Does background postnatal methyl mercury exposure in toddlers affect cognition and behavior?

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ABSTRACT

Because the toxicological effects of mercury (Hg) are more serious in the developing central nervous system of children than adults, there are growing concerns about prenatal and early childhood Hg exposure. This study examined postnatal methylmercury (MeHg) exposure and cognition and behavior in 780 children enrolled in the Treatment of Lead (Pb)-exposed Children clinical trial (TLC) with 396 children allocated to the succimer and 384 to the placebo groups. Mercury exposure was determined from analyses of blood drawn 1 week before randomization and 1 week after treatment began when succimer had its maximal effect on blood Pb (PbB). The baseline MeHg concentrations were 0.54 μg/L and 0.52 μg/L and post-treatment concentrations were 0.51 μg/L and 0.48 μg/L for placebo and succimer groups, respectively. Because the baseline characteristics in the two groups were balanced and because succimer had little effect on MeHg concentration and no effect on the cognitive or behavioral test scores, the groups were combined in the analysis of MeHg and neurodevelopment. The children's IQ and neurobehavioral performance were tested at age 2, 5 and 7 years. We saw weak, non-significant but consistently positive associations between blood MeHg and IQ test scores in stratified, spline regression and generalized linear model data analyses. The behavioral problem scores were constant or decreased slightly with increasing MeHg concentration. Additional adjustment for PbB levels in multivariable models did not alter the conclusion for MeHg and IQ scores, but did confirm that concurrent PbB was strongly associated with IQ and behavior in TLC children. The effects of MeHg on neurodevelopmental indices did not substantially differ by PbB strata. We conclude that at the present background postnatal MeHg exposure levels of US children, adverse effects on children's IQ and behavior are not detectable.

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1. Introduction

Mercury (Hg) is ubiquitous in the global environment and derives from both natural sources and human enterprise. Because the toxicological effects of this heavy metal are more serious in the developing central nervous system of children than adults, the greatest concern is about prenatal and early childhood mercury exposure (Stokstad, 2004). There are three forms of Hg that differ toxicologically: elemental Hg, organic Hg [mainly methylmercury (MeHg)], and inorganic Hg. Children are exposed to elemental Hg by inhaling the vapor from the elemental liquid or "quicksilver", folk medications, or dental amalgam; to inorganic Hg from old teething powders containing calomel, from certain religious rituals, and from metabolic oxidation of the elemental form. High elemental and inorganic Hg exposures produce acrodynia, proteinuria, hypertension, mood disturbances, cognitive declines, urticaria, insomnia and a painful rash (Clarkson et al., 2003). High prenatal exposure to MeHg produces a cerebral palsy-like illness accompanied by limb deformities, severe mental retardation and sensory handicaps in children. There have been two major outbreaks, one in Japan from contaminated fish (Harada, 1978), the other in Iraq (Amin-Zaki et al., 1976) from consumption of seed wheat grain preserved with MeHg.

In 2000, the U.S. National Research Council (NRC) concluded that chronic low-dose prenatal MeHg exposure from maternal consumption of fish (>0.1 μg/kg/day after accounting for uncertainty factors) may be associated with poorer performance on neurobehavioral tests, particularly those measuring attention,

fine-motor function, language, visual-spatial abilities, and verbal memory (NRC, 2000). This conclusion was supported mainly by results from studies in the Faroe Islands (Grandjean et al., 1997) and New Zealand (Kjellstrom et al., 1989) although a study conducted in the Seychelles did not confirm these observations (Davidson et al., 1998).

An integrative analysis of epidemiologic data from these 3 large scale cohort studies estimated the dose–response relationship of prenatal MeHg exposure and IQ in children. It concluded that, despite some inconsistencies, IQ was a useful end point for estimating cognitive effects of prenatal MeHg exposure (Axelrad et al., 2007). In the United States, several Federal agencies have examined data on MeHg exposure and its consequences, and have attempted to establish recommendations for safe exposures in children and pregnant women in the general population (NRC, 2000; Schober et al., 2003; ATSDR, 1999). However, most existing studies are of children with relatively high prenatal MeHg exposure owing to high volume consumption of finfish and/or sea mammals, and the results cannot be easily extrapolated to lower level prenatal as well as postnatal exposure conditions.

Concerns over early low level postnatal exposure to organic Hg from vaccines have been raised in recent years. For decades, vaccines such as pertussis, diptheria, tetanus, and influenza were preserved with thimerosal, an antimicrobial preparation composed of 50% ethylmercury. Assuming that the toxicity of ethylmercury is similar to MeHg, the exposure from a single vaccination would exceed Federal guidelines for that day and adherence to the usual pediatric vaccination schedule would deliver up to 188 μg by 6 months of age (Goldman and Shannon, 2001). However, ethyl mercury is believed to be eliminated more quickly than MeHg, resulting in lower concentrations in central nervous system tissues (Burbacher et al., 2005). Nevertheless, it has been speculated that the use of this preservative may have contributed to the "epidemic" number of cases of Autism Spectrum Disorder (ASD) observed in the United States and other developed countries (Bernard et al., 2001). However, the results of several large scale epidemiological studies have not supported this association (Rutter, 2005).

Because we conducted a large trial of the treatment of pediatric Pb poisoning that included detailed, prospective measures of cognition and behavior, and because we had blood samples available for further analysis, we were well positioned to study presumably low level postnatal Hg exposure and neurodevelopment. Thus we chose to assess the effects of Hg exposure at around age 2 years with cognitive and behavioral tests scores at baseline and up to 5 years later.

2. Materials and methods

2.1. Sample and study design

The data in this study came from the Treatment of Lead-exposed Children trial (TLC), a 4-site, placebo-controlled, randomized clinical trial. The TLC study was conducted between September 1994 and June 2003 in Philadelphia, PA; Newark, NJ; Cincinnati, OH; and Baltimore, MD. It was approved by the institutional review boards at the clinical sites, data coordinating center, the CDC, and the National Institute of Environmental Health Sciences.

The TLC study design is published elsewhere (TLC, 1998). Briefly, we accepted referral of children who were 12–33 months of age, had blood Pb levels between 20 μg/dL and 44 μg/dL and could be tested in English or Spanish. Children who had confirmed venous PbB concentrations between 20 μg/dL and 44 μg/dL and lived in cleanable housing had a second visit to confirm PbB levels. A total of 780 children entered the randomization phase, with 396 children allocated to succimer and 384 allocated to placebo.

In the last wave of follow-up, a total of 128 subjects were lost: 59 in the placebo group and 69 in the active drug group. Three subjects in the placebo group and 2 subjects in the succimer group were excluded from the final analysis because of conditions affecting mental or neuromotor status that are unlikely to be related to their exposure to Pb or mercury (autism spectrum disorder, epilepsy, retinal degeneration). The participant flow of TLC study is shown in Fig. 1.

2.2. IQ, cognitive, neuropsychological and behavioral tests

Before treatment began, we administered the Bayley Scales of Infant Development-II (BSID-II), the most widely used measure of infant intelligence (Bayley, 1993). The Mental Development Index (MDI) of the BSID-II is statistically analogous to an IQ score. After treatment, two waves of cognitive, behavioral, psychological and developmental assessment were administered at about age 5 and 7 years. Of all children who participated in the trial, 745 had caregiver's IQ measured with a short form of the Wechsler Adult Intelligence Scale-Revised (Silverstein, 1985). The administration of behavioral questionnaires to parents was completed by trained examiners reading questions aloud and providing clarification of items as necessary; all TLC examiners were blind to caregiver's IQ levels and their children's blood Hg and PbB concentrations. Table 1 presents TLC neurodevelopmental assessments by age of follow-up.

2.3. Blood mercury measurement

The Division of Laboratory Sciences at the National Center for Environmental Health of the Centers for Disease Control and Prevention analyzed the blood sample drawn about 1 week before randomization, and the sample drawn 1 week after treatment began (this sample showed the largest estimated mean difference between treatment groups in PbB level). Whole blood specimens were analyzed for Hg concentration. We measured total Hg in all samples. Since the lab's experience was that inorganic Hg was not detected in samples with less than 1 μg/L total Hg, we measured inorganic Hg only when the total Hg concentration was ≥ 1 μg/L, and in post-treatment samples with <1 μg/L if the baseline sample had been measured. In the US, 80–95% of Hg in blood is MeHg, while Hg in urine consists mostly of inorganic Hg (Mahaffey et al., 2004; Oken et al., 2008). These known distinctions assist in interpreting blood Hg levels in study subjects as well as patients in clinical studies (CDCP, 2005). Thus, we calculated blood MeHg as total Hg minus inorganic Hg, and assumed that all Hg was MeHg in samples with total Hg <1 μg/L or with undetectable inorganic Hg.

Specimens were analyzed using automated cold vapor atomic absorption spectrophotometry. The detection limit was 0.33 μg/L for total Hg and 0.35 μg/L for inorganic Hg. Throughout this study, all results of Hg given in μg/L can be converted to nmol/L by multiplying a conversion factor of 4.99.

2.4. Statistical analysis

Because more than 15% of the total Hg levels and more than 60% of inorganic Hg levels were below the limit of detection (LOD), we treated them with techniques for heavily left-censored data. In summary analyses, instead of simply replacing the non-detectable observations with LOD, in the dataset, the non-detectable values were read as being between zero and LOD (the "censoring interval"), then a maximum likelihood estimation (MLE) method was used to estimate mean, percentiles and variance. Censored data analysis results indicated that concentrations of total Hg, MeHg and inorganic Hg were lognormally distributed and logarithmic transformation of these dependent variables was used
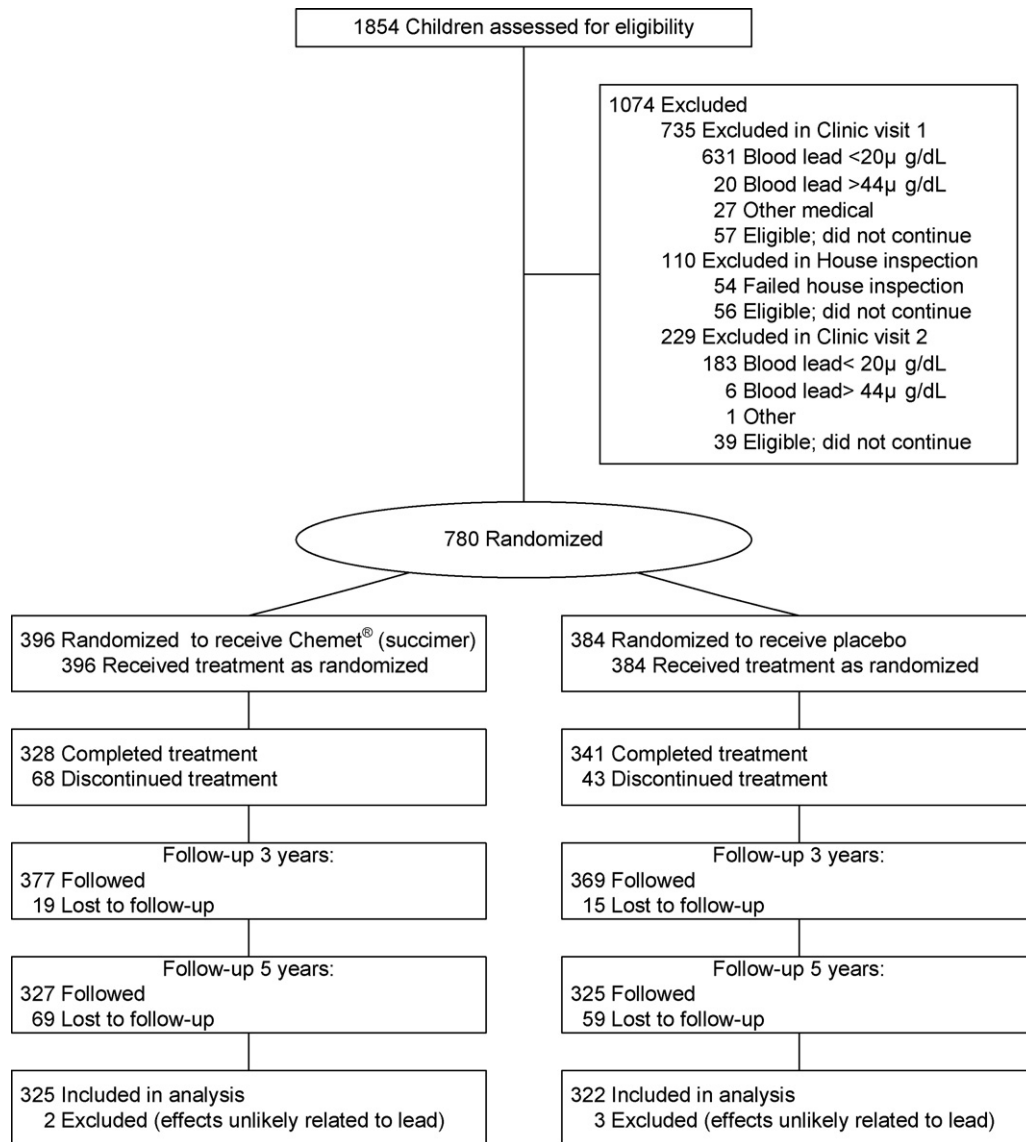
Fig. 1. Flow of patients through the TLC clinical trial. *Children who discontinued treatment and participated in the study through 7 years were included in the intent-to-treat analysis.

to conform to the requirement of a normal distribution. Censored data analyses were done with Minitab 15.1 software (Minitab Inc., State College, PA). For geometric mean (GM) estimation, however, we used the method of replacing non-detectable values with LOD.

We examined associations between 2 years blood MeHg, prospectively measured PbB and IQ or behavioral indices at 2, 5, and 7 years. For censored data, the non-parametric Kendall's tau-b correlation coefficient (ktau-b) was used, which makes no assumption of linearity between variables and accommodates multiple-censored data (Helsel, 2004). We examined the MeHg and IQ and behavior associations using scatter plot and cubic smoothing splines (which provided a fitted curve constructed by piecewise polynomials) with SPLUS 8.0 software (Insightful Corp, Seattle, WA).

Because spline regressions showed an approximately linear relation, we used a general linear model (GLM) for examining the MeHg effect. Based on the MeHg literature (Grandjean et al., 1997; Kjellstrom et al., 1989; Davidson et al., 1998; van Wijngaarden et al., 2006) and our previous work with these data (Chen et al., 2007; Chen et al., 2005), a priori covariates included exact age at IQ measurement, treatment group (not included in baseline model),

caregiver's IQ, clinical center, single parent (yes or no), language (English or Spanish), race (non-Hispanic black or others), gender, parent's employment (neither working or either working), parent's education (<12 years, 12 years or >12 years) and concurrent PbB level. Treatment per se was not associated with mercury, IQ or behavior scores in GLM analysis, and additional adjustment for treatment group did not markedly change the result; thus, the groups were combined in subsequent analyses. The same set of covariates was included for the regression models for 2 years blood MeHg and outcomes at baseline, 5 years and 7 years of age.

We analyzed the results for organic and inorganic Hg separately. All models were examined for statistical outliers and influential points. Each model was run first with outliers, then without outliers, and the results were compared. All of the results were essentially the same with or without outliers. The regression analyses for all measures were also repeated without influential points to determine whether the original results were dependent on such points. The results without influential points were consistent with the original analysis. Correlation and GLM analyses were done by Stata 10.0 software (Stata Corp, College Station, TX) and SAS 9.13 software (SAS Institute, Inc., Cary, NC).

Table 1
TLC neuropsychological and behavioral test instruments.

Neuropsychological and behavioral domains	Instruments (scale)
Age 5	
Cognition, learning, and memory	
Intellectual attainment	Wechsler Preschool and Primary Scales of Intelligence-Revised (WPPSI-R) (full-scale IQ) (Wechsler, 1989)
Attention/executive functions	Developmental Neuropsychological Assessment (NEPSY) (attention/executive function subscale) (Korkman et al., 1998)
Behavior	
Behavioral conduct	Conners' Parent Rating Scale-Revised (CPRS-R) (externalizing problems) (Conners, 1997)
Age 7	
Cognition, learning, and memory	
Intellectual attainment	Wechsler Intelligence Scale for Children-III (WISC-III) (full-scale IQ) (Wechsler, 1991)
Attention/executive functions	NEPSY (attention/executive function subscale) (Korkman et al., 1998)
	Conners' Continuous Performance Test (CPT) (d-prime) (Conners, 1995)
Verbal learning and memory	California Verbal Learning Test for Children (CVLTC) (list A memory and learning slope) (Delis et al., 1994)
Reading	Woodcock Language Proficiency Battery-Revised (WLPB-R) (broad reading score) (Woodcock, 1991)
Behavior	
Behavioral conduct	Behavioral Assessment System for Children–Parent Rating Scale (BASC-PRS) (externalizing problems) (Reynolds and Kamphaus, 1992)
Behavioral and academic conduct	Behavioral Assessment System for Children–Teacher Rating Scale (BASC-TRS) (adaptive skills, externalizing problems, school problems) (Reynolds and Kamphaus, 1992)
Neuromotor	
Neurological	Neurological Examination for Subtle Signs (NESS) (rapid sequential movements time) (Denckla, 1985)
Motor speed	CPT (hit reaction time) (Conners, 1995)

Table 2
Demographic characteristics and blood Hg levels of TLC children at baseline and follow-ups.

Characteristic	Placebo group ($N = 384$)	Succimer group ($N = 396$)
Clinic center-no. (%)		
Baltimore	104 (27.1)	109 (27.5)
Newark	102 (26.6)	106 (26.8)
Philadelphia	83 (21.6)	82 (20.7)
Cincinnati	95 (24.7)	99 (25.0)
Ethnic group or race-no. (%)		
White	42 (10.9)	46 (11.6)
African-American or Black	292 (76.1)	310 (78.3)
Other	50 (13.0)	40 (10.1)
Female sex-no. (%)	166 (43.2)	179 (45.2)
English-speaking-no. (%)	364 (94.8)	377 (95.2)
Parent's education <12 years—no. (%)	155 (40.4)	161 (40.7)
Neither parent working-no. (%)	220 (57.3)	234 (59.4)
Living with single parent-no. (%)	279 (73.4)	282 (72.3)
Baseline blood Hg (μg/L)		
Total[a]	0.56 (0.52, 0.61) ($N = 374$)	0.54 (0.50, 0.59) ($N = 393$)
Organic[a]	0.54 (0.50, 0.58) ($N = 328$)	0.52 (0.48, 0.56) ($N = 342$)
Inorganic[b]	0.28 (0.23, 0.33) ($N = 67$)	0.25 (0.21, 0.28) ($N = 76$)
Post-treatment blood Hg (μg/L)		
Total[a]	0.55 (0.52, 0.59) ($N = 379$)	0.52 (0.49, 0.55) ($N = 389$)
Organic[a]	0.51 (0.49, 0.54) ($N = 325$)	0.48 (0.45, 0.51) ($N = 335$)
Inorganic[b]	0.28 (0.24, 0.33) ($N = 71$)	0.30 (0.25, 0.36) ($N = 72$)
Age-years		
Baseline	2.0 ± 0.5[c]	2.0 ± 0.5
At Hg measurement	2.1 ± 0.5 ($N = 337$)	2.1 ± 0.5 ($N = 345$)
At MDI measurement	2.0 ± 0.5 ($N = 382$)	2.0 ± 0.5 ($N = 394$)
At 5 years IQ measurement	5.0 ± 0.5 ($N = 360$)	5.0 ± 0.5 ($N = 370$)
At 7 years IQ measurement	7.1 ± 0.2 ($N = 323$)	7.1 ± 0.2 ($N = 324$)
IQ score		
MDI (analogous to 2 years IQ)	81.5 ± 13.7 ($N = 375$)	82.8 ± 13.8 ($N = 390$)
5 years IQ	80.7 ± 13.1 ($N = 358$)	80.6 ± 13.4 ($N = 369$)
7 years IQ	86.5 ± 13.4 ($N = 321$)	86.9 ± 13.2 ($N = 323$)
Blood Pb—μg/dL		
Baseline	25.9 ± 4.8	26.5 ± 5.4
5 years	11.93 ± 4.9 ($N = 360$)	12.2 ± 5.5 ($N = 371$)
7 years[d]	8.0 ± 4.1 ($N = 312$)	8.0 ± 4.0 ($N = 310$)

[a] Geometric mean and 95% confidence interval, adjusted by age, gender, race and center.
[b] Unadjusted value, only include children with total Hg > 1.0 μg/L.
[c] Plus-minus values are means ± standard deviation.
[d] One child with a blood Pb concentration of 50.8 μg/dL was excluded from the analysis.

Table 3
Mean and standard deviation of blood MeHg concentrations, IQ and behavioral outcomes and correlations between baseline blood MeHg concentration and IQ and behavioral outcomes at specific ages.

Age	Variables	n	Median (Q1, Q3) or mean ± SD	Correlation with baseline blood MeHg (ktau-b)	Correlation with concurrent IQ (Pearson r)
2	Pretreatment baseline blood MeHg concentration (μg/L)	767	0.6 (0.4, 0.8)		−0.04[a]
	Post-treatment blood MeHg concentration (μg/L)	767	0.5 (0.4, 0.8)	0.58[*]	0.01[a]
	MDI	765	82.2 ± 13.7	−0.04	
5	IQ	727	80.7 ± 13.3	−0.01	
	CPRS-R				
	Oppositional index	721	58 ± 14	<−0.01	−0.14[*]
	Hyperactivity index	721	64 ± 14	<0.01	−0.14[*]
	ADHD index	721	60 ± 13	0.01	−0.23[*]
	Behavioral index	721	60 ± 12	0.01	−0.20[*]
7	IQ	644	87.1 ± 13	<0.01	
	BASC-TRS				
	Adaptive skills	531	46 ± 10	−0.04	0.39[*]
	Behavioral symptoms index	541	54 ± 12	0.04	−0.28[*]
	Externalizing problems	540	55 ± 13	0.03	−0.18[*]
	Internalizing problems	542	52 ± 10	0.01	−0.17[*]
	School problems	542	56 ± 12	0.03	−0.48[*]
	BASC-PRS				
	Adaptive skills	648	46 ± 11	0.03	0.33[*]
	Behavioral symptoms index	648	56 ± 15	0.02	−0.21[*]
	Externalizing problems	648	58 ± 15	0.03	−0.28[*]
	Internalizing problems	648	50 ± 12	0.03	−0.06

[a] ktau-b.
[*] $p < 0.05$.

3. Results

3.1. Patient characteristics

There were no differences between treatment and placebo groups in age, gender, race and socioeconomic status (parent's education, employment and life style). Overall, the children in the study were predominantly from low socioeconomic status families. The children were mostly of African-American heritage, spoke English, with a single parent. Girls accounted for 44% of children, 40% of children had parents with <12 years of education and more than half of children had neither parent employed. These and other baseline characteristics were balanced in the two groups (Table 2). A comparison of the baseline demographic characteristics and blood lead concentrations between children dropped or excluded in the last wave of TLC follow-up revealed few differences. Those not included were slightly younger (23 months vs. 25 months), and shorter in stature (84 cm vs. 86 cm) at baseline.

3.2. Blood mercury concentrations

Total Hg was detected and quantified in 657 (85%) baseline samples and in 623 (80%) post-treatment samples. Inorganic Hg was detected and quantified in 42 (29%) baseline samples and in 57 (40%) post-treatment samples. The higher percentage of detectable levels in the post-treatment samples arises from the requirement that any sample with a inorganic Hg detected in the baseline sample would also have a measurement done of the post-treatment sample, whether it was above 1 μg/L or not (that accounts for 9% of post-treatment detectable rate). Because more than 60% values were censored, we only estimate unadjusted inorganic Hg concentration for children with total Hg > 1.0 μg/L. The baseline and post-treatment inorganic Hg concentrations were quite similar between placebo and succimer groups. There were no differences between placebo and succimer groups for total Hg, inorganic Hg and MeHg concentrations of baseline or post-treatment (Table 2). All adjusted geometric means in Table 2

were adjusted for age, gender, race and center and based on complete cases. Thus, the number of children included depended upon the choice of exposure biomarker and covariates.

Almost all Hg in the blood (>80%) was MeHg, as was consistent with previous studies (Grandjean et al., 1997; Fok et al., 2007; Marsh et al., 1995). Because the inorganic Hg analyses were not sufficiently powered due to the small proportion of children in this sample with detectable values, the subsequent analyses of effects of mercury exposure on IQ and behavior were conducted with MeHg.

3.3. IQ, neuropsychological and behavioral tests

The mean (±SD) of children's IQ for placebo vs. succimer groups at 2 years, 5 years and 7 years of age were 82 ± 14 vs. 83 ± 14, 81 ± 13 vs. 81 ± 13 and 87 ± 13 vs. 87 ± 13 respectively. These scores did not differ by treatment, and the succimer and placebo groups were combined to study the effect of blood Hg concentrations on neuropsychological and behavioral test scores. The cognitive and behavioral test scores (mean ± SD) at 2, 5 and 7 years are shown in Table 3. These scores were found to be correlated with one another but not with baseline blood MeHg.

3.4. MeHg and IQ

First, we stratified the children by quartiles of baseline MeHg levels. Fig. 2 displays the mean IQ at age 2 (baseline), 5 and 7 years by quartiles of blood MeHg levels. Although MDI slightly decreased with increasing baseline MeHg, there is no consistent pattern for the IQ test scores at age 5 and 7 years by baseline MeHg levels.

We then plotted 2 years blood MeHg and IQ (at age 2, 5 and 7 years) and conducted spline regression analysis. At baseline, MDI did not change with MeHg, but at ages 5 and 7, IQ tended to increase with increasing MeHg concentration in the unadjusted data (after omitting two extreme blood MeHg values). Based on the approximately linear relationship, we conducted GLM analyses of MeHg and IQ at age 2 (baseline), 5 and 7 years by adjusting for a
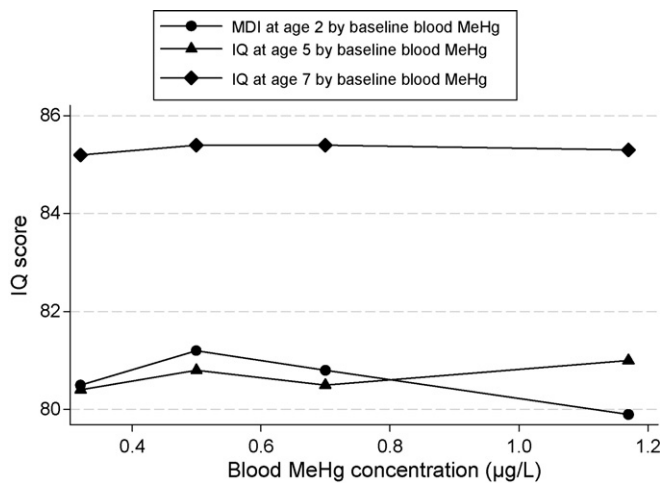
Fig. 2. IQ test scores by baseline blood MeHg level. Each data point shows the mean IQ test scores (adjusted for treatment group, exact age at IQ measurement, caregivers' IQ, clinical center, single parent, language, race, gender, parents' occupation, education and concurrent PbB) of children assessed at age 2, 5 and 7 years, grouped by quartiles of baseline blood MeHg concentration. The abscissa of each point is the median of each blood MeHg concentration category.

variety of covariates. The results are shown in Table 4. Although in none of the GLM models at 3 age points was the coefficient of MeHg statistically significant, it was consistently positive at all ages. The positive association between blood MeHg and IQ test scores remained constant in stratified data analysis and spline regression models. In GLM models, caregiver's IQ and concurrent PbB level

were significant at all ages as expected. Language, race and gender were significant but only at some ages.

3.5. MeHg and neuropsychological and behavioral test scores

The neuropsychological and behavioral test scores were not significantly associated with MeHg concentration (Table 3). Although not statistically significant, four behavioral problem scores at age 5 years and four teacher-rated behavior problems scores at age 7 years decreased (meaning more optimal) with increasing MeHg (after omitting two extreme MeHg values).

The scores of neuropsychological and behavioral tests at age 5 and age 7 stratified by baseline MeHg quartiles are shown in Table 5. Not all children completed all tests and so the numbers vary slightly. The differences between MeHg categories were compared by a Bonferroni method with the lowest MeHg concentration category as reference group. For the most part, there were no differences between MeHg categories among these scores. However, increasing blood MeHg categories were associated with a shallower learning slope on CVLTC (the slope of the increase in learning a list words over 5 trials) and slightly higher (i.e., less optimal) parent-reported behavioral symptoms index and externalizing behavior problems T scores on the BASC.

3.6. Relation to PbB

PbB concentrations in TLC children were part of the eligibility criteria and, thus, have a restricted spread and may have potential effects on the range of scores on IQ, neuropsychological and behavioral tests. The mean concentrations of PbB at baseline, 5 years of age and 7 years of age were 26.2 μg/dL, 12.0 μg/dL and

Table 4
GLM regression coefficients of MeHg and covariates on IQ at baseline and follow-up.

Independent variables	MDI		IQ at 5 years		IQ at 7 years	
	Coefficient	95% CI	Coefficient	95% CI	Coefficient	95% CI
Pretreatment baseline MeHg (μg/L, ln)	0.3	(−1.3, 1.9)	0.8	(−0.7, 2.3)	0.5	(−1.0, 2.1)
Treatment group			−0.5	(−2.3, 1.4)	0.3	(−1.7, 2.2)
Exact age at IQ measurement (in year)	−8.5[*]	(−10.6, −6.3)	−3.0[*]	(−4.9, −1.1)	−2.3	(−8.7, 4.1)
Caregiver's IQ	0.2[*]	(0.1, 0.3)	0.3[*]	(0.2, 0.4)	0.4[*]	(0.3, 0.5)
Center						
Baltimore	Reference					
Newark	−0.4	(−3.2, 2.4)	−5.6[*]	(−8.2, −2.9)	1.5	(−1.7, 4.6)
Philadelphia	−1.2	(−4.0, 1.7)	0.8	(−1.9, 3.5)	6.1[*]	(2.8, 9.4)
Cincinnati	−2.7	(−5.5, 0.2)	−4.7[*]	(−7.4, −2.0)	−1.4	(−4.6, 1.9)
Living with single parent						
No	Reference					
Yes	1.4	(−1.2, 4.0)	0.1	(−2.4, 2.6)	0.7	(−1.8, 3.3)
Language						
English	Reference					
Spanish	−2.5	(−7.8, 2.9)	−2.1	(−7.1, 2.9)	−6.7[*]	(−11.5, 1.8)
Race						
Black	Reference					
Non-black	0.4	(−2.5, 3.2)	3.0[*]	(0.2, 5.7)	4.8[*]	(2.0, 7.7)
Gender						
Boy	Reference					
Girl	2.0[*]	(0.1, 4.0)	−0.6	(−2.5, 1.2)	−1.0	(−2.9, 1.0)
Neither parent working						
Yes	Reference					
No	1.1	(−1.2, 3.5)	0.9	(−1.3, 3.1)	1.8	(−0.5, 4.2)
Parent's education						
=12 years	Reference					
<12 years	−0.6	(−2.9, 1.6)	−2.2[*]	(−4.4, −0.1)	−1.4	(−3.7, 0.8)
>12 years	−1.9	(−4.8, 1.0)	−1.5	(−4.3, 1.3)	1.3	(−1.6, 4.2)
Concurrent Pb level (μg/dL)	−0.3[*]	(−0.5, −0.1)	−0.4[*]	(−0.5, −0.2)	−0.4[*]	(−0.6, −0.3)

[*] $p < 0.05$.

Table 5
Neuropsychological and behavioral test scores at age 5 and age 7 by baseline MeHg level.

Neuropsychological and behavioral test	MeHg < Q1		MeHg ≥ Q1, <median		MeHg ≥ median, <Q3		MeHg ≥ Q3	
	N	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Age 5								
NEPSY								
Attention and executive	158	89 ± 17	161	88 ± 19	181	85 ± 18	147	87 ± 19
Language	160	85 ± 15	151	84 ± 14	173	83 ± 15	145	83 ± 14
Sensorimotor	161	87 ± 15	163	84 ± 15	185	86 ± 16	151	84 ± 16
Visuospatial	165	91 ± 13	167	90 ± 12	190	89 ± 12	154	90 ± 12
Memory	160	89 ± 14	162	87 ± 16	185	88 ± 16	153	88 ± 14
CPRS-R								
Oppositional index	176	58 ± 14	175	58 ± 13	198	58 ± 15	160	57 ± 14
Hyperactivity index	176	63 ± 14	175	64 ± 14	198	64 ± 14	160	63 ± 13
ADHD index	176	59 ± 13	175	60 ± 13	198	60 ± 13	160	60 ± 13
Behavioral index	176	60 ± 12	175	61 ± 12	198	61 ± 12	160	60 ± 12
Age 7								
NEPSY								
Attention and executive	145	86 ± 17	138	87 ± 17	161	87 ± 17	138	87 ± 17
CPT d-Prime	138	56 ± 11	134	57 ± 8	154	55 ± 10	135	55 ± 10
CVLTC								
List A memory	154	46 ± 12	154	44 ± 11	179	43 ± 12	148	42 ± 11
Learning slope	154	−0.2 ± 1.1	154	−0.5 ± 1.1[*]	179	−0.6 ± 1.1[*]	148	−0.4 ± 1.1
WLPB-R								
Broad reading score	146	95 ± 19	139	94 ± 17	164	94 ± 19	140	93 ± 18
CPT Hit response time	138	41 ± 14	134	44 ± 14	154	44 ± 12	135	41 ± 12
NESS								
Sequential movement time	140	0.9 ± 1.3	128	1.1 ± 1.3	152	0.9 ± 1.2	135	1.1 ± 1.3
BASC-TRS								
Adaptive skills	125	48 ± 10	124	46 ± 10	148	46 ± 9	126	45 ± 9
Behavioral Symptoms Index	127	53 ± 11	127	55 ± 12	153	55 ± 11	126	55 ± 12
Externalizing problems	127	54 ± 13	127	56 ± 13	151	55 ± 13	127	56 ± 14
Internalizing problems	128	52 ± 9	127	52 ± 11	153	53 ± 10	126	53 ± 11
School problems	127	55 ± 12	127	56 ± 12	153	57 ± 12	127	57 ± 12
BASC-PRS								
Adaptive skills	155	46 ± 10	154	45 ± 11	179	46 ± 11	149	46 ± 11
Behavioral Symptoms Index	155	54 ± 14	154	57 ± 17[*]	179	56 ± 15	149	55 ± 15
Externalizing problems	155	56 ± 15	154	60 ± 17[*]	179	59 ± 15	149	57 ± 14
Internalizing problems	155	49 ± 11	154	51 ± 13	179	51 ± 12	149	50 ± 12

[*] $p < 0.05$.

8.0 μg/dL, respectively. As we have previously reported, the associations between lower IQ and concurrent PbB were significant (Chen et al., 2007). Behavioral problems also tended to increase with increasing PbB concentration at both ages 5 and 7 in the unadjusted data (Chen et al., 2005).

Baseline PbB level was not correlated with baseline blood MeHG level (ktau-b = 0.02, p = 0.79), indicating that Hg exposure was independent of Pb exposure. For the test scores that were previously found to be associated with PbB, regression analyses were repeated with and without and with PbB as an independent variable. Although the regression coefficients for MeHg adjusted for PbB are different from those not adjusted, in both conditions, all three of the MeHg regression coefficients (at baseline, 5 years and 7 years of age) were not significant. However, concurrent PbB regression coefficients were significant in all models (Table 4). Additional adjustment for PbB level in multivariable models did not alter the conclusion of a negligible association between MeHg and IQ, but confirm a strong association between concurrent PbB concentration and IQ.

We also tested the blood MeHg by concurrent PbB interaction on IQ at 2, 5 and 7 years, including the same variables in the model previously presented in Table 4. The interaction was statistically significant only for MDI at 2 years. We also conducted an analysis of blood MeHg and IQ stratified by baseline PbB concentration (PbB ≤ 24.9 μg/dL, PbB > 24.9 μg/dL). In this analysis, higher blood MeHg was significantly associated with higher MDI scores at 2 years in the higher PbB stratum. Nearly identical results were obtained when stratified by concurrent PbB concentration. Similar stratified analyses were also conducted for behavioral outcomes,

including the BASC-TRS and BASC-PRS subscales. MeHg was not significantly associated with any of these behavioral outcomes within any baseline or concurrent PbB strata.

4. Discussion

This study presents results on prospectively studied neuropsychological performance in children with low but typical postnatal exposure to MeHg in the US. MeHg accounted for more than 80% of blood total Hg in our sample. This percentage of blood MeHg was consistent with the results from the National Health and Nutrition Examination Survey (NHANES) (Mahaffey et al., 2004), the Faroe Islands study (Grandjean et al., 1997) and the Hong Kong study (Fok et al., 2007). In those studies, greater than 90% of blood total Hg was estimated to be MeHg, and as the total Hg concentrations increase, the fraction of MeHg increases. Because MeHg exposure is of the greatest public health concern, our analyses were limited to the relationship between MeHg and child neurodevelopment.

After adjusting for effects of PbB and potential confounders, there were few and largely inconsistent associations between blood MeHg and tests of cognition or behavior; the contribution of MeHg to the regression models was very small (<1% explained variance). These findings were consistent at all three ages and in continuous and categorical analysis that take into account different combinations of covariates and multiple testing. The few significant blood MeHg associations observed in our study were limited to higher 2 years Bayley MDI scores for children within the higher baseline PbB stratum, and, at 7 years, a slightly shallower learning curve on a test of verbal memory and slightly

more optimal scores on parental ratings of their children's behavior.

As postnatal blood MeHg concentrations increased, IQ tended to increase and behavioral problems tended to decrease, although in most instances these associations were statistically non-significant. However, this counter-intuitive finding is consistent with results from the Seychelles study. In that study, four of the 6 measures showed better scores in the highest MeHg groups compared with lower groups for both prenatal and postnatal exposure (Davidson et al., 1998). In the Peru study, there also was no increase in the frequency of neurodevelopmental abnormalities associated with MeHg exposure in early childhood (Marsh et al., 1995). A possible mechanism for these findings is that exposure to MeHg comes mainly from ocean fish, which has other components such as selenium and amino acids that may buffer mercury's potential toxicity. In addition, ocean fish provide important nutrients, such as omega-3-fatty acids, which may improve brain performance to such an extent that any adverse effects from low levels of Hg are not apparent or mitigated (Ronchetti et al., 2006; Jedrychowski et al., 2006).

Compared with studies that found adverse effects for child development with prenatal and/or postnatal MeHg exposure, the MeHg concentrations in our study were much lower. In those studies that measured MeHg concentration in cord blood or children's blood samples, MeHg concentrations were from 0.88 to 22.9 μg/L, which were 1.5–40 times higher than those in this study (Jedrychowski et al., 2006; Debes et al., 2006). In the studies with higher levels of Hg exposure there were inverse associations between prenatal MeHg exposure and cognitive, psychological and scholastic test results. But, as was the case in our study, the Seychelles study did not find adverse neurobehavioral outcomes associated with either prenatal or postnatal MeHg exposure (Davidson et al., 1998).

According to the NRC's report on prenatal exposure to MeHg, the likelihood of subnormal scores on neurodevelopment tests increased as cord blood MeHg concentrations increased from levels as low as 5 μg/L. The children we studied had much lower Hg level, about 10% of NRC's reference dose. Compared with the data from NHANES (Mahaffey et al., 2004), the Hg level in our children was a little higher (Fig. 3). But it should be considered that NHANES reported mean blood Hg levels were significantly higher for African-Americans (non-Hispanic Black, 0.50 μg/L) than others (0.29–0.35 μg/L) (CDCP, 2004). Most children in our studies are non-Hispanic Black (77%), and the Hg levels (0.58 μg/L) were close to the NHANES non-Hispanic Black level (Fig. 3).

The major strengths of the present study include the relatively large sample size and the degree of testing and quality control that went into the measurement of both blood Hg and neuropsychological outcomes. The longitudinal nature of these data is a requirement for this kind of analysis, and the TLC study enjoyed remarkably high retention rates for a longitudinal investigation.

Study limitations include as-yet unmeasured mercury levels at 5 and 7 years of age. However, it is not clear that Hg at these low levels would affect cognition or behavior at school age. In our data, blood MeHg at age 2 years was not associated with deficits in neuropsychological outcomes. However, further research is needed to better understand the relationships between developmental exposure to MeHg in the womb, postnatally and neurodevelopment. We also did not have biomarkers of prenatal Hg exposure. While this makes it impossible for this study to address the issue of exposure in utero, most positive studies on this topic have recruited cohorts that obtain a significant proportion of their dietary protein from finfish or sea mammals (Grandjean et al., 1997; Davidson et al., 1998). It is unlikely that this was the case among the cohort of urban, inner-city families involved in TLC.

It could be argued that relatively high Pb exposure in TLC children may have masked possible adverse effects from low level Hg exposure. However, regression analyses that included a PbB by MeHg interaction term or stratified by baseline and concurrent PbB concentrations did not lend support to this as a factor in our results.

It has been widely speculated that exposure to organic Hg in utero and especially in vaccines may play a role in the etiology of Autism Spectrum Disorders (ASD). We did not study ASD, and our study did not attempt to designate children as being at risk for ASD. However, we can note that the Hg levels in the few untestable children in TLC, which likely included some with autistic disorders, were no different from those of the rest of the children. The baseline MeHg concentrations of IQ tested and untested subjects were 0.59 μg/L vs. 0.58 μg/L ($p > 0.95$).

5. Conclusions

In summary, although our study cannot determine a threshold above which postnatal Hg or MeHg exposure has detectable neurological and developmental consequences in children, at least we can conclude that at the present postnatal MeHg exposure level of U.S. children, adverse effects on children's cognition and behavior were not detectable, since they did not appear in a longitudinal study among children living in poverty in the U.S.

Conflict of interest

None.

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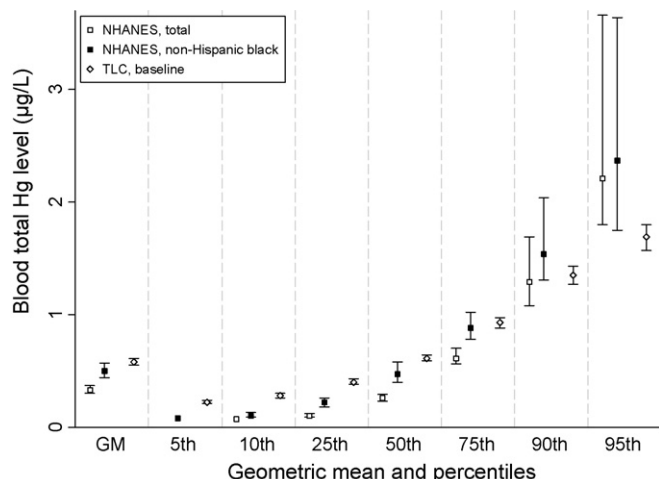

Fig. 3. Comparison of blood total Hg concentration between NHANES and TLC data.

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