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Abstract

Background: Polychlorinated biphenyls (PCBs), organochlorine pesticides, and methylmercury (MeHg) are environmentally persistent with adverse effects on neurodevelopment. However, especially among populations with commonly experienced low levels of exposure, research on neurodevelopmental effects of these toxicants has produced conflicting results.

Objectives: We assessed the association of low-level prenatal exposure to these contaminants with memory and learning.

Methods: 393 children, born between 1993-1998 to mothers residing near a PCB-contaminated harbor in New Bedford, Massachusetts were studied. Cord serum PCB, DDE, and maternal peripartum hair Hg levels were measured to estimate prenatal exposure. Memory and learning were assessed at age 8 (range: 7-11) years using the Wide Range Assessment of Memory and Learning (WRAML), age-standardized to a mean of 100 and standard deviation of 15. Associations with each WRAML index – Visual Memory, Verbal Memory, and Learning – were examined with multivariable linear regression, controlling for potential confounders.

Results: While cord serum PCB levels were low (sum of 4 PCBs, mean: 0.3 ng/g serum; range: 0.01, 4.4), hair Hg levels were typical of the U.S. fish eating population (mean: 0.6 µg/g; range: 0.3, 5.1). In multivariable models, each µg/g increase in hair Hg was associated with, on average, decrements of -2.8 on Visual Memory (95% CI: -5.0, -0.6, p=0.01), -2.2 on Learning (95% CI: -4.6, 0.2, p=0.08), and -1.7 on Verbal Memory (95% CI: -3.9, 0.6, p=0.14). There were no significant adverse associations of PCBs or DDE with WRAML indices.

Conclusions: These results support an adverse relationship between low-level prenatal MeHg exposure and childhood memory and learning, particularly visual memory.

Introduction

Polychlorinated biphenyls (PCBs) were formerly used as nonflammable dielectrics in electronic parts, vehicles for pesticide application, pigment suspension agents in carbonless copy paper, lubricants, and plasticizers in caulk and sealants (Korrick and Altshul 1998). Dichlorodiphenyldichloroethylene (DDE) is the major degradation product of dichlorodiphenyltrichloroethane (DDT), a pesticide that was widely used in the United States and is still in use elsewhere for malaria control. PCBs, DDE, and other organochlorines are lipophilic and therefore accumulate in fatty tissue, biomagnifying up the food chain. They readily cross the placenta, placing the developing fetus at risk for exposure.

Like organochlorines, methylmercury (MeHg) bioaccumulates and crosses the placenta. The usual source of MeHg is consumption of contaminated fish and marine mammals, particularly larger long-lived predators at the top of the food chain. There have been several mass human poisoning incidents resulting from high levels of prenatal MeHg or PCB exposures in which psychomotor and cognitive delays were observed in the children, while the mothers remained relatively unaffected (Amin-Zaki et al. 1974; Harada 1978; Lai et al. 2001). Thus, the developing nervous system of the fetus appears to be especially vulnerable to the effects of these neurotoxicants.

There is evidence supporting particular susceptibility of memory and learning abilities to early life PCB and MeHg exposure (Darvill et al. 2000; Jacobson and Jacobson 1996; Oken et al. 2008). Furthermore, memory and learning skills are important to school performance and later life productivity (Sheslow and Adams 1990). Existing epidemiologic literature, however, is inconclusive with respect to the effect of modest prenatal PCB exposure on childhood memory and learning among general population samples (Gladen and Rogan 1991; Jacobson and

Jacobson 1996; Jacobson et al. 1990; Jacobson et al. 1992; Longnecker et al. 2003; Vreugdenhil et al. 2004). Findings from studies of the effects of prenatal MeHg on childhood memory and learning, across a range of exposures, are also inconsistent (Davidson et al. 2006; Grandjean et al. 1997; Oken et al. 2008; Palumbo et al. 2000). While there is less literature investigating the effect of DDE exposure on memory and learning, results are inconclusive (Eskenazi et al. 2006; Gladen and Rogan 1991; Torres-Sanchez et al. 2013). There is evidence of effect modification between prenatal exposure to PCBs and MeHg in determining measures of childhood neurodevelopment, but this interaction has not been consistently observed (Grandjean et al. 2001; Stewart et al. 2003). Lastly, many previous studies of organochlorines and MeHg were done in populations with relatively high levels of exposure due to dietary habits such as frequent consumption of contaminated fish (Davidson et al. 2001; Jacobson and Jacobson 1996; Stewart et al. 2003) or marine mammals (Ethier et al. 2012; Grandjean et al. 2001), making the generalizability of results to more commonly experienced lower exposures uncertain.

Though PCBs have been banned in the United States since the late 1970s, reservoirs exist in building structures and in soil, often in locales such as public schools, frequented by children (Herrick et al. 2004). Additionally, while dietary fish consumption is the primary exposure route for MeHg, and an important source of PCBs, fish contains many nutrients that are beneficial for pregnant women and developing fetuses. These potential nutritional confounders have not been consistently considered in prior literature.

Therefore, our study considers the association of joint low-level prenatal organochlorine and MeHg exposures, adjusted for potential nutritional confounders, with school-aged assessments of memory and learning.

Methods

Study population

The New Bedford Harbor was highly contaminated with PCBs from industrial waste disposal by local electronics manufacturing beginning in the 1940s through 1977 (Choi et al. 2006). The New Bedford Cohort study was designed to assess the relation of prenatal PCB, pesticide, and metal exposure with child development. 788 mother-infant pairs were recruited from St. Luke's Hospital in New Bedford, Massachusetts at the time of the infant's birth, between 1993 and 1998. There are approximately 2000 births at the hospital per year, about 10% of which met the study eligibility criteria and occurred when study examiners were available for recruitment and examination of newborns. All mothers were aged 18 or older and resided in one of four towns surrounding the contaminated harbor - New Bedford, Acushnet, Fairhaven, or Dartmouth. Families were primarily English speaking, though Portuguese study materials were available for the 6 Portuguese speakers. Mothers who had a cesarean section birth or delivered infants requiring high-grade neonatal care or who were not available for neonatal examination were excluded. Study children were assessed at birth, later infancy, and age 8 years. This analysis focuses on the 607 children who were assessed at age 8.

Organochlorine exposure assessment

Prenatal organochlorine exposure was estimated by measuring levels of 51 PCB congeners and *p,p'*-DDE in cord serum collected at birth. Analyses were performed at the Harvard School of Public Health Organic Chemistry Laboratory using high resolution gas chromatography with a capillary column and electron capture detection (Korrick et al. 2000).

The limit of detection (LOD) for individual PCBs ranged from 0.001 to 0.04 ng/g serum, with most <0.01 ng/g (Korrick et al. 2000). For DDE, the LOD was 0.07 ng/g serum (Korrick et al. 2000). Quantifiable values below the LOD were used to optimize statistical power and avoid biased exposure estimates associated with censoring at the detection limit (Kim et al. 1995). Reproducibility was high, with a 3% within-batch coefficient of variation and a 20% between-batch coefficient of variation for the sum of PCBs and similar performance for DDE.

Two PCB measures were used in our analysis: (1) The sum of the four most prevalent PCBs ($\sum\text{PCB}_4$) (congeners 153, 118, 138, 180), which were measured with the least measurement error and are most frequently used to investigate congener-specific effects; and (2) the weighted sum (toxic equivalent or TEQ) (Van den Berg et al. 2006) of the dioxin-like PCBs (mono-ortho congeners 105, 118, 156, 167, and 189), measured to assess congeners that may have a different toxicological action than non-dioxin-like PCBs. Because of insufficient serum volume for sample-specific lipid measures, concentrations of the $\sum\text{PCB}_4$ were measured on a wet weight basis. We used the average serum lipid content of discarded cord blood from the study hospital to lipid adjust dioxin-like PCBs so their measurement scale would be comparable to other studies (Sagiv et al. 2010).

Methylmercury exposure assessment

Prenatal MeHg exposure was assessed by measuring the total mercury (Hg) concentration in maternal peripartum hair. Hair Hg is a valid biomarker of MeHg exposure, as MeHg enters hair follicles in quantities directly proportional to levels in the blood, is incorporated into the hair shaft (Who 1990), and does not appear to change in concentration once incorporated (Myers and Davidson 1998). Hair was collected approximately 10 days after delivery and the proximal 3 cm was analyzed to estimate exposure in the last trimester. The last trimester may be the most

important exposure window with regards to many aspects of neurodevelopment (Grandjean et al. 1999a; Kershaw et al. 1980). When the proximal (cut)-end of the hair could not be determined (45% of samples), a random 3 cm segment was analyzed. Of note, average hair Hg levels of those with and without a known cut end were comparable (mean: 0.63 $\mu\text{g/g}$, range 0.03-5.13 for those with a known cut end; mean: 0.64 $\mu\text{g/g}$, range: 0.07-3.04 $\mu\text{g/g}$ for those without a known cut end). Although the Hg concentration of hair may be affected by external contamination, hair treatment, and growth rate (Suzuki 1988), hair treatment is less likely to affect hair close to the scalp (Ohba et al. 2008). Samples were washed prior to analysis to remove any external contamination. Hair Hg analysis was performed using direct mercury analysis methods for atomic absorption at the Harvard School of Public Health Trace Metals Laboratory (Oken et al. 2005). Lab recovery rates for quality control standards were 90-110%, precision was >95%, and the average LOD was 0.05 $\mu\text{g/g}$ of hair. None of the hair Hg measures were below the LOD.

Memory and learning assessment

At age 8 years, 607 (78% of eligible) study children participated in neuropsychological testing at our study clinic. Testing included the Wide Range Assessment of Memory and Learning (WRAML) which assesses immediate and delayed recall of verbal and visual material, and yields an overall general memory score and domain-specific indices of visual memory, verbal memory, and learning (Sheslow and Adams 1990). The WRAML consists of nine subtests, which are age-scaled, summed and standardized using manual norms to create the Verbal Memory Index, the Visual Memory Index, and the Learning Index, each with a mean of 100 and a standard deviation of 15.

Covariate assessment

After birth, a trained study nurse reviewed hospital records to obtain infant race/ethnicity, birth weight, and gestational age (based on mother's last menstrual period) as well as information regarding the mother's pregnancy and delivery and the baby's initial pediatric evaluation. An obstetrical (OB) risk score was derived to summarize any adverse conditions prior to or during pregnancy, birth, and the neonatal period (Hobel et al. 1973; Sokol et al. 1977).

At a 2-week home visit, mothers were interviewed to obtain information on maternal diet, including consumption of fish before and during pregnancy using a food frequency questionnaire (Salvini et al. 1989); smoking, alcohol, and drug use; medical and reproductive histories; infant feeding; detailed demographic (race/ethnicity, income), and occupational and educational histories for both parents. An estimate of prenatal omega-3 intake was calculated using self-reported intake from the food frequency questionnaire and the average omega-3 content of reported foods.

The 8-year study assessment included a home visit to assess the quality of the home environment and parenting skills using the Home Observation for Measurement of the Environment (HOME) (Caldwell and Bradley 1985). Maternal IQ was measured using the Kaufman Brief Intelligence Test (KBIT) (Kaufman and Kaufman 1990) and maternal depression symptoms were assessed using the Beck Depression Inventory (BDI-II) (Beck et al. 1996). A questionnaire interview of the mothers or primary caregivers was conducted to update information obtained at infancy. Blood lead levels measured as part of routine pediatric screenings between the ages of 9 months and 4 years were abstracted from medical records.

Statistical analysis

Bivariate analysis was used to examine the relationship of the four primary exposure measures ($\sum\text{PCB}_4$, dioxin-like PCBs, DDE, and MeHg) with the three primary outcome measures (Verbal Memory Index, Visual Memory Index, and Learning Index). Residual plots were examined to assess assumptions of homoscedasticity and normality of the residuals, and Cook's distances were used to assess whether any observations were unusually influential. Results of these regression diagnostics confirmed the appropriateness of using multivariable linear regression models with untransformed variables for our analyses.

Potential covariates considered in our models included examiner (two examiners were used); child age at exam, sex, race/ethnicity, birth year, birth weight, gestational age, school grade, peak blood lead level from ages 1-3; parental education at birth; household income at birth; OB risk score; 8-year HOME score; and maternal parity, maternal age at birth, race/ethnicity, birth place, marital status at birth, breastfeeding, prenatal smoking, prenatal alcohol, drug use in the year prior to birth, prenatal omega-3 consumption, prenatal fish consumption, IQ, and depression (modeled as continuous or categorical variables as shown in Table 1).

Three strategies were used to select covariates to include in models: 1) age at exam and examiner were selected *a priori* to remove potential administration bias and any residual age effects; 2) variables were selected for which a partial F-test indicated that they predicted any of the three outcomes with $\alpha < 0.1$ when adjusting for all other potential covariates, thereby improving model fit; and 3) variables were selected whose presence in the model substantially changed the primary exposure effect estimate. This was defined as being ranked in the top three change-in-effect estimates for more than one of the twelve exposure-outcome association models. Covariates included in the final adjusted models were: examiner; child age at exam, sex, birth

year, school grade, prenatal smoke and alcohol exposure; household income; parental education; and maternal age at birth, IQ, birth place, and pregnancy omega-3 intake. A statistical significance criteria of $p < 0.05$ was used for all analyses, unless otherwise noted.

Among the 607 children included in the 8-year follow-up, 602 had complete WRAML data, 583 of these had cord serum organochlorine measures, 405 of these had hair Hg measures, and 393 of these had complete covariate data. Children with complete outcome, exposure, and covariate data ($n=393$) were included in these analyses. To examine exposure effect modification, we fit models which included interaction terms between pairs of exposure measures. For interpretability, the primary exposure (either the organochlorine measure or MeHg) was kept continuous and the secondary exposure was dichotomized into the higher 1/3 versus the lower 2/3 of exposure. Interactions were also examined between the primary exposures and the following dichotomized sociodemographic covariates: sex, maternal IQ (dichotomized at the median), prenatal smoking (ever versus never), parental education (less than versus greater than or equal to high school education), income (less than \$20,000 versus greater than or equal to \$20,000), and breastfeeding (ever versus never). Particular focus was given to interaction by sex, given the importance of potential sexual dimorphism in sensitivity to prenatal PCB or Hg exposures (Castoldi et al. 2008; Cordier et al. 2002; Sagiv et al. 2012a; Sagiv et al. 2012b).

The study protocol was reviewed and approved by the human subjects committees of the Harvard School of Public Health and Brigham and Women's Hospital (Boston, Massachusetts) and of Southcoast Hospitals Group (New Bedford, Massachusetts). Written informed consent was obtained from all participating families before study evaluation.

Results

Comparisons of children with complete outcome data who were included in this analysis or excluded because of missing covariate or exposure data are shown in Table 1. Outcome and exposure values were similar in the two groups. Children included in our analysis had higher maternal IQ, higher parental education, and higher rates of breastfeeding than those excluded. Both children and mothers who were included were more likely to be white than those who were not included. Although mothers of included children were also more likely to have smoked and consumed alcohol during pregnancy, there was considerable missing data for these covariates among those excluded. Sensitivity analyses showed that unadjusted associations between each exposure and outcome pair in the full dataset (those with complete outcome data) were comparable to associations observed in the analysis data set (those with complete outcome, exposure, and covariate data) (data not shown).

Our study sample had lower WRAML scores than the test standardization sample (mean 100, s.d. 15) consistent with sociodemographic disadvantage (Table 1). Girls tended to score higher than boys on the WRAML indices (see Supplemental Material, Table S1). Children in the 2nd grade or greater and those with higher household income and parental education, mothers who did not smoke while pregnant, and mothers with higher IQ also tended to perform better on the WRAML (see Supplemental Material, Table S1). Non-hispanic white children scored higher than children of other races or ethnicities (data not shown).

As expected, the three organochlorine measures were significantly correlated with each other, with Spearman correlation coefficients ranging from 0.59 to 0.89. Organochlorines were also significantly correlated with MeHg, though Spearman correlation coefficients were lower,

ranging from 0.33 to 0.41. The three WRAML indices were also significantly correlated with each other with Spearman correlation coefficients ranging from 0.41 to 0.47.

Table 2 compares the crude and final adjusted models. Prenatal MeHg exposure was associated with lower scores for all three measures of childhood memory and learning. The magnitude of the negative associations increased with adjustment for covariates, indicating negative confounding of the unadjusted estimates. While, the magnitude of the association was similar for all three WRAML indices, the MeHg associations were statistically significant only for the Visual Memory Index ($p=0.01$, compared with 0.14 and 0.08 for the Verbal Memory and Learning indices, respectively). No significant adverse associations of $\sum\text{PCB}_4$, dioxin-like PCBs, or DDE with memory and learning were observed (Table 2). Model diagnostics and scatterplots showed that there were a small number of observations ($n=1-3$) (see Table 2) with particularly high exposure values. Although there was a significant positive association between DDE and the Learning Index score before and after covariate adjustment, the adjusted estimate was essentially null after three children with particularly high exposures were excluded from the analysis (DDE levels of outliers: 9.74, 10.26, and 14.93 ng/g serum vs. DDE levels of non-outliers: mean 0.5, s.d. 1.1, range 0.02- 3.00 ng/g serum).

Given the associations between MeHg and the WRAML indices, the components of each index were examined to more precisely characterize the exposure-outcome relationships. Adjusted results are shown in Figure 1. Almost all of the subtests showed negative associations with MeHg exposure. In particular, the Finger Windows component of the Visual Index and the Visual Learning component of the Learning Index showed significant negative associations with prenatal MeHg exposure (Finger Windows: $\beta=-0.49$, 95% CI: -0.98, -0.01, p -value= 0.05; Visual Learning: $\beta=-0.50$, 95% CI: -1.01, 0, p -value= 0.05).

No significant interactions with the sociodemographic variables (maternal IQ, prenatal smoking, parental education, household income, breastfeeding) were observed (data not shown). Though there were also no significant interactions by sex, consistent with the potential increased sensitivity of boys to MeHg neurotoxicity observed elsewhere (Castoldi et al. 2008; Cordier et al. 2002; Sagiv et al. 2012b), the negative association between MeHg and the Learning Index was stronger in boys than girls ($\beta=-4.1$, 95% CI: -7.9, -0.7 for boys vs. $\beta=-0.7$, 95% CI: -3.7, 2.4 for girls, p for interaction=0.12). Associations between MeHg and the WRAML Indices were comparable when stratified by PCB levels, with the exception of the Verbal Memory Index wherein suggestive differences by PCB exposure were observed. Specifically, MeHg-Verbal Memory Index associations were negative among children with high (upper tertile) PCB levels ($\beta=-2.5$, 95% CI: -5.3, 0.3) and close to null among those with low (lower two tertiles) PCB levels ($\beta=0.4$, 95% CI: -3.3, 4.2) (p for interaction=0.21). PCB concentrations were not significantly associated with the memory and learning indexes, regardless of whether MeHg levels were high or low (data not shown).

Discussion

At the exposure levels in our study, after removing outliers, there were no significant associations between prenatal PCB or DDE exposures and memory and learning skills as assessed by the WRAML. Similarly, prenatal PCB exposure was not associated with several learning outcomes that were assessed among school-aged children in the Faroe Islands cohort (Grandjean et al. 2001) or in a cohort based in North Carolina (Gladden and Rogan 1991). However, a study conducted on children born to Lake Michigan fish eaters, reported that prenatal PCB exposure was associated with lower scores on the Sternberg, a test of working memory and

executive function, at age 4 (Jacobson et al. 1992), and the McCarthy memory scale at age 4, which assesses short-term recall (Jacobson et al. 1990).

The PCB exposure levels in our cohort were much lower than most other cohorts, including the Michigan cohort (Longnecker et al. 2003). Our null PCB findings may be due to non-linearity (e.g., a threshold effect) in the dose-response relationship at low exposure levels or perhaps a lack of power. Furthermore, exposure sources may be different in our cohort than in Michigan and other populations selected based on contaminated fish intake (Jacobson et al. 1990; Stewart et al. 1999). The New Bedford population was not selected based on fish consumption. Cord serum PCB levels were not only related to fish consumption, but also to consumption of organ meats, dairy, and demographic and lifestyle factors (Choi et al. 2006). Additionally, the congener profile of PCBs in New Bedford may contribute to differential toxicity as, for example, it is disproportionately high in congener 118 compared with other population-based samples (Korrick et al. 2000).

Hair Hg concentrations were associated with lower WRAML memory and learning index scores, and this negative association was statistically significant for Visual Memory (Table 2). With respect to the components of the Indices, hair Hg was associated with significantly lower scores on the Finger Windows subtest of the Visual Memory Index, in which the child is asked to manually reproduce a demonstrated spatial sequence, and the Visual Learning subtest of the Learning Index, in which the child is asked to recall a fixed number of abstract images on a grid, though associations with some of the other components of each index were non-significant but similar in magnitude. This was particularly the case for the Design Memory subtest of the Visual Memory Index and the Verbal Learning subtest of the Learning Index (Figure 1). The Finger Windows, Visual Learning, and Design Memory subtests primarily measure skills related to

visual-spatial memory, suggesting that this domain may be particularly sensitive to the effects of low-level prenatal MeHg exposure.

These results are consistent with a study conducted in Hong Kong in which low-dose prenatal MeHg exposure was adversely associated with a visual sequencing task among 6-10 year olds (Lam et al. 2013). Additionally, decreased performance on the Stanford-Binet copying tests, which measures visuospatial performance, was associated with maternal hair mercury in 5-6 year olds in French Guiana (Cordier et al. 2002), and with child hair mercury in a population of 7-12 year olds in the Amazon Basin (Grandjean et al. 1999b). In these latter two cross-sectional studies, children experienced substantial MeHg exposure (geometric mean hair Hg levels ranging from 1.4-11.0 $\mu\text{g/g}$) from consumption of fish contaminated with Hg pollution from gold mining. Furthermore, functional magnetic resonance imaging of Faroese boys (a subsample of a population with mean maternal hair Hg: 4.3 $\mu\text{g/g}$) showed that for tasks requiring visual processing, adolescents with higher prenatal exposure to MeHg had increased and more widespread brain activation than those with lower MeHg exposures, particularly in the primary visual cortex, suggesting toxicant-related damage to visual association areas (White et al. 2011). Likewise, a study of school-aged Inuit from Northern Quebec showed that prenatal mercury (mean cord blood Hg: 4.6 $\mu\text{g/dL}$) was associated with a reduction in the amplitude of specific visual evoked potentials, suggesting subclinical deficits in visual processing (Ethier et al. 2012). Indeed, the above findings, in combination with other evidence from the literature, suggest that centrally mediated visual functions may be important targets of MeHg toxicity (Burbacher et al. 2005; Dasari and Yuan 2009; Saint-Amour et al. 2006).

In contrast to the associations with visual memory found in our study and others (Cordier et al. 2002; Grandjean et al. 1999b; Lam et al. 2013), no significant MeHg associations were found

with the Design Memory subtest of the WRAML Visual Memory Index (Myers et al. 2003) in the Seychelles Islands (mean maternal hair Hg: 6.9 µg/g). However, potential confounding by the beneficial effects of polyunsaturated fatty acids were not taken into account in these Seychelles analyses which may, in part, account for null associations (Davidson et al. 2008; Oken et al. 2005).

Associations between hair Hg and outcomes were similar between strata defined by a range of sociodemographic factors. Although the interaction was not significant, MeHg exposure was associated with lower verbal learning scores in children with high PCB exposure, but not among children with low PCB exposure. Enhanced MeHg neurotoxicity in the setting of PCB exposure has been observed elsewhere. For example, in another study population with prenatal PCB and MeHg exposures comparable to New Bedford, MeHg was negatively associated with cognitive function at age 3 only among children with higher PCB exposure (Longnecker et al. 2003; Stewart et al. 2003).

The New Bedford cohort has been exposed to both PCBs and MeHg, allowing us to investigate potential interactions between these contaminants while accounting for potential nutritional confounders. Biomarkers were available for exposure measurement and, although we lacked individual cord serum lipid levels for our organochlorine analyses, the lipid content of cord serum is low and relatively constant (Denkins et al. 2000) so this is unlikely to have materially impacted our findings. Additionally, hair may be a less sensitive measure of MeHg than blood (Budtz-Jorgensen et al. 2004), which likely biased associations towards the null.

The New Bedford cohort includes a diverse population with a large representation of lower income families. Such populations are less studied and perhaps disproportionately exposed to

environmental hazards. Results of this study support the finding that prenatal MeHg exposure may be associated with decrements in memory and learning, particularly visual memory. This study contributes to the limited body of literature on the effect of prenatal toxicants on memory and learning, and suggests that further population-based research should be undertaken to better understand the effects of low-level prenatal MeHg exposures on child memory and learning, in general, and aspects of memory and learning related to visual processing, in particular.

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Table 1. Descriptive characteristics of included vs. excluded New Bedford Cohort children born 1993-1998 and assessed with the WRAML at age 8 years.

Descriptive characteristic	Included^a N (%)	Included^a Mean ± SD	Included^a Range	Excluded^b N (%)	Excluded^b Mean ± SD	Excluded^b Range
Outcome measures						
Verbal Memory Index	393	88.3 ± 13.2	48-126	209	87.4 ± 14.6	52-130
Visual Memory Index	393	91.2 ± 13.0	52-133	209	91.4 ± 12.4	57-119
Learning Index	393	97.5 ± 14.0	55-135	209	97.8 ± 13.8	60-142
Exposure measures						
∑PCB ₄ (ng/g serum) ^c	393	0.3 ± 0.3	0.01-4.4	192	0.3 ± 0.3	0-2.6
Dioxin-like PCBs (pg TEQ/g lipid) ^d	393	1.5 ± 2.9	0-42.8	192	1.4 ± 1.7	0.1-12.0
DDE (ng/g serum)	393	0.5 ± 1.1	0.02-14.9	192	0.5 ± 0.9	0-10.2
Hair Hg (µg/g)	393	0.6 ± 0.6	0.03-5.1	27	0.5 ± 0.3	0.1-1.2
Child characteristics						
Age at exam (years)	393	8.1 ± 0.6	7.1-11.0	209	8.2 ± 0.7	7.2-10.7
Birthweight (grams)*	393	3384 ± 431	2242-5221	209	3454 ± 451	1901-4767
Gestational age (weeks)	391	39.7 ± 1.3	33.0-42.5	209	39.8 ± 1.3	34.4-42.5
Gender (male)	196 (49.9)			110 (52.6)		
Child's race/ethnicity (white)*	287 (74.0)			124 (61.1)		
Birth year*						
1993-1994	112 (28.5)			81 (38.8)		
1995-1996	172 (43.8)			61 (29.2)		
1997-1998	109 (27.7)			67 (32.1)		
Grade (2 nd or higher)	340 (86.5)			182 (87.5)		
Breastfed > 1 month (yes)*	218 (55.5)			91 (44.0)		
Prenatal smoking (yes)*	131 (33.3)			34 (20.2)		
Prenatal alcohol (yes)	88 (22.4)			23 (18.6)		
Illicit drug in year prior to birth (yes)	54 (13.8)			20 (16.1)		
Prenatal omega 3 (grams/month)	393	28.3 ± 14.7	5.3-106.6	117	29.1 ± 15.8	7.3-97.1
Peak blood lead, age 1-3 (µg/dl)	374	6.8 ± 4.1	1.0-38.0	197	6.9 ± 3.6	1.0-22.8

Descriptive characteristic	Included^a N (%)	Included^a Mean ± SD	Included^a Range	Excluded^b N (%)	Excluded^b Mean ± SD	Excluded^b Range
Maternal characteristics						
Maternal age at birth (years)	393	26.9 ± 5.3	18.0-40.7	209	26.4 ± 5.6	18.1-41.3
Maternal race (white)*	322 (81.9)			94 (72.3)		
Maternal birth place (U.S.)	313 (79.6)			103 (81.8)		
Marital status at birth (married)*	240 (61.1)			98 (52.1)		
Parity (first birth)	246 (62.6)			129 (61.7)		
Parental education*						
Both less than HS	28 (7.1)			29 (14.4)		
At least one HS	153 (38.9)			79 (39.1)		
At least one greater than HS	212 (53.9)			94 (46.5)		
Household income						
Less than 20K	134 (34.1)			70 (34.0)		
20K to 40K	124 (31.6)			75 (36.4)		
Greater than 40K	135 (34.4)			61 (29.6)		
Maternal IQ (K-BIT)*	393	98.7 ± 10.1	57-124	206	96.6 ± 10.8	68-126
Maternal depression (BDI)	391	8.0 ± 8.4	0-49	209	8.8 ± 9.3	0-46
HOME score	382	45.7 ± 5.4	28-57	206	45.3 ± 5.4	30-57
OB risk score	393	15.0 ± 10.1	0.5-52.5	209	16.0 ± 10.8	0-52.8

Abbreviations: WRAML, Wide Range Assessment of Memory and Learning; PCBs, polychlorinated biphenyls; TEQ, toxic equivalent; DDE, dichlorodiphenyl dichloroethylene; MeHg, methylmercury; HS, high school; KBIT, Kaufman Brief Intelligence Test; BDI, Beck Depression Inventory; HOME, Home Observation for Measurement of the Environment; OB risk, obstetrical risk score.

^aChildren with complete exposure, outcome, and covariate data who were included in our analysis. ^bChildren who were excluded from the analysis because of missing exposure or covariate data. ^cCongeners 153, 118, 138, and 180. ^dCongeners 105, 118, 156, 167, and 189.

*Significant difference between means of included and excluded participants, $p < 0.05$.

Table 2. The change in WRAML score associated with a one unit increase in serum organochlorines or hair Hg levels: crude^a, adjusted^b, and adjusted without outliers^c.

Outcome and exposure measure	Crude β (95%CI)	Adjusted β (95%CI)	Adjusted β without outliers (95%CI)
Verbal Memory Index			
Σ PCB ₄ (ng/g) ^d	-1.3 (-5.3, 2.7)	-1.9 (-6.1, 2.3)	-1.4 (-7.3, 4.5)
TEQ (pg/g lipid) ^e	-0.2 (-0.6, 0.2)	-0.2 (-0.6, 0.3)	-0.1 (-0.8, 0.5)
DDE (ng/g)	0.2 (-1.0, 1.3)	0.1 (-1.1, 1.3)	1.8 (-5.4, 1.8)
MeHg (μ g/g)	-1.1 (-3.3, 1.1)	-1.7 (-3.9, 0.6)	-1.9 (-4.3, 0.5)
Visual Memory Index			
Σ PCB ₄ (ng/g) ^d	3.2 (-0.8, 7.1)	0.5 (-3.8, 4.8)	-0.2 (-6.2, 5.8)
TEQ (pg/g lipid) ^e	0.3 (-0.2, 0.7)	0.0 (-0.4, 0.5)	0.0 (-0.7, 0.6)
DDE (ng/g)	1.1 (0.0, 2.3)	0.6 (-0.6, 1.8)	0.1 (-3.6, 3.7)
MeHg(μ g/g)	-0.9 (-3.1, 1.3)	-2.8 (-5.0, -0.6)*	-3.1 (-5.5, -0.6)*
Learning Index			
Σ PCB ₄ (ng/g) ^d	3.2 (-1.1, 7.4)	-0.8 (-5.5, 3.8)	3.3 (-3.1, 9.7)
TEQ (pg/g lipid) ^e	0.2 (-0.3, 0.7)	-0.1 (-0.6, 0.4)	0.4 (-0.3, 1.1)
DDE (ng/g)	1.6 (0.4, 2.9)*	1.4 (0.1, 2.7)*	-0.3 (-4.2, 3.6)
MeHg(μ g/g)	-0.3 (-2.7, 2.0)	-2.2 (-4.6, 0.2)	-2.4 (-5.0, 0.3)

Abbreviations: MeHg, methylmercury; WRAML, Wide Range Assessment of Memory and Learning; PCBs, polychlorinated biphenyls; TEQ, toxic equivalent; DDE, dicholorodiphenyl dichloroethylene.

*p<0.05.

^aAdjusts for child's age at exam and examiner (n=393). ^bAdjusts for child's age at exam, examiner, sex, birth year, school grade, parental education, maternal age at birth, maternal birth place, household income, prenatal smoke exposure, prenatal alcohol exposure, prenatal omega 3 exposure, and maternal IQ (n=393). ^cAdjusts for same covariates as 'b'. For Σ PCB₄, TEQ and MeHg, n=392; for DDE, n=390. ^dCongeners 153, 118, 138, and 180. ^eCongeners 105, 118, 156, 167, and 189.

Figure legend

Figure 1. Associations (β and 95%CI) between a 1- $\mu\text{g/g}$ increase in maternal hair Hg and components of the WRAML indices. Estimates from multivariable linear regression models adjusted for child's age at exam, examiner, sex, birth year, school grade, parental education, maternal age at birth, maternal birth place, household income, prenatal smoke exposure, prenatal alcohol exposure, prenatal omega 3 exposure, and maternal IQ.

Figure 1.

