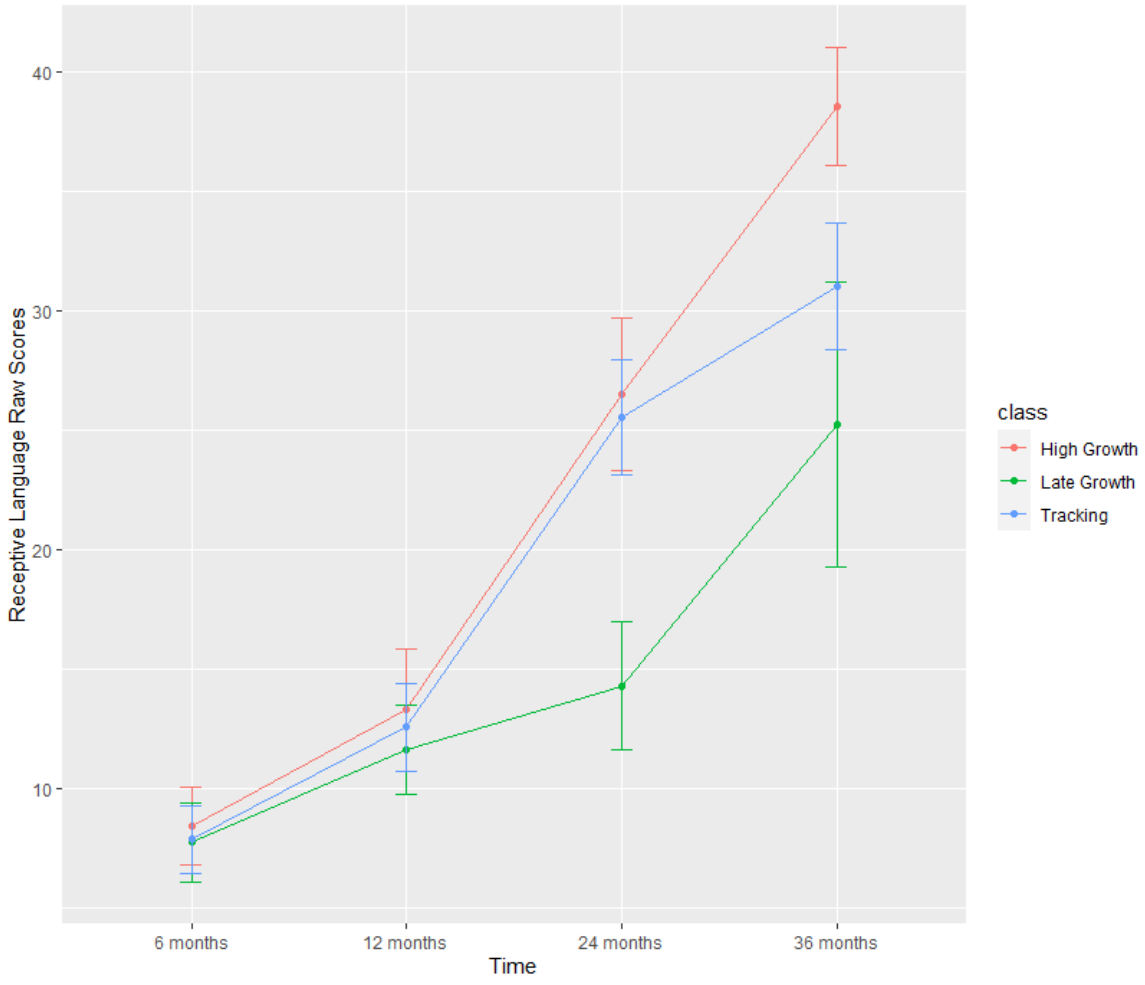
Footnotes for Figure 3.17: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.18: One-Step Latent Class Growth Analysis Plot for Receptive Language for mBP2



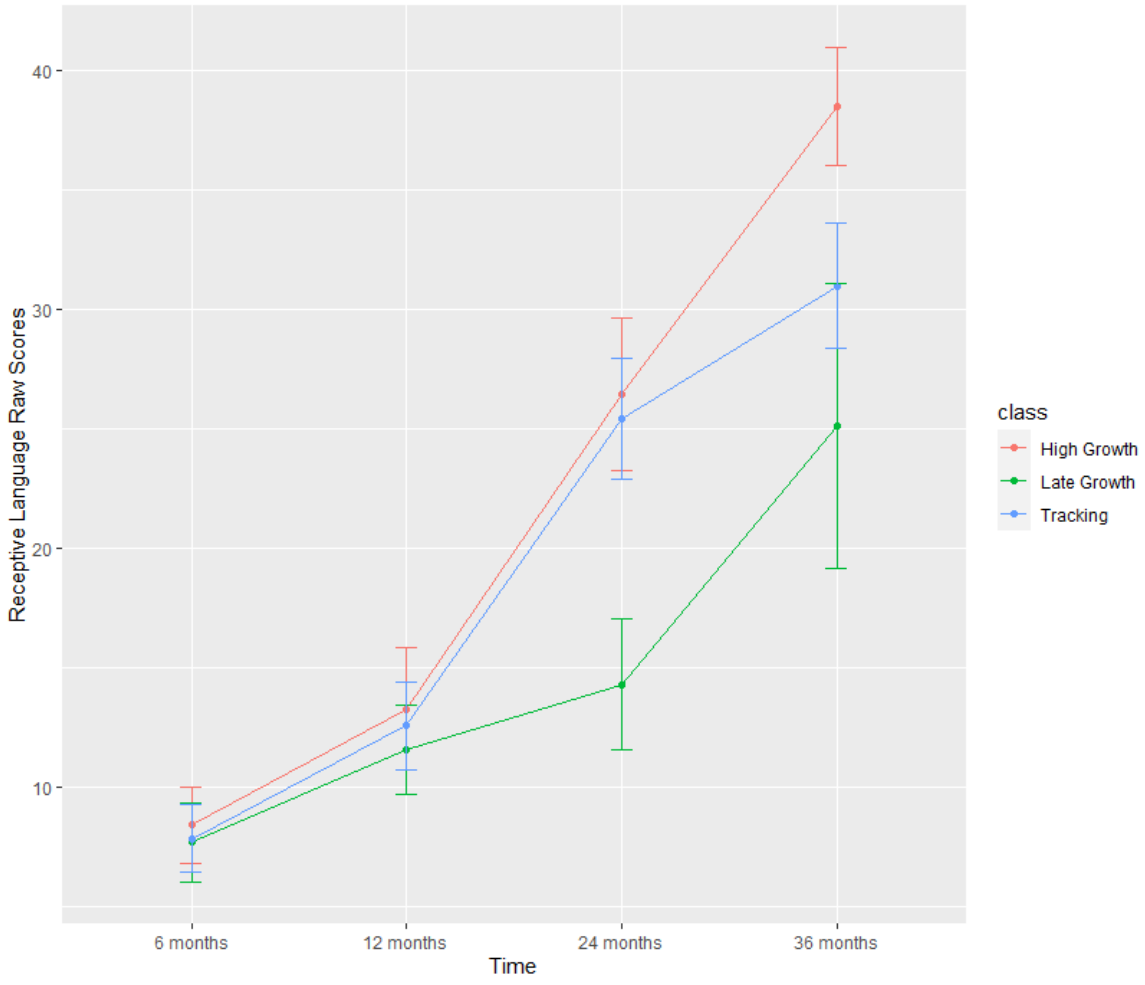
Footnotes for Figure 3.18: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.19: One-Step Latent Class Growth Analysis Plot for Receptive Language for mBzP2



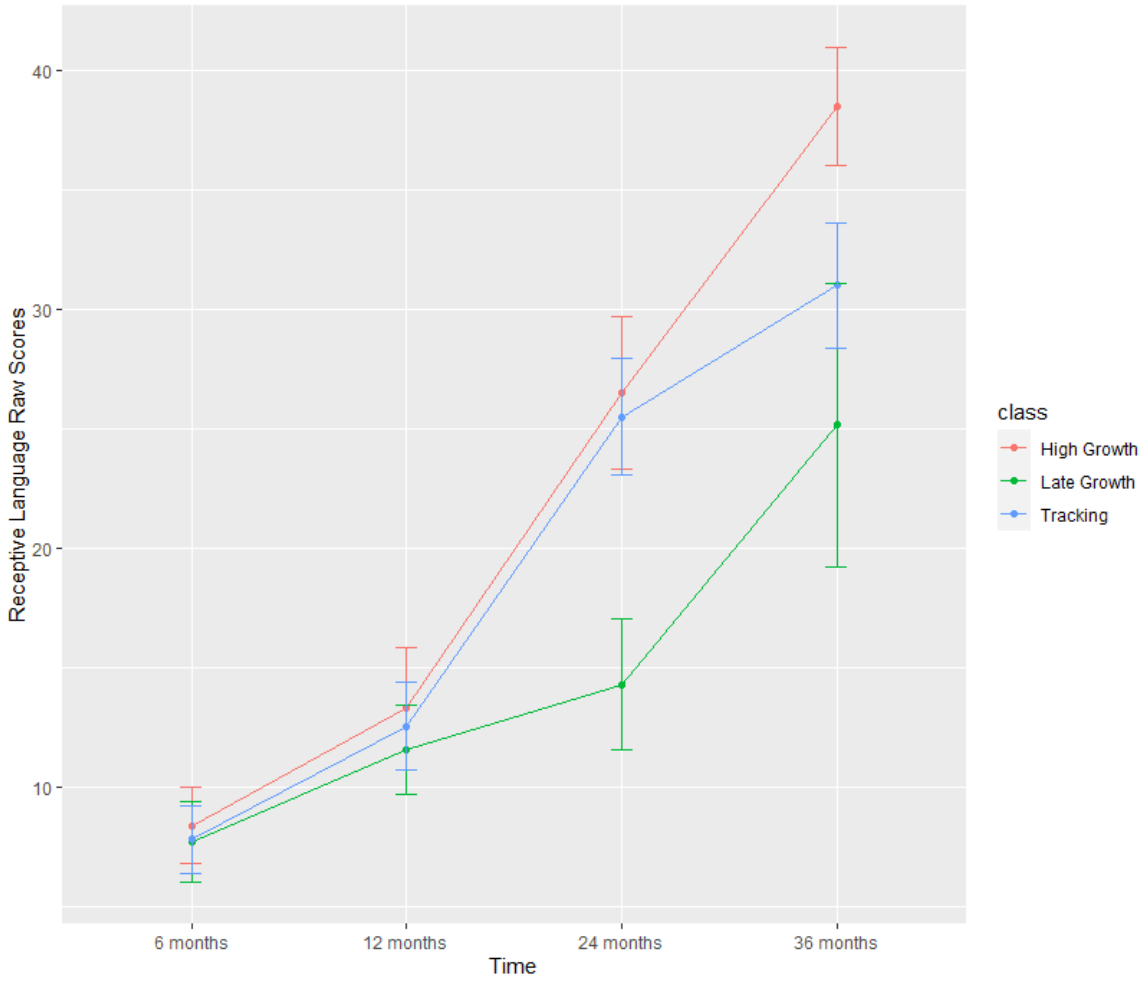
Footnotes for Figure 3.19: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.20: One-Step Latent Class Growth Analysis Plot for Receptive Language for mCNP



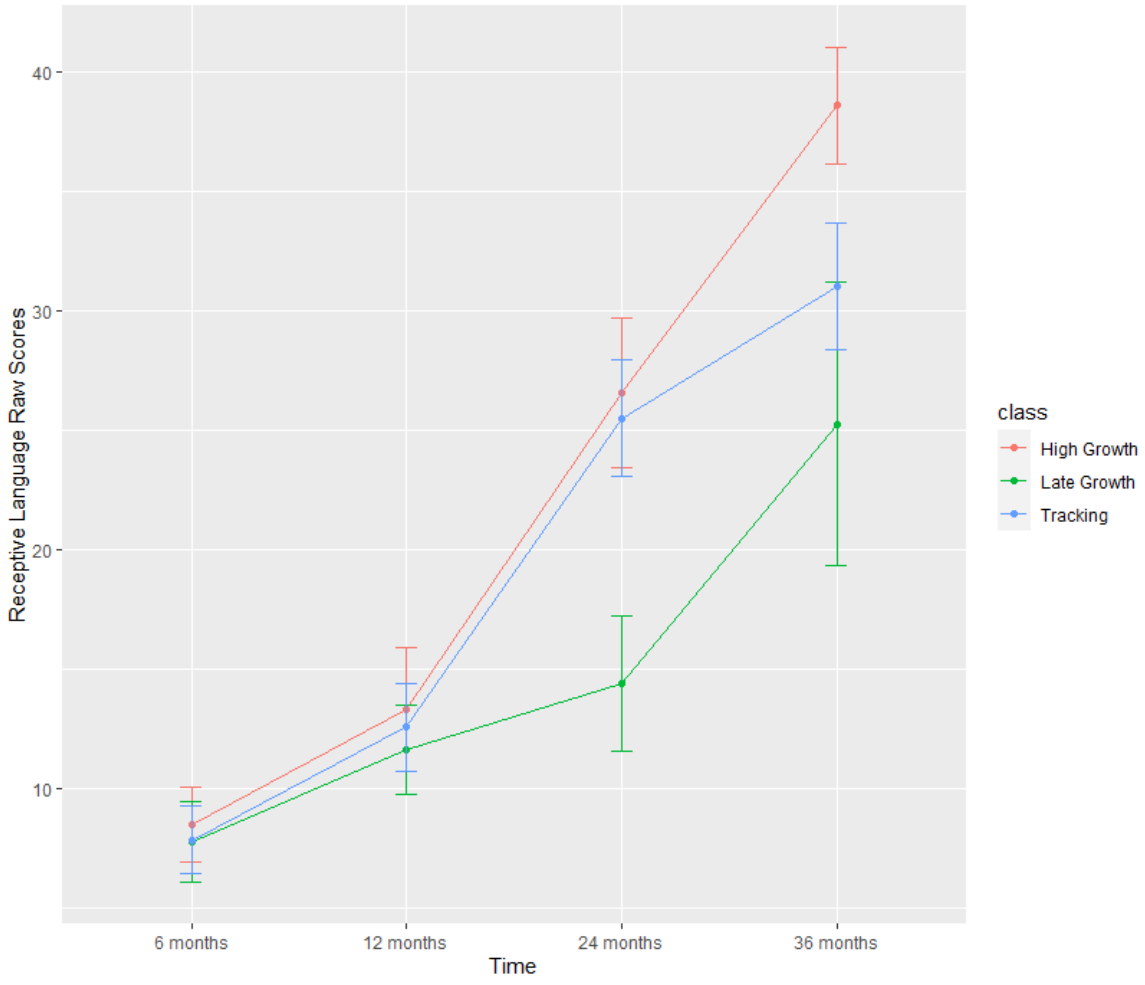
Footnotes for Figure 3.20: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.21: One-Step Latent Class Growth Analysis Plot for Receptive Language for mCOP



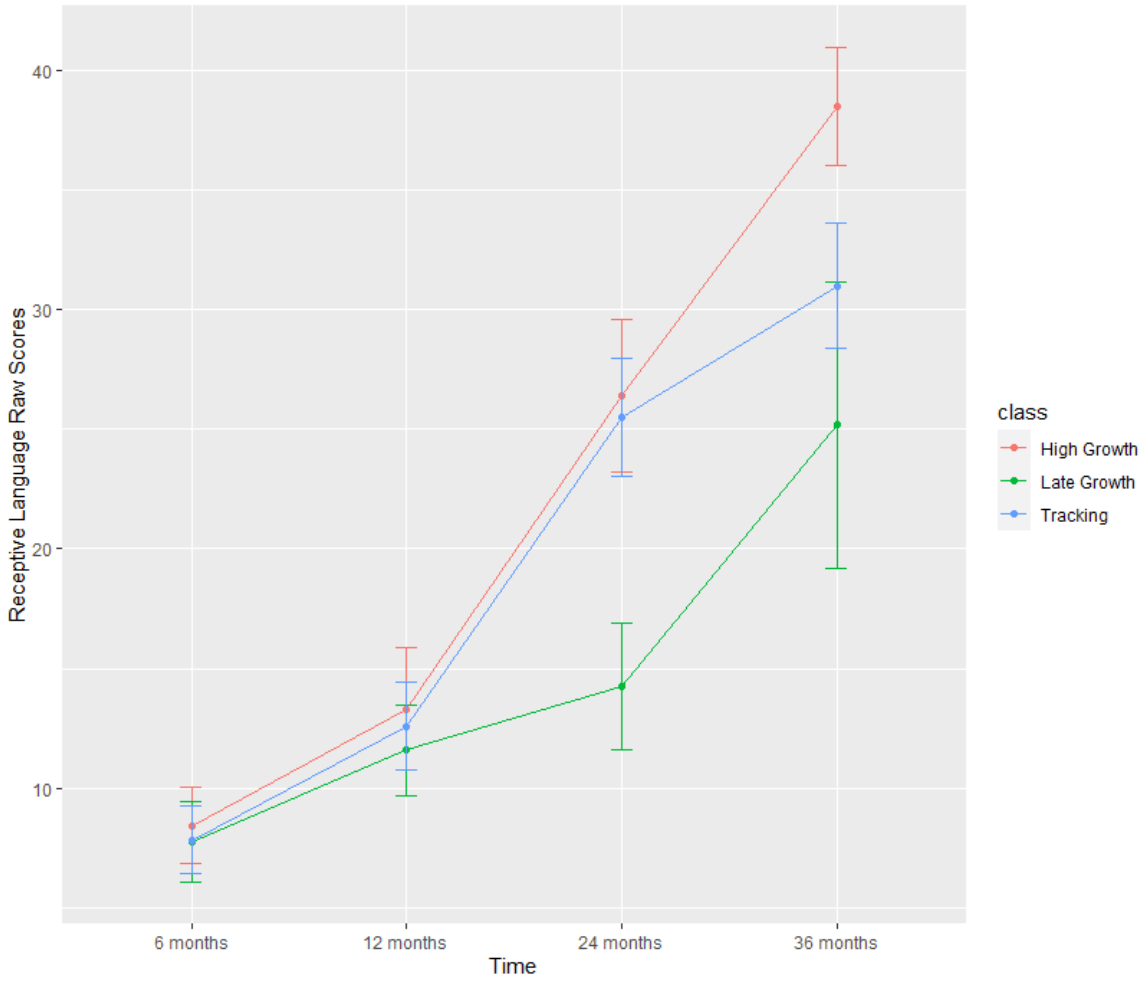
Footnotes for Figure 3.21: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.22: One-Step Latent Class Growth Analysis Plot for Receptive Language for mCPP



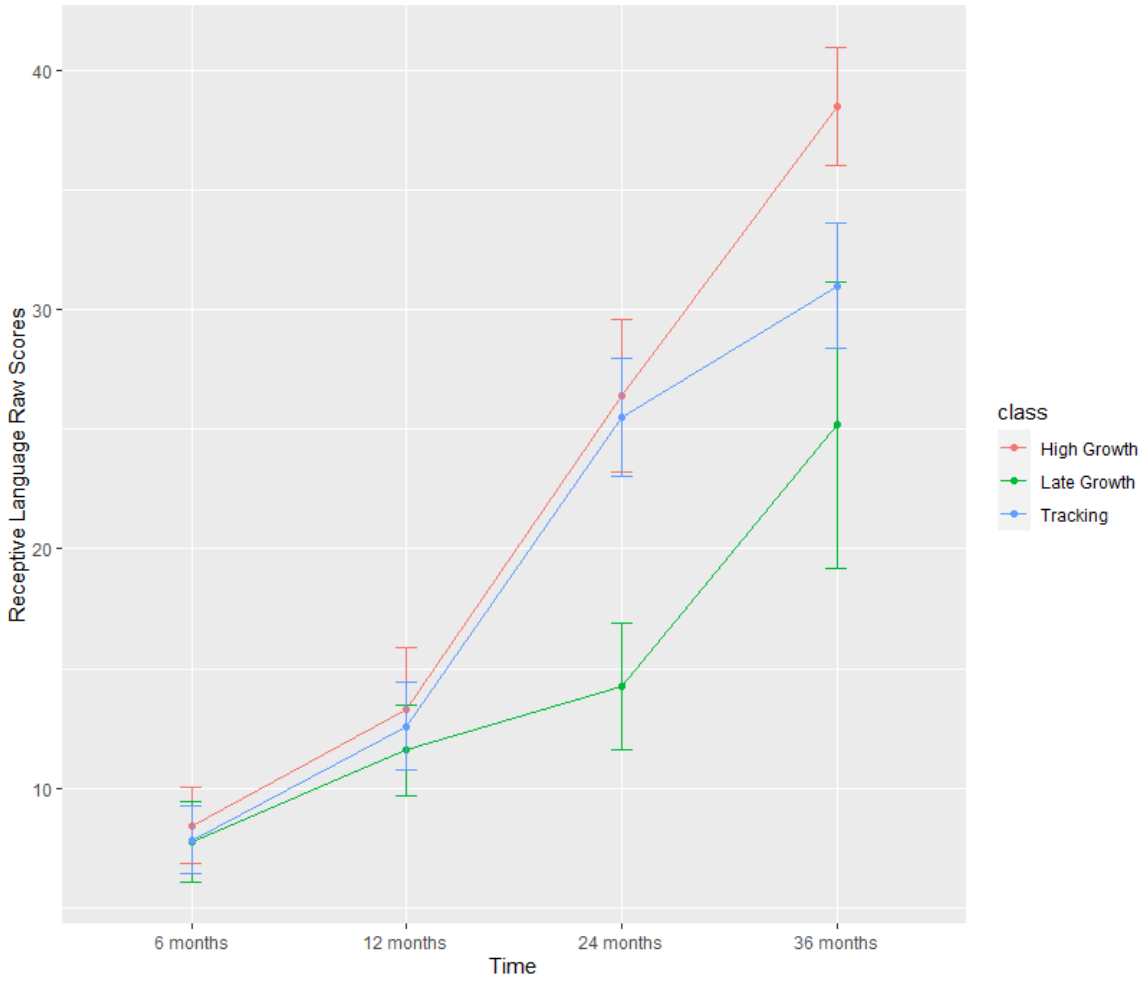
Footnotes for Figure 3.22: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.23: One-Step Latent Class Growth Analysis Plot for Receptive Language for mECPP



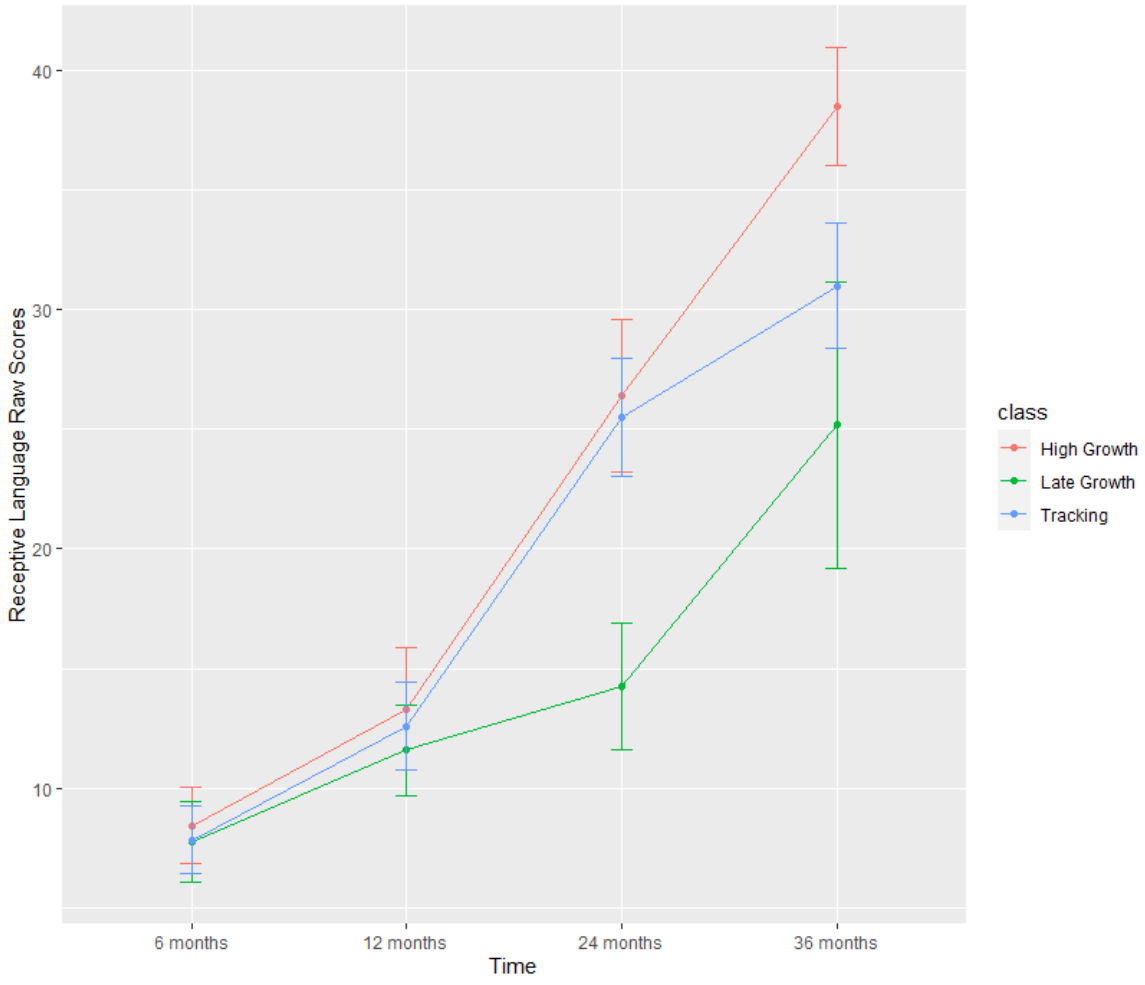
Footnotes for Figure 3.23: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.24: One-Step Latent Class Growth Analysis Plot for Receptive Language for mEHHP



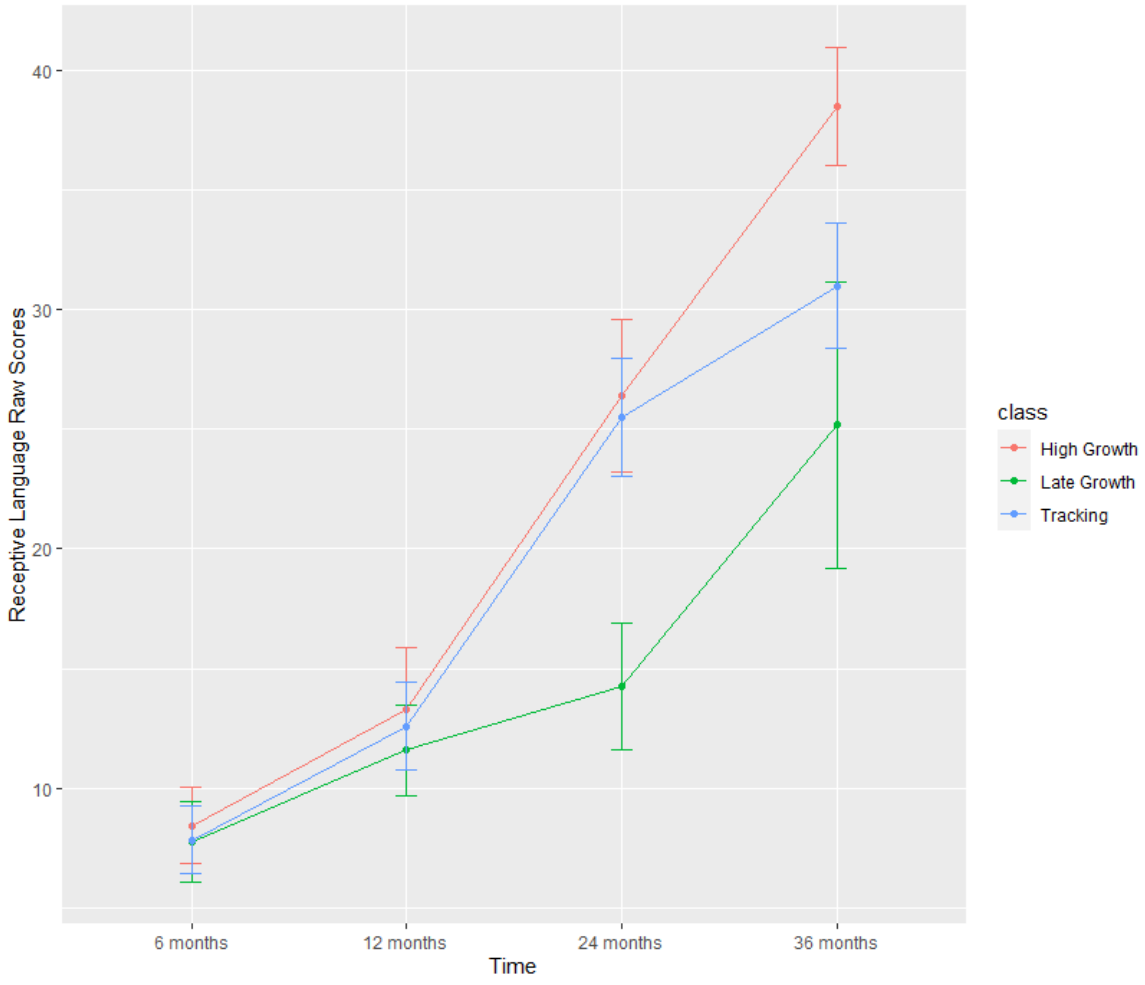
Footnotes for Figure 3.24: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.25: One-Step Latent Class Growth Analysis Plot for Receptive Language for mEHP



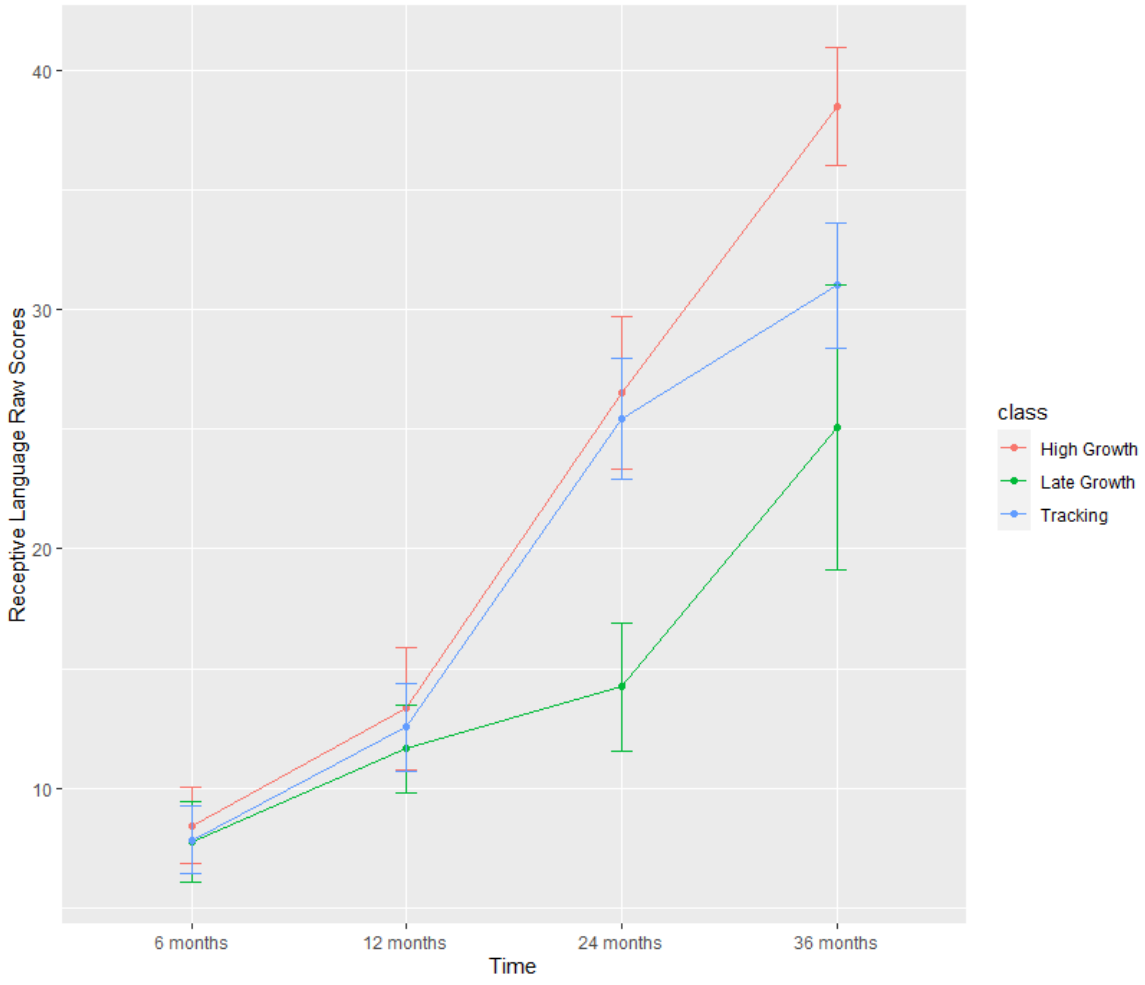
Footnotes for Figure 3.25: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.26: One-Step Latent Class Growth Analysis Plot for Receptive Language for mEOHP



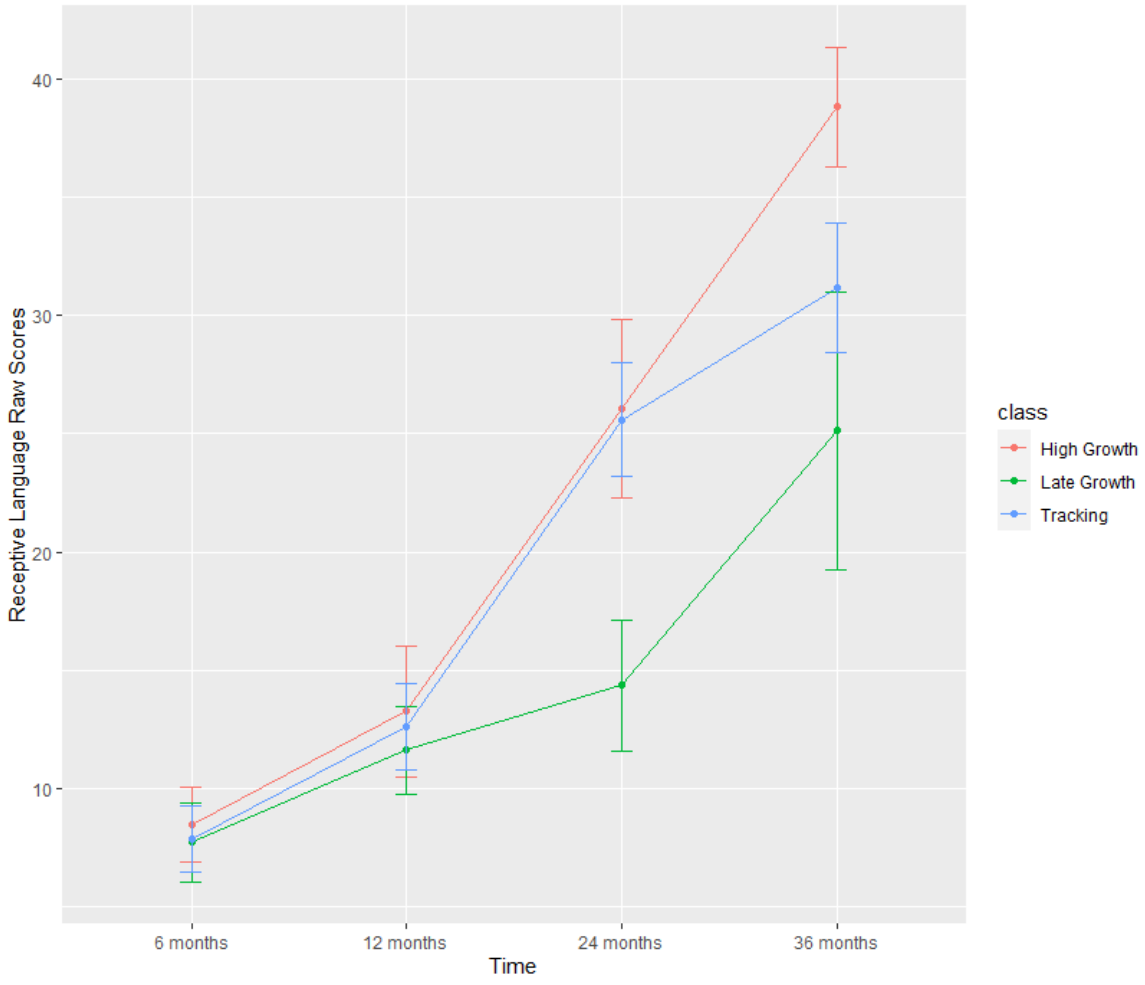
Footnotes for Figure 3.26: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.27: One-Step Latent Class Growth Analysis Plot for Receptive Language for mEP2



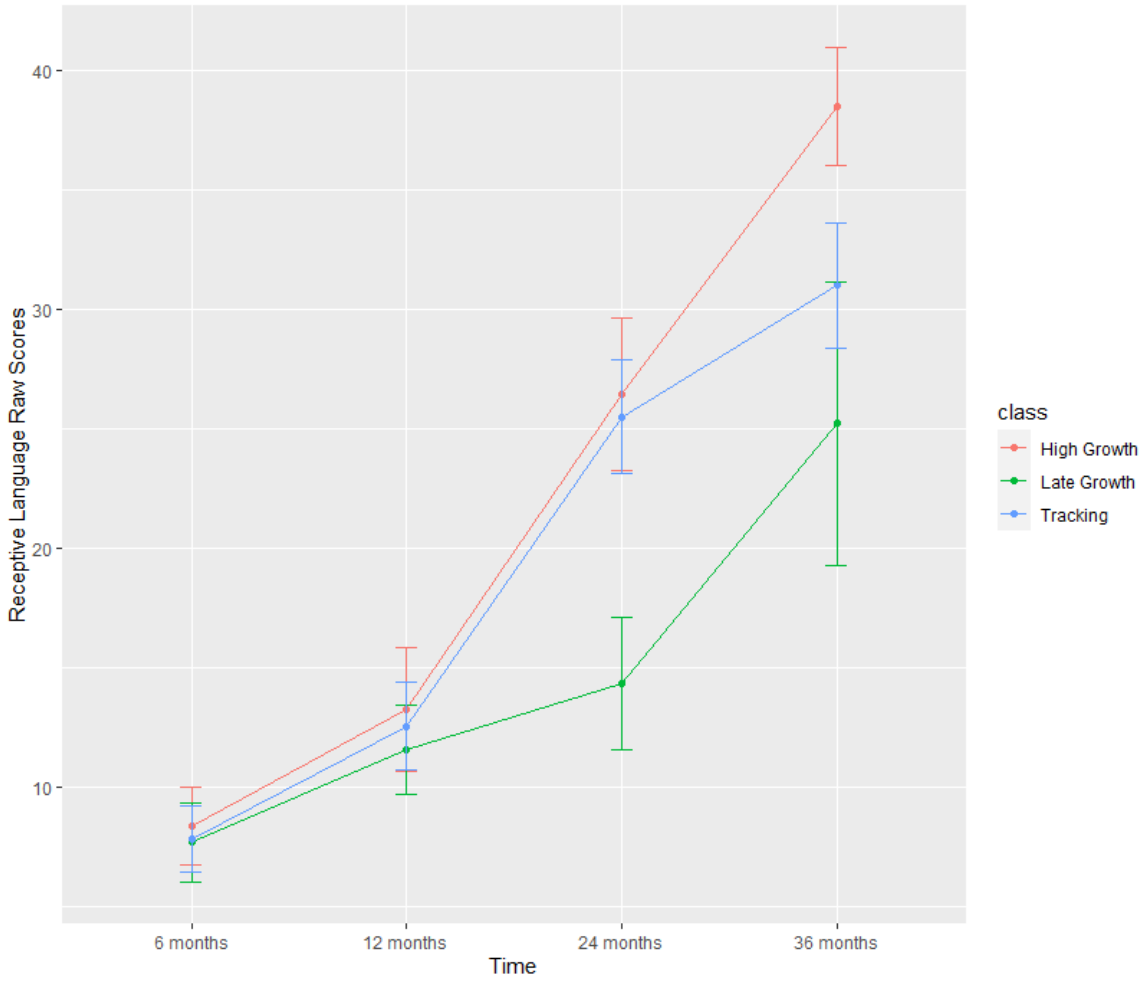
Footnotes for Figure 3.27: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.28: One-Step Latent Class Growth Analysis Plot for Receptive Language for MHBP



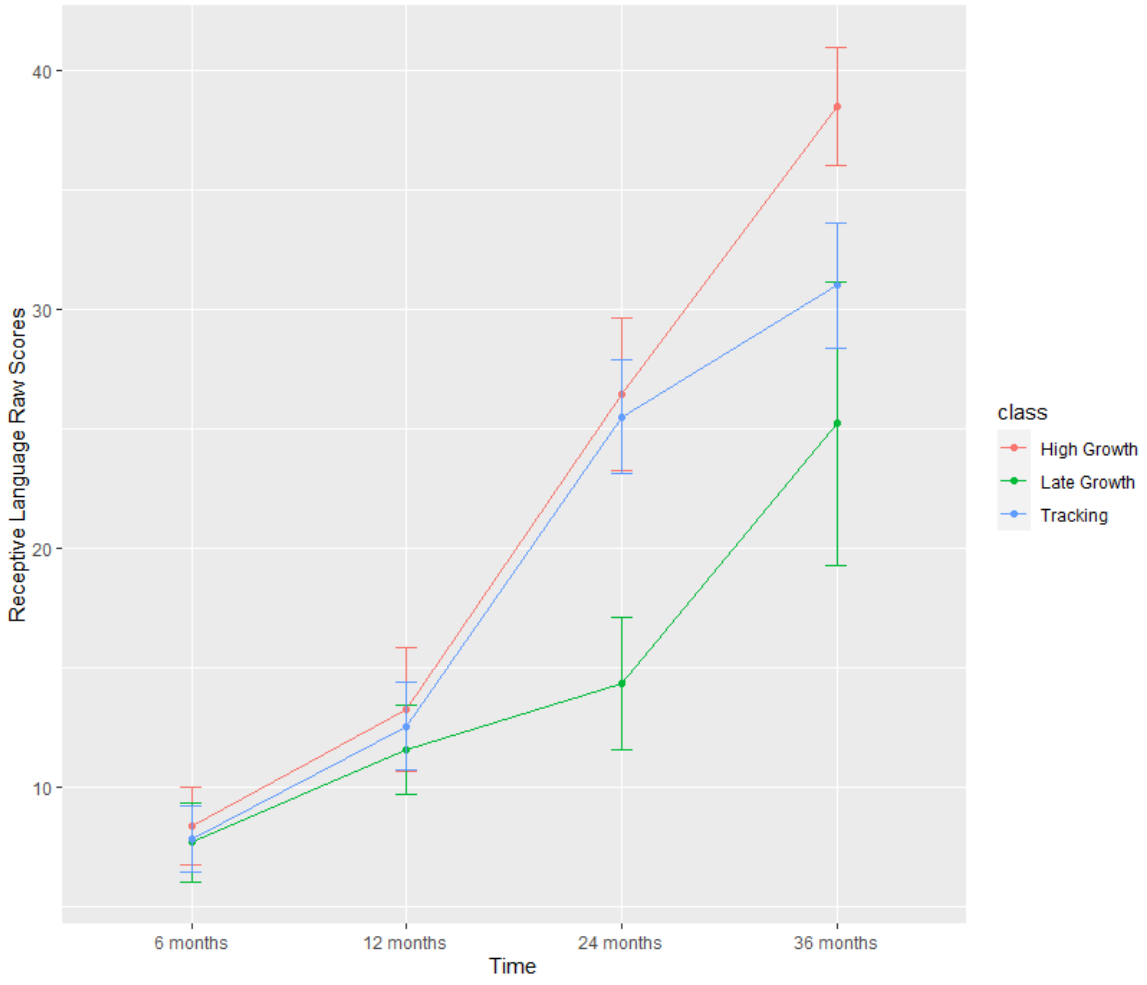
Footnotes for Figure 3.28: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.29: One-Step Latent Class Growth Analysis Plot for Receptive Language for MHiBP



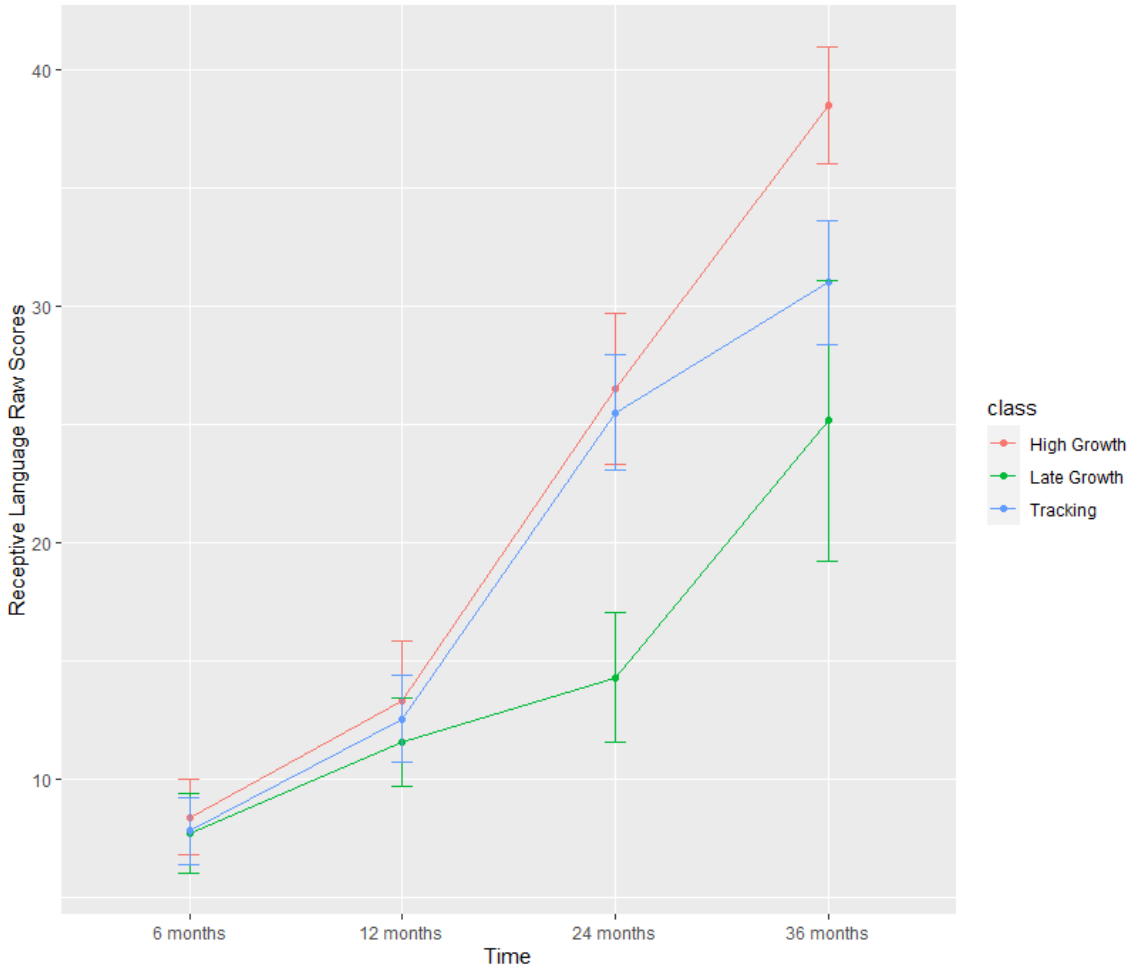
Footnotes for Figure 3.29: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.30: One-Step Latent Class Growth Analysis Plot for Receptive Language for miBP



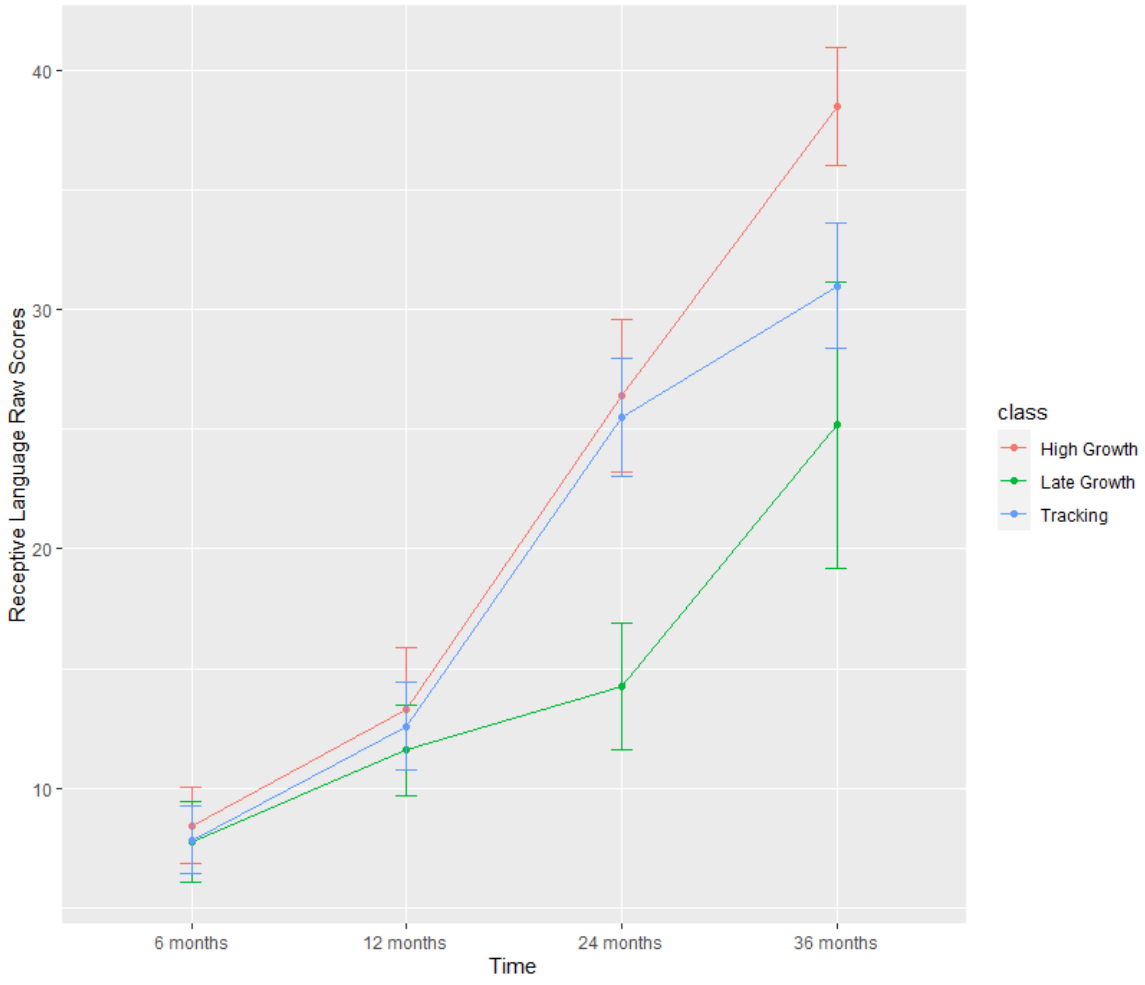
Footnotes for Figure 3.30: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.31: One-Step Latent Class Growth Analysis Plot for Receptive Language for mNP2



Footnotes for Figure 3.31: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Figure 3.32: One-Step Latent Class Growth Analysis Plot for Receptive Language for DEHP



Footnotes for Figure 3.32: Model was adjusted for child sex, maternal age at birth, maternal race/ethnicity, highest maternal educational attainment, and birth season; Points indicate mean scores at that age with 95 percent confidence intervals

Chapter 4 - The Impact of Prenatal Air Toxic Exposure on Language Development Trajectories in Siblings of Children with Autism

Abstract

Background: Language development is a critical part of neurodevelopment that is correlated with many neurodevelopmental disorders. We aimed to examine how prenatal air toxic exposure affects early childhood language development, utilizing a robust longitudinal analysis methodology.

Methods: Participants were drawn from the Early Autism Risk Longitudinal Investigation (EARLI) (n=251) and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) (n=393) cohorts that recruited pregnant mothers who previously had a child with autism (ASD). Expressive and receptive language development was measured using the Mullen Scales of Early Learning (MSEL) at ages 6,12, 24, and 36 months of age. A total of 53 air toxics were assessed by utilizing census tract data to assign exposures via the National Air Toxics Assessment (NATA) datasets from 2011 and 2014. We used latent class growth analysis (LCGA) to determine language trajectories based on MSEL receptive or expressive language raw scores, prenatal air toxic exposure, cohort, child sex, maternal age, maternal race/ethnicity, homeownership, and maternal educational attainment.

Results: We found 3 trajectories for both expressive and receptive language using both a two-step LCGA approach. Most air toxics were not statistically significant. Acetaldehyde showed significant protective effects for expressive language, decreasing the risk of falling into the Low Growth category for each IQR increase in concentration.

Conclusion: Most air toxics were not statistically significant, with only acetaldehyde showing significant protective effects. Observed trends in risk were

inconsistent with prior literature, which may be due to small sample size and differences in analysis methodology.

Introduction

Language development is a key milestone in early childhood development, playing important roles in later stages of life. Delays or impairments in language development can lead to atypical social and emotional development, poor academic performance, and increase risk of certain neurodevelopmental disorders (Beitchman & Brownlie, 2012; Howlin & Udwin, 2002; Irwin et al., 2002; Johnson et al., 1999; Roulstone et al., 2011; Snowling et al., 2006; Whitehouse et al., 2009). Common social factors that may influence language development include family history, child sex, maternal education, and home environment (28,187,188). However, environmental and chemical factors are still an area that requires additional exploration.

Certain systems are critical to early neurodevelopment, particularly the endocrine system. Because the hormones generated by the endocrine system play critical roles during fetal development, disruption of this system can lead to language development. Environmental pollutants that disrupt this system, known as endocrine disrupting chemicals (EDCs) are therefore of vital interest, having a higher *a priori* suspicion of causing language impairment. As such, understanding environmental causes that may act as EDCs will not only provide a better understanding behind the etiology of language disorders, but also provide stakeholders with vital knowledge to make impactful and lasting policy changes to reduce overall risk.

Air toxics, as defined by the United States Environmental Protection Agency (EPA), are toxic or hazardous air pollutants that cause or may cause serious health effects such as cancer, reproductive effects, and/or adverse environmental and ecological effects (116,117). Because of this broad definition, there are several classes

of chemicals that are considered to be air toxics; the Clean Air Act identifies a total of 187 air toxics the EPA is required to control, though there are many more (117). Polychlorinated biphenyls (PCBs), phthalates, polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), certain heavy metals, nitrogen oxides (NO_2 and NO_X), particulate matter (specifically $PM_{2.5}$), and certain volatile organic compounds (VOCs) are a few examples of air toxics with known endocrine disrupting effects (61,123–142,217–219). The mechanisms by which these air toxics cause these effects are varied, with some mimicking sex hormones (123,143–145), while others disrupt thyroid hormone pathways (123,124,146–149).

Because many air toxics are considered EDCs, their impact on neurodevelopment, and thus language development, must be considered. Several reviews have found that various EDCs that are considered air toxics impact neurodevelopment and language development specifically. Davis et al. found in their review that PCBs and certain pesticides were negatively associated with language delays, and Suades-Gonzalez et al. found PAHs to be negatively associated with language development (220,221). However, some studies have found that exposure to certain air toxics does not lead to language delays. Guxens et al. did a comprehensive study over six European cohorts, finding no association between NO_2, NO_X, particulate matter, and language development (222). Stingone et al. found that perchloroethylene did not influence standardized language test scores, and Huang et al. and Gascon et al. both found that certain phthalates did not negatively affect language (97,103,223). However, it should be noted that these studies did not specify the primary exposure

source for these EDCs. Rather, these studies examined all exposure, rather than focusing on airborne exposure to these EDCs.

There have been several studies that have primarily focused on airborne exposure to air toxics that are EDCs, rather than all exposure, and their possible connections with neurodevelopment. Exposure to airborne PAHs were found to be associated with a decrease in infant mental development across several studies using various measures of neurodevelopment, including the Bayley's Scales of Infant Development and Raven Coloured Progressive Matrices (21,61,62). Nitrogen dioxide was also found to be negatively associated with infant mental development in Freire et al. and resulted in a decrease in all subscales of the McCarthy Scales of Children's Abilities in Guxens et al. (224,225). Guxens additionally found that benzene was also negatively associated with infant mental development (225). There have also been multiple studies that have looked at hazardous air pollutants and their association with autism spectrum disorder, with some airborne EDCs being associated with ASD, such as certain heavy metals, methylene chloride, styrene, certain conjugates of xylene, and other aromatic solvents (118,119,159,160). A review by Volk et al. found comprehensive evidence that prenatal air pollution exposure is linked to various neurodevelopmental disorders and delays (226). However, there is a lack of literature utilizing trajectory analysis to assess this connection, as well as a lack of literature that specifically examines the impact of air toxics on language development.

We conducted a study based on data collected from two ASD sibling cohorts. We examined the association of prenatal air toxic exposure to language development

trajectories to explore potential associations between those trajectories with prenatal air toxic exposure.

Methods

Population

We included individuals in the Early Autism Risk Longitudinal Investigation (EARLI) and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) cohorts (173,174). Briefly, both studies recruited pregnant women who already had a child with a diagnosis of ASD and families whose fathers who had a biological child with ASD, with EARLI recruiting from northeast Maryland, southeast Pennsylvania, and northern California, while MARBLES recruited primarily from northern California. Both studies followed the mother and the expected child longitudinally, collecting demographic, neurodevelopmental, and exposure data at set time intervals. Children in EARLI were born between 2009 and 2013, while children in MARBLES were born between 2006 and 2023. Demographic information was obtained via in-person interviews and questionnaires.

Language Development and Neurodevelopment Measurement

The Mullen Scales of Early Learning (MSEL, 20) was used in order to assess language development, which was administered by trained study staff at 6, 12, 24, and 36 months of age at in-person study visits. Briefly, the MSEL is a standardized psychometric test that is used to measure cognitive development in children ages 3 to 60 months, providing scores on five different domains: gross motor, fine motor, expressive language, receptive language, and visual reception (175). Expressive

language involves the use of words and gestures to convey accurate and appropriate messages, thoughts, and ideas to others. Receptive language involves the ability to understand and process the meaning of language directed towards an individual. We used both expressive and receptive language raw scores to generate our language development trajectories, using the 6, 12, 24 and 36 month assessments.

Air Toxic Measurements

We assessed air toxic exposure by using an emissions-based model from the US EPA: the National Air Toxics Assessment (NATA). The NATA model is run every 3 years, and uses inputs from the National Emissions Inventory to generate the average ambient concentration of multiple air toxics. The national Emission Inventory provides estimate of air emissions of criteria pollutants and air toxics using emission sources, including point sources (e.g. industrial facilities, electric power plants), nonpoint sources (e.g. residential heating, commercial combustion), onroad sources (i.e. emission from onroad vehicles), nonroad sources (e.g. construction equipment, locomotives), and fire sources (227). A hybrid model that combines a regional air-quality model (CMAQ) and dispersion model (AERMOD) is then used to estimate annual average ambient air toxic concentrations across US census tracts (228,229). The NATA model has been shown to be reasonably accurate with its estimations, with one validation study confirming that NATA could predict national medians of most toxics well, though there are some instances where the model may overestimate or underestimate certain types of toxics from specific sources (230–232).

We used both the 2011 and 2014 NATA datasets. A total of 180 air toxics were assessed in 2011 and 181 air toxics in 2014. We selected 82 air toxics with known or

suspected endocrine disrupting effects based on prior literature (Table 4.6). We included air toxics that were measured in both the 2011 and 2014 datasets, leaving 53 total air toxics. Individuals who were born after 2012 (i.e. a birth year of 2013 to 2016) were assigned exposure corresponding to the 2014 NATA dataset, while those born in 2006 to 2012 were assigned exposure corresponding to the 2011 NATA dataset. The NATA datasets are publicly available on the US EPA website (https://www.epa.gov/national-air-toxics-assessment), and we used Total Concentration for all air toxics from both the 2011 and 2014 datasets, measured in ug/m^3 (233,234). Air toxic exposure was assigned via census tract. Home addresses were obtained from study participants at enrollment, which were then used to assign 2010 census tracts to individuals. These census tracts were then used to connect to state-specific NATA datasets for each air toxic exposure. Out of the original 621 individuals, 74 were not assigned a census tract, who were removed from the final analysis, as air toxic exposure could not be assigned.

To assist in the comparison of results and to better examine patterns that may arise due to correlations between air toxics, we grouped air toxics using agglomerative hierarchical clustering based on multiscale bootstrap resampling by using the pvclust function in the *pvclust* package in R. Briefly, agglomerative hierarchical clustering is a nesting algorithm that combines two variables, called clusters, that are the most similar into one larger cluster. This process is repeated until all variables are in one cluster, creating a dendrogram. We used Ward's Method for clustering and used Euclidian distance, defined as $\sqrt{\sum_i (x_i - y_i)^2}$, where x and y are two vectors (i.e. air toxic

measurements). Groupings were determined based on approximately unbiased test p-value, with an alpha of 0.95 or higher (235).

We performed an AIC analysis on several key air toxics to determine if a transformation was required. These air toxics were selected due to known toxicities (e.g. lead and mercury) or that were observed to have high magnitudes of risk in a preliminary examination, where they were transformed first by winsorizing to the 99th percentile and then dividing by their interquartile range. We calculated AIC based on the following transformations, along with their untransformed values: natural log, log base 2, log base 10, squared, root, winsorizing to the 99th percentile and dividing by interquartile range, and categorized based on quantiles (Table 4.8). Based on the AIC observed, we elected to transform all air toxics by winsorizing to the 99th percentile and dividing by their interquartile range.

Statistical Analysis

A total of 621 individuals were in the initial study populations. We excluded persons with 1 or no recorded language scores (n=89) or who were missing all air toxic measurements (n=74), yielding a sample size of 471 individuals (Table 4.1). The most frequently missing variables were expressive and receptive language scores at 24 months: 38.9% and 39.1% missing, respectively (Table 4.7). Other missing covariates include expressive and receptive Mullen scores at other time points (14.0% for 6 months, 5.9% for 12 months, and 6.2% and 6.4% for 36 months), maternal educational attainment (0.6%), maternal race (1.3%), and homeownership (4.3%). To include persons missing covariates or Mullen scores, we used multiple imputation by chained equations using the R package *mice* to impute missing values for variables listed above,

including Mullen scores (178). Imputed distribution of these variables can be seen in Table 4.7.

We used a latent class growth analysis (LCGA) approach to analyze the relationship between individual air toxics and language development trajectories, separately modeling expressive vs. receptive language. LCGA is a method to identify unmeasured (or latent) class membership using observed variables, with the goal of creating classes (or in our case, trajectories) so that individuals within a trajectory class are more similar than individuals between trajectory classes (179,180).

We assigned language trajectories to individuals via an LCGA with the expressive or receptive language raw scores as the inputs, and in a separate model regress these on air toxic exposures. We ran several LCGA models with different numbers of classes, ranging from 2 to 7 classes. While the following measures of fit were available to us (Bayesian information criteria (BIC), sample size adjusted BIC (SABIC), integrated complete likelihood (ICL), and entropy), we prioritized low BIC when selecting the best model, following recommendations by Diallo et al. (181). Additionally, we favored models with fewer classes to aid in interpretability and avoided models with low number of individuals within classes (<10% of the population). After individuals were assigned, a multinomial logistic regression was performed using the assigned classes as the outcome and air toxic exposure as our main predictor. We adjusted all models for cohort (EARLI vs. MARBLES) and also accounted for the following a priori potential predictors of language: child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership (as proxies for socioeconomic status) (27–30).

We assessed the coefficients and confidence intervals for each air toxic as a predictor of language trajectory class. Following each process, each person was assigned a probability of belonging to each trajectory class. For tables and figures, we assigned each person to their highest probability class. We selected class names based on the overall shape of the class in the plot, guided by designations used in prior literature. Because of the high number of statistical tests run, we also adjusted for multiple comparisons by using the Benjamini and Hochberg false discovery rate (236). The results of this adjustment can be found on Table 4.3.

In order to assess the potential impact of air toxic mixtures, we conducted a Bayesian kernel machine regression (BKMR). Briefly, BKMR utilizes a kernel function to represent the exposure-outcome response, which allows the model to make use of a large number of potential exposures (237,238). Because BKMR requires a binary outcome, if more than 2 trajectory classes are found to be ideal, then the "non-typically developing" trajectories will be combined to form a binary outcome for analysis. We then examined whether any interaction terms between exposures were statistically significant, along with the overall impact of the mixture model. Due to the high correlation between certain air toxics, we elected to use hierarchical variable selection, grouping air toxics that had a Spearman correlation of 0.6 or greater together prior to analysis (Figure 4.4).

All analyses were conducted using Rstudio version 2022.02.1 Build 461 with R Version 4.1.3 "One Push-Up". The LCGA was conducted using the *lcmm* package (182).

Results

Males had slightly lower MSEL raw scores than females at 36 months of age (Table 4.1). Children whose mothers were 30 or younger or who did not receive at least a college degree had lower MSEL raw scores than children who had mothers older than 30 or who received at least a college degree. Children of African American or Hispanic mothers also had lower MSEL raw scores than children from non-Hispanic white or multiracial mothers. Children whose parents rented had lower MSEL scores than children of homeowners. There was no appreciable difference in MSEL scores between birth seasons.

Hierarchical clustering denoted 7 total groupings of air toxics (Figure 4.3). These groupings are likely due to air toxics within the group sharing common sources, or that may act on the body using similar or identical pathophysiologies. It is also probable that some of the groups are due to those members belonging to the same class of chemicals (e.g. volatile organic compounds).

For expressive language, 3 trajectory classes were optimal for capturing patterns in language development. These trajectory classes were also named High Growth, Tracking, and Low Growth (Figure 4.1). We used High Growth as the referent class for our multinomial regression. In general, we observed that associations of many air toxics with expressive language trajectories were not statistically significant (Table 4.2). However, lead, naphthalene, and acetaldehyde were statistically significant, decreasing risk of falling into the Low Growth trajectory class for every IQR increase in concentration.

For receptive language, 3 trajectory classes were optimal for capturing patterns in language development, which we named High Growth, Tracking, and Low Growth

(Figure 4.2). We used High Growth as the referent class for our multinomial regression. We observed that none of the air toxics examined were not statistically significant (Table 4.2).

The posterior probabilities of inclusion for air toxics groups (Group PIP) and each individual air toxic (Conditional PIP) can be found in Tables 4.4 and 4.5, which indicate the relative importance of the air toxic, both within their defined groups and individually, to the mixture model. Our BKMR analysis indicates that there is a slight increase in overall risk of falling into a non-typically developing trajectory for both expressive and receptive language as overall air toxic concentration increases, though there is a drop in risk at higher quantiles (Figures 4.5 and 4.8). However, this increase in risk was not statistically significant. In addition, for both expressive and receptive language, interactions between air toxics do not appear to be statistically significant (Figures 4.7 and 4.10), although there is an indication that a general direction may be present for some interactions (Figures 4.6 and 4.9).

Discussion

The goal of this study was to characterize language development trajectories in two cohorts of children with enhanced risk for autism and evaluate if prenatal air toxic exposure was associated with delayed or abnormal language development. Results indicated that most air toxics were not associated with impaired language trajectories. Prior to adjusting for multiple comparisons, lead, naphthalene, and acetaldehyde were statistically significant for expressive language, leading to a decrease in risk of falling into the Low Growth trajectory class for each IQR increase. Following adjustment for multiple testing, only acetaldehyde remained statistically significant.

Acetaldehyde is a volatile organic compound (VOC) that's commonly used as a solvent in industrial applications. Our results indicated that acetaldehyde had a protective effect on language development; that is, higher levels of prenatal exposure led to a decreased risk of belonging to an abnormal language trajectory. Prior literature has found mixed results regarding exposure to airborne acetaldehyde and its effect on neurodevelopment. While von Ehrenstein et al. found that exposure increased the odds for ASD, Kalkbrenner et al. did not find this result (24,160). The results from Kalkbrenner et al. showed decreased odds for ASD, as well as slightly higher scores on the Social Responsiveness Scales, though results were not statistically significant (24). Further, Madaniyazi et al. also found that acetaldehyde did not significantly impact various measures of neurodevelopment as reported by the Ages and Stages Questionnaire, which included a measure of language (239).

Lead was found to be protective in our results; that is, higher exposure to lead was associated with a decreased risk of belonging to an abnormal trajectory class for both expressive and receptive language. While these results were not statistically significant when adjusted for multiple testing, it nonetheless is inconsistent with prior studies that show that lead is neurotoxic. Several prior studies have also shown that higher levels of lead exposure were associated with worse language development. Hou et al. found that blood lead levels were associated with worse neurodevelopmental outcomes in children, including language development as measured by the Gesell Development Schedules (240). Lin et al. found that prenatal exposure to lead was associated with lower language quotients as measured by the Comprehensive Developmental Inventory for Infants and Toddlers within 2 year olds (241). Campbell et

al. found that higher bone lead levels in male teenagers from 11 to 14 years old decreased language processing performance, lending evidence that lead exposure continues to negatively impact language past childhood into adolescence (242).

One of the groups created via hierarchical clustering included the toxics toluene, xylene, ethylbenzene, and hexane. These toxics fall under a class of chemicals known as volatile organic compounds, or VOCs. All toxics within this group were shown to have protective effects for both expressive and receptive language, although the effects were not statistically significant; that is, an increase in prenatal exposure led to a decreased risk of falling into an abnormal language trajectory for all four of the toxics. This result is contradictory to prior literature that has shown xylene, toluene, and ethylbenzene to be neurotoxic in nature. Madaniyazi et al. found that increases in the concentration of certain forms of xylene were associated with lower scores in the Ages and Stages Questionnaire, while Grandjean and Landrigan found in a review that toluene exposure can lead to deficits in neurodevelopment (239,243). Additionally, Von Ehrenstein et al. found that ethylbenzene, toluene, and xylenes were associated with a greater risk for autism spectrum disorder (160). Kalkbrenner et al. also found that ethylbenzene, hexane, toluene, and xylenes all increased the risk of autism, though only ethylbenzene and xylenes were statistically significant. However, our results show that all four toxics had protective effects, although our results were not statistically significant. This may be due to random chance due to multiple testing, or it may be due to the relatively small sample size, which led to comparatively smaller populations that fall into each of the trajectory classes.

Multiple testing is of particular concern in this study, due to the large number of air toxics examined; in total, we examined 53 air toxics over two different measures of language across three classes. In order to help address the problem of multiple comparisons, we adjusted p-values using the Benjamini and Hochberg false discovery rate (236). After adjusting for multiple comparisons, only acetaldehyde remained statistically significant for expressive language. In addition, some of the groups had a small number of individuals, despite our efforts to choose classes that had greater than 10 percent of the study population. Because of these two factors, it is possible that we found false negatives in our analysis; that is, the fact that we did not find many statistically significant associations does not mean that air toxics are not without risk.

Most prior studies that examined airborne air toxics and neurodevelopment found that some toxics were linked to measures of neurodevelopment. However, in our study, after adjusting for multiple comparisons, we only found that acetaldehyde was statistically significant. Furthermore, we found that acetaldehyde decreased the risk of falling into an abnormal language trajectory, which runs counter to our hypothesis that exposure to air toxics would increase risk of neurodevelopmental delays or disorder. While our smaller sample size may explain some of this discrepancies, there may be further reasons for our disparate results. Because we used NATA to collect exposure data, we may not be capturing all sources of airborne exposure to these air toxics, since NATA focuses mostly on outdoor sources of air pollution. As such, our exposure may be underrepresenting the true exposure for some air toxics. It is also possible that our findings were influenced by the uniqueness of our sample, where children were at enhanced risk for autism.

We examined the potential effects of air toxic mixtures on language development trajectories by utilizing BKMR analysis. Our results found that there was an increase in risk in a mixture model as concentrations increased from the 50th quantile up until the 60th quantile, though risk then decreased past the 65th percentile. This change in risk was also not statistically significant. In addition, when we examined interactions between any single air toxic and all other air toxics, we found that there were no statistically significant interactions, though some trends could be seen with some air toxics. It should be noted that our sample size is relatively small, which limits our ability to assess interactions, even within the BKMR framework.

Strengths of our study include the use of language development trajectories as our outcome, which allows for a more detailed and accurate examination of how air toxic affects language development when compared to a single time point outcome. In addition, because we made use of repeated measures for our outcome in order to develop these trajectories, there is a decreased risk of exposure misclassification, as these measures would decrease the probability of incorrect assignment of trajectories. Second, the use of cohorts with heightened familial risk of autism allows for a greater ability to detect abnormal language development trajectories that may be less common in the general population, as these cohorts are at a heightened prevalence for neurodevelopmental delays.

Our results are not applicable to the general population, as both cohorts used were at heightened risk of autism. In addition, while the MSEL is considered to be a suitable psychometric measure of expressive and receptive language (185) and has been shown to have good validity (186), language is a complex phenotype, with multiple

facets to consider. Our analysis is limited to expressive and receptive language only, and only through the methodology of the MSEL. Future research should explore other facets of language, such as vocabulary, grammatical development, or semantics. We are also unable to extrapolate language trajectories after 36 months of age. Future studies should aim to examine language development over a longer period of time, particularly because language can develop rapidly within the first few years of life. Due to the nature of LCGA analysis, our results are sensitive to the number of classes selected. While we followed recommendations outlined in Diallo et al., we may have selected for a non-ideal number of classes, which may have resulted in different results (181). However, we are confident that the overall direction of effect would remain consistent between various class choices. Finally, our study focused on airborne exposure of air toxics, but it is not the only route of exposure for many of the toxics examined. Our study can't account for those additional routes of exposure, nor did it account for the actual body burden the mother experienced. In addition, because NATA is not published every year, we used datasets closest to each child's birth year, which may not reflect the actual concentration of air toxics absorbed during the entire prenatal period.

Tables and Figures

Table 4.1: Mean and Standard Deviation of 36 Month Mullen Scores for Included Population by Child Characteristics

	Expressive language raw score (36 months)	Receptive language raw score (36 months)
Child Sex		
Female (N=200)	32.2 (5.25)	31.6 (4.63)
Male (N=271)	30.2 (6.84)	29.7 (5.90)
Maternal age		
20 to 30 years (N=92)	29.5 (6.81)	29.3 (5.19)
31 to 35 years (N=186)	31.4 (6.03)	30.6 (5.62)
36 to 40 years (N=140)	31.2 (6.12)	31.0 (5.47)
40 to 49 years (N=53)	32.0 (6.44)	31.0 (5.30)
Maternal Race/Ethnicity		

Non-Hispanic White (N=262)	31.7 (5.92)	31.0 (5.30)
Black/African American (N=31)	29.8 (6.28)	29.3 (3.54)
Hispanic (N=90)	29.9 (6.60)	28.7 (6.24)
Other/Multiracial (N=88)	30.7 (6.88)	31.3 (5.36)
Maternal Educational Attainment		
High School/GED or Less (N=43)	28.8 (6.86)	28.0 (4.96)
Some college (N=170)	30.1 (6.28)	29.4 (5.53)
Bachelor's degree (N=149)	32.4 (5.65)	31.4 (5.19)
Graduate or Professional degree (N=109)	31.6 (6.48)	31.9 (5.34)
Homeownership		
Rent (N=178)	30.3 (7.12)	29.6 (6.31)
Own (N=293)	31.5 (5.69)	31.1 (4.83)

Birth Season		
Spring (n=103)	31.1 (5.84)	30.3 (5.03)
Summer (n=141)	30.7 (6.57)	30.0 (5.65)
Fall (n=120)	31.5 (6.47)	31.0 (5.82)
Winter (n=107)	31.0 (6.19)	30.8 (5.27)
Overall (N=471)	31.0 (6.29)	30.5 (5.48)

Footnotes for Table 4.1: Mean and standard deviations calculated after removing missing values

Table 4.2: Relative Risk Ratios for each IQR increase of Air Toxics

Air Toxic	Expressive Language		Receptive Language	
	Tracking	Low Growth	Tracking	Low Growth
Group 1				
Cresol/Cresylic Acid	1.00 (0.78, 1.28)	1.00 (0.70, 1.42)	1.00 (0.67, 1.49)	1.00 (0.64, 1.56)
Phenol	1.00 (0.78, 1.28)	1.00 (0.70, 1.42)	1.00 (0.67, 1.49)	1.00 (0.64, 1.56)
Group 2				
DEHP	0.97 (0.76, 1.25)	0.96 (0.67, 1.38)	0.99 (0.67, 1.48)	0.97 (0.62, 1.52)
Carbon Disulfide	0.99 (0.77, 1.27)	0.98 (0.68, 1.41)	1.00 (0.67, 1.49)	0.99 (0.63, 1.55)
Group 3				
Xylenes	0.90 (0.71, 1.16)	0.76 (0.53, 1.10)	0.97 (0.65, 1.43)	0.69 (0.44, 1.08)
Toluene	0.88 (0.69, 1.12)	0.74 (0.52, 1.07)	0.99 (0.67, 1.47)	0.76 (0.49, 1.19)
Ethylbenzene	0.85 (0.67, 1.09)	0.77 (0.54, 1.11)	1.01 (0.68, 1.50)	0.79 (0.50, 1.23)
Hexane	0.90 (0.71, 1.16)	0.76 (0.53, 1.10)	0.97 (0.65, 1.43)	0.69 (0.44, 1.08)
Group 4				
Dibenzofuran	1.25 (0.97, 1.60)	0.92 (0.65, 1.30)	1.01 (0.68, 1.50)	1.13 (0.72, 1.76)
Polychlorinated Biphenyls	1.01 (0.79, 1.29)	0.99 (0.70, 1.42)	0.98 (0.66, 1.47)	1.01 (0.65, 1.58)
Group 5				
Hexachlorobenzene	1.00 (0.78, 1.28)	1.01 (0.71, 1.44)	1.00 (0.67, 1.49)	1.00 (0.64, 1.56)
Trichlorobenzene	1.20 (0.94, 1.53)	1.24 (0.86, 1.77)	0.91 (0.61, 1.36)	0.97 (0.62, 1.52)
Group 6				
2,4,6-Trichlorophenol	0.92 (0.72, 1.18)	0.91 (0.64, 1.29)	0.79 (0.53, 1.19)	0.99 (0.63, 1.56)
Trifluralin	0.97 (0.76, 1.25)	1.03 (0.73, 1.47)	0.88 (0.59, 1.31)	1.00 (0.64, 1.55)
Arsenic	1.10 (0.86, 1.40)	1.05 (0.74, 1.50)	0.90 (0.60, 1.35)	0.96 (0.61, 1.50)
Carbaryl	0.96 (0.75, 1.23)	0.92 (0.64, 1.31)	1.08 (0.72, 1.60)	1.03 (0.66, 1.61)
2,4-Dichlorophenoxyacetic Acid Salts And Esters	0.90 (0.71, 1.15)	0.97 (0.68, 1.38)	1.00 (0.67, 1.49)	0.87 (0.56, 1.36)
Group 7				
Manganese	1.00 (0.78, 1.28)	1.15 (0.80, 1.64)	0.91 (0.61, 1.36)	1.02 (0.65, 1.59)
Nickel	0.98 (0.77, 1.26)	0.98 (0.69, 1.40)	1.03 (0.69, 1.53)	0.93 (0.59, 1.45)
Dimethyl Phthalate	1.09 (0.85, 1.40)	1.08 (0.76, 1.54)	1.01 (0.68, 1.52)	1.13 (0.73, 1.77)

Acrylonitrile	1.07 (0.84, 1.37)	1.01 (0.71, 1.44)	1.44 (0.95, 2.20)	1.40 (0.88, 2.23)
Vinyl Chloride	0.99 (0.78, 1.27)	0.77 (0.54, 1.11)	0.95 (0.64, 1.42)	0.83 (0.53, 1.30)
Methyl Iodide	1.04 (0.81, 1.32)	0.94 (0.66, 1.34)	1.03 (0.69, 1.54)	0.99 (0.64, 1.55)
Aniline	1.01 (0.79, 1.30)	0.95 (0.67, 1.36)	1.03 (0.70, 1.54)	1.03 (0.66, 1.61)
Ethylene Dibromide	1.09 (0.85, 1.39)	1.09 (0.77, 1.56)	1.05 (0.70, 1.57)	1.11 (0.71, 1.74)
Mercury	1.16 (0.91, 1.49)	1.07 (0.75, 1.53)	1.10 (0.73, 1.64)	1.08 (0.69, 1.70)
Methyl Chloride	1.01 (0.79, 1.29)	1.01 (0.71, 1.45)	1.01 (0.68, 1.50)	1.01 (0.65, 1.58)
Nitrophenol	1.01 (0.79, 1.29)	0.96 (0.67, 1.37)	1.31 (0.88, 1.96)	1.40 (0.89, 2.19)
No Group				
Acetaldehyde	0.91 (0.71, 1.16)	0.53 (0.36, 0.77)	0.81 (0.55, 1.20)	0.71 (0.46, 1.10)
Acrolein	1.08 (0.84, 1.37)	0.70 (0.48, 1.02)	1.06 (0.71, 1.58)	0.93 (0.59, 1.45)
Acrylamide	1.00 (0.78, 1.27)	0.96 (0.67, 1.37)	0.99 (0.66, 1.48)	0.97 (0.62, 1.52)
Chlorobenzene	1.01 (0.79, 1.29)	0.99 (0.70, 1.41)	0.99 (0.66, 1.47)	0.99 (0.63, 1.55)
Dibutyl phthalate	1.11 (0.87, 1.42)	0.98 (0.68, 1.41)	1.41 (0.91, 2.17)	1.37 (0.85, 2.21)
Dichlorobenzene	1.01 (0.79, 1.29)	0.75 (0.52, 1.09)	1.24 (0.82, 1.87)	1.01 (0.64, 1.61)
Dichlorvos	0.99 (0.78, 1.27)	0.98 (0.68, 1.39)	1.02 (0.68, 1.53)	1.01 (0.64, 1.58)
Dimethyl Formamide	0.92 (0.72, 1.18)	0.89 (0.62, 1.27)	1.22 (0.81, 1.85)	1.04 (0.66, 1.64)
Ethylene Oxide	0.97 (0.76, 1.24)	0.99 (0.70, 1.41)	1.01 (0.67, 1.50)	0.99 (0.63, 1.55)
Hydrazine	1.00 (0.78, 1.28)	1.01 (0.71, 1.44)	0.98 (0.66, 1.46)	0.98 (0.63, 1.53)
Methyl Bromide	1.01 (0.79, 1.29)	0.96 (0.67, 1.37)	0.95 (0.64, 1.42)	0.99 (0.63, 1.54)
Methyl Tertbutyl Ether	1.10 (0.86, 1.41)	1.04 (0.73, 1.49)	0.97 (0.65, 1.44)	0.92 (0.59, 1.43)
Naphthalene	0.97 (0.76, 1.23)	0.60 (0.42, 0.88)	0.99 (0.67, 1.46)	0.69 (0.44, 1.08)
Nitrobenzene	1.00 (0.78, 1.28)	1.00 (0.70, 1.42)	1.00 (0.67, 1.50)	1.00 (0.64, 1.56)
Propylene Dichloride	1.21 (0.95, 1.55)	0.98 (0.69, 1.39)	0.84 (0.56, 1.26)	0.96 (0.61, 1.50)
Styrene	0.93 (0.73, 1.19)	0.76 (0.53, 1.09)	0.97 (0.65, 1.45)	0.79 (0.51, 1.24)
Trichloroethylene	0.98 (0.77, 1.25)	0.78 (0.54, 1.12)	1.29 (0.85, 1.96)	0.91 (0.57, 1.45)
Triethylamine	0.95 (0.74, 1.21)	0.77 (0.53, 1.11)	0.87 (0.59, 1.28)	0.69 (0.45, 1.08)
Vinyl Acetate	1.01 (0.79, 1.29)	0.79 (0.55, 1.13)	0.92 (0.62, 1.38)	0.93 (0.59, 1.45)
Vinylidene Chloride	1.02 (0.79, 1.30)	1.02 (0.72, 1.46)	1.10 (0.72, 1.67)	1.12 (0.70, 1.78)
Cobalt	0.92 (0.72, 1.18)	0.97 (0.68, 1.39)	0.99 (0.67, 1.48)	0.91 (0.58, 1.43)
Cyanide	1.01 (0.79, 1.29)	1.04 (0.73, 1.49)	1.02 (0.68, 1.52)	1.01 (0.65, 1.57)

Lead	0.95 (0.74, 1.22)	0.64 (0.44, 0.92)	0.84 (0.56, 1.25)	0.77 (0.50, 1.21)
Selenium	0.86 (0.67, 1.10)	0.76 (0.53, 1.09)	1.02 (0.68, 1.51)	0.81 (0.51, 1.26)
Tetrachloroethylene	0.95 (0.75, 1.21)	0.84 (0.58, 1.22)	1.05 (0.70, 1.57)	0.97 (0.62, 1.53)

Footnotes for Table 4.2: All models were adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership; each relative risk is per IQR increase for each air toxic; High Growth was used as the referent group for both models

Table 4.3: False Discovery Rate Adjusted P-Values

Air Toxic	Expressive Language				Receptive Language			
	Tracking		Low Growth		Tracking		Low Growth	
	Non-corrected	Corrected	Non-corrected	Corrected	Non-corrected	Corrected	Non-corrected	Corrected
Group 1								
Cresol/Cresylic Acid	0.998	0.998	0.995	0.995	0.998	0.998	0.998	0.998
Phenol	0.998	0.998	0.995	0.995	0.998	0.998	0.998	0.998
Group 2								
DEHP	0.836	0.998	0.832	0.995	0.973	0.998	0.904	0.998
Carbon Disulfide	0.924	0.998	0.928	0.995	0.994	0.998	0.962	0.998
Group 3								
Xylenes	0.423	0.998	0.144	0.678	0.864	0.998	0.106	0.998
Toluene	0.294	0.998	0.108	0.678	0.954	0.998	0.227	0.998
Ethylbenzene	0.562	0.998	0.181	0.678	0.567	0.998	0.239	0.998
Hexane	0.201	0.998	0.163	0.678	0.961	0.998	0.29	0.998
Group 4								
Dibenzofuran	0.079	0.998	0.627	0.995	0.968	0.998	0.594	0.998
Polychlorinated Biphenyls	0.936	0.998	0.974	0.995	0.935	0.998	0.97	0.998
Group 5								
Hexachlorobenzene	0.982	0.998	0.97	0.995	0.997	0.998	0.994	0.998
Trichlorobenzene	0.151	0.998	0.245	0.811	0.641	0.998	0.885	0.998
Group 6								
2,4,6-Trichlorophenol	0.512	0.998	0.586	0.995	0.259	0.998	0.98	0.998
Trifluralin	0.836	0.998	0.851	0.995	0.54	0.998	0.995	0.998
Arsenic	0.468	0.998	0.78	0.995	0.615	0.998	0.854	0.998
Carbaryl	0.758	0.998	0.635	0.995	0.721	0.998	0.895	0.998
2,4-Dichlorophenoxyacetic Acid Salts And Esters	0.409	0.998	0.852	0.995	0.996	0.998	0.553	0.998
Group 7								

Manganese	0.988	0.998	0.451	0.995	0.662	0.998	0.928	0.998
Nickel	0.892	0.998	0.927	0.995	0.89	0.998	0.738	0.998
Dimethyl Phthalate	0.48	0.998	0.662	0.995	0.944	0.998	0.584	0.998
Acrylonitrile	0.571	0.998	0.961	0.995	0.089	0.998	0.154	0.998
Vinyl Chloride	0.95	0.998	0.158	0.678	0.819	0.998	0.414	0.998
Methyl Iodide	0.784	0.998	0.744	0.995	0.87	0.998	0.97	0.998
Aniline	0.907	0.998	0.797	0.995	0.867	0.998	0.882	0.998
Ethylene Dibromide	0.497	0.998	0.622	0.995	0.806	0.998	0.635	0.998
Mercury	0.228	0.998	0.71	0.995	0.649	0.998	0.722	0.998
Methyl Chloride	0.938	0.998	0.94	0.995	0.966	0.998	0.955	0.998
Nitrophenol	0.928	0.998	0.831	0.995	0.183	0.998	0.142	0.998
No Group								
Acetaldehyde	0.452	0.998	0.001	0.044	0.295	0.998	0.126	0.998
Acrolein	0.55	0.998	0.061	0.678	0.792	0.998	0.743	0.998
Acrylamide	0.97	0.998	0.815	0.995	0.953	0.998	0.897	0.998
Chlorobenzene	0.941	0.998	0.953	0.995	0.944	0.998	0.969	0.998
Dibutyl Phthalate	0.405	0.998	0.917	0.995	0.125	0.998	0.196	0.998
Dichlorobenzene	0.918	0.998	0.132	0.678	0.315	0.998	0.951	0.998
Dichlorvos	0.949	0.998	0.89	0.995	0.912	0.998	0.971	0.998
Dimethyl Formamide	0.522	0.998	0.51	0.995	0.34	0.998	0.872	0.998
Ethylene Oxide	0.79	0.998	0.975	0.995	0.978	0.998	0.964	0.998
Hydrazine	0.984	0.998	0.962	0.995	0.928	0.998	0.94	0.998
Methyl Bromide	0.949	0.998	0.82	0.995	0.796	0.998	0.963	0.998
Methyl Tertbutyl Ether	0.442	0.998	0.819	0.995	0.874	0.998	0.711	0.998
Naphthalene	0.776	0.998	0.009	0.236	0.95	0.998	0.102	0.998
Nitrobenzene	0.996	0.998	0.993	0.995	0.996	0.998	0.994	0.998
Propylene Dichloride	0.127	0.998	0.898	0.995	0.396	0.998	0.843	0.998
Styrene	0.588	0.998	0.135	0.678	0.887	0.998	0.306	0.998
Trichloroethylene	0.86	0.998	0.173	0.678	0.223	0.998	0.687	0.998
Triethylamine	0.684	0.998	0.161	0.678	0.482	0.998	0.104	0.998
Vinyl Acetate	0.924	0.998	0.192	0.678	0.691	0.998	0.733	0.998

Vinylidene Chloride	0.895	0.998	0.895	0.995	0.665	0.998	0.639	0.998
Cobalt	0.531	0.998	0.887	0.995	0.977	0.998	0.692	0.998
Cyanide	0.944	0.998	0.828	0.995	0.933	0.998	0.968	0.998
Lead	0.686	0.998	0.015	0.262	0.379	0.998	0.261	0.998
Selenium	0.243	0.998	0.135	0.678	0.941	0.998	0.348	0.998
Tetrachloroethylene	0.686	0.998	0.372	0.995	0.816	0.998	0.895	0.998

Footnotes for Table 4.3: An FDR of 0.1 was used

Table 4.4: Posterior Inclusion Probabilities for Expressive Language

Air Toxic	Group PIP	Conditional PIP
Group 1		
Acetaldehyde	0.47	0.32
Acrylamide	0.47	0
Hydrazine	0.47	0.28
Methyl Tertbutyl Ether	0.47	0.40
Group 2		
Acrolein	0.59	0.04
Dibutyl Phthalate	0.59	0.003
Dichlorobenzene	0.59	0.18
Dimethyl Formamide	0.59	0.10
Hexachlorobenzene	0.59	0.42
Naphthalene	0.59	0.24
Trichloroethylene	0.59	0.003
Vinyl Acetate	0.59	0.01
Tetrachloroethylene	0.59	0
Group 3		
Acrylonitrile	0.66	0.61
Vinyl Chloride	0.66	0.39
Group 4		
DEHP	0.42	0.20
Ethylbenzene	0.42	0.04
Xylenes	0.42	0.09
Hexane	0.42	0.27
Styrene	0.42	0
Toluene	0.42	0
Triethylamine	0.42	0.01
Cobalt	0.42	0.21

Selenium	0.42	0.19
Group 5		
Chlorobenzene	0.48	0
Dimethyl Phthalate	0.48	0
Nitrophenol	0.48	0.01
Arsenic	0.48	0.66
Manganese	0.48	0.26
Nickel	0.48	0.07
Group 6		
Cresol/Cresylic Acid	0.74	0.42
Phenol	0.74	0.58
Group 7		
Methyl Bromide	0.89	0.55
Trifluralin	0.89	0.45
Group 8		
Ethylene Dibromide	0.79	0.53
Propylene Dichloride	0.79	0.47
No Group		
Aniline	0.80	1
2,4,6-Trichlorophenol	0.68	1
Carbaryl	1	1
Carbon Disulfide	1	1
2,4-Dichlorophenoxyacetic Acid Salts And Esters	0.22	1
Dibenzofuran	0.49	1
Dichlorvos	1	1
Ethylene Oxide	0.86	1
Methyl Chloride	1	1
Methyl Iodide	1	1
Nitrobenzen3	1	1
Polychlorinated Biphenyls	0.92	1

Trichlorobenzene	1	1
Vinylidene Chloride	0.79	1
Cyanide	0.93	1
Lead	0.58	1
Mercury	1	1

Footnotes for Table 4.4: All models were adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership; Tracking and Low Growth classes were combined into a “non-optimal” class for comparison

Table 4.5: Posterior Inclusion Probabilities for Receptive Language

Air Toxic	Group PIP	Conditional PIP
Group 1		
Acetaldehyde	0.88	0.08
Acrylamide	0.82	0.76
Hydrazine	0.88	0.15
Methyl Tertbutyl Ether	0.88	0.01
Group 2		
Acrolein	0.86	0
Dibutyl Phthalate	0.86	0.12
Dichlorobenzene	0.86	0.02
Dimethyl Formamide	0.86	0.18
Hexachlorobenzene	0.86	0.08
Naphthalene	0.86	0.05
Trichloroethylene	0.86	0.20
Vinyl Acetate	0.86	0.14
Tetrachloroethylene	0.86	0.21
Group 3		
Acrylonitrile	1	0.69
Vinyl Chloride	1	0.31
Group 4		
DEHP	0.72	0.12
Ethylbenzene	0.72	0.13
Xylenes	0.72	0
Hexane	0.72	0.22
Styrene	0.72	0.02
Toluene	0.72	0.25
Triethylamine	0.72	0
Cobalt	0.72	0.17

Selenium	0.72	0.09
Group 5		
Chlorobenzene	1	0.23
Dimethyl Phthalate	1	0.03
Nitrophenol	1	0.05
Arsenic	1	0.28
Manganese	1	0.04
Nickel	1	0.37
Group 6		
Cresol/Cresylic Acid	1	0.52
Phenol	1	0.48
Group 7		
Methyl Bromide	1	0.62
Trifluralin	1	0.38
Group 8		
Ethylene Dibromide	1	0.25
Propylene Dichloride	1	0.75
No Group		
Aniline	1	1
2,4,6-Trichlorophenol	1	1
Carbaryl	1	1
Carbon Disulfide	0.84	1
2,4-Dichlorophenoxyacetic Acid Salts And Esters	1	1
Dibenzofuran	1	1
Dichlorvos	0.85	1
Ethylene Oxide	0.96	1
Methyl Chloride	1	1
Methyl Iodide	0.81	1
Nitrobenzene	1	1
Polychlorinated Biphenyls	1	1

Trichlorobenzene	0.51	1
Vinylidene Chloride	0.98	1
Cyanide	1	1
Lead	1	1
Mercury	1	1

Footnotes for Table 4.5: All models were adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership; Tracking and Low Growth classes were combined into a “non-optimal” class for comparison

Table 4.6: Air Toxics with Evidence of Endocrine Disrupting Activity

Acetaldehyde (244)	o-Cresol (245)	Xylenes (isomers and mixture) (246,247)	Methyl tert butyl ether (248)
Acrolein (249,250)	m-Cresol (245)	Ethylene oxide (251)	4,4'-Methylenedianiline (252)
Acrylamide (253)	p-Cresol (245)	Ethylene thiourea (254)	Naphthalene (255)
Acrylonitrile (256)	2,4-D, salts and esters (257)	Heptachlor (258,259)	Nitrobenzene (260)
4-Aminobiphenyl (261)	DDE (262)	Hexachlorobenzene (263)	4-Nitrophenol (264,265)
Aniline (266)	Dibenzofurans (267,268)	Hexachlorocyclopentadiene (269)	Ethylene dibromide (Dibromoethane) (270)
2,4,6-Trichlorophenol (271)	1,2-Dibromo-3-chloropropane (272)	Hexachloroethane (273)	N-Nitrosomorpholine (274)
Bis(2-ethylhexyl)phthalate (DEHP) (275)	Dibutylphthalate (276,277)	Hexane (278,279)	Parathion (280)
Carbaryl (281)	1,4-Dichlorobenzene(p) (282)	Hydrazine (283,284)	Pentachloronitrobenzene (Quintobenzene) (285)
Carbon disulfide (286)	Dichlorvos (287)	Lindane (all isomers) (288)	Pentachlorophenol (289)
Chlordane (288,290)	Dimethyl formamide (291)	Methoxychlor (292,293)	Phenol (294,295)
Chlorobenzene (263)	Dimethyl phthalate (77,296)	Methyl bromide (Bromomethane) (297)	Polychlorinated biphenyls (Aroclors) (123,143,267)
Chlorobenzilate (298,299)	2,4-Dinitrophenol (300,301)	Methyl chloride (Chloromethane) (302)	Propoxur (Baygon) (303)
Cresols/Cresylic acid (isomers and mixture) (245)	Ethyl benzene (246,247)	Methyl iodide (Iodomethane) (304)	Propylene dichloride (1,2-Dichloropropane) (305)
o-Xylenes (246,247)	Styrene (306)	Arsenic Compounds (inorganic including arsine) (307)	2,4-Toluene diamine (308)
m-Xylenes (246,247)	2,3,7,8-Tetrachlorodibenzo-p-dioxin (309)	Chromium Compounds (310)	Toxaphene (chlorinated camphene) (311)
p-Xylenes (246,247)	Toluene (312)	Cobalt Compounds (313)	1,2,4-Trichlorobenzene (314,315)
Cyanide Compounds (316)	Trichloroethylene (317)	Mercury Compounds (318)	Trifluralin (319)
Lead Compounds (320)	2,4,5-Trichlorophenol (321)	Nickel Compounds (322)	Vinyl acetate (323)
Manganese Compounds (324)	Triethylamine (325)	Selenium Compounds (326)	Vinyl chloride (327)
Tetrachloroethylene (Perchloroethylene) (328)	Vinylidene chloride (1,1-Dichloroethylene)	Tetrachloroethylene (Perchloroethylene) (328)	

Footnotes for Table 4.6: All toxics have shown potential evidence of endocrine disruption in either animal or epidemiological studies

Table 4.7: Comparison of characteristics of included versus excluded individuals

	Included (N=471)	Excluded (N=150)	Overall (N=621)
Child Sex			
Female	200 (42.5%)	80 (57.6%)	280 (45.9%)
Male	271 (57.5%)	59 (42.4%)	330 (54.1%)
Missing	0	11	11
Maternal Age			
20 to 30 years	92 (19.5%)	34 (23.9%)	102 (16.6%)
31 to 35 years	58 (12.3%)	8 (5.6%)	244 (39.8%)
36 to 40 years	186 (39.5%)	58 (40.9%)	177 (28.9%)
40 to 49 years	135 (28.7%)	42 (29.6%)	66 (10.8%)
Missing	0	8	8
Maternal Race/Ethnicity			
Non-Hispanic White	260 (55.9%)	43 (44.3%)	303 (53.9%)
Black/African American	29 (6.3%)	10 (10.3%)	39 (6.9%)
Hispanic	89 (19.1%)	23 (23.7%)	112 (19.9%)
Other/Multiracial	87 (18.7%)	21 (21.7%)	108 (19.3%)
Missing	6	53	59

	Included (N=471)	Excluded (N=150)	Overall (N=621)
Maternal Educational Attainment			
High School/GED or less	148 (31.6%)	23 (25.3%)	36 (6.4%)
Some college	109 (23.3%)	24 (26.4%)	201 (36%)
Bachelor's degree	43 (9.2%)	11 (12.1%)	171 (30.6%)
Graduate or Professional degree	168 (35.9%)	33 (36.2%)	133 (23.8%)
Missing	3	59	62
Homeownership			
Rent	175 (38.5%)	43 (48.3%)	218 (40.1%)
Own	280 (61.5%)	46 (51.7%)	326 (59.9%)
Missing	16	61	77
Expressive language raw score (6 months)			
Mean (SD)	6.26 (1.20)	6.74 (1.65)	6.32 (1.28)
Median [IQR]	6.00 [1.00]	6.00 [1.00]	6.00 [1.00]
Missing	66	89	155
Receptive language raw score (6 months)			
Mean (SD)	7.84 (1.51)	8.62 (1.59)	7.95 (1.55)

	Included (N=471)	Excluded (N=150)	Overall (N=621)
Median [IQR]	8.00 [2.00]	9.00 [2.00]	8.00 [2.00]
Missing	66	89	155
Expressive language raw score (12 months)			
Mean (SD)	11.8 (2.61)	13.1 (2.44)	12.0 (2.62)
Median [IQR]	12.0 [4.00]	13.0 [3.00]	12.0 [4.00]
Missing	28	92	120
Receptive language raw score (12 months)			
Mean (SD)	12.4 (2.11)	12.7 (1.80)	12.5 (2.07)
Median [IQR]	13.0 [3.00]	13.0 [2.00]	13.0 [3.00]
Missing	28	92	120
Expressive language raw score (24 months)			
Mean (SD)	20.6 (4.76)	20.7 (5.58)	20.6 (4.85)
Median [IQR]	21.0 [6.00]	21.0 [6.50]	21.0 [6.00]
Missing	183	115	298
Receptive language raw score (24 months)			

	Included (N=471)	Excluded (N=150)	Overall (N=621)
Mean (SD)	23.2 (5.47)	22.7 (5.88)	23.2 (5.51)
Median [IQR]	25.0 [6.50]	25.0 [8.50]	25.0 [7.00]
Missing	184	115	299
Expressive language raw score (36 months)			
Mean (SD)	31.1 (6.29)	27.0 (8.22)	31.0 (6.36)
Median [IQR]	32.0 [6.75]	26.0 [11.5]	32.0 [7.00]
Missing	29	139	168
Receptive language raw score (36 months)			
Mean (SD)	30.5 (5.54)	27.1 (8.65)	30.4 (5.65)
Median [IQR]	31.0 [5.00]	28.0 [6.00]	31.0 [5.00]
Missing	30	139	169

Footnote for Table 4.7: Percentages were calculated after removing missing individuals from the sample

Table 4.8: Comparison of Imputed versus Non-Imputed Covariates

	Non-imputed (N=471)	Imputed (N=471)
Child Sex		
Female	200 (42.5%)	200 (42.5%)
Male	271 (57.5%)	271 (57.5%)
Missing	0	
Maternal Age		
20 to 30 years	92 (19.5%)	92 (19.5%)
31 to 35 years	58 (12.3%)	186 (39.5%)
36 to 40 years	186 (39.5%)	140 (29.7%)
40 to 49 years	135 (28.7%)	53 (11.3%)
Missing	0	
Maternal Race/Ethnicity		
Non-Hispanic White	260 (55.9%)	262 (55.6%)
Black/African American	29 (6.3%)	31 (6.6%)
Hispanic	89 (19.1%)	90 (19.1%)
Other/Multiracial	87 (18.7%)	88 (18.7%)
Missing	6	

	Non-imputed (N=471)	Imputed (N=471)
Maternal Educational Attainment		
High School/GED or less	148 (31.6%)	43 (9.1%)
Some college	109 (23.3%)	170 (36.1%)
Bachelor's degree	43 (9.2%)	149 (31.6%)
Graduate or Professional degree	168 (35.9%)	109 (23.1%)
Missing	3	
Homeownership		
Rent	175 (38.5%)	178 (37.8%)
Own	280 (61.5%)	293 (62.2%)
Missing	16	
Expressive language raw score (6 months)		
Mean (SD)	6.26 (1.20)	6.26 (1.21)
Median [IQR]	6.00 [1.00]	6.00 [1.00]
Missing	66	
Receptive language raw score (6 months)		
Mean (SD)	7.84 (1.51)	7.80 (1.54)

	Non-imputed (N=471)	Imputed (N=471)
Median [IQR]	8.00 [2.00]	8.00 [2.00]
Missing	66	
Expressive language raw score (12 months)		
Mean (SD)	11.8 (2.61)	11.9 (2.58)
Median [IQR]	12.0 [4.00]	12.0 [4.00]
Missing	28	
Receptive language raw score (12 months)		
Mean (SD)	12.4 (2.11)	12.4 (2.10)
Median [IQR]	13.0 [3.00]	13.0 [3.00]
Missing	28	
Expressive language raw score (24 months)		
Mean (SD)	20.6 (4.76)	20.3 (4.79)
Median [IQR]	21.0 [6.00]	20.0 [7.00]
Missing	183	
Receptive language raw score (24 months)		

	Non-imputed (N=471)	Imputed (N=471)
Mean (SD)	23.2 (5.47)	22.9 (5.46)
Median [IQR]	25.0 [6.50]	25.0 [7.00]
Missing	184	
Expressive language raw score (36 months)		
Mean (SD)	31.1 (6.29)	31.0 (6.29)
Median [IQR]	32.0 [6.75]	32.0 [7.00]
Missing	29	
Receptive language raw score (36 months)		
Mean (SD)	30.5 (5.54)	30.5 (5.48)
Median [IQR]	31.0 [5.00]	31.0 [5.00]
Missing	30	

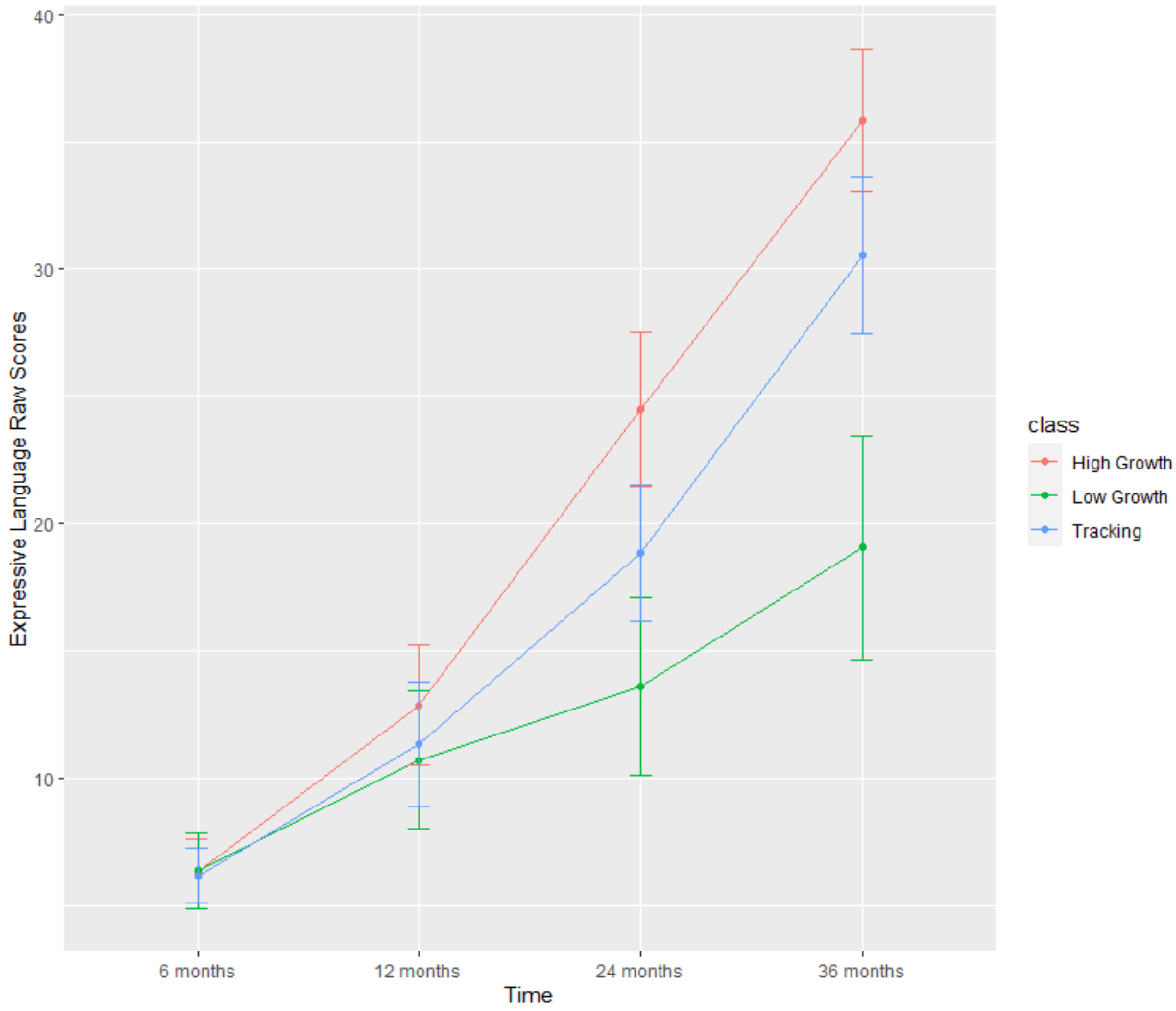
Footnote for Table 4.8: Percentages were calculated after removing missing individuals from the sample

Table 4.9: Comparison of Transformation of Air Toxics

Transformation	Lead	Mercury	Methyl Chloride	Acrylonitrile	Dibutyl Phthalate	Acetaldehyde	Dichlorobenzene	Naphthalene	Xylenes	Hexane
Unaltered	2436.87	2438.52	2439.32	2434.7	2437.89	2440.35	2440.12	2440.23	2439.64	2439.17
Natural log	2433.44	2437.60	2439.13	2436.78	2438.17	2440.15	2439.34	2440.09	2438.13	2437.74
Log base 2	2433.44	2437.60	2439.13	2436.78	2438.17	2440.15	2439.34	2440.09	2438.13	2437.74
Log base 10	2433.44	2437.60	2439.13	2436.78	2438.17	2440.15	2439.34	2440.09	2438.13	2437.74
Squared	2436.56	2434.82	2440.13	2435.85	2439.06	2440.62	2440.55	2437.66	2441.28	2441.10
Root	2434.95	2437.80	2439.22	2435.04	2437.43	2440.26	2439.69	2440.47	2439.05	2438.57
Winsorized and divided by IQR	2435.49	2436.75	2439.11	2434.26	2437.31	2440.30	2439.40	2440.44	2439.83	2439.27
Quantile	2436.65	2433.46	2440.26	2433.00	2438.76	2442.27	2440.92	2441.98	2442.84	2440.70

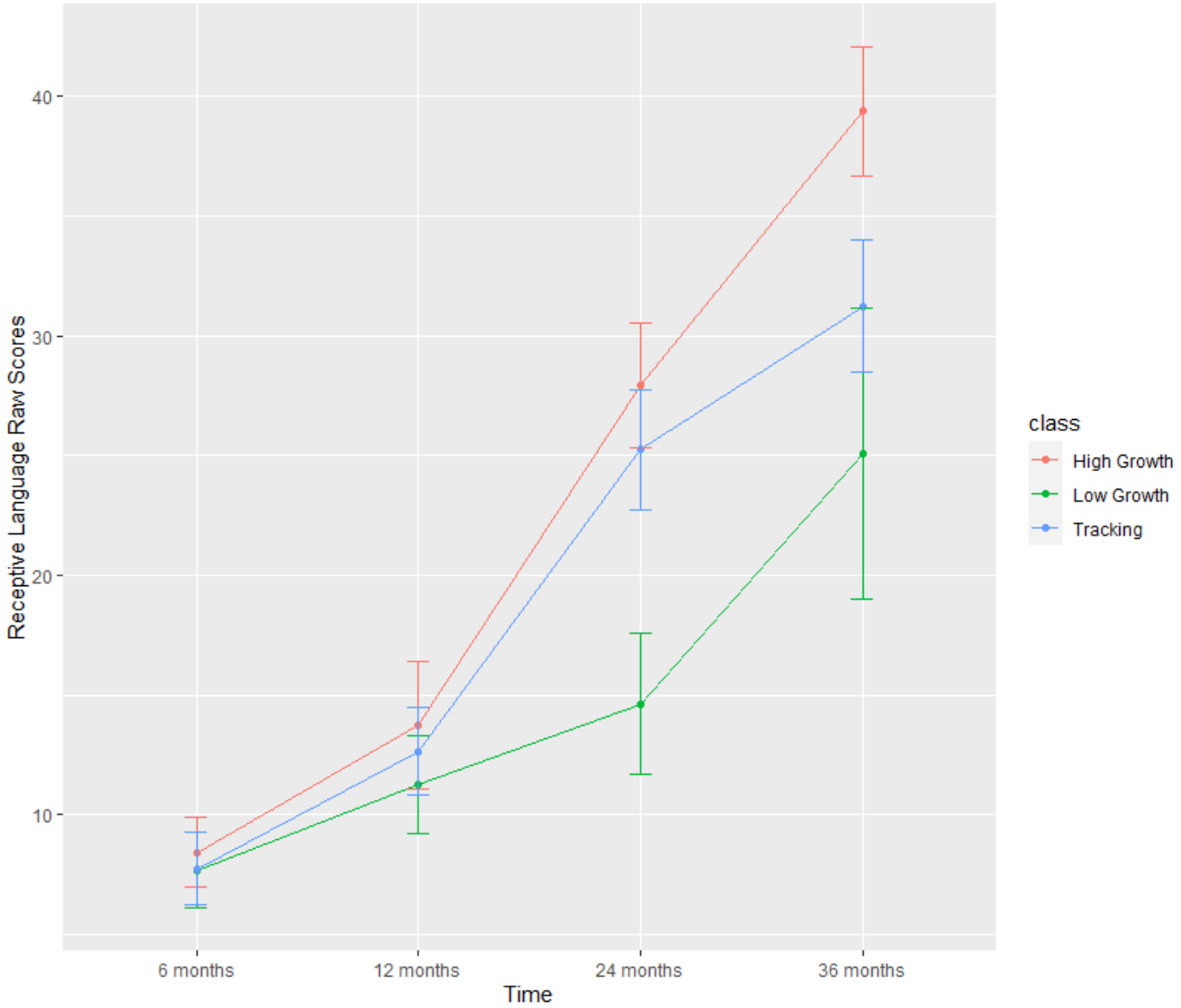
Footnotes for Table 4.9: AIC values are presented and rounded to the nearest hundredth; lower values indicate better fit

Figure 4.1: Language Development Trajectories from Latent Class Growth Analysis for Expressive Language



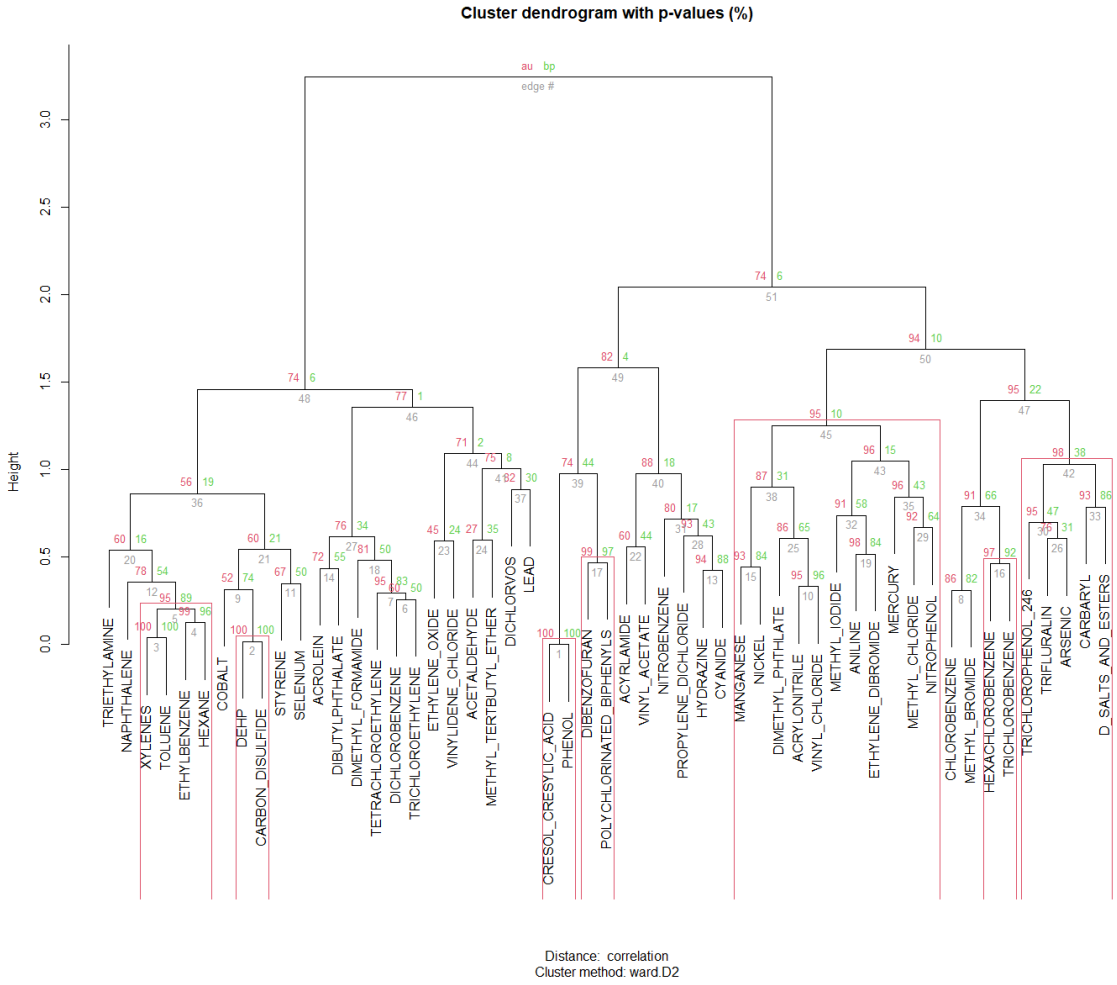
Footnotes for Figure 4.1: Points indicate mean scores at that age with 95 percent confidence intervals

Figure 4.2: Language Development Trajectories from Latent Class Growth Analysis for Receptive Language



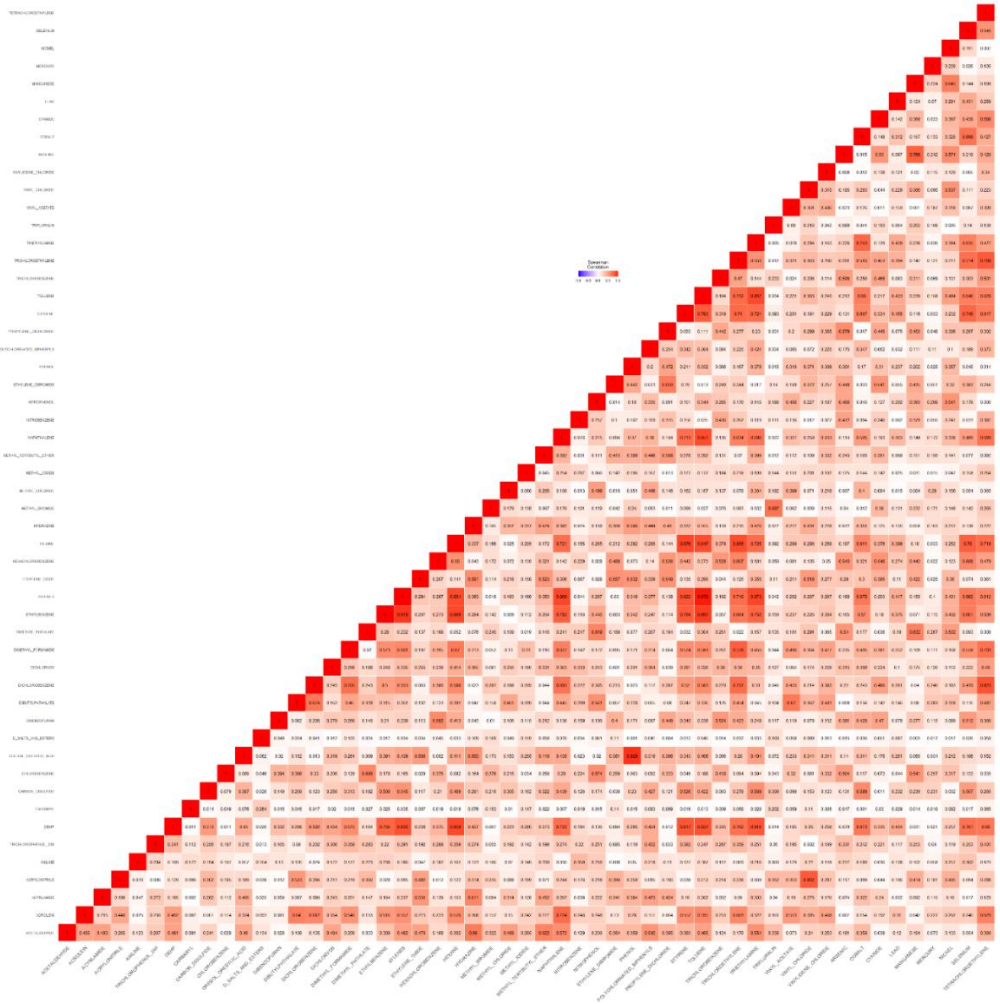
Footnotes for Figure 4.2: Points indicate mean scores at that age with 95 percent confidence intervals

Figure 4.3: Cluster Dendrogram for Air Toxics



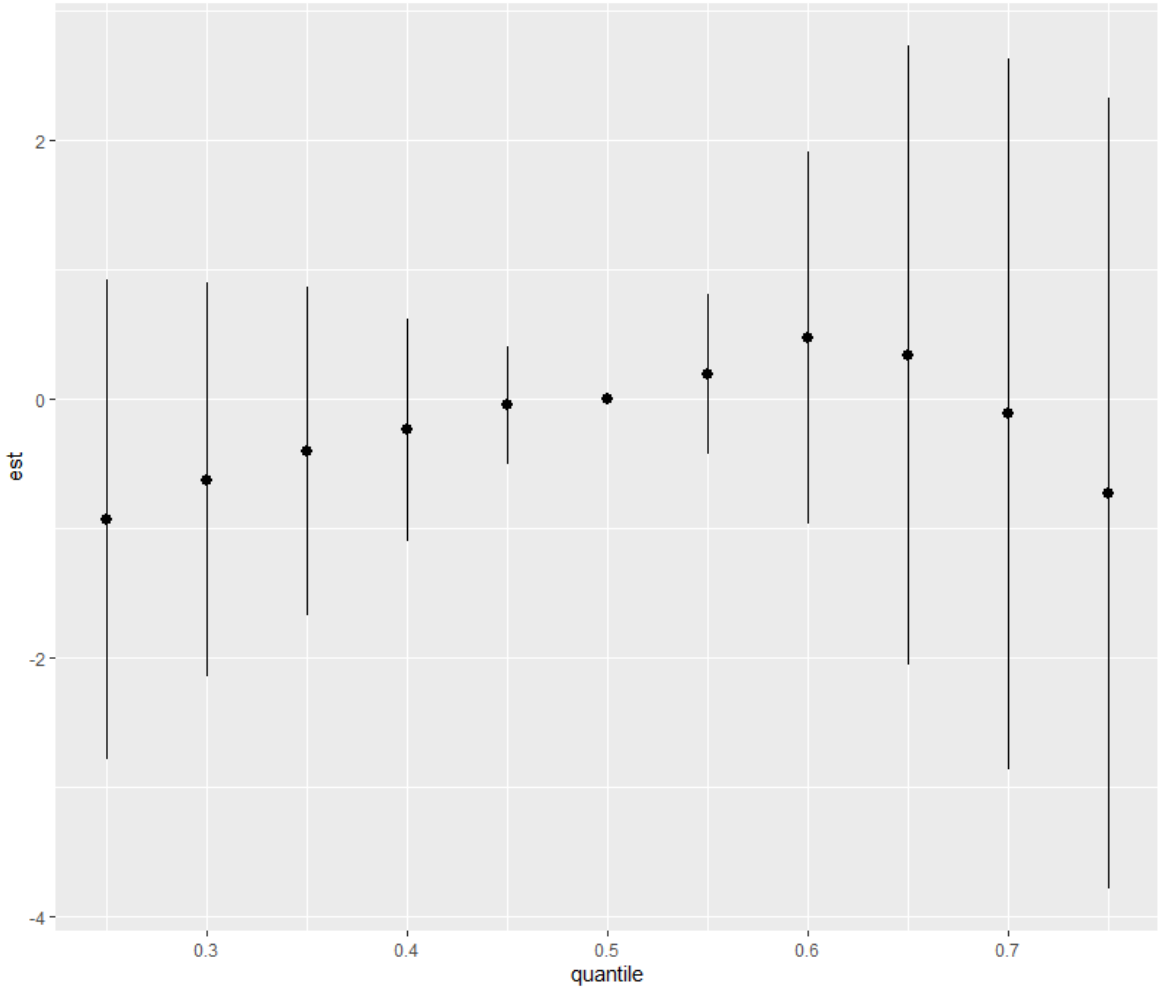
Footnotes for Figure 4.3: Red boxes indicate clusters; Values at nodes indicate p-value percentages for clustering (higher percentage indicates tighter clustering); clusters were drawn on neighboring nodes with p-values greater than or equal to 95

Figure 4.4: Spearman Correlation between Air Toxics Heat Map



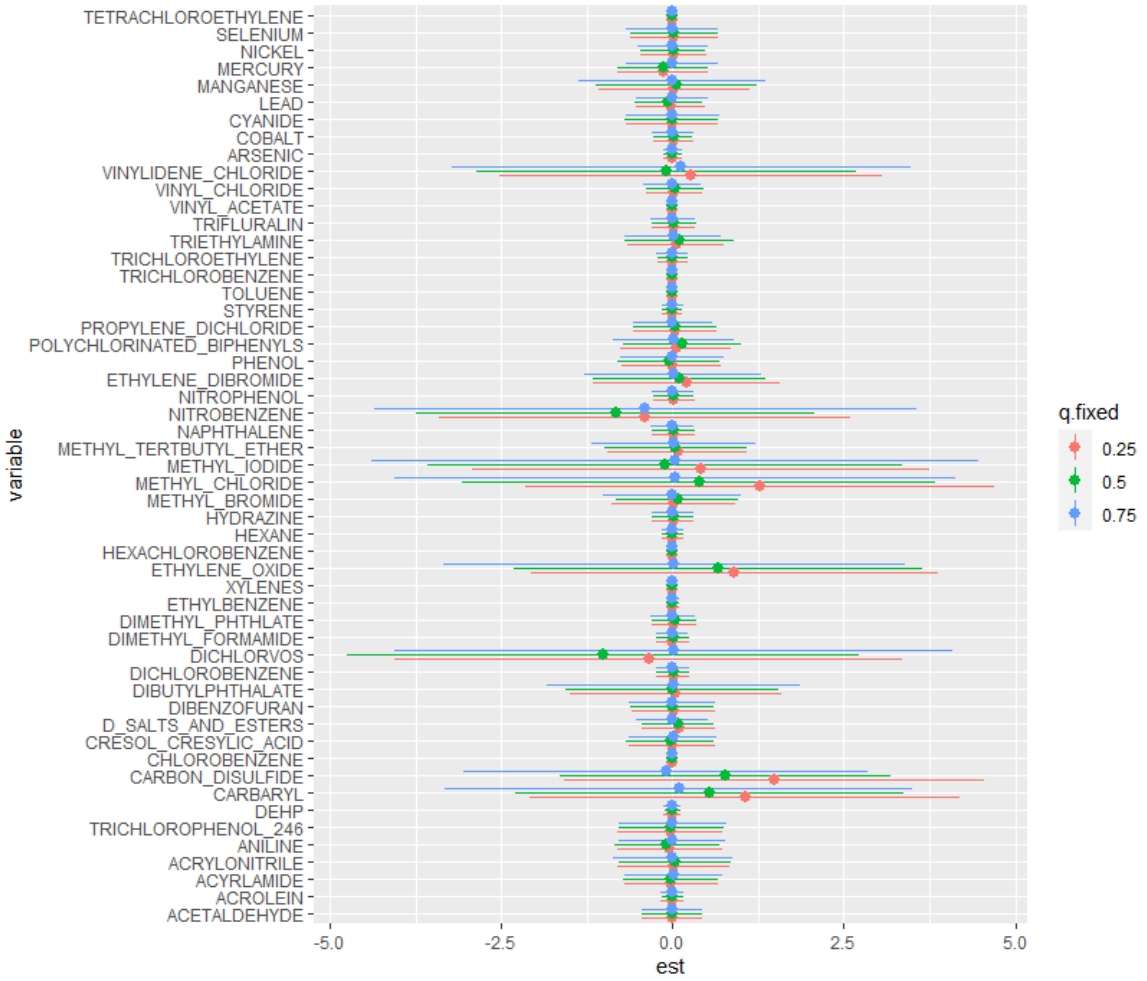
Footnotes for Figure 4.4: Spearman correlations were used between each pairwise combination; redder squares indicate greater positive correlation (i.e. greater concentration of one toxic leads to greater concentration in the other)

Figure 4.5: Bayesian Kernel Machine Regression Overall Risk – Expressive Language



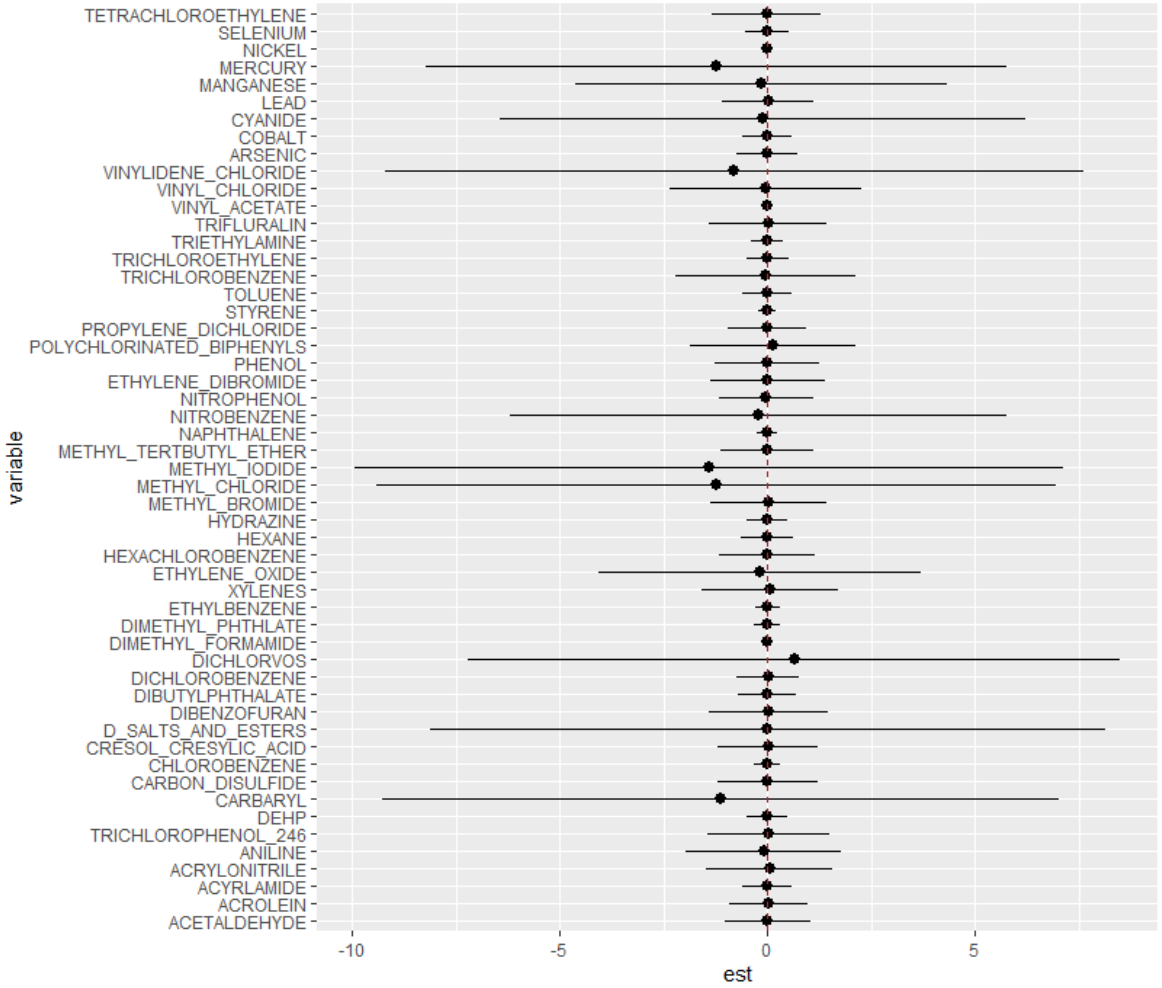
Footnotes for Figure 4.5: Overall effect of chemical mixture of all air toxics on effect of belonging to non-optimal language trajectory; 50th percentile is used as baseline comparison; adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership

Figure 4.6: Bayesian Kernel Machine Regression Single Exposure Effects – Expressive Language



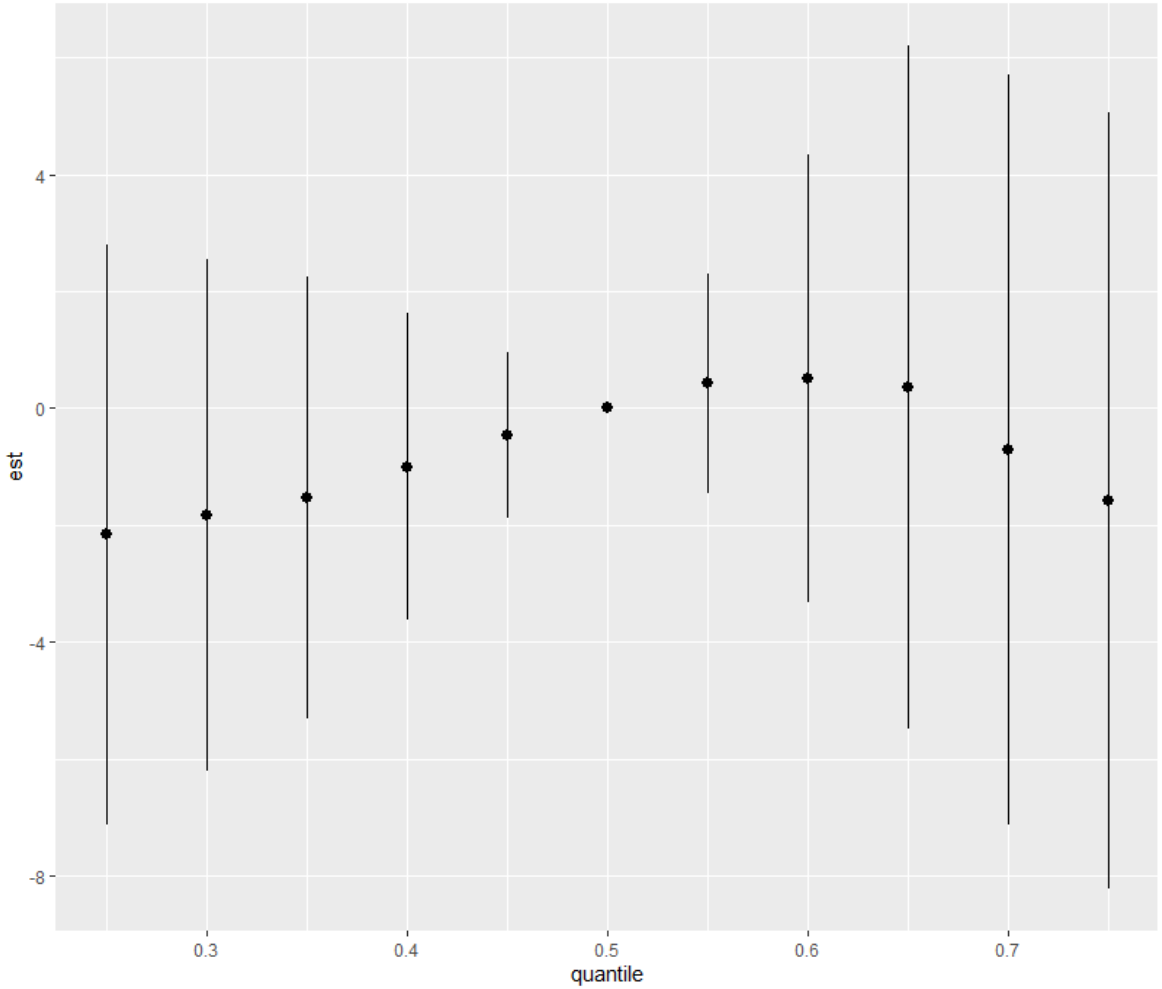
Footnote for Figure 4.6: Effect of single air toxic and probability of belonging to a non-optimal language trajectory associated with the exposure at 75th versus 25th percentile, while holding other air toxics at their 25th, 50th, and 75th percentiles; adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership

Figure 4.7: Bayesian Kernel Machine Regression Interactive Effects - Expressive Language



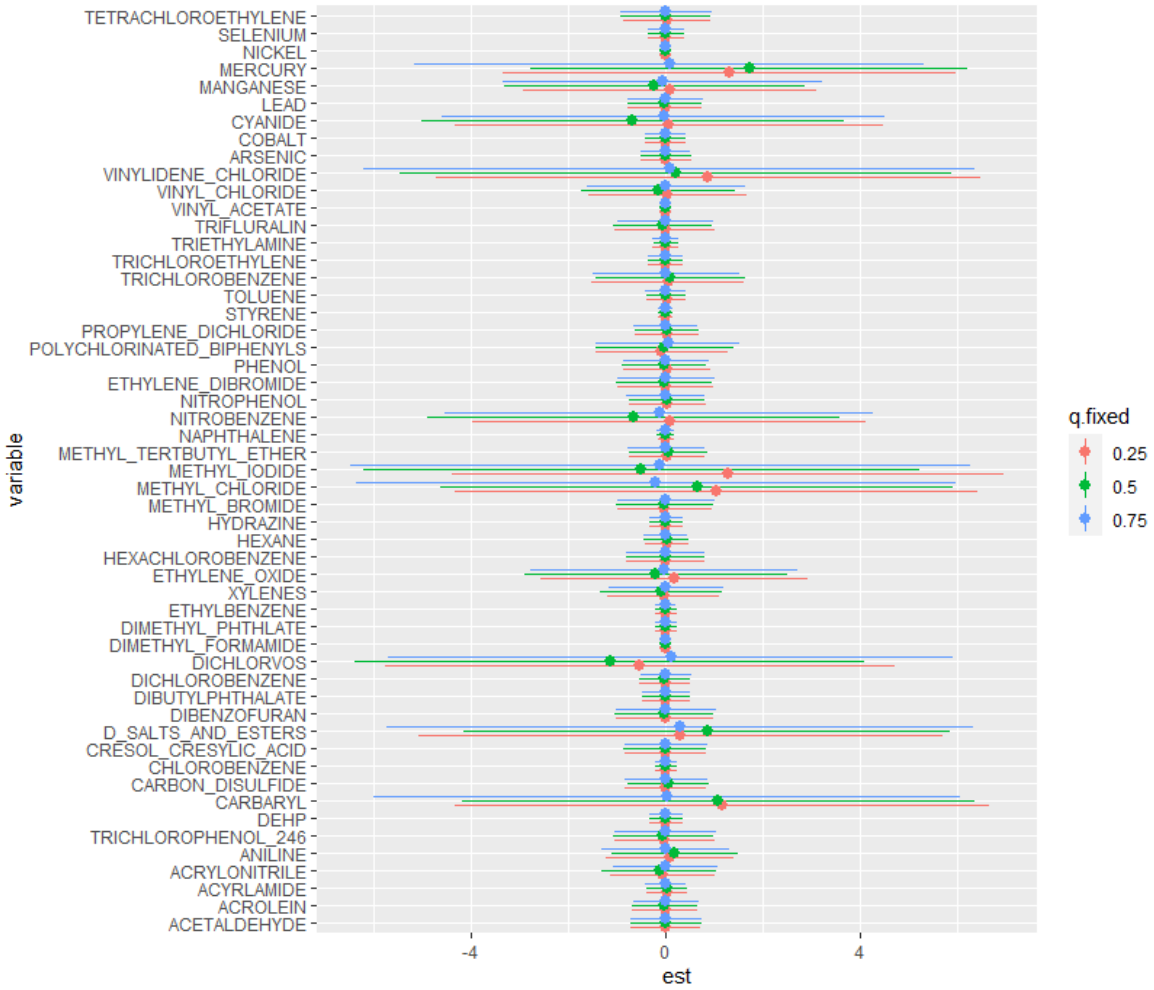
Footnote for Figure 4.7: Each point shows difference between effect size of air toxic when all other air toxics are held at 75th percentile and when all other air toxics are held at 25th percentile, including 95 percent confidence intervals; adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership

Figure 4.8: Bayesian Kernel Machine Regression Overall Risk – Receptive Language



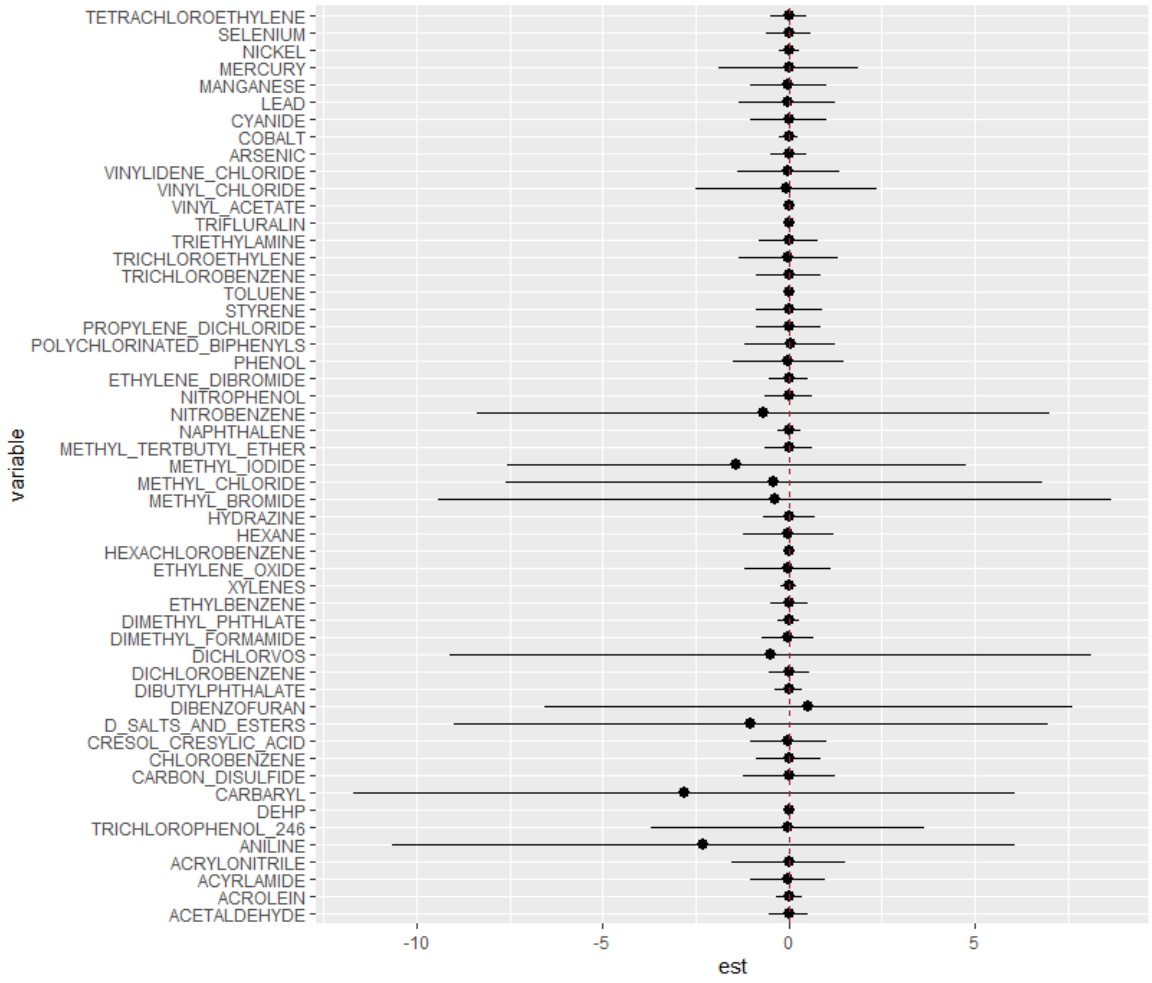
Footnotes for Figure 4.8: Overall effect of chemical mixture of all air toxics on effect of belonging to non-optimal language trajectory; 50th percentile is used as baseline comparison; adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership

Figure 4.9: Bayesian Kernel Machine Regression Single Exposure Effects – Receptive Language



Footnote for Figure 4.9: Effect of single air toxic and probability of belonging to a non-optimal language trajectory associated with the exposure at 75th versus 25th percentile, while holding other air toxics at their 25th, 50th, and 75th percentiles; adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership

Figure 4.10: Bayesian Kernel Machine Regression Interactive Effects - Receptive Language



Footnote for Figure 4.10: Each point shows difference between effect size of air toxic when all other air toxics are held at 75th percentile and when all other air toxics are held at 25th percentile, including 95 percent confidence intervals; adjusted for child sex, maternal age, maternal race/ethnicity, maternal education and home-ownership

Chapter 5 – Discussion, Conclusions, and Future Directions

Mixtures Analysis

Prior research has used a variety of methods to examine chemical mixtures. Loftus et al. used a weighted quantile sum regression (WQS) to examine the effects of a phthalate mixture on neurodevelopment, and Ramos et al. used a combination of principal component analysis (PCA) with structural equation modeling (SEM) to assess the effects of phthalates, organophosphate esters, and organophosphorous pesticides on language ability (107,206). WQS is also used in air toxics literature in order to assess air toxic mixtures (239,329) However, WQS has several limitations, namely that by using quantiles, information about the exposure is lost. In addition, while WQS may be used to assess the effects of a mixture, as well as determine which individual exposure contribute the most, it is unable to generate any measure of risk (208). Finally, the WQS assumes that all exposures in the weighted index have associations in the same direction with the outcome, an assumption that is highly unlikely to occur in an environmental mixture (330).

While we performed a mixtures analysis in our third analysis with air toxic exposures, we did not perform a mixtures analysis for our second analysis with phthalate exposures due to constraints on time. If we were to repeat this analysis, then a mixtures analysis should be performed, as exposure to phthalates rarely occurs in isolation. Because phthalates can degrade into multiple metabolites, it may also be prudent to use a mixture analysis in order to completely capture the effect of the parent compound. However, the metabolites may have differing mechanisms of actions that may lead to different physiological responses, so if this approach and rationale were to be used, then one must be certain that the metabolite in question all act via similar

pathophysiologies. In addition, while our analysis did not show that an air toxic mixture significantly influences language development trajectories, this may not be because a mixture does not have an effect. Our sample size was relatively small, which may have affected our ability to statistically resolve our BKMR, which can be sensitive to sample size. In addition, while none of the interactions were statistically significant, there was evidence of trends for certain air toxics, which indicates that a mixture of certain air toxics may impact language development trajectories.

Future research should focus on correcting the shortcomings of our mixture analysis. A mixtures analysis should be performed on all phthalate metabolites, ideally with a larger sample size in order to achieve adequate statistical power and resolution to observe any small effect sizes that may arise. Different methodology may be prudent to use as well; we utilized BKMR for its advantages over other methodologies. First, because it conducts variable selection and effect estimates simultaneously, it is more capable of capturing uncertainty in the exposure-response function compared to other methods. Second, it can examine both main effect and interaction components of the model while also accounting for uncertainty within the model (237,331). However, other methods may prove useful to answer other questions or to help support the results from a BKRM analysis. For example, weighted quantile sum regression may be able to provide a clearer picture on the effect of an overall mixture, and might be able to corroborate results taken from a BKMR analysis.

Use of High/Enhanced Risk Cohorts

The population used for this research consisted of families where the pregnant mother or father had a biological child that had already been diagnosed with autism.

The mother and the younger sibling were then followed through time. As a result, this population would be considered an enhanced risk autism cohort due to the heritable nature of autism. This also allowed us to observe a greater variability of potentially neurodivergent phenotypes, giving us greater power and ability to detect and characterize language development trajectories. However, this also limits the generalizability of our results. While enhanced risk cohorts have been used in the past to examine neurodevelopmental disorders and delays (195,196,332–334), we run the risk of not being able to appropriately apply our results to the general population, limiting the overall utility of our results. Future research should look at the general population in order to improve overall generalizability.

Use of Trajectory Analysis (Latent Class Growth Analysis)

The statistical method used for this research was latent class growth analysis (LCGA), a type of growth curve modeling that tries to predict latent classes using observed variables. This methodology may provide better insight into how prenatal exposure to endocrine disruptors impacts neurodevelopment. While not performed in this research, a comparison of this method to other cross-sectional methods would be prudent to observe any differences in risk, as well as assess precision (e.g. comparison of risk ratios obtained via LCGA to odds ratios from a more traditional logistic regression using a subscore from a specific time point). Ideally, we would see greater precision with the use of LCGA compared to a logistic regression.

Beyond comparison to cross-sectional methods, there are also other longitudinal methods that may be employed to assess risk using repeat measures. Such methods include mixed effect models, group based trajectory methods, and growth mixture

modeling. Prior studies have made use of such methods to assess the effects of environmental toxics on neurodevelopmental (335–337). Each method has their own strengths and weaknesses, but it is important to note that the trajectories determined in each study may be unique to that study's population (169). While trajectory analysis may prove useful in better assessing and describing health outcomes over time, they are not without their limitations, particularly when it comes to interpretability and generalizability of results.

Sex Differences in the Effect of Phthalates

We examined whether sex acted as a modifier of prenatal phthalate exposure and language, finding that it did not act as a modifier for expressive language, but did act as a modifier for some metabolites for receptive language. Our results showed that for the metabolites of di-isononyl phthalate (DiNP), females may have a higher risk of falling into the Tracking trajectory compared to males. For certain metabolites of DEHP, females may be at a lower risk for falling into the Late Growth trajectory compared to males, although both sexes see a decreased risk of falling into the trajectory in general.

This research is one of the few that has examined potential sex differences in the effects of phthalate exposure on language development. Olesen et al. performed a stratified analysis on boys and girls, but did not examine specifically whether sex was a modifier (105). However, the results indicated that there may be a sex difference present for some phthalate metabolites, as difference between effect measures were observed between girls and boys. For example, the metabolites for DEHP were shown to result in increased risk for boys, but decreased risk for girls, a result that is reflected in our own study. Dewey et al. also performed a stratified analysis, with their results

indicating that DEHP decreased language composite scores on the Bayley Scales of Infant Development – Third Edition for males, but increased it in females (338). While they did not compare the stratified results directly, it can be inferred that a sex difference is present.

Our study examined modification more directly by including a sex-phthalate interaction term and examining whether that term was statistically significant. We did not stratify by sex like Dewey and Olesen; rather, we kept all individuals in the analysis. Our results with DEHP largely agreed with both studies, namely that DEHP or metabolite for DEHP affected males more strongly than females, with males generally having heightened risk of falling into a suboptimal language trajectory compared to females. Thus, this study supports prior literature, and the use of an interaction term rather than stratification provides stronger evidence that a sex difference is present. Future studies should strongly consider performing a similar analysis in order to better separate the differential effects phthalates have on males versus females.

Discussion on Environmental Exposure

We examined prenatal phthalate exposure in two different studies using two different exposure pathways and methodologies. In one study, we assigned prenatal phthalate exposure through metabolite biomarker concentrations determined in urine samples provided by the pregnant mother, adjusting for creatinine or specific gravity depending on the specific cohort the child belonged to. In another study, certain phthalate exposures were included in the model as air toxics, where total concentration was assigned to each individual based on their birth year and census tract. Two

phthalates were common between both studies: dibutyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP).

While results were consistent for DEHP across both studies, showing that DEHP decreased risk of falling into an abnormal language trajectory for both expressive and receptive language, the results for DBP differed between the two studies. When examined as an air toxic, DBP appeared to increase risk for falling into an abnormal language trajectory for both expressive and receptive language. However, when examined via biomarkers, DBP appeared to decrease the risk for falling into an abnormal language trajectory for receptive language.

This finding highlights the potential differences in association that may be found for any environmental exposure depending on the route of exposure taken as well as the measurement method used. Biomarkers are often considered to be the “gold standard” for measuring overall environmental exposure, though the nature of the toxicant and the biomarkers being used must be taken into consideration. In this instance, the use of urinary biomarkers to measure phthalate exposure is commonly regarded to be among the more accurate methods of measuring phthalate exposure, though the timing of urine collection is an important factor in the overall accuracy of this method (339). Since our study using urinary biomarkers consisted of multiple measurements, and because the parent cohorts had specified instructions for the collection of urine samples, the overall accuracy of our phthalate exposure is likely to be high. As a result, the study that used the urinary biomarkers is the stronger study when examining the impact of prenatal phthalate exposure on language development.

However, it still must be considered that these urinary biomarkers are measuring the metabolites of the phthalates, rather than the parent compound. The metabolites have their own toxicity when compared to their parent compound, and as such, we must consider the possibility that our results may be due to the effects of the metabolites, rather than the parent compound. This is counter to the study utilizing air toxics, which is measuring the effects of the parent compound. The different result with dibutyl phthalate may be a result of this.

In our third study, we examined the effects of air toxics that act as EDCs on language development by using the National Air Toxics Assessment. It logically follows that the route of exposure for our toxics was purely airborne, and that we did not consider other possible exposure routes for those phthalates. While this may appear to be a weakness of our study, and it can be considered one, we were interested in examining the effects of prenatal airborne exposure to these toxics, rather than all possible exposure.

While there have been many studies that have examined the same air toxics in the past, many of them do not specify airborne exposure only. For example, there are many studies that have examined the effects of pesticides on neurodevelopment. However, pesticide exposure can occur via multiple mediums, such as airborne exposure (i.e. inhalation), ingestion (e.g. via contaminated food or water), or dermal exposure (e.g. handling of pesticide containers or sprayers). As such, a study that examines a pesticide such as dichlorvos, trifluralin, or parathion may be capturing all possible routes of exposure, rather than just airborne exposure.

Our study is one of the few studies that specifically examined airborne exposure of air toxics and its potential relationship with language development. Of the air toxics that have been examined, airborne PAHs were found to be associated with a decrease in infant mental development across several studies using various measures of neurodevelopment, including the Bayley's Scales of Infant Development and Raven Coloured Progressive Matrices (21,61,62). Guxens found that benzene was negatively associated with infant mental development (225), and there have been multiple studies that have linked some airborne EDCs with an increased risk of ASD, including certain heavy metals, methylene chloride, styrene, certain conjugates of xylene, and other aromatic solvents (118,119,159,160). However, while some of the measures above measure some degree of language, none of the studies specifically looked at language. Our study therefore provides vital information on the impact of airborne exposure to EDCs and their potential impact on language development.

When examining and measuring environmental exposures, it is prudent to consider the route we are assuming the exposure is taking and whether this route is the prevalent or biologically viable pathway. As mentioned above, air toxics are not found solely in the air; lead, pesticides, and some VOCs are found in other mediums, such as water, food, or personal care products. These additional routes may result in an underestimation of total exposure to the toxic in question. However, airborne exposure may be the primary route for some toxics, even if there are other routes of exposure. As a result, airborne exposure may be the most important route to examine. Furthermore, it may not always be viable to collect exposure data to the level of detail and granularity that would give us the true level of exposure for an individual. In these instances, it may

be more prudent to use exposure data that is more readily available, even at the cost of accuracy. The findings from such a study are not invalid, but may be used to justify further examination and study, which may lead to more accurate exposure data collection. This, in turn, will lead to stronger studies that can help guide and inform new policies to reduce the exposure and impact of these environmental contaminants.

Future Examination of Language Development as an Outcome

Our primary outcome of interest was language development. Because our population included two cohorts that had an enhanced familial risk for autism, we were able to detect more nuanced differences in neurodevelopment than a more generalized cohort. Future studies should aim to examine language in a more generalizable cohort in order to determine if the patterns observed in this research can be found in a cohort that is not enriched for autism or other neurodevelopmental disorders.

Furthermore, the use of language impairment as an outcome may help to better identify individuals who may go on to develop other neurodevelopmental disorders. Some studies have noted that individuals that present with language impairments go on to develop other neurodevelopmental disorders such as autism (340,341). Identification of individuals with language development impairments may be crucial to improve the gender gap in neurodevelopmental diagnoses, such as in autism, where females are often under-diagnosed (342,343). This is due to language milestones being relatively gender agnostic which may result in a more objective assessment on neurodevelopment. Pediatricians, parents, and caretakers may then use these assessments in order to refer a child for further psychometric or diagnostic testing in order to determine if a child may possess a neurodevelopmental disorder, or that the

child may need to be reassessed at a later time to determine if sub-clinical symptoms worsen such that a diagnosis can be made.

Conclusions

We performed three studies in order to assess the effects of endocrine disruptors on language development. The first analysis examined if child neurodevelopmental diagnosis was related to overall language development trajectory. We found that diagnostic status was related to language trajectories, with children who were classified as ASD or non-TD being more likely to fall into trajectories that would indicate deficits in language development.

The second analysis examined if prenatal exposure to various phthalates affected language development. Most of the phthalates examined failed to statistically resolve. However, trends were observed among the phthalates based on their weights, with low molecular weight phthalates generally showing an increased risk of falling into an abnormal language development trajectory and high molecular weight phthalates generally showing a decreased risk instead. We further found that sex was not a modifier for expressive language, but was a modifier for receptive language for the metabolites of DiNP (mCOP and mNP2) and the metabolites of DEHP (mEHP, mEHHP, mEOHP, and mECPP), showing a stronger protective effect for females versus males for falling into the Late Growth trajectory.

The third analysis examined if prenatal exposure to specific air toxics affected language development. After adjusting for multiple testing via false discovery rate, only acetaldehyde was statistically significant, showing an overall decrease in risk for

belonging to an abnormal language trajectory. Further, we found that a model that contained all air toxics did not appear to have an impact on overall language trajectory, and that there did not appear to be any statistically significant interactions between the multiple air toxics, although some trends were observed with some air toxics, with mixtures with those air toxics showing a protective effect.

In summary, our findings indicate that most EDCs had a null effect on language development trajectories. Of the EDCs that had a significant effect, most were protective in nature, decreasing the risk of falling into a non-optimal language trajectory with increasing concentration. Furthermore, we found that sex may be acting as a modifier for phthalates with respect to receptive language, and that air toxic mixtures do not significantly impact the risk of falling into a non-optimal trajectory. Future research should focus on examining a larger and more representative population, ideally with multiple outcome measurements in order to continue to better capture the progression of language and neurodevelopment.

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Appendix

Supplemental Table 1: Median and Interquartile Ranges of Phthalate Metabolites (ng/mL) by Child Characteristics for Included Individuals – File Supplemental 1

Supplemental Table 2: AIC for Transformations of Phthalates – File Supplemental 2