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Neurodevelopmental Outcomes at 5 Years in Children Exposed Prenatally to Maternal Dental Amalgam: The Seychelles Child Development Nutrition Study

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Abstract

Limited human data are available to assess the association between prenatal mercury vapor (Hg^0) exposure from maternal dental amalgam restorations and neurodevelopment of children. We evaluated the association between maternal dental amalgam status during gestation and children's

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neurodevelopmental outcomes at 5 years in the Seychelles Child Development Nutrition Study (SCDNS). Maternal amalgam status was determined prospectively in a longitudinal cohort study examining the associations of prenatal exposure to nutrients and methylmercury (MeHg) with neurodevelopment. A total of 236 mother-child pairs initially enrolled in the SCDNS in 2001 were eligible to participate. Maternal amalgam status was measured as number of amalgam surfaces (the primary metric) and number of occlusal points. The neurodevelopmental assessment battery was comprised of age-appropriate tests of cognitive, language, and perceptual functions, and scholastic achievement. Linear regression analysis controlled for MeHg exposure, maternal fatty acid status, and other covariates relevant to child development. Maternal amalgam status evaluation yielded an average of 7.0 surfaces (range 0–28) and 11.0 occlusal points (range 0–40) during pregnancy. Neither the number of maternal amalgam surfaces nor occlusal points were associated with any outcome. Our findings do not provide evidence to support a relationship between prenatal exposure to Hg^0 from maternal dental amalgam and neurodevelopmental outcomes in children at 5 years of age.

Keywords

Mercury; Methylmercury; Dental Amalgam; Neurodevelopment; Prenatal

1. INTRODUCTION

Dental amalgam remained the preferred restorative material for posterior teeth for over one and one half centuries. Women of current and future childbearing potential have been frequent recipients of dental amalgam restorations despite questions regarding its safety during pregnancy. For example, 40,469 out of 48,989 women (82.6%) enrolled in the Norwegian Mother and Child Cohort Study between 2000 and 2007 reported the presence of amalgam restorations in their teeth during pregnancy (Lygre et al., 2010). Over 58% of mothers with amalgams reported its presence in 5 or more of their teeth while pregnant, with 1% reporting placement and 3.4% removal of amalgam during their child's gestation. Recent clinical characteristics of dentists participating in practice-based research networks have confirmed use of dental amalgam in female patients continues in the United States (US), in particular for restoration of posterior teeth (DeRouen et al., 2010; Nascimento et al., 2010; Makhija et al., 2011). Dental practitioners participating in the Dental Practice-Based Research Network (DPBRN) chose amalgam about 50% of the time when restoring posterior teeth in female patients (Makhija et al., 2011). The patients' mean (SD) age for both sexes combined was 31.1 (16.4) years, suggesting a substantial number of dental amalgams were placed in women of childbearing age (defined as women 15 to 44 years of age). Moreover, the United States Food and Drug Administration (FDA) estimates 1.6 million additional amalgam restorations will be placed in pregnant and lactating women in the US in 2013 (FDA, 2009). Studies of the longevity of amalgam restorations suggest nearly half survive more than 15 years in the mouth (Reviewed by Soares and Cavalheiro, 2010). It is generally not recommended to replace clinically sound restorations without cause, since unnecessary damage to remaining tooth structure can occur during amalgam removal.

Amalgam restorations present in the oral cavity continuously release small amounts of mercury vapor (Hg^0) for the life of the restoration (WHO, 1991). Modern, ready-to-mix encapsulated dental amalgam contains between 42.5–47% metallic mercury by weight (Kerr Corporation) and exposure to Hg^0 from restorations begins immediately upon placement. Inhaled Hg^0 is effectively absorbed by the maternal lungs, distributed throughout the mother's body, and crosses the placenta. Hg^0 enters the fetal kidneys and can cross the blood brain barrier and enter the fetal brain, where in adequate dosage it is a neurotoxicant (WHO,

1991). Given the current utilization of dental amalgam in women and its longevity as a restoration, a significant number of developing fetuses continue to be exposed prenatally to Hg^0 via this mechanism. Chronic inhalation of high concentrations of Hg^0 is known to produce disturbances of sensation, motor function, cognition, and personality. Evidence in adults and older children suggests that inhalation of Hg^0 solely from dental amalgams produces exposure levels below those found occupationally, and is unlikely to be associated with these adverse neurological manifestations (Brownawell et al., 2005; CETS, 1997; European Commission, 2004; Health Canada, 2008; WHO, 1997; Bellinger et al., 2006; DeRouen et al., 2006). There is a paucity of human data regarding the impact on the developing brain from *in utero* exposure to Hg^0 from maternal dental amalgam. The FDA's recent classification of dental amalgam recognized the potential for enhanced vulnerability of susceptible populations to Hg^0 exposure. Dental amalgam was classified as a Class II dental device with special controls, in part to take into account that "the developing neurological system in fetuses and young children may be more sensitive to the neurotoxic effects of mercury vapor" (FDA, 2009).

We investigated whether prenatal exposure to Hg^0 from maternal dental amalgam was associated with adverse neurodevelopmental outcomes in a cohort of children participating in the Seychelles Child Development Nutrition Study (SCDNS). At 9 and 30 months of age we found no evidence for an association of maternal amalgam surfaces present during gestation and performance on standard mental and psychomotor developmental tests administered to the children (Watson et al., 2012). Secondary analysis of that data using an alternative metric of exposure, occlusal points, also found a lack of evidence for an association, but suggested possible detriment in mental development of girls only present at 9 months of age. The primary aim of the current study was to examine this same cohort of children at 5 years of age to extend our findings from this earlier study. Because the children were concurrently exposed prenatally to the organic form of mercury, MeHg, through maternal consumption of ocean fish during pregnancy, we also tested the hypothesis that co-exposure to both chemical forms of mercury resulted in an additional adverse impact on test performance.

2. METHODS

2.1. Subjects

The SCDNS Cohort is a prospective, double-blind, longitudinal study of mother-infant pairs residing in the Republic of Seychelles (Bonham et al., 2008; Davidson et al., 2008; Strain et al., 2008, Strain et al., 2012; McAfee et al., 2012). Inclusion and exclusion criteria have been reported previously (Davidson et al., 2008). Of the women recruited with apparently healthy pregnancies at their initial visit, 24 were excluded prior to or at birth (4 were not pregnant, 14 had a miscarriage, termination of pregnancy or stillbirth, one withdrew, one delivered premature twins, two delivered abroad, one mother had preeclampsia and one baby had trisomy 21). Therefore, there were 276 subjects eligible for this study. Gestational amalgam status was available on 236 mothers. The study was reviewed and approved by the Research Subjects Review Board of the University of Rochester, Rochester, NY, and the Ethics Committee of the Republic of Seychelles.

2.2. Determination of Maternal Dental Amalgam Status (Hg^0 Exposure)

The primary metric of Hg^0 exposure was the total number of amalgam surfaces present in the mother's mouth during gestation. This metric takes into account all surfaces of amalgam available for release of Hg^0. This exposure measure has been used previously for this cohort (Watson et al., 2012) and by numerous other investigators (Bellinger et al., 2006, 2007; DeRouen et al., 2006; Factor-Litvak et al., 2003; Luglie et al., 2005; Kingman et al., 1998,

2005; Maserejian et al., 2008; Pesch et al., 2002). Reliability of amalgam status was assessed independently for approximately 5% of mothers and found to be 100% concordant. A secondary metric of Hg^0 exposure was an occlusal point score as described previously (Watson et al., 2011; Watson et al., 2012). This metric may better capture enhanced release of Hg^0 from the occlusal amalgam surface that occurs during brushing and chewing (Abraham et al., 1984; Gay et al., 1979; Sallsten et al., 1996; Vimy and Lorscheider, 1985). The measure includes an estimate of area of each occlusal surface using a modification of the 'amalgam points' scoring system developed by Olstad and colleagues (Olstad et al., 1987). Each occlusal surface is assigned a score of 1 point for small size occlusal amalgams such as pits, 2 points for medium size such as on premolars, and 3 points for large size on molars.

2.3. Developmental Assessment

The children's test battery included: finger tapping [(FT) dominant and non-dominant hands]; three subtests of the Preschool Language Scale-Revised Edition [Total Language Score (PLS-TL), Verbal Ability Score (PLS-VA), and Auditory Comprehension (PLS-AC)]; two subtests of the Woodcock-Johnson Scholastic Achievement Test, second edition (Letter-Word Identification and Applied Problems); and two subtests of the Kaufman Brief Intelligence Test [Verbal Knowledge (KBIT-VK) and Matrices (KBIT-M)]. Mothers completed the Child Behavior Checklist (CBCL), the Kaufman Brief Intelligence Test [Matrices (KBIT-M)], and the Pediatric Review of Children's Environmental Support and Stimulation (PROCESS). Higher scores indicate improved performance, except for FT and CBCL. All tests were available in English and Seychellois Creole, and the preferred version was administered by an experienced team of trained maternal and child health nurses.

2.4. Covariates

We accounted for covariates known or predicted to influence neurodevelopmental outcomes consistent with our previous reports. These included sex, child age, family status at 5 years (1 if living with both parents, 0 if not), maternal age, birth weight, maternal intelligence (assessed by KBIT-M), socioeconomic status (SES) (measured by the Hollingshead Four-Factor SES), and the PROCESS. All covariates were treated as continuous variables except for sex and family status.

In addition to being exposed prenatally to Hg^0 from maternal dental amalgam, cohort children were also exposed prenatally to MeHg and nutrients from maternal consumption of fish and other seafood during pregnancy. Cohort mothers consumed on average 76g/day of fish while pregnant and did not consume marine mammals (Bonham et al., 2009). Metrics of prenatal MeHg exposure and maternal nutrients derived from fish were therefore included as covariates. The prenatal MeHg exposure metric was the average total mercury (THg) concentration in the longest available segment of maternal hair representing growth during gestation (Cernichiari et al., 1995a). Previous studies of pathological specimens from Seychelles have shown maternal hair THg correlates highly with infant brain THg levels (Cernichiari et al., 1995b). A recent study has reported the stable isotope ratio of total mercury in the hair to be consistent with exposure to MeHg from regular fish consumption (Sherman et al., 2013). Fish are high in polyunsaturated fatty acids (PUFA) and we measured total omega-3 (n-3) and omega-6 (n-6) as total lipids (including phospholipids) in maternal serum samples taken at 28 weeks and at delivery as described previously (Strain et al., 2008).

2.5. Statistical Analysis

The primary analysis used linear regression models to examine the covariate-adjusted relationships between amalgam surfaces and each of the outcomes. All analyses were done

with the R analysis system (R Development Core Team, 2011). Each model was run first with and then without an amalgam surface by sex interaction term, because we previously found evidence for differential effects on males and females (Watson et al., 2011, Watson et al., 2012). As mentioned above, all regression models adjusted for the covariates included in earlier analyses (Strain et al., 2012). Models were fit both with and without adjustment for prenatal MeHg, n-3 and n-6 PUFA.

Secondary analyses were all identical to the primary regression analyses except that a different exposure metric was used, occlusal points, or an alternate PUFA status metric was used. Model assumptions were checked using standard methods, including checking for constant variance, nonlinearity, and normally distributed residuals (Weisberg, 2005). Potential outliers and influential observations were examined and none were found to be extreme; all observations were included in all model results.

3. Results

Measured prenatal dental amalgam status and complete covariates at 5 years of age were available on 236 subjects. Of the 242 subjects reported previously at 9 and 30 months (Watson et al., 2012) there were 6 that lacked outcome data at age 5 years. There were 194 mothers (82%) with at least one amalgam restoration present during pregnancy among the 236 subjects. Table 1 presents the descriptive data on the children's developmental outcomes, exposures, and covariates. The mean number of prenatal amalgam surfaces for all subjects used in the analysis was 7.0 surfaces (SD 6.6). The mean number of prenatal occlusal points for all subjects used in the analysis was 11.0 (SD 9.4). The correlation between amalgam surfaces and occlusal points (including subjects with no amalgams) was 0.93. The correlation between amalgam surfaces and MeHg exposure was 0.10. Prenatal dental amalgam surfaces were not significantly (bivariately) correlated with any of the ten outcomes (correlations ranged from −0.06 to 0.10; p-values were all greater than 0.10).

Results from our primary regression analyses examining the association of amalgam surfaces as the exposure metric with developmental outcomes are shown in Tables 2a and 2b. The interaction of Hg^0 with sex was not significant in any model. We report here the model results without the interaction. All models were significant except for finger tapping non-dominant hand indicating that the covariates including exposures significantly predicted all outcomes. Prenatal dental amalgam surfaces were not associated with any developmental outcome. As expected, the child's age and sex were associated with outcomes in a number of models, as were maternal age at delivery, maternal K-BIT, PROCESS score, and prenatal status of n-6.

As a secondary analysis we examined the association of occlusal points as the exposure measure and developmental endpoints. All of the models were significant apart from finger tapping non-dominant hand. Prenatal occlusal points were not associated with any of the developmental outcomes. We also examined models using either amalgam surfaces or occlusal points with an alternate metric for prenatal PUFA status (DHA and AA instead of n-3 and n-6). The use of these alternate metrics for PUFA status did not meaningfully change the exposure-response relationship.

4. DISCUSSION

Using our primary metric of exposure to Hg^0, amalgam surfaces, we found no significant sex by amalgam surfaces interactions, and no significant associations with neurodevelopmental outcomes at 5 years of age. This is the third evaluation of Hg^0 in this prospectively studied cohort and extends our earlier findings. At 9 and 30 months of age we reported no evidence of an association between the number of maternal amalgam surfaces

present during pregnancy and scores on the Mental (MDI) and Psychomotor (PDI) developmental indices of the Bayley Scales of Infant Development-II (BSID-II) (Watson et al., 2012). However, a secondary analysis of the SCDNS data using our secondary metric of exposure, occlusal points, did suggest a possible adverse association present only in girls on the PDI at 9 months of age. We used occlusal points as a secondary metric of Hg^0 exposure from amalgam because studies suggest it may have a better correlation with Hg^0 release from amalgam restorations, and mercury levels in tissues and other biomarkers (Vimy and Lorscheider, 1985; Maserejian et al., 2008; Eggleston and Nylander, 1987; Guzzi et al., 2006). In this study using occlusal points as the metric of Hg^0 exposure, we found no significant associations with any outcome.

We examined concurrent *in utero* exposure in humans to MeHg from maternal fish consumption and Hg^0 from maternal amalgam since few data are available addressing potential increased risk (Brownawell et al., 2005; European Commission, 2004). Experimental studies of combined prenatal exposure to Hg^0 and MeHg in animals are equivocal, but overall suggest increased risk. Fredriksson et al. (1996) found concurrent exposure to MeHg and Hg^0 resulted in higher brain concentrations of total mercury in rat offspring than seen with individual exposures. More recently, Ishitobi et al. (2010) reported Hg^0 exposure was associated with elevated brain concentrations only in the presence of little or no co-exposure to MeHg. Fredriksson et al. (1996) reported that prenatal co-exposure to MeHg and Hg^0 enhanced behavioral changes in rat offspring. Yoshida et al. (2011), however, did not find this in mice. In this SCDNS cohort the maternal hair MeHg levels during gestation, a biomarker of MeHg exposure to the fetus, were nearly 12 times higher than reported for females of childbearing age in the 1999–2000 U.S. National Health and Nutrition Examination Survey (5.6 ppm vs. 0.47 ppm) (McDowell et al., 2004). These differences are consistent with higher levels of fish intake by SCDNS mothers (76g/day), compared with estimates of lower fish consumption (mean of 11.3g/day of finfish and shellfish) by women of childbearing age (15–44) in the US at approximately the same time (EPA, 2002). It should be noted, however, that the estimates for US women included those who consumed no fish during the reporting period. When the subset of those women of childbearing age who ate fish during the reporting period was considered separately, estimates of fish consumption (mean 82.5g/day) were comparable to SCDNS mothers (EPA, 2002). In this regard, the SCDNS cohort represents a sentinel population in which to examine neurodevelopmental risk from concurrent prenatal exposure to two chemical forms of mercury. Taking into consideration the high prenatal MeHg exposure of SCDNS cohort children and the findings from animal studies, we hypothesized that prenatal co-exposure to both chemical forms of mercury might result in elevated neurodevelopmental risk. Instead, we could find no evidence that exposure to Hg^0 from maternal amalgams was associated with increased risk for adverse neurodevelopmental outcomes at 5 years of age after controlling for MeHg exposure.

Previous studies in this cohort suggest that the beneficial effects associated with fish consumption may offset or surpass any adverse effects associated with prenatal MeHg exposure (Strain et al., 2008; Strain et al., 2012). PUFA present in fish are vital for brain growth and development (Innis, 2008) and the ALSPAC study in the United Kingdom found that children born to mothers who consumed more fish scored higher on IQ testing (Hibbeln, 2007). Scientific evidence supporting a beneficial role of PUFA in modifying potential adverse effects of prenatal Hg^0 exposure is absent. In earlier evaluations of this cohort at 9 and 30 months, similar associations of prenatal Hg^0 exposure with outcomes were found both in the presence and absence of adjustment for prenatal PUFA status (Watson et al., 2012). Moreover, at 9 months the beneficial association of n-3 PUFA on the Psychomotor Developmental Index (PDI) did not change when accounting for Hg^0 exposure (Strain et al., 2008). Our findings at 5 years of age are analogous, in that accounting for prenatal exposure

to PUFA using either metric (n-3/n-6 or DHA/AA) and the full dataset, does not change the lack of relationship of prenatal Hg^0 exposure (surfaces or occlusal points) with any outcome.

Our study has a number of strengths, including a well-defined cohort of adequate size to detect associations with covariates known to influence neurodevelopment, precise biological markers of exposure, and the use of standard neurodevelopmental assessments. Significant statistical associations were found with covariates known to influence child developmental outcomes, but not with the exposures we were studying. These findings suggest that there was adequate power to detect associations if they were present. The study also has limitations. The cohort may not have been large enough to detect associations if the effect size was small. Although we used standard measures of exposure and neurodevelopment, other measures of exposure or outcome might have resulted in different findings. We believe the measures were reasonable and chosen with a good rationale.

In 2009 the FDA classified dental amalgam to a Class II dental device. That decision was partially based on the lack of available evidence regarding the potential for risk to the developing fetus from the mother's amalgam restorations. Since that time, we have now examined associations of maternal dental amalgam with neurodevelopmental outcomes in two separate cohorts (Watson et al., 2011; Watson et al., 2012). In the main cohort study we had a retrospective measure of Hg^0 vapor exposure and reported that maternal amalgams present during pregnancy were adversely associated with one of six neurodevelopmental tests. The adverse association was present only in boys on the Letter-Word Identification subtest of the Woodcock-Johnson Tests of Achievement administered at 66 months of age (Watson et al., 2011). That study was limited by uncertainties related to the amalgam status and inconsistencies in the findings. In the SCDNS cohort we have studied amalgams prospectively and on primary analyses have found no evidence of an association between the number of maternal amalgam surfaces present during pregnancy and scores on the Mental (MDI) and Psychomotor (PDI) developmental indices of the Bayley Scales of Infant Development-II (BSID-II) (Watson et al., 2012). Taken together with our current findings, these studies do not support the hypothesis that there is an adverse association of prenatal mercury vapor exposure from maternal dental amalgams with neurodevelopment in children up to 5 years of age.

Conclusion

We find no evidence supporting an adverse association of maternal dental amalgam with neurodevelopmental outcomes in children at age 5 years, even in the presence of elevated MeHg exposure from fish consumption. Because the sensitivity of testing children for neurodevelopmental deficits increases with age, additional insight may be gained from future evaluations of this cohort. Given continued placement of amalgam restorations in females and their extended longevity, comparable studies on larger cohorts of mothers and children are warranted.

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Highlights

- We measured prenatal maternal amalgam status as a metric of mercury vapor exposure.
- We administered age appropriate neurodevelopmental tests to the children at 5 years.
- Prenatal mercury vapor exposure was not associated with any outcome.
- We found no evidence that maternal amalgams harm children's neurodevelopment.

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Table 1

Descriptive Data: Children's Developmental Outcome Scores, Maternal Mercury and PUFA Concentrations, and Covariates

(n = 236)	Mean	Median	SD	Min	Max
Finger Tapping Dominant Hand	23.43	23.50	5.62	5.40	39.60
Finger Tapping Non-dominant Hand	21.35	21.50	4.82	8.60	34.80
PLS-Total Language	118.79	120.00	5.32	100	128
PLS-Auditory Comprehension	55.63	56.00	2.70	47	60
PLS-Verbal Ability	63.16	63.50	3.22	51	68
Woodcock Johnson- Applied Problems	15.14	16.00	4.07	2	24
Woodcock Johnson - Letter Word	10.96	11.00	6.00	1	24
CBCL - Total Score	59.29	60.00	8.59	25	77
Child KBIT - Verbal Knowledge	11.87	12.00	2.77	6	18
Child KBIT - Matrices	7.74	8.00	1.20	2	9
Prenatal Hair MeHg (ppm)	5.63	4.91	3.65	0.19	18.49
Maternal mean DHA (mg/mL)	0.028	0.027	0.007	0.011	0.051
Maternal mean AA (mg/mL)	0.099	0.097	0.021	0.059	0.171
Maternal serum omega-3 (mg/mL)	0.032	0.032	0.009	0.013	0.058
Maternal serum omega-6 (mg/mL)	1.216	1.219	0.207	0.656	1.723
Occlusal points score	11.01	9.00	9.38	0	40
Amalgam surfaces	6.96	5.00	6.58	0	28
Mother's age at Delivery	27.77	27.00	6.03	16	43
Maternal KBIT	86.40	88.00	13.90	48	117
Hollingshead SES at 5 years	31.53	30.00	10.95	8	63
PROCESS score	151.78	151.50	14.91	113	190
Child's age	5.62	5.54	0.30	5.14	6.33
Child's birth weight (kg)	3.23	3.22	0.49	1.65	4.45

Data are for n=236 children of whom 118 (50%) are males and 85 (36%) are living with both parents. PUFA, polyunsaturated fatty acids; PLS, Preschool Language Scale; CBCL, Child Behavior Checklist; KBIT, Kaufman Brief Intelligence Test; MeHg, methylmercury; DHA, docosahexaenoic acid; AA, arachidonic acid; SES, Socioeconomic status; PROCESS, Pediatric Review of Children's Environmental Support and Stimulation

Table 2a

Primary Analysis using Amalgam Surfaces: Linear regression coefficients and confidence intervals for covariate-adjusted models. Each model had a sample size of n=236.

	Finger Tapping Dominant Hand			Finger Tapping Non-Dominant Hand			PLS Total Language Score			PLS Auditory Comprehension			PLS Verbal Ability		
	Slope	**95% CI**	**p**	**Slope**	**95% CI**	**p**	**Slope**	**95% CI**	**p**	**Slope**	**95% CI**	**p**	**Slope**	**95% CI**	**p**
Amalgam surfaces	−0.02	(−0.14, 0.09)	0.668	−0.03	(−0.13, 0.07)	0.549	−0.07	(−0.17, 0.03)	0.160	−0.02	(−0.07, 0.03)	0.473	−0.05	(−0.11, 0.01)	0.096
Prenatal MeHg	0.01	(−0.20, 0.21)	0.946	−0.10	(−0.29, 0.08)	0.285	0.18	(−0.01, 0.36)	0.060	0.09	(−0.00, 0.18)	0.060	0.09	(−0.03, 0.20)	0.136
Maternal serum omega-3	−40.28	(−131.04, 50.47)	0.383	−24.04	(−105.10, 57.03)	0.560	42.61	(−37.21, 122.43)	0.294	12.49	(−27.74, 52.72)	0.541	30.12	(−19.87, 80.11)	0.236
Maternal serum omega-6	**4.04**	**(0.34, 7.74)**	**0.032**	0.45	(−2.86, 3.75)	0.790	−1.18	(−4.44, 2.07)	0.474	−0.50	(−2.14, 1.14)	0.548	−0.68	(−2.72, 1.36)	0.510
Family Status at 5 years	−0.30	(−1.84, 1.25)	0.706	−0.04	(−1.42, 1.33)	0.952	0.02	(−1.33, 1.38)	0.974	0.33	(−0.35, 1.01)	0.344	−0.31	(−1.16, 0.54)	0.478
Mothers age at Delivery	**0.13**	**(0.00, 0.26)**	**0.042**	0.06	(−0.06, 0.17)	0.331	0.09	(−0.02, 0.20)	0.118	0.05	(−0.01, 0.10)	0.103	0.04	(−0.03, 0.11)	0.235
Maternal KBIT	−0.01	(−0.06, 0.05)	0.827	0.02	(−0.03, 0.07)	0.439	0.03	(−0.02, 0.08)	0.262	0.02	(−0.00, 0.04)	0.112	0.01	(−0.02, 0.04)	0.610
Hollingshead SES at 5 years	−0.06	(−0.13, 0.02)	0.140	−0.01	(−0.08, 0.06)	0.746	−0.00	(−0.07, 0.07)	0.969	0.01	(−0.03, 0.04)	0.700	−0.01	(−0.05, 0.03)	0.710
PROCESS score	0.01	(−0.04, 0.06)	0.680	0.01	(−0.04, 0.05)	0.773	0.04	(−0.00, 0.09)	0.079	0.01	(−0.01, 0.03)	0.380	**0.03**	**(0.00, 0.06)**	**0.036**
Child's age	−1.70	(−4.19, 0.78)	0.179	−1.17	(−3.39, 1.05)	0.302	**6.52**	**(4.34, 8.71)**	**<0.001**	**3.80**	**(2.70, 4.90)**	**<0.001**	**2.72**	**(1.35, 4.09)**	**<0.001**
Child's birth weight (kg)	0.15	(−1.34, 1.65)	0.842	0.44	(−0.90, 1.77)	0.519	−0.23	(−1.55, 1.08)	0.729	0.09	(−0.57, 0.76)	0.783	−0.32	(−1.15, 0.50)	0.438
Sex	**−2.49**	**(−3.94, −1.04)**	**0.001**	−0.88	(−2.17, 0.42)	0.183	**1.44**	**(0.17, 2.72)**	**0.026**	0.30	(−0.34, 0.94)	0.360	**1.14**	**(0.35, 1.94)**	**0.005**
Model p-value		**0.010**			0.860			**<0.001**			**<0.001**			**<0.001**	

Positive slopes indicate improved performance, except for Finger Tapping where negative slopes indicate improved performance. PLS = Preschool Language Scale; CBCL = Child Behavior Checklist; KBIT = Kaufman Brief Intelligence Test; MeHg = methylmercury; SES = Socioeconomic status; PROCESS = Pediatric Review of Children's Environmental Support and Stimulation; Sex (male = 0, female = 1).

Table 2b

Primary Analysis using Amalgam Surfaces: Linear regression coefficients and confidence intervals for covariate-adjusted models. Each model had a sample size of n=236.

	Woodcock Johnson Applied Problems			Woodcock Johnson Letter Word Recognition			Child Behavior Checklist Total Score			Child KBIT Verbal Knowledge			Child KBIT Matrices		
	Slope	**95% CI**	**p**	**Slope**	**95% CI**	**p**	**Slope**	**95% CI**	**p**	**Slope**	**95% CI**	**p**	**Slope**	**95% CI**	**p**
Amalgam surfaces	0.05	(−0.02, 0.12)	0.199	−0.01	(−0.10, 0.09)	0.849	0.09	(−0.08, 0.25)	0.317	−0.04	(−0.09, 0.02)	0.168	0.00	(−0.02, 0.03)	0.821
Prenatal MeHg	0.05	(−0.08, 0.18)	0.439	−0.09	(−0.27, 0.09)	0.316	−0.04	(−0.35, 0.28)	0.819	0.02	(−0.08, 0.12)	0.665	−0.00	(−0.05, 0.04)	0.938
Maternal serum omega-3	20.81	(−36.07, 77.70)	0.472	−1.54	(−78.21, 75.13)	0.969	−23.8	(−159.87, 112.28)	0.731	6.40	(−36.58, 49.38)	0.769	9.40	(−10.00, 28.80)	0.341
Maternal serum omega-6	−0.05	(−2.37, 2.27)	0.969	1.36	(−1.77, 4.48)	0.393	−0.09	(−5.64, 5.46)	0.975	1.21	(−0.54, 2.96)	0.176	0.25	(−0.54, 1.05)	0.528
Family Status at 5 years	0.10	(−0.87, 1.06)	0.843	0.42	(−0.88, 1.73)	0.522	0.15	(−2.16, 2.46)	0.898	0.20	(−0.53, 0.93)	0.590	−0.24	(−0.57, 0.09)	0.152
Mothers age at Delivery	0.02	(−0.06, 0.10)	0.705	0.09	(−0.01, 0.20)	0.085	−0.13	(−0.32, 0.06)	0.194	0.05	(−0.01, 0.11)	0.117	−0.00	(−0.03, 0.02)	0.811
Maternal Kbit	0.03	(−0.00, 0.07)	0.059	0.03	(−0.02, 0.08)	0.226	**−0.13**	**(−0.21, −0.04)**	**0.003**	0.00	(−0.02, 0.03)	0.795	0.01	(−0.00, 0.02)	0.160
Hollingshead SES at 5 years	−0.01	(−0.05, 0.04)	0.819	0.00	(−0.06, 0.07)	0.903	−0.11	(−0.22, 0.00)	0.060	0.03	(−0.01, 0.06)	0.150	0.01	(−0.01, 0.02)	0.470
PROCESS score	0.02	(−0.01, 0.05)	0.224	0.03	(−0.01, 0.08)	0.138	−0.05	(−0.13, 0.03)	0.219	−0.00	(−0.03, 0.02)	0.812	−0.01	(−0.02, 0.01)	0.308
Child's age	**7.27**	**(5.71, 8.83)**	**<0.001**	**12.26**	**(10.16, 14.36)**	**<0.001**	−2.22	(−5.95, 1.51)	0.241	**3.04**	**(1.86, 4.22)**	**<0.001**	**0.98**	**(0.44, 1.51)**	**<0.001**
Child's birth weight (kg)	−0.09	(−1.03, 0.84)	0.843	0.61	(−0.65, 1.87)	0.341	−0.42	(−2.67, 1.82)	0.710	0.39	(−0.32, 1.09)	0.283	0.13	(−0.19, 0.45)	0.430
Sex	0.31	(−0.60, 1.22)	0.499	1.18	(−0.05, 2.40)	0.059	−1.57	(−3.74, 0.60)	0.156	**0.72**	**(0.03, 1.40)**	**0.041**	0.12	(−0.19, 0.43)	0.436
Model p-value		**<0.001**			**<0.001**			**<0.001**			**<0.001**			**0.015**	

Positive slopes indicate improved performance, except for CBCL where a negative slope indicates improved performance. PLS, Preschool Language Scale; KBIT, Kaufman Brief Intelligence Test; MeHg, methylmercury; SES, Socioeconomic status; PROCESS, Pediatric Review of Children's Environmental Support and Stimulation; Sex (male = 0, female = 1).