Total blood mercury and serum measles antibodies in US children, NHANES 2003–2004

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ABSTRACT

Background: Environmental toxins, pathogens and host susceptibility cofactors may interact to contribute to disease. In vitro mercury exposure inhibited antiviral cytokines in human cells; however, little is known about the relationship between mercury and viruses in children. Children are susceptible to mercury toxicity; lower vitamin B-12 and folate levels and higher homocysteine levels may represent susceptibility cofactors. This study aimed to evaluate associations between total blood mercury (Hg) and measles antibodies in children, and the influence of these susceptibility cofactors.
Design: Cross-sectional data on serum measles antibodies, Hg, homocysteine, methylmalonic acid (MMA, indicator of B-12 deficiency), and folate were obtained from the 2003–2004 NHANES for children aged 6–11 years with measles seropositivity (n = 692). We used linear regression to evaluate relationships between measles antibodies and Hg, stratified by sex, MMA ≥, folate <, and homocysteine ≥ sample medians, adjusted for demographic, nutritional and environmental cofactors.
Results: Hg (range: 0.10–19.10 μg/L) was inversely associated with measles antibodies (range: 1.00–28.24 units) in non-stratified analysis (n = 692), yet positively associated among the subset of boys with higher MMA and lower folate (n = 98). Among this subset with higher homocysteine levels (n = 61), correlations were positive across all Hg quartiles relative to Q1 (Hg ≤ 0.20 μg/L): Q2:β = 6.60 (3.02, 10.19); Q3:β = 8.49 (6.17, 10.81); Q4 (Hg > 0.80 μg/L):β = 4.90 (2.12, 7.67) (p_{trend} = 0.077).
Conclusion: Stratification by susceptibility cofactors revealed opposing directionality for correlations between Hg and measles antibodies, with positive effect estimates at lowest exposures only among boys with higher MMA, lower folate and higher homocysteine levels.

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1. Introduction

Environmental toxins, microbial pathogens and host susceptibility are cofactors that may interact to contribute to disease risk, and therefore, it has recently been proposed that environmental epidemiological research integrate toxicological and infectious disease models to evaluate potential interactions (Feingold et al., 2010). In vitro exposures of human peripheral blood mononuclear cells (PBMCs) to organic mercury compounds inhibited type II interferon (IFN-γ) (de Vos et al., 2007; Gardner et al., 2010), an antiviral cytokine that protects against persistent measles-virus infection of the central nervous system (CNS) (Finke et al., 1995; Patterson et al., 2002; Reuter and Schneider-Schaulies, 2010). Little is known, however, about the potential for mercury to interact with the measles virus in human in vivo studies.

Findings from experimental studies of the relationships between mercury and viruses are mixed. 2-Furylmercury chloride, an organic mercury derivative, was found to inhibit human rhinovirus (Verheyden et al., 2004). Mice infected with coxsackievirus B3 showed increased mercury in brain tissue and decreased mercury in serum relative to control mice (Ilback et al., 2007), heart viral titers were elevated in coxsackievirus B3 infected mice when first exposed to mercuric chloride (inorganic mercury) compared to unexposed infected mice (South et al., 2001), and liver viral titers were increased in herpes simplex virus type 2 infected mice when first exposed to mercuric chloride compared to unexposed infected mice (Christensen et al., 1996). The relationship between mercury exposures and serum viral antibody titers in humans, however, has not been previously investigated.

Feingold et al. (2010) specifically identified a gap in research using the US National Health and Nutrition Survey (NHANES) concerning interactions between environmental toxicants such as mercury and viral pathogens. Mercury is a known neurotoxicant to which children are particularly susceptible (ATSDR, 1999). As a sulfhydryl metal, mercury's general mechanism of toxicity is to bind with thiols of

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exposed cysteine residues on proteins (Klaassen, 2008), and has been shown to act as an immunotoxicant by dysregulating immune response (Gardner et al., 2010). NHANES uses a cross-sectional study design to collect interview, medical examination and laboratory data from a probability sample of the U.S. population. Measles antibodies were measured in serum of children aged 6–11 years, along with total blood mercury levels for NHANES 2003–2004 (CDC, 2003–2004). Other years did not contain these data. The aim of the current study was to investigate whether there is a correlation between total blood mercury levels and measles antibody titers in children.

Our previous findings suggested that boys aged 3–5 with lower folate and higher methylmalonic acid (MMA, an indicator of B-12 deficiency) levels may be susceptible to mercury-associated alterations in amino acids, specifically, decreases in homocysteine levels (Gallagher and Melliker, 2011) potentially indicative of a metabolic disruption hypothesized to underlie cellular hypomethylation (Deth et al., 2008; Lee et al., 2009). Persistent infection of rat glioma cells with measles virus (subacute sclerosing panencephalitis, SSPE strain) has also been reported to induce hypomethylation (Munzel and Koschel, 1982). Therefore, to integrate possible host susceptibility cofactors with toxicant and pathogen exposures, our primary objective was to evaluate the relationship between total blood mercury (Hg) and serum measles antibodies stratified by these same susceptibility factors, sex, MMA and folate levels. Because we previously found that boys with higher MMA and lower folate had higher homocysteine levels (Gallagher and Melliker, 2011) and experimental results suggested that homocysteine inhibits metallothionein (Barbato et al., 2007), a protein that reduces mercury's bioavailability (Aschner, 1997; ATSDR, 1999) and cytotoxicity (Rising et al., 1995; Vitarella et al., 1996; Yao et al., 1999), a secondary objective was to evaluate the effect of additional stratification by homocysteine levels.

2. Methods

We conducted a secondary data analysis of cross-sectional data on serum measles antibodies, total whole blood mercury (Hg), plasma methylmalonic acid (MMA), serum folate, and plasma homocysteine were obtained from the 2003–2004 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2003–2004) for children aged 6–11 years who tested seropositive for measles antibodies (CDC, 2006a) and whose parents dietary recall was categorized as reliable by NHANES (CDC, 2007). NHANES uses a complex, multistage, probability sampling design to select participant's representative of the civilian, non-institutionalized US population (CDC, 2010a). Each sample person is assigned a sample weight that reflects adjustments for complex survey design (including oversampling), survey nonresponses (with adjustments for age and race), and post-stratification, in order to ensure that calculated estimates are US population representative (CDC, 2010a). Whole blood mercury measurements were performed by the Division of Laboratory Sciences, National Center for Environmental Health, of the Centers for Disease Control and Prevention using inductively coupled plasma mass spectrometry (CDC, 2006b, 2011a). Although total blood mercury is a biomarker of both inorganic and organic mercury exposure over the past several weeks to months (ATSDR, 1999), it is primarily a measure of organic mercury (CDC, 2010b). Levels of measles IgG antibody were measured by enzyme immunoassay that used a lysate of measles virus-infected human fetal diploid lung cells (HFDL) as the viral antigen and a lysate of uninfected HFDL as the control antigen, with a value of ≥1 representing seropositivity (McQuillan et al., 2007; CDC, 2011b). Measles vaccination status was not recorded, however, it is likely that the measles titers for most children in this study represent vaccination rather than wild type virus exposures (CDC, 2004a, 2005).

Increased MMA levels in serum are considered direct measures of vitamin B-12 tissue stores and the first indication of B-12 deficiency (Moelby et al., 1990). Blood samples from children were non-fasting

samples (CDC, 2004b). Because human and animal studies indicate that omega-3 polyunsaturated fatty acids (PUFAs) can have immuno-modulatory effects (Ergas et al., 2002; Simopoulos, 2002), for example, eicosapentaenoic acid (EPA) (Makino et al., 2001; Ergas et al., 2002; Simopoulos, 2002) and docosahexaenoic acid (DHA) (Ergas et al., 2002; Simopoulos, 2002), and certain fish are high in both PUFAs and mercury (Oken et al., 2005; Mahaffey et al., 2008), it is important to control for possible confounding effects of PUFAs (Grotto et al., 2010). In consideration of scientific findings that DHA may be less specific to fish than EPA due to the presence of DHA in eggs (Beynen, 2004), we created a variable for EPA dietary intake using NHANES estimated 24-hour dietary EPA intake based upon reliable parental recall of their child's intake of specific foods over the past 24 h (CDC, 2007), i.e., reference group = none; above and below the median of 0.007 g for the sample subset of children aged 6–11 years. For this same sample subset, dichotomous variables were created using median cut-points for homocysteine ≥ 4.66 μmol/L, MMA ≥ 0.108 μmol/L and folate < 14.8 ng/mL. Additionally, the following median-cut dichotomous variables were created per scientific findings regarding associations with immune function: serum vitamin D ≥ 24 ng/mL (Arnson et al., 2007), serum cotinine ≥ 0.080 ng/mL (Avanzini et al., 2006) and whole blood lead ≥ 1.4 μg/dL (Mishra, 2009). Because cadmium has been associated with suppression of immediate hypersensitivity immune response in children (Ritz et al., 1998), we also created a dichotomous variable for whole blood cadmium; however, the smallest cutoff point between lowest and higher exposures was greater than the median, i.e., ≥ 0.20 μg/L, and therefore, this dichotomous variable represents a frequency distribution of 64% < 0.20 μg/L and 36% ≥ 0.20 μg/L. A dichotomous variable was created to adjust for below and above the poverty index ratio.

Our objective was to evaluate the relationship between Hg and measles virus antibodies in children with laboratory confirmed measles virus exposure, and therefore, the analytic sample domain was restricted to survey participants aged 6–11 years with measles seropositivity (≥ 1 unit) and without missing values for data on blood mercury, cadmium and lead, plasma homocysteine and methylmalonic acid, serum folate and cotinine, and the poverty index ratio. Subjects were further restricted to those with reliable status of parental 24-hour dietary recall (n = 692). There were 702 observations with both Hg and measles antibody levels measured; of these, 10 observations were excluded due to measles seronegativity, for an overall sample of 692 children. Continuous blood mercury (Hg) (μmol/L) measures were log-transformed, and scatterplots and residual plots visually inspected for linearity of the relationships between Hg and measles antibodies. Based upon weighted frequency distributions for the subsample of children aged 6–11 years, the following categorical variables were created for total blood mercury quartiles (Q): Q1: Hg ≤ 0.20 μg/L; Q2: 0.20 < Hg ≤ 0.40 μg/L; Q3: 0.40 < Hg ≤ 0.80 μg/L; Q4: Hg > 0.80 μg/L.

We used linear regression analyses to evaluate the relationships between continuous and categorical Hg and measles antibodies, stratified by sex, MMA ≥ and folate < sample medians, as well as homocysteine ≥ the sample median, fully adjusted for continuous age, race (non-Hispanic white compared to non-white), poverty, body mass index, serum vitamin D, and NHANES-estimated 24-h dietary intake of EPA and selenium. Bivariate regression analysis was used to evaluate each covariate's relationship with measles antibody levels in each of the following subgroups: boys with high MMA and low folate, all other boys, girls with high MMA and low folate, and all other girls. Only the cotinine covariate showed no statistical evidence of association, so was omitted from all models. Variables determined a priori for model inclusion because of their potential relationships with measles antibody titers and mercury levels included age (CDC, 2004a, 2005) and body weight (Ball et al., 2001), respectively; we used body mass index to proxy the latter as there was evidence of multicollinearity between body weight in kilograms and age in years. EPA was also determined a priori for model inclusion because of its relationship

with mercury (Oken et al., 2005; Mahaffey et al., 2008; Grotto et al., 2010). A test for interaction between mercury, homocysteine and measles titers was conducted by entering a statistical interaction term for the interaction between homocysteine and mercury into the model with all covariates except cotinine. We used backward elimination to identify covariates without evidence of a statistical correlation with measles. As a result, the fully-adjusted models presented do not include blood lead and cadmium. Statistical analysis was conducted using SAS version 9.2. Primary sampling units, strata and dietary intake survey subsample weights were incorporated to calculate sample means and 95% confidence intervals, to determine statistically significant differences among subset sample means and distributions of sample characteristics, and to perform linear regression procedures in accordance with complex survey design using the Taylor linearization procedure, which is robust against the likelihood of correlated errors and heteroscedasticity (Kott, 1991). A separate analysis was also conducted using unweighted robust regression in order to evaluate robustness of results in unweighted analysis while maintaining stability against heteroscedasticity (Carroll and Ruppert, 1982). Additionally, the relationship between mercury, adjusted for covariates, and measles titers were graphically depicted by plotting a residualized mercury variable by measles titers. The plotted mercury variable was comprised of the residual values generated by modeling log-transformed mercury as the dependent variable and the remaining covariates as independent variables. The Rao-Scott Chi-square test used to determine statistically significant differences in proportions for weighted sample characteristics among subsets, and 95% confidence intervals were calculated for the difference between weighted means to determine statistical significance. To convert Hg reported as µmol/L to µg/L, µmol/L was divided by 4.99 (CDC, 2006b). Statistical significance was defined as an alpha level ≤ 0.05.

3. Results

We show in Table 1 that measles antibody titers for the overall sample ranged from 1.00 to 28.24 units, and that Hg ranged from 0.10 to 19.10 µg/L. Mean measles antibody levels were not statistically different between boys with high MMA and low folate (9.83; SD = 0.88) and all other children (10.60; SD = 0.33). Mean Hg levels were not statistically different between boys with high MMA and low folate (0.64 µg/L; SD = 0.11) and all other children (0.69 µg/L; SD = 0.08). Hg levels were similar for excluded observations. Boys with higher MMA and lower folate had significantly higher mean homocysteine (5.39 µmol/L; SD = 0.24) compared to all other children (4.74 µmol/L; SD = 0.04).

Table 2 presents linear regression results for the relationship between continuous log-transformed Hg (µmol/L) and measles virus antibody titer, for the overall sample (n = 692), and for the following subsets: boys with high MMA and low folate (n = 98), all other boys (n = 231), girls with high MMA and low folate (n = 89), all other girls (n = 274), and for the overall sample less boys with high MMA and low folate (all other children, n = 594). There were no statistically significant associations between Hg and measles antibodies in unadjusted analysis; however, in multivariable analysis, boys with higher MMA and lower folate showed a positive association (β = 1.62; 95% CI = 0.06, 3.19), whereas the overall sample showed an inverse association (β = −0.87; 95% CI = −1.70, −0.03), as did the overall sample less boys with higher MMA and lower folate (β = −1.14; 95% CI = −1.88, −0.39), as well as all other boys (β = −1.39; 95% CI = −2.74, −0.04). Although continuous Hg was positively associated with measles antibodies among boys with higher MMA and lower folate, relationships were not statistically significant comparing higher Hg quartiles to the lowest Hg quartile.

We also examined the possibility of interaction between homocysteine and mercury. A covariate for the interaction between continuous mercury and homocysteine above/below the sample median was

Table 1
Sample subset weighted mean (standard error) values, frequency distributions and proportions for boys with lower folate and higher MMA (n = 98) and for all other children (n = 594), aged 6–11 years with seropositive measles viral antibody titers and reliable parental dietary recall report (n = 692), NHANES 2003–2004.

	Boys with high MMA and low folate	All other children
Total blood mercury (µg/L)[a,b]	0.64 (0.11)	0.69 (0.08)
Overall sample min, max: 0.10, 19.10		
Measles antibody titer[a,c]	9.83 (0.88)	10.60 (0.33)
Overall sample min, max: 1.00, 28.24 units		
Homocysteine (µmol/L)[a,d]	5.39 (0.24)[e]	4.74 (0.04)
Overall sample min, max: 2.07, 24.51		
Methylmalonic acid (µmol/L)[a,d]	0.15 (0.007)[e]	0.12 (0.004)
Overall sample min, max: 0.04, 0.53		
Folate (ng/mL)[a,c]	11.99 (0.29)[e]	16.80 (0.32)
Overall sample min, max: 3.10, 58.10		
Vitamin D (ng/mL)[a,c]	26.87 (0.97)	26.32 (0.68)
Overall sample min, max: 3.00, 44.00		
Selenium[f] (mcg)	110.39 (6.22)	92.31 (3.22)
Overall sample min, max: 10.90, 326.40		
EPA[f] estimated dietary intake past 24 h:		
None	29 (30%)	199 (34%)
≤0.007 g	28 (29%)	202 (34%)
>0.007 g	41 (42%)	193 (32%)
Overall sample min, max: 0, 0.82		
Age (years)	8.48 (0.23)	8.63 (0.12)
Body mass index (kg/m²)	18.43 (0.43)	18.83 (0.25)
Non-Hispanic white	24 (24%)	154 (26%)
Below poverty index	26 (27%)	221 (37%)

[a] Non-fasting blood sample.
[b] Whole blood.
[c] Serum.
[d] Plasma.
[e] Statistically significant difference at α = 0.05 level, calculated based upon weighted complex survey design.
[f] NHANES-estimated 24-h dietary intake of selenium and eicosapentaenoic acid (EPA) based upon parental 24-h recall.

statistically significant in the multivariable model for the overall sample (p = 0.043), thus supporting stratification by the dichotomous homocysteine covariate. Table 3 presents linear regression results for only those sample subsets with evidence of a statistically significant relationship between measles virus antibody titer and mercury, stratified by higher and lower homocysteine levels. Subsets shown include models for boys with higher MMA, lower folate and higher homocysteine (n = 61), all other boys with lower homocysteine (n = 119), and all other children, both boys and girls, with lower homocysteine (n = 308). Using a continuous measure of Hg, in unadjusted models, none of the sample subsets showed a statistically significant relationship between measles antibody titers and Hg; however, multivariable regression results showed a positive association among boys with higher MMA, lower folate and higher homocysteine (β = 1.67; 95% CI = 0.39, 2.95), whereas inverse associations were evident among all other boys with lower homocysteine (β = −1.87; −3.22, −0.52) and among all other children with lower homocysteine (β = −1.85; −3.06, −0.65). Figs. 1, 2, and 3 depict these relationships graphically using a residualized mercury variable adjusted for covariates. Using Hg quartiles, in unadjusted analysis, the latter two subgroups showed inverse associations, with statistically significant relationships evident for Hg quartiles 2 and 4 (relative to the first quartile) among all other boys with lower homocysteine, and for the 4th Hg quartile among all other children with lower homocysteine; these inverse relationships were consistent with multivariable results (p-value for trend = 0.001 and 0.015, respectively). Among boys with

Table 2
Linear regression coefficients (95% CI) for the relationship between continuous log-transformed total blood mercury (μmol/L) and measles virus antibody titer, for overall sample and sample subsets, children aged 6–11 years with seropositive measles viral antibody titers and reliable parental dietary recall report, NHANES 2003–2004.

	Overall sample (n=692)	Boys with high MMA and low folate (n=98)	All other boys (n=231)	Girls with high MMA and low folate (n=89)	All other girls (n=274)	Overall sample less boys with high MMA and low folate (n=594)
Unadjusted model[a]: continuous mercury	−0.55 (−1.47, 0.36) R^2<0.01	0.95 (−1.33, 3.24) R^2=0.01	−1.53 (−3.26, 0.20) R^2=0.03	−1.82 (−5.07, 1.43) R^2=0.03	0.01 (−1.70, 1.73) R^2<0.01	−0.84 (−1.78, 0.10) R^2<0.01
Fully adjusted[a] model: continuous mercury	−0.87[b] (−1.70, −0.03) R^2=0.12	1.62[b] (0.06, 3.19) R^2=0.21	−1.39[b] (−2.74, −0.04) R^2=0.30	−1.76 (−4.84, 1.32) R^2=0.20	−1.27 (−2.88, 0.62) R^2=0.13	−1.14[a,c] (−1.88, −0.39) R^2=0.16

[a] Statistically adjusted for age, body mass index, NHANES-estimated dietary intake of eicosapentaenoic acid (EPA) and selenium, serum vitamin D, poverty, race/ethnicity, and plasma homocysteine. (Models for overall sample and overall sample less boys with high MMA and low folate also adjusted for sex, MMA and folate).
[b] p≤0.05.
[c] p≤0.01.

higher MMA, lower folate and higher homocysteine, although Hg quartiles did not show evidence of a statistically significant relationship with measles antibody titers in unadjusted analysis, in the statistically-adjusted model, relative to the lowest Hg quartile, each of the higher Hg quartiles showed a significantly positive association with measles antibody levels (p-value for trend = 0.077).

In unweighted multivariable analysis, the positive associations between Hg and measles antibody titers were robust among boys with higher MMA, lower folate and higher homocysteine, as were the inverse associations between Hg and measles antibody titers among all other boys with lower homocysteine; however, results were not robust for all other children with lower homocysteine. Also in unweighted analysis, a statistically significant trend (p = 0.006) was evident only for boys with higher MMA, lower folate and higher homocysteine.

4. Discussion

This research presents one of the first investigations of the relationship between mercury and measles antibodies in a human population. Overall sample findings suggest an inverse relationship between Hg and measles antibodies; however, among boys with higher MMA and lower folate a positive relationship was observed. Further, among boys with higher MMA, lower folate and higher homocysteine, this positive relationship was also evident using both continuous and categorical Hg measures, and showed stability in unweighted analysis. Unadjusted analyses did not consistently show significant associations between mercury and measles titers. Including age, BMI and the potential confounding variable EPA in the model, however, resulted in stronger and more consistent associations between mercury and measles titers. Investigations such as this one into the relationship between mercury and viral antibodies are needed as we begin to learn how mercury can induce both suppressive and stimulating effects on the immune system (Havarinasab and Hultman, 2005; Havarinasab et al., 2005; de Vos et al., 2007; Gardner et al., 2010).

The findings of a positive association between continuous Hg and measles virus antibodies only among boys with lower folate and higher MMA, as well as an association present at very low Hg levels among the subset with higher homocysteine levels, suggests a subpopulation that may be uniquely susceptible to Hg immuno-modulation by virtue of their MMA, folate and homocysteine levels; an interpretation further supported by similar mean Hg levels in these children compared to all other children. A biologically plausible mechanism of action underlying a positive association between Hg and measles antibodies among boys with higher MMA and lower folate might be suggested by in vitro findings in human PBMCs of the inhibitory effects of both subcytotoxic doses of ethylmercury (Gardner et al., 2010) and higher doses of methylmercury (de Vos et al., 2007) on IFN-γ, a cytokine required to overcome persistent measles virus-induced CNS infection (Reuter and Schneider-Schaulies, 2010). On the other hand, IFN-γ is also considered a pro-inflammatory cytokine, and in vitro studies indicate variable IFN-γ levels over time in ethylmercury-treated mice. Evidence is not currently available in support of or against these biological mechanisms in children. Further, in the current study, the strength of the positive Hg effect estimate was somewhat attenuated at the highest Hg quartile, and confidence intervals overlapped among mid- and highest quartiles, leaving open questions about dose–response effects among boys with higher MMA, lower folate and higher homocysteine.

Among all children with lower homocysteine, Hg was inversely associated with measles antibody levels, particularly at the highest Hg exposure. One interpretation is that the highest Hg levels inhibited measles virus replication, analogous to Verheyden et al. (2004) experimental findings that 2-furylmercury, an organic mercury derivative, inhibited late, but not early, human rhinovirus RNA synthesis. Reverse

Table 3

Survey weighted linear regression coefficients (95% CI) for the relationships between continuous total blood mercury (Hg), Hg quartiles (Q) and measles virus antibody titers, for boys with higher MMA, lower folate and higher homocysteine; all other boys with lower homocysteine; and all other children with lower homocysteine: children aged 6–11 years with seropositive measles viral antibody titers and reliable parental dietary recall report, NHANES 2003–2004.

	Boys with higher MMA, lower folate and higher homocysteine (n = 61)	All other boys with lower homocysteine (n = 119)	All other children with lower homocysteine (n = 308)
Unadjusted, continuous log-transformed Hg (µg/L)	1.23 (−1.21, 3.68) R^2 = 0.03	−1.72 (−4.12, 0.67) R^2 = 0.06	−1.56 (−3.13, 0.02) R^2 = 0.03
Fully-adjusted[a] continuous log-transformed Hg (µg/L)	1.67[b] (0.39, 2.95) R^2 = 0.41	−1.87[c] (−3.22, −0.52) R^2 = 0.35	−1.85[c] (−3.06, −0.65) R^2 = 0.19
Unadjusted, Hg quartiles:			
Q1: Hg ≤ 0.20 µg/L	Reference	Reference	Reference
Q2: 0.20 < Hg ≤ 0.40 µg/L	0.89 (−3.36, 5.14)	−5.20[c] (−8.98, −1.42)	−2.34 (−5.84, 1.17)
Q3: 0.40 < Hg ≤ 0.80 µg/L	3.04 (−0.59, 6.66)	−1.89 (−5.86, 2.08)	−0.81 (−4.06, 2.43)
Q4: Hg > 0.80 µg/L	1.36 (−4.38, 7.10)	−4.81[b] (−9.57, −0.06)	−3.61[b] (−7.06, −0.17)
R^2 (p value for trend)	0.03 (0.586)	0.20 (0.096)	0.05 (0.093)
Fully-adjusted[a], Hg Quartiles:			
Q1: Hg ≤ 0.20 µg/L	Reference	Reference	Reference
Q2: 0.20 < Hg ≤ 0.40 µg/L	6.60[c] (3.02, 10.19)	−4.12[c] (−6.63, −1.61)	−1.87 (−5.02, 1.28)
Q3: 0.40 < Hg ≤ 0.80 µg/L	8.49[d] (6.17, 10.81)	−0.24 (−2.42, 1.94)	−0.37 (−3.10, 2.35)
Q4: Hg > 0.80 µg/L	4.90[c] (2.12, 7.67)	−4.90[d] (−7.32, −2.49)	−3.83[c] (−6.30, −1.35)
R^2 (p value for trend)	0.56 (0.077)	0.44 (0.001)	0.20 (0.015)

Note: Sample size by Hg quartiles (Q):
(i) Boys with higher MMA, lower folate and higher homocysteine − Q1, n = 16; Q2, n = 20; Q3, n = 10; Q4, n = 15.
(ii) All other boys with lower homocysteine − Q1, n = 32; Q2, n = 40; Q3, n = 30; Q4, n = 17.
(iii) All other children with lower homocysteine − Q1, n = 80; Q2, n = 79; Q3, n = 86; Q4, n = 63.
[a] Statistically adjusted for age, body mass index, NHANES-estimated dietary intake of eicosapentaenoic acid (EPA) and selenium, serum vitamin D, poverty, and race/ethnicity. (Models for all other boys and for all other children also adjusted for MMA and folate; and model for all other children also adjusted for sex.).
[b] p ≤ 0.05.
[c] p ≤ 0.01.
[d] p ≤ 0.001.

causality may also play a role, as higher levels of measles antibodies may reduce levels of blood mercury via induction of metallothionein (MT). MT is known to bind with Hg to minimize its cellular bioavailability (Aschner, 1997; ATSDR, 1999) and protect against cytoxicity (Rising et al., 1995; Vitarella et al., 1996; Yao et al., 1999). Although it is unknown whether the measles virus induces MT production, findings from a study of HIV+ subjects during chronic viremic episodes indicated a positive relationship between sustained HIV virus replication and increased MT gene expression; the latter finding was absent in healthy, uninfected controls (Raymond et al., 2010). MT induction was found in mice infected with coxsackievirus B3, with subsequent decreased serum Hg and increased brain Hg (Ilback et al., 2007). Thus, a biologically plausible mechanism for the inverse association between measles virus and total blood mercury may be MT induction and relatively unimpeded MT function with resultant decreased bioavailability of total blood mercury as measles antibody levels increase. Among all other boys with lower homocysteine, however, the 2nd Hg quartile also showed a statistically significant inverse

association with measles antibody levels, but only the highest Hg quartile's inverse correlation with measles antibodies was robust in unweighted analysis, suggesting a possible threshold, rather than a dose–response. These hypothesized mechanisms notwithstanding, the cross-sectional study design limits interpretations of causality in either direction.

Additionally, a broader perspective that considers epigenetic effects is merited in light of mercury's inhibitory effects on methionine synthase (Waly et al., 2004) and hypothesized DNA hypomethylation (Deth et al., 2008) with consequent alterations in gene expression (Lee et al., 2009). For example, Toker and Huehn (2011) reviewed emerging scientific knowledge regarding the role of epigenetics in controlling expression of Foxp3 which is required for regulatory T cell (T$_{reg}$) development and immune function. Further, Sellin et al. (2009) showed that measles virus affects Foxp3T$_{reg}$ homeostasis in mice, and speculated that the interplay between measles virus and T$_{reg}$ cells may play a role in persistent measles virus infections in subacute
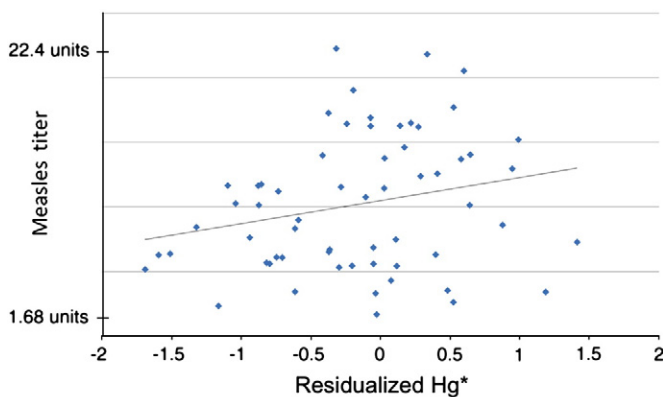
*Log-transformed and adjusted for age, BMI, EPA, selenium, vitamin D, poverty, race/ethnicity.

Fig. 1. Residualized Hg vs. measles titer: boys with low folate, high MMA, high homocysteine (n = 61).
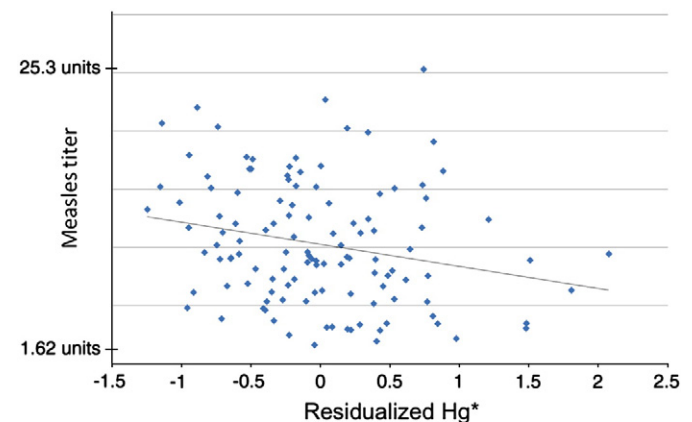
*Log-transformed and adjusted for age, BMI, EPA, selenium, vitamin D, poverty, race/ethnicity.

Fig. 2. Residualized Hg vs. measles titer: boys with high folate, low MMA, low homocysteine (n = 119).

*Log-transformed and adjusted for age, BMI, EPA, selenium, vitamin D, poverty, race/ethnicity.
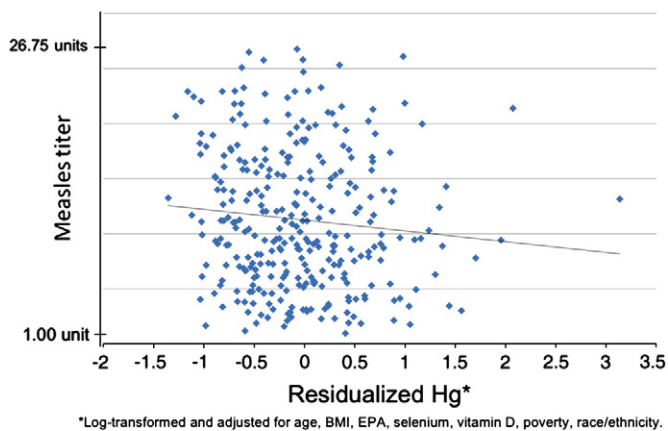
Fig. 3. Residualized Hg vs. measles titer: all other children with high folate, low MMA, low homocysteine (n = 308).

sclerosing panencephalitis (SSPE). Future research regarding a potential relationship between mercury and Foxp3T$_{reg}$ may yield additional insights.

This study is limited by lack of data on specific sources, forms, tissue distributions and timing of mercury exposures in the study children. Methylmercury is an organic mercury compound found in fish (Oken et al., 2005; Mahaffey et al., 2008) and ethylmercury is an organic mercury compound found in the vaccine preservative thimerosal. Thimerosal has not been used in the measles vaccine, and has been removed from most vaccines (FDA, 2010). The current study measured total blood mercury, which includes primarily organic mercury and, in the majority of the population, undetectable levels of inorganic mercury (CDC, 2010b); however, this measure does not differentiate between different forms of organic mercurials, and consequently, interpretations are limited regarding sources of exposures. Because the clearance half-time of organic mercurials in the blood is relatively brief (<3 months) (ATSDR, 1999; Burbacher et al., 2005), Hg represents recent, but not historical exposures. Additional studies are needed to tease out the relative importance of different organo-mercurial exposures in relation to measles virus antibody levels.

The cross-sectional study design hinders our understanding of the dynamics between mercury and measles virus antibodies and how other factors, e.g., genetic, epigenetic, metallothionein, nutrients, amino acid metabolism, might influence this relationship. As in any epidemiologic study, the presence of additional unmeasured confounders or cofactors is a possibility; however, every effort was made to account for potential confounding variables. For example, in the adjusted model for boys with higher MMA, lower folate and higher homocysteine levels, adding estimated beneficial fish nutrient intake (EPA) into the model resulted in an increased effect estimate for each Hg quartile by more than 75% and each of these estimates was statistically significant, whereas without EPA-adjustment, the 2nd and 4th quartiles showed borderline significance. These results suggest that including even a crude estimate of EPA from a 24-hour dietary intake survey helped control for confounding of mercury by beneficial aspects of fish intake.

In summary, using a U.S. representative sample of children aged 6–11 years, we present early evidence of an association between levels of mercury in blood and measles antibodies. Novel findings are reported of a positive association between very low levels of Hg exposure and measles antibodies among boys with lower folate, higher MMA and higher homocysteine, but inverse associations between higher levels of Hg exposure and measles antibodies among all other children. These findings highlight the importance of considering dynamics between toxicant exposures, pathogens and host susceptibility. Further research is warranted to discern sources and timing of concurrent Hg and measles virus exposures, biological interactions with nutritional and metabolic factors, related epigenetic processes, and correlations with immune and neurologic endpoints among susceptible infants and children.

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