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PII: S0013-9351(26)01566-5

DOI: https://doi.org/10.1016/j.envres.2026.125235

Reference: YENRS 125235

To appear in: *Environmental Research*

Received Date: 26 March 2026

Revised Date: 8 July 2026

Accepted Date: 9 July 2026

Please cite this article as: Khan, D., Eckel, S.P., Hernandez-Castro, I., Hu, H., Yang, T., Farzan, S.F., Breton, C.V., Bastain, T.M., Malin, A.J., Prenatal fluoride exposure and motor development among infants in Los Angeles, California, *Environmental Research*, https://doi.org/10.1016/j.envres.2026.125235.

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Abstract

Background: Prenatal fluoride exposure, at United States (US) population relevant levels, has been associated with poorer child neurodevelopmental outcomes; however, no US study has examined whether these associations manifest in infancy. This study investigated associations between prenatal fluoride exposure and infants' neurodevelopment in Los Angeles, California.

Methods: Participants were from the Maternal and Developmental Risks from Environmental and Social Stressors (MADRES) cohort. MADRES is a predominately Hispanic prospective pregnancy and birth cohort in urban Los Angeles. Third trimester specific gravity-adjusted maternal urinary fluoride (MUF_{SG}) concentration was a biomarker of fluoride exposure. Infant motor development, problem solving, communication and personal-social functioning at 6, 9, 12, and 18-months were assessed with the Ages and Stages Questionnaire-3 (ASQ-3). ASQ-3 scores were reverse coded, such that higher scores indicate worse performance. Longitudinal negative binomial regression examined associations between MUF_{SG} and ASQ-3 scores adjusted for maternal age, race/ethnicity, income, BMI, infant sex and corrected infant age at ASQ-3 assessment.

Results: Among 346 mother-child pairs, the median (IQR) MUF_{SG} was 0.79 (0.54–1.07) mg/L. Higher MUF_{SG} was associated with worse gross motor performance (IRR=1.22, 95% CI: 1.03–1.45, p=0.02). No significant associations were observed between MUF_{SG} and fine motor: IRR=1.15 (95% CI: 0.96–1.39), problem solving: IRR=1.15 (95% CI: 0.90–1.48), personal-social: IRR=1.04 (95% CI: 0.83–1.31), or communication: IRR=0.90 (95% CI: 0.71–1.14) scores.

Conclusions: Third trimester fluoride exposure may increase risk of developmental neurotoxicity manifesting in infancy. Additional studies are needed to further explore this association across other US-based regions and the nation.

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Key words: Prenatal fluoride exposure; Infant motor development; MADRES cohort; Ages and Stages Questionnaire-3 (ASQ-3); Maternal urinary fluoride

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1. Background

Early childhood neurodevelopment is a complex and dynamic process shaped by genetic, biological, and environmental factors (Black et al. 2017; Mualem et al. 2024). It unfolds through a sequence of rapidly progressing processes that begin in gestation and continue through early childhood. During these periods, multiple interconnected brain regions undergo substantial structural and functional maturation, creating critical windows in which developing neural systems are particularly sensitive to environmental influences (Yoshida et al. 2022; Posar and Visconti 2022). Considerable attention has been paid to adverse impacts of prenatal psychosocial factors such as maternal stress (Wu et al. 2022), as well as chemical exposures such as lead (Jia et al. 2023) and air pollution (Peuters et al. 2024) on infant cognitive and behavioral outcomes. Furthermore, concern has grown regarding the potential for adverse impacts of prenatal fluoride exposure on neurobehavioral development, particularly given that it is widely distributed in drinking water and dental products. Fluoride readily crosses the placenta (Valdez Jiménez et al. 2017; Mullenix et al. 1995), and recent epidemiological evidence shows that higher prenatal fluoride exposure is associated with lower IQ, worse executive function, and symptoms of neurobehavioral problems among children in North America (Bashash et al. 2017, 2018; Green et al. 2019; Dewey et al. 2023; Malin et al. 2024). While a limited number of studies outside of the US have investigated the effects of fluoride on infant neurodevelopment (Valdez Jiménez et al. 2017; Cantoral et al. 2021), no US study has examined the whether prenatal fluoride exposure is associated with neurodevelopmental outcomes in infancy. This is an important area of investigation because neurodevelopomental delays in infancy may serve as early indicators of broader neurodevelopmental disruption (Posar and Visconti 2022). Therefore, the present study examined whether third-trimester maternal urinary fluoride concentrations are associated with neurodevelopment in infants under two years of age living in Los Angeles, California.

2. Methods

2.1. Participants

The MADRES cohort is a prospective pregnancy and birth cohort of 1,065 primarily Hispanic women with low income living in Los Angeles, California. A detailed description of the MADRES cohort and protocol has been published elsewhere (Bastain et al. 2019). Briefly, between 2015 and 2020, pregnant participants were recruited prior to 30 weeks of gestation at three partner community health clinics and one private obstetrics and gynecology practice in Los Angeles. Eligible cohort participants were over the age of 18 years and spoke English or Spanish fluently. Women were ineligible if they had a multiple gestation pregnancy, had a mental, physical, or cognitive disability which prevented staff from obtaining informed consent, were HIV positive, or were incarcerated. Written informed consent was obtained at cohort entry for each participant. Figure 1 outlines the selection of participants for the analytic sample. Among the 490 mother–child pairs with third-trimester MUF_{SG} measurements, 350 had available MUF_{SG} data and at least one ASQ-3 outcome. We excluded participants with missing maternal income and race (n = 3), and one extreme MUF_{SG} value, resulting in a final analytic sample of 346 pairs. Primary analyses incorporated all available longitudinal ASQ-3 domain scores across 6, 9, 12, and 18 months, while secondary analyses used timepoint-specific ASQ-3 scores at each age. ASQ-3 sample sizes varied across study visits time points, ranging from 346 participants at the earliest timepoint (6 month) to 68 at the latest (18 months). In addition, we compared characteristics of the analytic sample (n = 346) with the full MADRES cohort (n = 1,065). The two groups were similar across key demographic and exposure characteristics, suggesting that the analytic sample was representative of the larger cohort (**Table S1**). This study was approved by the Institutional Review Boards (IRBs) at the University of Southern California (HS-21-

00718) and The University of Florida (IRB202400997), and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2. Measures

2.2.1. Third Trimester Fluoride Exposure

Single spot urine samples were collected from participants during third trimesters of pregnancy. Maternal urinary fluoride (MUF) was measured at the Oral Health Research Institute at the Indiana University School of Dentistry using the Martinez-Mier et al modification (Thomas et al. 2016; Martínez-Mier et al. 2011) of the hexamethyldisiloxane microdiffusion method. MUF measurements were adjusted for specific gravity (MUF_{SG}) (see the **eMethods**). (Malin et al. 2024). Urine samples were collected in both the first and third trimesters. Participants were asked to fast prior to their visit if they were willing, so third-trimester samples were more often collected under fasting conditions compared with the first trimester (Malin et al. 2024, 2023). Because fasting status affects urinary concentrations, including fluoride, we used the third-trimester sample as our exposure measure to enhance comparability across participants. In addition to fasting considerations, we prioritized the third-trimester MUF_{SG} measure because late gestation is a biologically sensitive period when the developing brain is especially responsive to environmental exposures. During this stage, the cerebellum, central to motor coordination as well as cognitive, social, and language functions, undergoes its most rapid growth (Spoto et al. 2021). Concurrently, widespread cortical and subcortical regions show accelerated synaptogenesis, dendritic expansion, and circuit organization beginning around 22 weeks and intensifying through late gestation (Leibovitz et al. 2022; Shukla et al. 2026).

Urinary fluoride was selected as the exposure biomarker because it reflects total ionic fluoride intake from all sources and is widely used in population-based fluoride research. Although spot

urine samples show within-person variability, recent findings from the Canadian MIREC cohort (Malin et al. 2023) showing that MUF_{SG} has moderate intraclass correlation across pregnancy (ICC = 0.46; 95% CI: 0.32–0.57), supporting its use in epidemiologic analyses.. It is also recognized as a standard metric in epidemiological investigations, particularly those exploring associations with neurodevelopmental outcomes (Tylenda 2003; Taylor et al. 2025).

2.2.2. Infant Neurodevelopment

The ASQ-3 is a validated, widely used, and standardized parent-reported screening measure. It contains five developmental sub-scales examining neurodevelopmental milestones across gross motor, fine motor, problem solving, personal-social, and communication domains among children 3–61 months old (Squires et al. 2009). ASQ-3 assessments were administered by trained bilingual research staff during in-person study visits, following standardized protocols (Squires et al. 2009; Bastain et al. 2019; Hernandez-Castro et al. 2022). Research staff did not have access to laboratory-measured exposure biomarkers, including maternal urinary fluoride, at the time of assessment. Thus, assessors were blinded to MUF_{SG} concentrations The ASQ-3 has high test-retest reliability (92%), high interrater reliability (93%) between parents and professionals, and strong concurrent validity (Gollenberg et al. 2010; Squires et al. 1997, 2009). The ASQ-3 was administered at each follow-up using age-appropriate forms tailored to the child's developmental stage, which reduces the likelihood of recall and repeated response bias across repeated assessments (Squires et al. 2009). To ensure consistent administration and minimize potential influences of parental literacy or interpretation, bilingual trained research staff guided parents through each questionnaire (Bastain et al. 2019). ASQ-3 scores are known to stabilize as children grow older; however, our study intentionally focused on early infancy (6–18 months), when motor trajectories begin to emerge and early disruptions may be most detectable. At the 6-month

MADRES study visit, only the ASQ-3 gross and fine motor subscales were administered; however, all developmental domains of the ASQ-3 were administered at the 9-, 12-, and 18-month study visits. ASQ-3 administration occurred within an approximate 6-week window for each of the study visits, except for the 12-month visit which was administered within an 8-week window. All five ASQ-3 domains were administered and scored according to the ASQ-3 manual (Squires et al. 2009). ASQ-3 scores are discrete scores with 5- to 10- point increments summed together to represent infants developmental domains. Scores range from 0 to 60..

2.3. Covariates

Covariates were identified based on previous literature (Hernandez-Castro et al. 2024; Hernandez-Castro et al. 2022; Malin et al. 2024) and included in our analysis if justified using a directed acyclic graph (**eFigure 1**), as well as if their inclusion in the the exposure–outcome–only model resulted in a 10% change or more in model estimates for MUF_{SG}. Covariates included maternal age (continuous variable measured in years), maternal race/ethnicity (White, Black, Hispanic, and others) where others included multiracial, non-Hispanic and other non-Hispanic, maternal income (categorized as less than $30,000, more than $30,000, and unknown), pre-pregnancy body mass index (continuous variable calculated as weight in kilograms divided by height in meters squared), infant sex (male or female), and infant age at the administration of the ASQ-3 (continuous variable measured in weeks). Infant age at the administration of the ASQ-3 was adjusted for preterm infants whereby if infants were born pre- term (<37 weeks of gestation at birth) age at ASQ-3 administration was subtracted by the number of weeks born preterm to calculate a corrected age (Hernandez-Castro et al. 2022; Squires et al. 2009) (for formula see the eMethods). Furthermore, we conducted sensitivity analyses further adjusting for infant birth order (first-born, second-born, or higher), the mode of delivery (vaginal delivery,

cesarean section, and others) where vaginal delivery includes both induced /not induced, cesarean section includes planned cesarean section / unplanned/emergency cesarean section, and others included vaginal birth after cesarean, or vacuum and forceps assisted vaginal birth and maternal smoking status during pregnancy (yes/no). These variables were included in sensitivity analyses because mode of delivery, and maternal smoking during pregnancy are associated with child neurodevelopmental outcomes (Chen et al. 2022; Zar et al. 2025). Smoking can also influence fluoride metabolism(Laisalmi et al. 2003). Additionally, infant birth order (i.e., parity) can influence child birth outcomes and is commonly adjusted for in studies of prenatal fluoride exposure and neurodevelopment (Tsuchida et al. 2026; Green et al. 2019). Furthermore, prenatal lead exposure is a well-established developmental neurotoxicant that may co-occur with fluoride in some populations(Grandjean 2019; Maas et al. 2007; Jia et al. 2023); therefore, we also considered lead as a potential confounder in sensitivity analyses . Data on maternal Pb was only available in the first-trimester (n = 152); however, prior research shows that blood lead levels are consistent between the first and third trimesters; therefore, first-trimester values can reliably represent third-trimester levels (Rygiel et al. 2021).

2.4. Statistical analysis

Descriptive statistics (means, medians, frequencies, and percentages) were calculated for MUF_{SG} and sociodemographic variables. Since ASQ-3 scores were left skewed, they were operationalized with a previously utilized (Hernandez-Castro et al. 2022; Hernandez-Castro et al. 2024) reverse scored, integer count version of the ASQ-3, which used the natural 5- to 10-point increment increase of the scores to reverse score and convert each 5-point increment into a single count. Therefore, each 1-point increase in reverse scored integer count ASQ-3 score was equivalent to a 5-point decrease on the original ASQ-3 scale, enabling us to use statistical models

appropriate for a count outcome with the interpretation that higher integer values corresponded to worse scores (Hernandez-Castro et al. 2022; Hernandez-Castro et al. 2024) (for formula see the eMethods). For the primary analysis, we conducted longitudinal negative binomial models adjusted for covariates (maternal age, race/ethnicity, income, BMI, infant sex, and corrected infant age at ASQ-3) to examine associations of third trimester MUF_{SG} with ASQ-3 scores including all study time points (6, 9, 12 and 18 months). Because ASQ-3 scores were collected repeatedly, we used longitudinal models to capture within-child change over time and to incorporate all available observations. This approach appropriately accounts for within-subject correlation and permits pooling of ASQ-3 scores across ages, consistent with standard repeated-measures methodology (Fitzmaurice et al. 2011; Little and Rubin 2019). Specifically, this approach allows each infant to contribute all available ASQ-3 observations (6, 9, 12, and 18 months), increases statistical power, and appropriately handles unbalanced data where children have different numbers of assessments. Longitudinal analyses followed an available-case strategy, including all participants with at least one ASQ-3 score. Infants contributed data for any combination of the four assessment timepoints; those with incomplete records were not excluded, ensuring all valid observations were utilized in the final models.This strategy is consistent with standard longitudinal modeling approaches, which allow individuals with incomplete outcome data to contribute all available observations to generalized linear models under a missing-at-random assumption (Fitzmaurice et al. 2011; Little and Rubin 2019). In secondary analyses, we conducted cross-sectional negative binomial models, adjusted for covariates (maternal age, race/ethnicity, income, BMI, infant sex, and corrected infant age at ASQ-3), to explore associations between third trimester MUF_{SG} and ASQ-3 scores at each individual study visit time-point (6, 9, 12 and 18 months) separately. Negative binomial

regression provided the lowest Akaike's information criteria (AIC) and Bayesian information criteria (BIC) scores when compared to other count modelling approaches (i.e., Poisson regression, zero-inflated Poisson regression), indicating better model fit. In addition, the negative binomial model accounts for over-dispersion by estimating a dispersion parameter, making it less restrictive than models that assume the variance equals the mean. Given previous literature (Green et al. 2020; C. V. Goodman et al. 2023) suggesting potential sex-specific biological impacts of MUF_{SG}, we additionally evaluated whether infant sex modified the association between MUF_{SG} and ASQ-3 scores by including a statistical interaction term in models. When the interaction term was not statistically significant, final models were estimated without the interaction. Model evaluation and diagnostics were performed. Cook's distance and DfBeta > 2 / sqrt (n) were calculated to detect outliers and test for influential data points (Bollinger et al. 1981). This procedure identified several influential observations, including MUF_{SG} = 7.99mg/L, 3.21mg/L, and 3.73 mg/L. The MUF_{SG} concentrations of 3.21 mg/L and 3.73 mg/L were retained in the analyses, as they reflect authentic and plausible, albeit statistically atypical, variation within the study population rather than spurious or erroneous values (C. Goodman et al. 2022; Malin et al. 2023). However, we conducted sensitivity analyses excluding these observations. In contrast, the extreme observation for MUF_{SG} = 7.99 mg/L was excluded from all models given that it reflects atypical fluoride exposure for North America and could disproportionately influence model estimates (Malin et al. 2024).

For all models, estimated associations (regression slopes) were exponentiated to incidence rate ratios (IRR) and 95% confidence intervals (CI) were computed for all models. All IRRs represent the change in ASQ domain scores per 1 mg/L increase in MUF_{SG}. Primary models were adjusted for maternal age, race/ethnicity, income, BMI, infant sex, and corrected infant age

at ASQ-3. To assess the robustness of the primary models, sensitivity analyses were conducted in which models were additionally adjusted for infant birth order, mode of delivery, maternal smoking status, and first-trimester blood lead levels. Statistical analyses were performed using STATA/MP version 18.0 (StataCorp LLC. 2023). The criterion for statistical significance was $\alpha < .05$.

3.Results

Descriptive statistics for study participants are summarized in **Table 1**. The mean (SD) age of participants was 29.28 (5.98) years, and the median (IQR) MUF_{SG} was 0.79 (0.54–1.07) mg/L. Overall, 52 % of infants in the sample were females.

3.1. Primary analysis: Longitudinal associations between third trimester MUF_{SG} and ASQ-3 scores

Longitudinal associations between third trimester MUF_{SG} and ASQ-3 scores across study time-points (6, 9, 12 and 18 months) are presented in **Table 2**. Higher MUF_{SG} was significantly associated with poorer gross motor performance (IRR=1.22, 95% CI: 1.03–1.45, p=0.02) but not with scores on any other domains; fine motor: IRR=1.15 (95% CI: 0.96–1.39, p=0.12), problem solving: IRR=1.15 (95% CI: 0.90–1.48, p=0.26), personal-social: IRR=1.04 (95% CI: 0.83–1.31, p=0.71), or communication: IRR=0.90 (95% CI: 0.71–1.14, p=0.39). Although the associations were not statistically significant, the IRRs were still notably elevated and suggestive in magnitude, indicating a potential underlying pattern.

3.2. Secondary analyses

3.2.1. Cross-sectional associations between third trimester MUF_{SG} and ASQ-3 scores at each study visit

Cross-sectional associations between third trimester MUF_{SG} and domain-specific scores on the ASQ-3 at each developmental time point are presented in **Table 2**. While no associations were

statistically significant, the estimated IRR of third trimester MUF_{SG} with ASQ-3 gross motor scores were consistently positive and of similar magnitudes at each time point: 6-month IRR = 1.18, 95% CI: 0.98–1.41, p = 0.07; 9-month IRR = 1.19, 95% CI: 0.73–1.93, p = 0.48; 12-month IRR = 1.20, 95% CI: 0.86–1.67, p = 0.29; 18-month IRR = 1.30, 95% CI: 0.40–4.23, p = 0.66. There was less consistency in estimated IRR for the other ASQ-3 domains across infant ages.

3.2.2. Sensitivity analyses

After excluding the two observations with MUF_{SG} concentrations of 3.21 mg/L and 3.73 mg/L, results from primary longitudinal models were unchanged **(Table S2)**. In addition, results from the primary longitudinal analysis were generally consistent after adjusting for infant birth order, mode of delivery, or maternal smoking status **(Table S3)**. When models were adjusted for first-trimester blood lead, the magnitude of association remained the same; however, the sample size was reduced to 161 participants (less than half of the original sample**)** and associations were no longer statistically significant **(Table S3)**. Lastly, infant sex was the only effect modifier tested in this analysis, and did not significantly modify the association between third-trimester MUF_{SG} and ASQ-3 scores in the adjusted longitudinal models or at any individual time point; all MUF_{SG} × sex interaction terms were non-significant (**Table S4**)

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4. Discussion

This is the first US-based study to examine associations between prenatal fluoride exposure and infant neurodevelopment. Furthermore, it assessed mutiple infant neurodevelopmental domains. We observed that mothers with higher levels of fluoride in their urine during the third trimester of pregnancy tended to have infants with worse gross motor development across the first 18 months of life. In contrast, prenatal fluoride exposure was not associated with fine motor, problem-solving, personal-social or communication development in infancy; however, the IRRs for fine motor and problem-solving were elevated and suggestive of a potential underlying pattern. The significant association between prenatal fluoride exposure and infant motor development was observed in the longitudinal analysis, which pools ASQ-3 scores from 6–18 months, whereas time-specific models were underpowered and did not reach statistical significance. Nevertheless, the association between prenatal fluoride exposure and 6-month infant gross motor scores (i.e., the time-specific model with the largest samples size) was trending toward significance. Furthermore, the consistent pattern of effect estimates across ages in time-specific models supports the longitudinal finding that higher prenatal MUF_{SG} is associated with poorer gross motor development.

These findings suggest that motor function may be a particularly sensitive domain for potential fluoride-related prenatal developmental toxicity manifesting in infancy. Specifically, the observed association with gross motor scores, in the absence of associations across other ASQ-3 domains, may reflect differential vulnerability of motor circuits during late gestations. Alternatively, motor function may be easier to reliably assess during infancy than other domains. For example, communication skills in infancy are still emerging and highly variable, making them challenging to assess with precision (Ferreira and Cruz-Santos 2021). In addition, the

personal-social and problem-solving subdomains of the ASQ-3 tended to have weaker internal consistency at the younger developmental stages (Velikonja et al. 2017; Squires et al. 2009). This reduced reliability likely reflects the considerable variability and context-dependence of early social-emotional and cognitive behaviors (Velikonja et al. 2017), and may explain the absence of associations in our analyses for these domains. Furthermore, the smaller sample sizes available for certain domains may have limited statistical power, thereby contributing to the lack of detectable associations. Conversely, as previously noted, longitudinal models leverage repeated observations from each child, thereby providing greater statistical power than timepoint-specific analyses. This may explain why associations emerged more clearly in the longitudinal results (Fitzmaurice et al. 2011; Twisk 2013).

Additionally, although ASQ-3 missingness was assumed to be missing at random, it is possible that children with lower gross motor scores might have been more likely to attend multiple follow-ups. However, we compared motor development trajectories of the full study sample with the subsample that completed all four assessments (n=55) and they were nearly identical (**eFigure 2**). This suggests that children contributing more repeated measures did not differ meaningfully from the overall cohort, and that differential follow-up is unlikely to have biased the longitudinal findings. It is also important to note that all gross motor cross-sectional estimates were elevated, consistent with the longitudinal findings. For fine motor and problem-solving, the elevated IRRs at 18 months, despite the limited sample size, suggest that age at assessment may play an important role in detecting early developmental differences in relation to fluoride exposure.

There are few non-US based studies that have examined potential impacts of fluoride exposure on neurodevelopment during infancy and toddlerhood; however, findings from those studies are

generally consistent with the current study. For example, a study based in Mexico City found that higher dietary fluoride intake during pregnancy was associated with lower cognitive and language scores in toddlers at the ages of 12 and 24 months, though motor outcomes were not specifically assessed (Cantoral et al. 2021). Additionally, in a Mexico-based study that included high fluoride exposure levels, in utero fluoride exposure was associated with cognitive developmental delays in infants aged 3–15 months (Valdez Jiménez et al. 2017). Cantoral et al. reported a median (IQR) dietary fluoride intake of 1.12 mg/day (0.54). While we did not assess food fluoride intake directly in this study, other studies assessing urinary fluoride levels among pregnant women in Mexico City have reported levels comparable to MADRES (Bashash et al. 2017; Malin et al. 2023). Conversely, Valdez-Jiménez et al. reported mean (SD) maternal urinary fluoride concentrations ranging from 1.9 ± 1.0 mg/L to 2.7 ± 1.1 mg/L across pregnancy, which is considerably higher than the median (IQR) MUF_{SG} of 0.79 (0.54–1.07) mg/L in MADRES. These findings collectively suggest that fluoride exposure during gestation may interfere with neurodevelopmental processes across multiple domains, in addition to motor development, even at comparatively lower exposure levels.

There are several plausible biological pathways by which prenatal fluoride exposure can impact infant motor development. Fluoride readily crosses the placenta, and is implicated in oxidative stress, excitotoxicity, as well as in the disruption of thyroid hormone regulation (Strunecka and Strunecky 2020; Mancini et al. 2016; Morshed and Davies 2020). The developing motor system is particularly sensitive to biological stressors. Oxidative stress and excitotoxicity can impair neuronal survival, synaptic organization, and myelination, while thyroid hormones are essential for axonal growth, neuronal differentiation, and motor circuit development. Disruptions in these processes during gestation may adversely impact motor system function development (Strunecka

and Strunecky 2020; Mancini et al. 2016; Morshed and Davies 2020). Animal studies have also shown that fluoride exposure can alter hippocampal morphology, reduce synaptic density, and impair neurotransmitter balance, particularly in glutamatergic and dopaminergic pathways, which are essential for motor coordination and executive function (Taylor et al. 2024).

The association observed between third-trimester MUF_{SG} and motor development reflects that late pregnancy represents a critical window of vulnerability for the developing fetus. This sensitivity likely relates to the rapid differentiation of cerebellar and basal ganglia circuits occurring during the third trimester and early postnatal life (Spoto et al. 2021). These regions undergo accelerated maturation and reorganization during this period. Therefore, they are highly susceptible to environmental insults, such as fluoride exposure, which may manifest as delays in achieving gross motor milestones.

Motor development also plays a critical role in shaping later cognitive and behavioral outcomes. Motor signs often precede cognitive and social symptoms in neurodevelopmental disorders such as autism spectrum disorder (ASD), suggesting that dysfunction in motor pathways may serve as early indicators of broader neurotoxic effects (Posar and Visconti 2022). Indeed, longitudinal studies have shown that atypical motor trajectories can be detected by the end of the first year of life among children later diagnosed with an ASD, frequently before social-communication deficits emerge (Zwaigenbaum et al. 2013). Specifically, children later diagnosed with ASD tend to exhibit early impairments in gross and fine motor skills, postural control, and movement patterns (Lim et al. 2021). Emerging evidence also indicates that motor functioning may moderate the severity of core ASD symptoms, highlighting its role in shaping developmental outcomes (Fulceri et al. 2019). In addition, neurobiological studies support these findings, linking motor impairments to cerebellar and basal ganglia dysfunction, which are also implicated

in social and cognitive deficits (Papadopoulos et al. 2014). Together, the results suggest that fluoride exposure during the third trimester of pregnancy may interfere with neurodevelopmental pathways across diverse domains, with motor function reflecting an early manifestation of such disruption.

4.1. Strengths and Limitations

This study has several methodological strengths. The prospective design establishes clear temporality between prenatal fluoride exposure and subsequent neurodevelopmental outcomes, reducing the risk of reverse causation. The use of the ASQ-3, a well-validated and reliable screening tool for infant and child developmental outcomes, including among Hispanic populations, enhances robustness of the outcome measurement. Additionally, the use of maternal urinary fluoride from pregnant women who predominately fasted before collection as an individual biomarker provides a comprehensive estimate of fluoride intake from all sources, including water, the diet, and dental products. Furthermore, our analytic models accounted for a breadth of covariates related to fluoride exposure, metabolism, and neurodevelopment, strengthening the internal validity of the observed associations.

This study also has some limitations. First, the urinary fluoride measurements capture total ionic fluoride, and we did not assess the specific chemical forms in which fluoride may be present. Therefore, we did not evaluate whether different fluoride species exert distinct biological effects. Although speciation was not evaluated, total urinary fluoride remains the standard exposure biomarker in population-level fluoride research (Taylor et al. 2025). In addition, fluoride exposure was estimated from spot urine samples rather than 24-hour collections, which may introduce random error due to variability in daily behaviors such as fluid intake and use of fluoridated products. However, the predominance of fasting samples, particularly in the third

trimester, likely mitigates some of this variability. Also, the number of infants assessed at each individual time point, particularly at 9 and 18 months, was smaller than would be ideal for fully powered age-specific analyses, although this was partially addressed through longitudinal modeling which has improved power over cross-sectional analysis. Finally, certain ASQ-3 domains, such as 'communication', may be more difficult to interpret in early infancy due to developmental variability and measurement constraints, which could affect the validity of domain-specific findings. These limitations underscore the need for refinement in future research.

5. Conclusion

When examined longitudinally, by pooling ASQ-3 scores from 6–18 months, we observed that higher fluoride exposure during the third trimester was associated with poorer gross motor performance in infancy. This highlights a potentially sensitive window for fluoride-related neurodevelopmental outcomes manifesting in infancy. Future studies with larger, more diverse cohorts in different populations are needed to confirm these associations and clarify the mechanisms underlying fluoride's potential impacts on early motor development.

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CRediT authorship contribution statement

Durdana Khan: Writing – original draft, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – review & editing. **Sandrah P. Eckel**: Methodology, Investigation, Formal analysis, Writing – review & editing. **Ixel Hernandez-Castro**: Methodology, Investigation, Data curation, Formal analysis, Writing – review & editing. **Howard Hu**: Methodology, Writing – review & editing. **Tingyu Yang**: Methodology, Investigation, Formal analysis, Writing – review & editing. **Shohreh F. Farzan:** Methodology, Investigation, Writing – review & editing. **Carrie V. Breton:** Methodology, Investigation, Writing – review & editing, Supervision, Funding acquisition, Resources.**Theresa M. Bastain:** Methodology, Investigation, Writing – review & editing, Supervision, Funding acquisition, Resources. **Ashley J. Malin:**Writing – original draft, Writing – review & editing, Supervision, Funding acquisition, Resources, Methodology, Investigation, Conceptualization.

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Funding

This work was supported in part by funding from the National Institute of Environmental Health Sciences and National Institute on Minority Health and Health Disparities (grant Nos. P30ES007048, R00ES031676, P50MD015705, P50ES026086, R24ES035954) and the US Environmental Protection Agency (grant No. 83615801–0).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential. Deidentified data are available upon reasonable request and review and approval by the USC Institutional Review Board.

Acknowledgements

We are indebted to the MADRES study families, nurses, midwives, physicians, and staff at each of our study sites for their cooperation and participation and especially to the members of the MADRES study team for their efforts to improve the health of underserved communities.

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Table 1: Summary statistics of study participants with complete data on third trimester MUF_{SG} and covariates

Study variables	Number (n)	Mean (SD) or Frequency (%)	Median (p25–p75)*
Adjusted third trimester urinary fluoride MUF_{SG} (mg/L)	346	0.91 (0.54)	0.79 (0.54-1.07)
Maternal age (years) at consent	346	29.28 (5.98)	29.11 (24.35-33.86)
Household income			
Less than $30,000	158	45.66	--
$30,000 and more	85	24.57	--
Don't know	103	29.77	--
Maternal race/ethnicity			
White	24	6.94	--
Black	38	10.98	--
Hispanic	279	80.64	--
Others	5	1.45	--
Maternal pre-pregnancy BMI (kg/m^2)	346	28.74 (6.63)	27.84 (24.53-31.64)
Infant sex			
Female	180	52.02	--
Male	166	47.98	--
Infant birth order			
First-born	112	32.37	--
Second born or higher	223	64.45	--
Missing	11	3.18	--
Mode of delivery			
Vaginal delivery	225	65.03	--
C- section (planned/emergency)	90	26.01	--
Others	30	8.67	--
Missing	1	0.29	--
Maternal smoking status during pregnancy			
No	338	97.69	
Yes	8	2.31	
First trimester blood lead level, µg/dL) (n=152)	152	0.52 (0.63)	0.36 (0.26-0.59)
Infant age at administration of the ASQ-3 (in months)			
Infant Age in months at 6 month ASQ-3	346	6.57 (0.81)	6.45 (6.22-6.77)
Infant Age in months at 9 month ASQ-3	114	9.51 (0.39)	9.42 (9.22-9.70)
Infant Age in months at 12 month ASQ-3	223	11.79 (0.55)	11.57 (11.38-12.26)
Infant Age in months at 18 month ASQ-3	68	18.56 (0.34)	18.47 (18.34-18.81)

MUF_{SG}, Specific gravity-adjusted maternal urinary fluoride; ASQ-3, Ages and Stages Questionnaire-3; BMI, Body Mass Index
*Continuous variables are presented as median (p25–p75), where p25–p75 represents the interquartile range.(IQR)

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Table 2: Associations between third trimester MUF_{SG} concentrations and ASQ-3 scores at different time points

Longitudinal associations between MUF_{SG} (mg/L) concentrations and ASQ-3 scores among infants between 6-18 months of age					
Third trimester MUF_{SG} (mg/L)	Gross motor IRR (95% CI) n=378	Fine motor IRR (95% CI) n=378	Problem solving IRR (95% CI) n=237	Personal social IRR (95% CI) n=237	Communication IRR (95% CI) n=237
Third trimester MUF_{SG} (mg/L)	**1.22 (1.03-1.45)** ***p=0.02***	1.15 (0.96-1.39) *p=0.12*	1.15 (0.90-1.48) *p=0.26*	1.04 (0.83-1.31) *p=0.71*	0.90 (0.71-1.14) *p=0.39*
Timepoint-specific associations between third trimester MUF_{SG} concentrations and ASQ-3 scores at different time points					
6-month ASQ-3 scores (n=346)	1.18 (0.98-1.41) *p=0.07*	1.19 (0.88-1.60) *p=0.27*	--	--	--
9-month ASQ-3 scores (n=114)	1.19 (0.73-1.93) *p=0.48*	0.59 (0.28-1.23) *p=0.16*	1.16 (0.65-2.06) *p=0.90*	1.28 (0.90-1.82) *p=0.16*	0.87 (0.58-1.32) *p=0.52*
12-month ASQ-3 scores (n=223)	1.20 (0.86-1.67) *p=0.29*	1.05 (0.73-1.51) *p=0.80*	1.05 (0.81-1.36) *p=0.72*	0.97 (0.76-1.23) *p=0.81*	0.91 (0.70-1.19) *p=0.51*
18-month ASQ-3 scores (n=68)	1.30 (0.40-4.23) *p=0.66*	1.47 (0.77-2.79) *p=0.24*	1.23 (0.75-2.01) *p=0.41*	0.79 (0.43-1.47) *p=0.46*	0.84 (0.55-1.27) *p=0.40*

IRR, Incidence Rate Ratio; MUF_{SG}, specific gravity adjusted maternal urinary fluoride; ASQ-3, Ages and Stages Questionnaire-3
Models are adjusted for maternal age, race, income, BMI, infant sex and corrected age at ASQ-3 administration
IRRs reflect the relative change in ASQ scores per 1 mg/L increase in MUF_{SG}
Statistically significant results are shown in bold ($p < .05$)
The p-values shown represent the MUF_{SG} term from the adjusted negative binomial models.

636 Figure 1 caption:

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Figure 1: Flow diagram of the study participants
Note: Sample sizes for the longitudinal analysis exceed the final analytic sample because repeated-measures models incorporate all available ASQ-3 observations contributed by each child across the 6-, 9-, 12-, and 18-month visits

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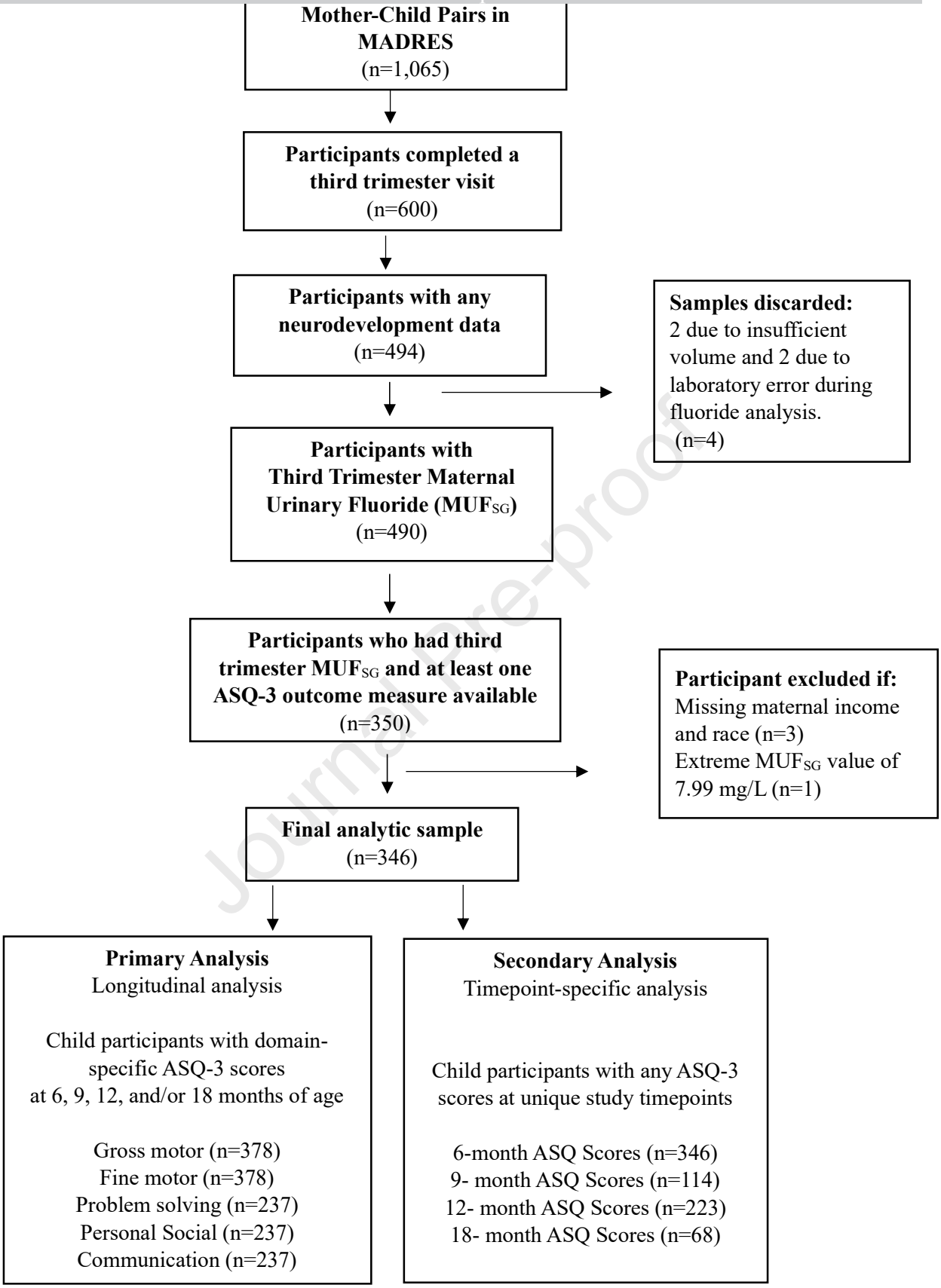

Figure 1: Flow diagram of the study participants

Note: Sample sizes for the longitudinal analysis exceed the final analytic sample because repeated-measures models incorporate all available ASQ-3 observations contributed by each child across the 6-, 9-, 12-, and 18-month visits

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Highlights

- This is the first US study on prenatal fluoride exposure and infant neurodevelopment
- Higher maternal urinary fluoride was linked with poorer infant gross motor scores
- There were no significant associations for fine motor, social, or cognitive scores
- Third-trimester fluoride exposure may affect early gross motor development

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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