Published in final edited form as:

Environ Res. 2008 May ; 107(1): 79–88. doi:10.1016/j.envres.2007.08.015.

Scalp Hair and Urine Mercury Content of Children in the Northeast United States: The New England Children's Amalgam Trial

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Abstract

Children may be at particular risk from toxic effects of mercury (Hg). Previous studies of hair (organic) and urine (inorganic) Hg levels in US children were unable to assess Hg levels while accounting for exposure to amalgam dental restorations. This analysis describes, over a 5-year period, levels and correlates/predictors of scalp hair (H-Hg) and urinary (U-Hg) mercury in 534 New England Children's Amalgam Trial (NECAT) participants, aged 6–10 years and without exposure to dental amalgam at baseline. Results: Mean H-Hg levels were between 0.3 and 0.4 µg/g over 5 years. 17–29% of children had H-Hg levels ≥0.5µg/g, and 5.0 to 8.5% of children had levels ≥1µg/g, in any given study year. In adjusted models, fish consumption frequency was the most robust predictor of high H-Hg. U-Hg mean levels were between 0.7 to 0.9 µg/g creatinine over two years. The percentage of those with U-Hg≥2.3µg/g creatinine ranged from 4 to 6%. Number of amalgam restorations had a significant dose-response relationship with U-Hg level. Daily gum chewing in the presence of amalgam was associated with high U-Hg.

Keywords

Child; Fishes; Dental Amalgam; Mercury; Methylmercury

INTRODUCTION

Inorganic mercury (Hg) from environmental sources can be changed to organic (methyl) Hg in aquatic sediment and enter the food chain. Human exposure to methyl mercury (MeHg) (National Research Council 2000) is thought to occur primarily through fish consumption, with some species of fish (e.g. swordfish, tilefish, shark, king mackerel) containing higher levels of mercury than others (US Dept. of Health and Human Services). Inorganic Hg exposure may occur from environmental exposures, dental amalgam, and household products, paints or skin-lightening creams in some regions (Kazantzis 2002). Hair Hg (H-Hg) is used for biological monitoring of exposure to methyl mercury, since MeHg is incorporated into hair in proportion

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to MeHg in blood. Normal hair grows approximately 1cm per month, so H-Hg may reflect organic Hg exposure during the past year, depending on hair length (National Research Council 2000). Urinary Hg (U-Hg) is widely used for assessment of exposure to inorganic Hg. It reflects Hg in the kidney, and, at steady state, total body burden of inorganic Hg (Barregard 1993). Since some MeHg is demethylated to inorganic Hg in vivo, MeHg may also contribute to U-Hg levels.

While US population values for hair mercury in children 1–5 years were recently reported (McDowell et al. 2004), we are unaware of any North American data reporting mercury levels in either hair (organic) or urine (inorganic) in older children, save for certain special populations known to be at high risk of exposure (Dumont et al. 1998). Nor have we found results from repeated sampling in the same children over extended periods, permitting possible longitudinal trends by age to be examined.

The New England Children's Amalgam Trial (NECAT) (2003; Bellinger et al. 2006) includes 534 children from two sites, rural Maine (predominantly white) and the metro-Boston area, which is racially and ethnically diverse. In addition to measurements of H-Hg and U-Hg at several time points, data are also available for variables including demographics, socioeconomic characteristics, fish consumption, and number of amalgam dental restorations. While it is not a community sample, availability of both H-Hg and U-Hg measurements over multiple timepoints, ability to account for amalgam restorations, and its racial and ethnic diversity make NECAT a unique and valuable data source.

The objective of this analysis is to describe levels and predictors of scalp hair and urine Hg in 534 NECAT participants, aged 6–10 years at baseline, over a 5-year period. Predictors of high levels of H-Hg and U-Hg will also be described.

MATERIALS AND METHODS

A detailed description of the NECAT study methods and protocol is given elsewhere (2003). Briefly, the NECAT was a two-arm randomized safety trial designed to compare the neuropsychological and renal function of children whose dental caries were restored using amalgam, with those receiving mercury-free composite restorations (Bellinger et al. 2006).

Recruitment

Children were recruited for the NECAT from one urban (Boston/Cambridge, MA) and one rural (Farmington, ME) geographic area in the United States. Eligibility criteria for participating children were: 6–10 years of age, no prior amalgam restorations, 2+ posterior teeth with caries on the occlusal (i.e., chewing) surfaces, English speaking, and no major health disorders, as reported by the primary caregiver. Dental screening examinations were performed by a dentist or a dental hygienist.

Of 5,116 children screened, eligibility was confirmed for 598, and parental or guardian consent and child assent obtained for 534 (89%). After completing baseline data collection visits, these 534 children were randomized to a study treatment arm (amalgam or composite), and received standard dental care every 6 months. Over the course of the study, additional dental restorations were placed as clinically indicated, and according to randomized treatment arm assignment (Bellinger et al. 2006). All study procedures were reviewed and approved by Institutional Review Boards at NERI and the respective clinical sites.

Data collection

The baseline clinical examination included a dental examination, dental X-rays and standard preventive dental care, anthropometric measures (height, weight, percent body fat), collection

of scalp hair and urine samples, health interviews, and neuropsychological testing of both child and parent/guardian. Data were collected annually from primary caregivers on family demographics and habits of study participants (e.g. dietary habits including fish consumption and gum chewing that might influence mercury exposure). Follow-up data included semiannual dental examinations and treatment, annual collection of urine samples, and biennial collection of scalp hair samples.

Hair samples (50–100 hairs, cut as close to the scalp as possible) were collected at baseline (September 1997–February 2000) and years 1 (January 1999–December 2000), 3 (February 2001–April 2004), and 5 (February 2003–March 2005). Urine samples were collected annually. Initially, timed overnight urine samples were collected but compliance became increasingly problematic, so the protocol was amended mid-trial to collect spot samples at the dental clinic. Both sets of samples were sent to a central laboratory (University of Rochester Medical School Department of Environmental Medicine, Rochester, NY) for analysis.

Analytical Methods

Total mercury (Hg) was measured in urine and hair by cold vapor atomic absorption spectrometry (CVAAS) (Magos and Clarkson 1972). This technique is well known, and the laboratory has performed well in inter- (Boischio and Cernichiari 1998) and intra-laboratory (Boischio and Cernichiari 1998) comparisons. Comparisons have also been performed between the CVAAS method and independent methods (X-ray fluorescence spectrometry, and gas chromatography with atomic fluorescence detection), with good agreement between the three methods (Cernichiari et al. 1995).

The detection limit for urine, initially 1.5 ng/mL, was reduced to 0.45 ng/mL after February 1, 2000 as a result of increasing the volume of urine analyzed from each child. For this reason, U-Hg values are not directly comparable between samples collected before and after this date, so only urine data from years 3–5 (subsequent detection limit) will be included in this analysis. U-Hg was expressed in µg/g creatinine, in order to take urinary flow rate into account (Barber and Wallis 1986).

For hair, the detection limit was 0.75 ng Hg. Hair samples were dissolved in 10 ml volume, with an aliquot (typically 3 ml) removed for analysis. Detectable concentration (µg Hg/g hair) varies with hair mass, with larger samples (e.g. longer hair) having a lower detectable concentration. About half of the samples were below the detectable concentration (0.28 µg/g on average). Due to inaccuracy of imputing from very high detection limits, samples with hair mass < 6.6 mg (N=282 out of 1741 samples) are excluded from all analysis and descriptive statistics. A hair mass of 6.6 mg corresponds to a detection limit of 0.38 µg/g, the median H-Hg content of the detectable samples.

For study participants with H-Hg or U-Hg below the detectable limit, values were imputed for non-detectable mercury as detection limit/sqrt(2) (Hornung and Reed 1990).

Statistical Methods

Repeated measures models were fit to determine significant correlates/predictors of hair and urine mercury. Predictors were: age, gender, race (non-Hispanic White, non-Hispanic Black, Hispanic, Other), site (Boston vs. Maine), primary caregiver education, (<high school, high school, >high school), family income (<20K, 20–40K, >40K), primary caregiver and child immigration (born outside 50 US states), maternal smoking (current, past, never), fish consumption (daily, weekly, bimonthly, less), number of amalgam fillings (documented number of tooth surfaces filled with amalgam, not by treatment group), and chewing gum on amalgam fillings (daily, occasionally, never). Outcome variables were (1) 75th percentile of

mercury values, as calculated across all study years (0.5 µg/g for hair and 1.0 µg/g creatinine for urine); 2) 95th percentile of mercury values for urine (2.3 µg/g creatinine), and H-Hg value of 1.0 µg/g. The latter value falls close to the 95th percentile and corresponds roughly to the reference dose (RfD) for methyl mercury (0.1 µg/kg per day) recommended by the US EPA (Rice et al. 2003); (3) H-Hg or U-Hg level as a continuous variable, log-transformed. Repeated measures logistic regression was used for the first two outcomes, with repeated measures analysis of covariance for mercury level. A multivariate model was fit using a backwards elimination procedure starting with all twelve predictors. In all models, a compound symmetric variance structure was assumed.

Statistical analysis was performed using the SAS Version 9.1 statistical package.

RESULTS

Descriptive Data

Baseline demographic characteristics of the NECAT participants and their parents/primary caregivers are shown in Table 1. The sites differed considerably in racial/ethnic composition. Almost all children in Maine were non-Hispanic White with US-born parents; children in Boston were racially diverse, often with immigrant parents. Of the 61 children in the “other” race category, 26% self-identified themselves as belonging to two or more race/ethnic categories, 20% as either Asian-Pacific Islander or with a specific Asian ethnicity, 5% as Native American or Alaskan Native, and the remainder by ethnicity or country of origin. Fifty-six percent of children in the “other” category had parents born outside the US, compared to 34% for non-Hispanic Black, 6% for non-Hispanic White, and 74% for Hispanic.

Fish consumption frequency, gum-chewing habits, and mean number of amalgam fillings of NECAT participants are shown, by study visit, in Table 2. The proportion of frequent fish consumers (once a week or more) declined from 39% at baseline to 22% at Year 5, while the proportion who ate fish at most once a month, increased from 41% to 50%. Gum chewing habits remained fairly stable over time, with 82–88% of children reporting occasional (less than daily) use of chewing gum, and prevalence of daily use fluctuating between 6 and 12%. The decrease in mean number of amalgam fillings in years 3–5 reflects shedding of deciduous teeth. The number of fillings placed was greatest shortly after entry into the study due to unmet dental needs, but the children did have moderate recurrence of treatment needs.

Figure 1 shows hair and urine mercury values by study year. Imputation of values below the detection limit was not attempted for baseline urine Hg due to the small proportion (6.4%) of detectable values; thus the baseline values for urine Hg are not shown.

There were only 313 children with all four years of H-Hg measurements and 343 children with all three years of urine Hg measurements. Including only these children does not change the numbers in Figure 1 appreciably.

Both hair and urine Hg were significantly correlated over study timepoints within children. Correlation coefficients (r_p) over study years ranged from 0.2–0.6 ($p<0.001$) for H-Hg and 0.3–0.5 ($p<0.001$) for urine Hg.

H-Hg Cozrrelates

Table 3a shows predictors of H-Hg, across all study years. Fish consumption was a highly significant predictor of all three measures of H-Hg (Figure 2a). The large standard error for daily fish consumption is due to the small number of children ($N=11$) in this category. In models, fish consumption of ‘never’ and ‘once a month or less’ are grouped together, as we consider the latter to be a very low level of fish consumption.

Other significant predictors of H-Hg include the Boston (vs. Maine) site (0.42 vs. 0.25 $\mu\text{g/g}$) and being of “other” race. Having a primary caregiver born outside the 50 US states was associated with increased (but not high) H-Hg. H-Hg also decreased slightly with age.

Samples with hair weight < 6.6 mg were excluded from analysis due to imprecision in imputation. Low hair weight (ie, very short hair) was prevalent in Maine at year 5, especially for boys. To confirm that this exclusion did not influence results, two subset analyses were conducted: (1) Boston only, and (2) excluding year 5. Both subset analyses were consistent with the main analysis, except that immigration status of the primary caregiver was no longer significantly associated with higher H-Hg. This may have been due to reduced sample size, as hair weight did not differ by primary caregiver immigration.

Table 4 shows mean and % high hair Hg by race/ethnicity (with “other” race/ethnicity broken down into constituent categories), and by caregiver region of birth. Race/ethnicities included in the “other” category for analysis are shaded. The highest mean hair Hg values were seen in Asian/Pacific Islanders and those whose caregivers reported race as Cape Verdean ethnicity. These 2 groups also had the highest percentage of hair Hg observations $\geq \mu\text{g/g}$, followed by those whose caregivers reported race as a Caribbean ethnicity, or as more than one race and/or ethnicity. All the high H-Hg values for Caribbean ethnicity came from children whose caregivers identified as Haitian.

While contributing a small number of observations (N=14), children with caregivers born in Cape Verde, an archipelago off the west coast of Africa, accounted for the highest mean H-Hg and the greatest percent of high values, followed by those whose caregivers were born in Asia. The high H-Hg values for those reporting Caribbean as caregiver’s country of birth came from children with caregivers born in the Dominican Republic, Haiti, and Jamaica.

U-Hg correlates

Table 3b shows predictors of U-Hg, across study years 3–5. Number of amalgam fillings (tooth surfaces) and use of chewing gum with amalgam fillings were directly associated with each of the U-Hg measures. No significant interaction was found between number of amalgam-restored surfaces and frequency of chewing gum (data not shown), although there was a positive trend, suggesting that U-Hg may increase with chewing gum on increasing numbers of restored surfaces.

Figure 2b shows mean U-Hg levels by number of amalgam fillings (tooth surfaces) for years 3–5 combined. A clear association is evident, with mean U-Hg rising above 1.0 $\mu\text{g creatinine}$ when more than 5 filled surfaces are present. Each child contributes several observations, sometimes in different strata of number of amalgam surfaces. However, using year 5 data (with only one observation per child) yields very similar results.

Being of “other” race was associated with increasing U-Hg (0.74 $\mu\text{g/g creatinine}$ vs 0.61 for Whites in the adjusted model), but not with prevalence of the highest levels. Urinary Hg and odds of high ($\geq 2.3 \mu\text{g/g creatinine}$) U-Hg also decreased slightly with age.

To determine the combined effects of fish consumption and amalgam exposure on H-Hg and U-Hg, additional models were fit that included the interaction between fish consumption and number of amalgam fillings. No significant interactions were found. Additionally, H-Hg levels were similar in children with weekly fish consumption and many amalgam fillings, compared to children with weekly fish consumption and few/no amalgams, supporting the conclusion that amalgam does not affect H-Hg. However, the 9 children with both high (weekly to daily) fish consumption and ≥ 16 amalgam fillings was 3.8 $\mu\text{g/g creatinine}$, compared to 2.79 $\mu\text{g/g}$

creatinine for those with ≥ 16 amalgam fillings, irregardless of fish consumption. This suggests the possibility of an additive effect of fish consumption on U-Hg. .

DISCUSSION

Hair Hg

The mean values for H-Hg over the five years of the NECAT study (0.3–0.4 $\mu\text{g/g}$ hair) were higher than the mean of 0.22 $\mu\text{g/g}$ seen in younger children (ages 1–5) in the 1999–2000 NHANES (McDowell et al. 2004). This is likely due to the higher levels of fish consumption in the NECAT children, relative to the national sample of NHANES. For example, only 25% of the children in the NHANES sample reported consuming fish 3 or more times in the past 30 days (the highest consumption category) (McDowell et al. 2004), while nearly 40% of the NECAT children reported consuming fish at least once a week or more often.

The NECAT H-Hg values fall within the range seen in other studies of children (Benes et al. 2002; Pesch et al. 2002) and adults (Bjornberg et al. 2003; Lindow et al. 2003) in Northern Europe and the United Kingdom, but are lower than values reported from heavy fish-consuming populations in the UK, Asia, and Mediterranean regions (Batista et al. 1996; Ip et al. 2004; Murata et al. 2004).

Consistent with other studies in children (Batista et al. 1996; Ip et al. 2004; McDowell et al. 2004; Pesch et al. 2002) and adults (Airey 1983; Hightower and Moore 2003; McDowell et al. 2004; Morrisette et al. 2004), fish consumption was strongly associated with H-Hg such that children who ate fish weekly or more often were over eight times as likely to have high (≥ 1 $\mu\text{g/g}$) levels of H-Hg than children who ate fish less than twice a month.

Even adjusting for fish consumption, the Boston site, “other” race, and immigrant status of primary caregiver were additionally associated with higher values of H-Hg. This suggests that either the Hg content of the fish consumed by the children differs systematically, or that some other source(s) of organic Hg exist that are associated with these variables. It is plausible that the species of fish consumed might differ between families with origins in different countries; however, logistical considerations prevented us from collecting detailed data on fish species consumed. Children in the “other” race category were over 3 times as likely to have high H-Hg, and 56% of the “other” category were children with primary caregivers born outside the 50 US states. The high H-Hg levels in this category may be caused by special fish consumption habits in Asian Americans and Pacific Islanders (6), which may not be completely captured by our question on fish consumption. As noted earlier, 20% of the children in the “other” category self-identified as Asian-American or Asian/pacific Islander.

It is also instructive to note characteristics of the 58 NECAT children with high (≥ 1.0 $\mu\text{g/g}$) H-Hg at one or more visits. Of these 58 children, 42 had H-Hg ≥ 1 $\mu\text{g/g}$ at one visit only; 11 at 2 visits, 4 at 3 visits, and 1 at all 4 visits. Seven (12%) had parents born in Asia (6 of them either China or Taiwan), and 15 (26%) had parents born in the Caribbean (13 of them from the island of Hispaniola (Haiti or the Dominican Republic)). This corresponds to high H-Hg in 47% of the NECAT children with families of Asian origin (and 60% of those with Chinese/Taiwanese origin), 25% of those of Caribbean origin, and 33% of those specifically from the island of Hispaniola, compared to 4.8% of the children with parents born in the US. Also, as noted earlier, high H-Hg was prevalent in the small number of observations from children with caregivers born in the island nation of Cape Verde. Among the 20 children (3.8%) with at least one H-Hg observation ≥ 2 $\mu\text{g/g}$, those with US-born caregivers were also underrepresented (25%, compared to 77% of the total NECAT study population), and those with caregivers from Hispaniola were overrepresented (50%, compared to 7.3% in NECAT). . This would appear to support ethnic and cultural differences in fish consumption patterns as a possible cause of

higher H-Hg in children of “other” race/ethnicity and/or foreign-born parents. Lack of association of child’s birthplace with H-Hg in adjusted models argues against Hg exposure prior to immigration.

For Blacks and Hispanics, our data do not agree with those for children age 1–5 in the NHANES study, for whom H-Hg levels were about twice as high as in Non-Hispanic Whites. This may reflect regional differences in fish consumption, or other differences (such as ethnicity) between the NECAT participants and the NHANES sample. The slight decrease in H-Hg with age (about 2% per year) may be due to unmeasured variation in fish consumption frequency or type, or may reflect biologic processes. Multivariate analyses found no effect of parental education or family income, though these may be associated with fish consumption, and hence, indirectly, with H-Hg. Amalgam fillings did not appear to contribute to H-Hg in these children. This is to be expected since inorganic Hg does not incorporate into hair in significant amounts.

Finally, the H-Hg levels seen in NECAT children should be examined in the context of existing recommendations by the US EPA aiming at keeping exposure to methyl mercury low enough to prevent adverse health effects. The US EPA reference dose (RfD, 0.1 µg/kg per day) corresponds to a hair Hg concentration of about 1.2 µg/g. The reference dose is ten times lower than the lower confidence limit of the benchmark dose (BMDL), derived by the National Research Council in the analyses of data from in utero exposure, mainly in the Faroe Island study {Rice, 2003 #177}. Although the analyses were based on estimated risks for the fetus, the US EPA extends the recommendations to other groups as well, including young children (www.epa.gov) as it is likely that children are more sensitive to methyl mercury neurotoxicity than adults (Rice 2003). There are relatively few data available on the associations between mercury burden and health status in young children. One study with 7 to 12 year old Amazonian children with high (mean 11 µg/g; 80% >10 µg/g) H-Hg levels found inverse associations between H-Hg levels and performance on several neuropsychological tests. (Grandjean et al., 1999). An analysis from the 7-year Faroe Islands study found an inverse relationship between children’s H-Hg levels (mean of 3 µg/g) and neuropsychological test performance, but the association did not persist after adjustment for prenatal exposure (Grandjean et al., 1997), and at the 14 year evaluation, H-Hg level (mean of 1 µg/g) was not significantly associated with concurrent test scores (Debes et al., 2006). In the Seychelles islands study, some adverse associations between postnatal exposure (mean of 6 µg/g) and test scores were found in 9-year olds, but only in girls (Myers et al., 2003).

In year five of NECAT, about five percent (4.4%) of girls aged 11 to 15 (mean 13.4) years had hair mercury ≥ 1 µg/g. Even if there was a tendency towards decreasing H-Hg with age, it is likely that many of them will still have an exposure level above the US EPA RfD when they enter child-bearing age. This RfD was derived using an uncertainty factor of ten from the BMDL, i.e. from the level statistically consistent with the specified excess risk observed. There are, however, many sources of uncertainty, and it seems clear that exposure should be kept well below the level corresponding to the BMDL (Rice 2004).

Urinary Hg

Consistent with other studies (Levy et al. 2004; Trepka et al. 1997), amalgam exposure was positively associated with U-Hg. Our data also indicate that daily use of chewing gum in the presence of amalgam is associated with higher mean U-Hg and a 3-fold increase in odds of high U-Hg. This is consistent with case reports of higher levels of Hg uptake from amalgam fillings associated with chewing gum in adults (Barregard et al. 1995; Sallsten et al. 1996).

Age also appeared to have a small to moderate inverse association with U-Hg levels (about 4% difference in U-Hg per year of age) and prevalence of high U-Hg. This may be caused by a constant absolute Hg uptake per amalgam filling, but a lower body burden per kg body weight,

or, as expressed in our study, per g creatinine. It is difficult to compare this finding with others since few studies have reported U-Hg in US children of this age range. However, a small (N=60) study of children aged 4–8 years also found higher U-Hg in younger, as well as physically smaller, children, adjusting for amalgam exposure and fish consumption (Levy et al. 2004).

The mean U-Hg level at baseline (e.g. under conditions of no amalgam exposure) was well below the 1.5 µg/L level of detection (only 6% of samples had detectable levels), and for children with no amalgam fillings in years 3–5, imputing values for those below the detection limit, it was 0.6 µg/g creatinine. In comparison, the mean U-Hg levels for a similarly designed randomized trial of dental amalgam exposure in children in Portugal (Casa Pia) were 1.3 µg/L at baseline, and 1.8 µg/L after one year (Evens et al. 2001). A likely reason for this is the much higher fish consumption in the Mediterranean coastal areas compared to the Northeast USA. While methyl mercury in fish is primarily reflected in increased H-Hg levels, some methyl mercury can be demethylated to inorganic Hg and excreted in urine (Johnsson et al. 2005), which is reflected in higher average U-Hg in areas of high fish consumption (Barregard et al. 2006). This study found no significant association between fish consumption and U-Hg, either overall or in children with no amalgam fillings. However, there were only two cases of daily fish consumption with no amalgam fillings, which limits our ability to detect an association.

The association between increased U-Hg and being of “other” race may be explained by a slight effect of differing patterns of fish consumption. Of the 12 children reporting daily fish consumption at any visit, half were in the “other” race/ethnic group. It is also possible that the “other” group, which had a high percentage of foreign-born parents, may have had differential exposure to other sources of inorganic Hg that were not accounted for in this study.

There were 57 samples from 44 children who had high (>2.3 µg/g creatinine) U-Hg at some point during the study (10% of those who supplied urine samples). Of these 44 children, 34 had high U-Hg at one visit, 7 at 2 visits, and 3 at all 3 visits. Of these 3 children, 2 were girls from Boston and one was a boy from Maine. All were white with US-born caregivers, were 9 years old at the year 3 U-Hg observation, and consumed fish with frequency of monthly or less. They had 7–16 amalgam fillings and all occasionally chewed gum.

There are no specific guidelines for U-Hg in children. However, other analyses of data from the NECAT study did not find any consistent effects of either amalgam exposure or U-Hg on a wide array of neuropsychological and developmental test scores over 5 years of follow-up. (Bellinger et al. 2007)

Strengths and Limitations

To our knowledge, this is the only study published to date to examine H-Hg and U-Hg levels, measured concurrently, over several years time in US children with well-characterized dental amalgam exposure. It is also the largest US study in this age group. However, these children, who met stringent eligibility criteria for a clinical trial, by no means constitute a community sample. The eligibility criteria for the NECAT study, including the presence of 2 or more dental caries in need of restoration at baseline, resulted in a study population with lower socioeconomic status than the general population. While this study population might not be representative of all US children, it provides important data on a population that may be underserved in access to health care and thus potentially vulnerable. The racial and ethnic diversity of this population allowed investigation of the impact of race and the immigrant status of primary caregiver, which to our knowledge has not been examined previously.

An obvious limitation of the study was the detection limit of both Hg measures. Because this population had generally low levels of Hg, roughly half of the samples were below the level

of detection. Imputation of these samples limits the accuracy of the mean Hg levels. Imputation also may affect the results of the logistic regression models. However, similar results were obtained when using only detectable levels in the analyses. Omitting hair sample with weight <6.6 grams, in order to improve accuracy of imputation, might also have meant that boys, or others more likely to have very short hair, would be more likely to have missing samples for analysis than girls. However, since only 5 children had missing samples from all visits due to low sample weights, we feel this is unlikely to result in significant bias. Also, the change in detection limits for U-Hg precludes the use of baseline (e.g. pre-amalgam exposure) U-Hg levels for comparison.

CONCLUSIONS

Over a 5-year period, children in the NECAT study had mean H-Hg values of 0.3–0.4 µg/g hair, and mean U-Hg values of 0.7–0.9 µg/g creatinine. After adjustment for demographic and other variables, frequency of fish consumption, study site (Boston vs. Maine), and self-reported race/ethnicity other than black, white, or Hispanic remained significantly associated with higher H-Hg. Age was associated with slightly decreased H-Hg. For U-Hg, number of amalgam-restored surfaces and use of chewing gum in presence of amalgam were the most robust predictors in the adjusted model.

These findings should be interpreted in the proper context. Avoiding fish consumption may be unwarranted, given its health benefits (Mozaffarian and Rimm 2006); however, consumers should choose fish with lower Hg content when possible (US Dept. of Health and Human Services; US Environmental Protection Agency 2004). Our data also suggest the advisability of providing fish and seafood advisory information in languages and locations accessible to recent immigrants.

While exposure to amalgam has not been associated with detrimental neuropsychological effects in the NECAT study (Bellinger et al. 2007; Bellinger et al. 2006), our data suggest that amalgam-associated Hg exposure might be reduced by avoidance of gum-chewing in the presence of amalgam fillings.

Acknowledgements

This study was supported by a cooperative agreement (U01 DE11886) between the New England Research Institutes and the National Institute of Dental and Craniofacial Research.

The study was reviewed and approved by the Institutional Review Boards of New England Research Institutes and participating clinical sites.

Susan Assmann, Ph.D. (New England Research Institutes), Elsa Cerniciari, MS (University of Rochester School of Medicine, Rochester, NY), David Daniel, Ph.D. (University of Maine at Farmington), Michael Doherty MS (New England Research Institutes) and Joan Landon, MPH (New England Research Institutes), contributed to earlier versions of these analyses, parts of which were presented at the 2001 Congress of Epidemiology, Toronto, CA.

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Figure 1.

Figure 1a. Distribution of Hair Hg by Study Year. New England Children's Amalgam Trial. "Mean" = "Arithmetic Mean". "GM" = "Geometric Mean"

Figure 1b. Distribution of Urinary Hg by Study Arm and Study Year. New England Children's Amalgam Trial. "Mean" = "Arithmetic Mean". "GM" = "Geometric Mean"

Figure 2.

(a) H-Hg by fish consumption, and (b) U-Hg by number of amalgam-restored tooth surfaces. N= number of samples. Bars show standard error. New England Children's Amalgam Trial.

a) H-Hg by participant's fish consumption, across all study years.

b) U-Hg by number of amalgam-restored tooth surfaces; Years 3–5 combined.

Table 1
Baseline demographic characteristics, New England Children's Amalgam Trial
(N=534)*

Age, mean (SD)	N	7.9 (1.4)	%
Site			
Boston	291		54.5
Maine	243		45.5
Gender			
Female	287		53.8
Male	247		46.3
Race			
Non-Hispanic White	323		62.1
Non-Hispanic Black	98		18.9
Hispanic	38		7.3
Other	61		11.9
Household income			
≤ \$20,000	160		31.2
\$20,001 - \$40,000	222		43.3
> \$40,000	131		25.5
Education of Primary Caregiver			
< HS	72		13.9
HS graduate	391		75.3
College/Post-college degree	56		10.8
Primary Caregiver Birthplace			
US (50 states)	408		77.7
Caribbean	59		11.2
Europe/UK	18		3.4
Asia	15		2.9
Africa/Mid-East	14		2.7
South or Central America	11		2.1
Child (study participant) Born Outside 50 US States	28		6.7
Maternal Smoking			
Never	167		35.6
Past only	123		26.2
Current	179		38.2

* For race, N=520. For income, N=513. For education N=519.

For primary caregiver birthplace, N=525. For child birthplace, N=420.

For maternal smoking, N=469. For H-Hg analyses, N=521. For U-Hg analysis, N=455.

** Race was self-reported by the children's primary caregivers.

*** Included Asian or Pacific Islander (N=9), Native American/Alaskan Native (N=2), Multiracial (N=20), 28 who answered the question with one or more ethnicities other than Hispanic, and 2 "refused or missing".

Fish consumption frequency, gum chewing, and amalgam fillings by study year; New England Children's Amalgam Trial.

Table 2

	Baseline	Year 1	Year 2	Year 3	Year 4	Year 5
Fish Consumption * (N,%)						
every day	5	4	0	4	1	0
at least once a week	197	115	68	127	97	86
at least twice a month	104	85	51	87	78	107
once a month or less	214	177	122	186	188	195
Gum Chewing (N,%)						
None	42	37	15	31	35	30
Occasionally (<daily)	424	315	212	323	303	332
Daily	54	28	14	49	26	26
Amalgam surfaces ** (mean ± sd)	0 ± 0	3.5 ± 4.9	3.6 ± 5.1	3.1 ± 4.6	2.4 ± 3.9	2.0 ± 3.4

* includes tuna and all other types of fish

** one tooth can have several filled surfaces. The number of amalgam surfaces decreased over time due to exfoliation of primary teeth. None of the children had amalgam fillings at baseline, as per NECAT study design.

Table 3

Predictors of hair (3a) and urinary (3b) mercury content; the New England Children's Amalgam Trial. Repeated measures analysis across study years as noted below; backwards elimination starting with all variables[†].

A. Predictors of Hair Hg; across all study years.			H-Hg $\geq 0.5 \mu\text{g/g}$			H-Hg $\geq 1 \mu\text{g/g}$			H-Hg level η^2		
	OR [95% CI]	p-value		OR [95% CI]	p-value		OR [95% CI]	p-value	Slope* [SE]	p-value	
Age											
Race											
Ref. = White	0.9 [0.8,0.9]	<0.001							-0.02 [0.01]	0.003	
Black	0.7 [0.4,1.2]										
Hispanic	0.4 [0.1,1.1]	<0.001							-0.13 [0.09]	<0.001	
Other	2.3 [1.2,4.4]								-0.25 [0.16]		
									0.30 [0.11]		
Site											
Boston vs. Maine	2.5 [1.5,4.0]	<0.001							0.20 [0.08]	0.009	
Primary caregiver											
born outside 50											
US States.		NS							0.22 [0.09]	0.018	
Fish Consumption											
Ref. = <2x/month		<0.001									
Daily	1.4 [0.3,7.3]								0.00 [0.28]	<0.001	
Weekly	3.1 [2.1,4.4]								0.26 [0.05]		
2x/month	1.9 [1.3,2.9]								0.16 [0.05]		
B. Predictors of Urinary Hg; Study years 3-5.											
	OR [95% CI]	p-value	U-Hg $\geq 1 \mu\text{g/g creatinine}$			U-Hg $\geq 2.3 \mu\text{g/g creatinine}$			U-Hg level η^2		
	OR [95% CI]	p-value		OR [95% CI]	p-value		OR [95% CI]	p-value	Slope* [SE]	p-value	
Age											
Race											
Ref. = White		NS							-0.04 [0.02]	0.013	
Black		NS									
Hispanic									-0.07 [0.07]	0.025	
Other									0.30 [0.16]		
									0.19 [0.09]		
# Amalgam surfaces									0.09 [0.01]	<0.001	
Chewing gum on	1.2 [1.2,1.3]	<0.001									
amalgam fillings											
Ref. = Never		0.004									
Daily	2.9 [1.6,5.5]								0.34 [0.12]	0.029	
Occasionally	1.2 [0.8,1.8]								0.07 [0.06]		

[†] age, gender, race/ethnicity, study site, education of primary caregiver, family income, primary caregiver born outside 50 US states (yes/no), child born outside of 50 US states (yes/no), maternal smoking, fish consumption frequency, number of amalgam fillings. Variables with $p \geq .05$ are not retained in the model and are indicated as "NS" in the table.

η^2 values below detectable limit are imputed. OR=odds ratio; CI=confidence interval; SE=standard error; NS=not significant.

* slopes of log-transformed Hg levels.

Table 4
Hair Hg by caregiver-reported race/ethnicity and Birth Country of Primary Caregiver. The New England Children's Amalgam Trial. Categories included in the "other" race/ethnicity group are shaded.

	# samples	Mean Hair Hg	% High (≥ 1 $\mu\text{g/g}$)
Race/Ethnicity			
Asian or Pacific Islander	37	1.0	38%
Black	288	0.3	2%
Cape Verdean *	18	1.0	39%
Caribbean *	23	0.8	22%
Hispanic	125	0.5	8%
Indian *	16	0.4	13%
Multiracial	67	0.5	16%
Native American	8	0.1	0%
Other ethnicity *	11	0.3	0%
White	1132	0.3	2%
Caregiver Region of Birth			
Africa	22	0.3	0%
Asia	46	0.9	33%
Cape Verde	14	1.1	50%
Caribbean **	172	0.6	12%
Central America	13	0.2	0%
Europe	63	0.5	5%
Middle East	8	0.3	0%
South America	16	0.7	25%
USA	1385	0.3	2%

* the caregivers of 28 children reported one or more ethnicities only, and not race.

** Caribbean Includes Haiti, Dominican Republic, Puerto Rico, Jamaica, and Trinidad.