HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2020 April 01.

Published in final edited form as:

Environ Res. 2019 April ; 171: 523–529. doi:10.1016/j.envres.2019.01.026.

Prenatal exposure to mercury in relation to infant infections and respiratory symptoms in the New Hampshire Birth Cohort Study

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Abstract

Background: Mechanistic studies support the potential for mercury (Hg) to alter immunity, including via *in utero* exposure. As yet, there are few prospective studies of *in utero* Hg exposure and subsequent immune-related outcomes, especially in infancy.

Objectives: We investigated the association of biomarkers of prenatal Hg exposure and maternal silver-mercury dental amalgams with the occurrence of infant allergy, respiratory infection, and respiratory symptoms in the first year of life.

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Conflict of interest

The authors declare no competing financial interests or conflicts of interest.

Methods: The New Hampshire Birth Cohort Study (NHBCS) ascertained information on infant allergies, infections and symptoms through telephone interviews at 4, 8 and 12 months postpartum and measured total Hg in maternal toenails collected at ~28-30 weeks gestation. Information on maternal fish consumption and presence of dental amalgams was obtained from a questionnaire administered at study enrollment at 24-28 weeks. A total of 1,321 NHBCS mother-infant pairs had at least one Hg exposure measure (toenail Hg or information on dental amalgams) and information on dietary fish intake. Generalized linear models and generalized estimating equation models with Poisson regression adjusted for potential confounders (maternal age, level of education, parity, smoking, alternative Healthy Eating Index-2010, infant sex, gestational age, feeding mode, and day care attendance) were used to assess the association between infant outcomes and prenatal toenail Hg levels. We subsetted this analysis on mothers who consumed fish (n=706) as a measure of *in utero* methylmercury (MeHg) exposure. Associations between infant outcomes and dental amalgams as a measure of *in utero* inorganic Hg exposure were assessed among mothers who did not consume fish (n= 218).

Results: Among women who ate fish during pregnancy, higher maternal toenail Hg concentrations were associated with an increased risk of lower respiratory infections and respiratory symptoms requiring a doctor visit among infants age 9 to 12 months (relative risk (RR) 1.4 (95% CI: 1.1, 1.9) and 1.2 (95% CI: 1.0, 1.4) respectively), whereas a reduced risk of lower respiratory infections was observed among infants 0 to 4 months of age (RR = 0.7 (95% CI: 0.5, 1.0). We found little to no evidence of associations of toenail Hg with upper respiratory infections, allergy or eczema at any age to one year. Among infants of mothers who did not consume fish, we found an elevated risk of upper respiratory infections requiring a doctor visit in relation to having dental amalgams during pregnancy (RR = 1.5 (95% CI: 1.1, 2.1)). Overall, weaker associations were observed with lower respiratory infections, respiratory symptoms, and medically confirmed allergies, and there was no association with eczema.

Conclusions: Our analyses of a US birth cohort, along with prior mechanistic work, raise the possibility that gestational Hg exposure through fish/seafood consumption and dental amalgams may alter respiratory infections and respiratory symptoms in infants.

Keywords

cohort study; prenatal exposure; mercury; infection; immunity; allergy; atopy

1. Introduction

Mercury (Hg) is among the top 10 toxicants of greatest public health concern listed by the World Health Organization [1]. Methylmercury (MeHg) contamination in fish and shellfish is due predominantly to industrial contamination of aquatic ecosystems and is a major source of low-level MeHg exposure in the general population [2-4]. Human exposure to elemental or inorganic Hg can occur through artisanal mining and Hg-containing dental amalgams [5]. Hg can penetrate physiologic barriers including blood-brain, blood-testes and placenta [6,7]. Experimental studies and epidemiologic data on adults suggest immunotoxic effects of Hg [8-13]. However, as yet there are few epidemiologic studies that investigate the impacts of prenatal Hg exposures on immune function [14,15]. Prenatal or peripartum blood Hg concentrations were associated with increased cord blood IgG in Brazil [16], and decreased

naïve T helper cells, reduced IgM production by neonatal B cells and impaired clonal expansion after stimulation in cell culture in an Indigenous Canadian population [17]. In a study of children in the Faroe Islands where MeHg exposure occurs via high seafood consumption and whale meat intake, cord blood Hg concentrations were associated with increased IgM autoantibodies to neural antigens and reduced anti-keratin (non-neural) IgG autoantibodies at age 7 years [18] as well as reduced grass specific IgE [19] Less is known about the impacts of elemental Hg, especially during pregnancy. However, there is some evidence of decreased B cell responsiveness from a randomized amalgam clinical trial [20] and increased IgE in a cross-sectional study from Bavaria [21] related to dental amalgams in children. Prospective studies have reported inconsistent findings between Hg (measured largely in cord blood) and occurrence of wheeze, atopy status and eczema among children [19,22-24]. To our knowledge, there are few prior studies on association between prenatal Hg exposure and infant infections. Therefore, as part of the New Hampshire Birth Cohort Study, we investigated the association of measures of prenatal Hg exposure (toenail Hg as a biomarker of MeHg, and presence of dental amalgams as a measure of elemental Hg) with clinical manifestations of altered immune function –respiratory infection, allergy and atopy – in the first year of life.

2. Methods

2.1 Study Population

Since January 2009, the NHBCS has been recruiting pregnant women (ages 18-45 years) receiving prenatal care at study clinics in New Hampshire, USA as previously described [25,26]. Briefly, women included in our study were screened for eligibility at an initial prenatal care visit and enrolled around gestational week 24-28 if they reported using water from a private, unregulated well in their home since their last menstrual period and were not planning a change in residence prior to delivery. Only singleton births were included in the cohort. All protocols were approved by the Committee for the Protection of Human Subjects at Dartmouth College. All participants provided written informed consent.

2.2 Outcome Assessment

Telephone interviews were conducted at four, eight and twelve months postpartum with infants' caregivers to determine whether they had any respiratory infections or respiratory symptoms, atopic dermatitis (eczema) or allergy in the preceding four months. Questions were asked regarding a range of infections and symptoms related to respiratory and gastrointestinal illnesses; our analysis was focused on those involving the respiratory tract. Diagnoses or symptoms of upper respiratory infections (i.e. rhinorrhea, colds, nasal congestion, otitis media, conjunctivitis), lower respiratory infections (i.e. respiratory syncytial virus, pertussis, bronchitis, bronchiolitis, pneumonia), other respiratory symptoms (i.e. cough, difficulty breathing, wheeze), or atopic dermatitis (eczema) were ascertained. For each type of infection or respiratory condition reported, caregivers were asked whether the infant saw a doctor, and if yes, whether medication was prescribed. Additionally, caregivers were asked if infants had allergies to: cats or dogs, pollen, dust, latex, insect bites, grass and plants, antibiotics, or food (peanuts, nuts, eggs, other), and for each reported allergy, whether it was confirmed by a physician.

2.3 Exposure Assessment

Participants were asked to complete a medical history and lifestyle questionnaire upon enrollment, which ascertained sociodemographic factors (e.g., age, race/ethnicity, marital status, and education), reproductive history (e.g., previous pregnancies, pregnancy complications, birth outcomes), and health history. Smoking during pregnancy was derived from the prenatal as well as post-partum questionnaires. Participants were asked whether they had smoked cigarettes during the 1st, 2nd or 3rd trimesters of their pregnancy. From the combined answers, a dichotomous variable was created to indicate whether a mother had ever smoked cigarettes during one or more trimesters (yes/no). As part of the enrollment prenatal questionnaire, mothers were asked whether or not they had silver-mercury dental amalgam fillings and the number of amalgam fillings. Delivery type, and birth outcomes were obtained from a review of prenatal, delivery and infant birth medical records.

2.4 Maternal Fish Consumption

At enrollment, participants were asked to complete a written, validated, semi-quantitative food frequency questionnaire (FFQ) [27,28] to quantify diet over the previous year. The FFQ asked about the consumption of 120 different food items including five questions regarding fish and seafood including tuna “canned tuna fish (3-4 oz)”, breaded fish “breaded fish cakes, pieces, or fish sticks (1 serving, store bought)”, dark meat fish “e.g., tuna steak, mackerel, salmon, sardines, bluefish, swordfish (3–5oz)”, shell fish “shrimp, lobster, scallops as a main dish”, and other white fish “e.g. cod, haddock, halibut (3–5 oz)”.

2.5 Maternal Toenail Collection and Mercury Exposure Assessment

At the enrollment visit, mothers were provided the instructions and materials to collect and mail back a toenail clipping sample (separating the big toenail clippings from the other nails) in a pre-paid postage envelope. Prenatal toenail samples were collected from mothers at a median of 28.6 weeks of gestation (mean = 29.5 weeks, SD=4.2 weeks). Toenails take approximately 6 to 12 months to grow out so clippings reflect past exposures; however, toenail concentrations of trace metals such as Hg appear to be stable over time [29]. Sources of both elemental and MeHg exposure have been associated with increased toenail concentrations, although about 90% of toenail Hg is considered to be from MeHg exposure [30,31].

After careful washing to remove external contaminants, clippings from the big toe were analyzed for total Hg at the Trace Element Analysis Core (Dartmouth College, Hanover, New Hampshire, USA), using inductively coupled plasma mass spectrometry (ICP-MS) using methods described previously [30]. In each analysis batch, five replicate reference materials were run for quality control. The percent recovery for Hg in hair reference material (#13, NIES, Tsukuba, Japan) was 94 ± 10% across all batches (n=134). The majority of participants (89%) had toenail total Hg levels above the limit of detection (LOD) of 0.10 ng/g. Samples that were below the detection limit were assigned the LOD divided by the square root of 2.

2.6 Statistical Analysis

We used natural log (ln) transformed prenatal toenail Hg concentrations (treated as a continuous variable) because of the skewed distribution. As above, toenail Hg largely reflects MeHg exposure, with the primary exposure route being fish or seafood consumption. Therefore we examined toenail total Hg concentrations among women who ate fish or seafood to estimate MeHg exposure and to control for the potential beneficial effects of other constituents of these foods such as n-3 fatty acids [32-35]. In our main analyses, we examined any respiratory infections or symptoms that resulted in a doctor visit or any allergy that was medically confirmed. To estimate relative risk in each of the three time windows: 0 to 4 months, 5 to 8 months, and 9 to 12 months, we used generalized linear models (GLM) with Poisson regression with a robust error variance [36] for single events (eczema, wheeze and allergy) and generalized estimating equation (GEE) models [37] with a log-link function with specification of a Poisson distribution and a robust error variance [36] for grouped events where each single event is treated as a repeated measurement. To estimate the population average effect of Hg exposure on respiratory infections, symptoms or allergy/eczema over all three time points of longitudinal data, we used GEE models with Poisson regression with a robust error variance [36] and an autoregressive working correlation structure of one which accounts for the dependency of observations.

We further modeled infant outcomes in relation to maternal self-reported silver-Hg dental amalgam fillings (yes/no) as a proxy for inorganic, elemental Hg exposure [38] and restricted these analyses to women who did not report eating fish or seafood. The number of subjects with dental amalgams was too small to evaluate each time period (infant age) separately. Thus, we computed occurrence of infant outcomes over the first year of life using GEE models [37] with Poisson regression [36] with an autoregressive correlation matrix, and robust error variances where each single event is treated as a repeated measurement.

Factors that *a priori* could influence respiratory infection, symptoms or allergy risk: maternal age at enrollment (years), parity (0, 1-2, ≥3), maternal alternative Healthy Eating Index-2010 (derived from the FFQ using the methods of Chiuve and colleagues [39] on a continuous scale), maternal educational attainment (high school or less, junior college/technical college/ come college, college graduate, postgraduate school), cigarette smoking during pregnancy (yes/no), infant sex, gestational age (continuous), birth weight (continuous), infant feeding mode at 4, 8, and 12 months (breast, formula, or mixed breast and formula) and day care attendance at 4, 8, and 12 months (yes/no), were considered as potential confounders and included as model covariates. Gestational age was calculated using first trimester ultrasound gestational age estimates or, if an ultrasound was unavailable, date of last menstrual period. The results of these fully adjusted models are presented in Tables 2 and 3 and Table A.1.

Models were additionally adjusted for fish/seafood intake (servings per day) to account for potential residual confounding by potentially beneficial nutrients in fish/seafood. To obtain the total number of servings of fish or seafood consumed per day, we summed consumption rates across all five fish questions using the mid-point of consumption categories on the FFQ. To interpret the change in outcome per doubling of toenail Hg, we exponentiated the beta coefficients multiplied by the natural log of 2 (i.e. $e^{\beta*\ln(2)}$). Because Hg concentrations were modeled on the natural log scale, we multiplied the beta coefficient by ln(2) so that our

relative risk estimate would reflect association with a two-fold increase in exposure. The change in outcome due to the presence of dental amalgam fillings was computed as the exponential of the beta estimate (i.e. e^{β}). Descriptive analyses were performed with the software package SAS (version 9.1 for Windows; SAS Institute, Cary, NC) and analytic models were run with RStudio Version 1.1.419. For all analyses, we used a *p*-value of 0.05 (2-sided) to define statistical significance.

3. Results

3.1 Population Characteristics

Of the 1,788 mothers enrolled in the NHBCS by January 2018, a total of 1,329 had information on fish/seafood consumption and 1,327 had either prenatal toenail Hg measured or information on the presence of dental amalgams. Data on infant sex was missing for 6 mother-infant pairs and were therefore omitted from the final study sample (n=1,321). The study sample was representative of the entire cohort on baseline maternal and infant characteristics (data not shown).

Mothers in our study had a mean age of 31.4 years at the time of enrollment with the majority white, married, and multiparous with at least some college education (Table 1). Cigarette smoking exposure was low (6% of mothers reported smoking cigarettes during pregnancy) (Table 1). Most infants were term, normal birth weight, and delivered vaginally; the average gestational age at birth was 39.4 weeks with 6% born prematurely and 4% low birth weight (Table 1). Twenty to thirty percent of infants were ever in daycare in the first year of life, 39% were exclusively breast fed at 4 months and 17% of mothers reported still breast feeding (and not using formula) at 12 months (Table 1).

3.2 Prenatal Mercury Exposure Through Fish Consumption or Silver Mercury Amalgam Fillings in the NHBCS

One-fifth of mothers in our study reported that they had never consumed fish/seafood in the past year. Among women who did not consume fish (n=298), geometric mean (95% CI) levels of prenatal toenail Hg were 0.01 (0.004, 0.01) μg/g whereas women who consumed fish (n=1,023) had geometric mean (95%CI) levels of 0.02 (0.02, 0.03) μg/g ($p<0.0001$). The number of fish/seafood servings per day correlated with maternal toenail Hg levels (Spearman $r=0.33$, $p<.0001$; Figure A.1a). Nearly half (47%) of the study participants reported having silver-Hg amalgam fillings with a median of 4.0 fillings per woman (range 1-20). Having silver-Hg amalgam fillings did not correlate with maternal toenail Hg levels (Spearman $r=-0.04$, $p=0.37$; Figure A.1b).

3.3 Prenatal Mercury Exposure and Infant Respiratory Infections, Symptoms and Allergies

Among mothers who consumed fish, higher maternal toenail Hg levels related to a greater relative risk of both lower respiratory infections and respiratory symptoms requiring a doctor visit at 9 to 12 months 1.4 (95% CI: 1.1, 1.9) and 1.2 (95% CI: 1.0, 1.4, respectively, for a doubling of toenail Hg), while a reduced relative risk of lower respiratory infection was observed among infants 0-4 months of age 0.7 (95% CI: 0.5, 1.0) (Table 2). There was little

to no evidence of associations of toenail Hg with upper respiratory infection, allergy or eczema at any interval (Table 2). The presence of amalgam fillings was not included as a covariate in our final models as they did not correlate with toenail Hg (Figure A.1b); adjustment for this variable did not alter our findings (data not shown).

In sex-stratified analyses, the toenail Hg associated increased relative risk of lower respiratory infections at the 9-12 month interval was slightly more pronounced in boys than girls (boys: 1.6 (95% CI: 1.2, 2.4, girls: 1.3 (95% CI: 0.9, 1.9); p for interaction = 0.11) and relative risk of respiratory symptoms was slightly more pronounced in girls than boys (boys: 1.1 (95% CI: 0.9; 1.4; girls: 1.3 (95% CI: 1.0; 1.6); p for interaction = 0.73). See Table A.1.

Among mothers who did not eat fish/seafood (n=218), presence of silver-Hg amalgam fillings (n=102) was associated with an increased relative risk of infant upper respiratory infections in the first year of life 1.5 (95% CI; 1.1, 2.1) in comparison to infants born to mothers without dental amalgams (n=116). The dental amalgam associated relative risks of lower respiratory infections, respiratory symptoms, wheeze, and allergy were also increased but not statistically significantly so (Table 3). There was no apparent association of amalgams with eczema. However, stratified analyses suggested potential inverse amalgam-eczema associations in girls 0.2 (95% CI: 0.1, 0.8) but not boys (p for interaction = 0.75). Conversely, maternal amalgams were associated with elevated relative risk of respiratory symptoms 2.3 (95% CI: 1.3, 4.3) and allergy 5.5 (95% CI: 1.7, 17.9) in girls but not boys (p for interaction = 0.27 for respiratory symptoms and p for interaction = 0.15 for allergy) (Table 3). Inclusion of maternal toenail total Hg slightly increased the strength and statistical significance of the association of amalgam with upper respiratory infections (RR=1.6 (95% CI: 1.1, 2.2), but it also reduced the size of our sample to 182 due to missing measures of toenail Hg in this subgroup.

In sensitivity analyses, results were generally similar by type of delivery. For example, at 9 to 12 months, each doubling of toenail Hg was associated with a relative risk of lower respiratory infection of 1.6 (95% CI: 1.0, 2.8) for C-section deliveries and 1.4 (95% CI: 1.0, 2.0) for vaginal deliveries. However, toenail Hg associations with respiratory symptoms were observed among C-section deliveries (RR=1.4(95% CI: 1.1, 1.8) but attenuated and not statistically significant in vaginal deliveries (RR=1.1(95% CI: 0.9, 1.3), p for interaction 0.13. Dental amalgam associated relative risk of upper respiratory infections in the C-section stratum was not significant 1.2 (95%CI: 0.7, 1.9) whereas it was for vaginal delivery 1.7(95% CI: 1.1, 2.6), but again the interaction was not statistically significant (p=0.26).

4. Discussion

In a prospective pregnancy cohort study, we found evidence that higher Hg exposure *in utero* may alter risk of immune-related outcomes in the first year of life. Among infants whose mother's consumed fish/seafood, prenatal toenail Hg was associated with an increased risk of lower respiratory infection and respiratory symptoms at ages 9-12 months but not earlier; indeed, a decreased risk was observed for lower respiratory infections during the first 4 months (Table 2). Among infants whose mothers did not consume fish/seafood, but had silver-Hg amalgam fillings, we found an increased risk of upper respiratory infections

compared to infants of mothers without amalgams; similar trends were observed for lower respiratory infections and respiratory symptoms, wheeze and allergy, but with limited statistical precision (Table 3). However, in sex stratified analyses, maternal amalgams fillings were associated with a significantly increased risk of respiratory symptoms and allergies in girls, but not boys (Table 3). Conversely, amalgams were associated with a lower risk of eczema in girls (Table 3). In sensitivity analyses, some differences in associations were noted by type of delivery although not consistently so. However, the impact of C-section delivery on our outcomes is of interest, as we have previously demonstrated that this mode of delivery impacts the infant gut microbiome and thus may impact immune function early in life[40].

In Minamata Disease, the neurotoxic impacts of prenatal MeHg poisoning were more severe in boys than girls supporting possible sex differences in susceptibility to Hg toxicity[41] . We assessed potential sex differences in associations but none were statistically significant. This is consistent with limited power due to the modest sample size and small number of events in sex stratified analyses, especially for dental amalgam exposure (Table 3). In this context, it is possible that the unexpected inverse association of amalgams with eczema in girls is spurious. Similarly, inverse associations of toenail Hg with lower respiratory infection at 0-4 months (Table 2), may be a consequence of the small number of events leading to unstable effect estimates.

While limited data exist on the impacts of the presence of dental amalgam during pregnancy and offspring immune outcomes, a few studies have investigated post-natal exposure. A cross sectional study of early school aged children in southern Germany reported adverse associations between urinary Hg levels, serum total IgE levels and acute atopic eczema[21]. In this analysis, children with silver-Hg amalgam fillings (n=47) had higher urinary Hg concentrations than children without amalgams or with alternative filling materials. Finally, the New England Children's Amalgam Randomized Trial conducted an immune function substudy (n=59) using repeated measures over 5-years and reported reduced B-cell response to poke weed mitogen (PWM) stimulation in children who received silver-Hg amalgam fillings compared to their baseline measurements [20]. Both of these studies are consistent with increased risk of altered immune responses associated with Hg exposure from dental amalgams.

Findings from prospective studies of infant or child immune outcomes and biomarkers of *in utero*/peripartum exposure to Hg have been mixed (summarized in Table A.2). No clear associations of biomarkers of prenatal Hg exposure (cord blood or maternal Hg levels) with wheeze, eczema, or atopic status have been observed in young children (1.5-5 years of age) from the Avon Longitudinal Study of Parents and Children [23], mother/child pairs from Krakow, Poland[22], or the Osaka Maternal and Child Health Study[24]. In a large Korean prosective study of children age 7-8, blood Hg concentrations were associated with increased risk of asthma, wheezing and airway hyperresponsiveness up to age 11-12[42]. Cross sectional studies have reported conflicting results on effects of low-level Hg on asthma in school age children including inverse associations[43,44].

Studies have also observed Hg-associated changes in biomarkers of both humoral and cellular immunity. In the Faroe Islands, Grandjean and colleagues reported an inverse association between high levels of prenatal MeHg exposure (assessed via cord blood Hg) and allergen-specific IgE levels in 7 year old children [19]. In a smaller sub-study of 7-year-old children from the Faroe Islands, cord blood Hg levels were positively associated with IgM autoantibodies to neural antigens, but negatively associated with IgG autoantibodies to non-neural antigens (i.e., keratin) [18]. Cross-sectional studies of newborn cord blood Hg levels have observed subtle changes in the developing immune system (Table A. 1). Two smaller cohorts of mother-infant pairs in a Brazilian mining community [16] and a subsistence fishing community on the St. Lawrence River [17] observed cord blood Hg associated changes in antibody levels and lymphocyte activity. In our previous analysis of the NHBCS, a doubling of maternal peripartum toenail Hg was associated with decreased cord blood monocyte proportion in all newborns, but an increase in B cells in female infants only [45].

In utero Hg exposure has also been associated with differential epigenetic and genetic regulation in newborns and children. In the NHBCS, a multiplicative interaction between postpartum maternal toenail Hg and prenatal urinary arsenic after controlling for main effects was associated with increased methylation was observed near CpG islands of gamma-glutamyltransferase 7 (GGT7), a gene involved in metabolism of the redox regulator glutathione (GSH) [45], an essential antioxidant, involved in metal detoxification, and inhibiting autoimmunity [46]. Another study of childhood exposure to MeHg found associations of childhood hair Hg levels with increased trascription of JAK2 and decreased trascription of HLAdrb5 - genes involved in immunologic and neurologic function [43]. These results provide preliminary insight into possible mechanisms whereby early Hg exposure (and its potential interaction with other chemical exposures) might alter immune response in children.

The impact of low level Hg in women of childbearing age has also has been investigated. An NHANES study of young women indicated a positive cross-sectional association between hair Hg levels and antinuclear autoantibodies (odds ratio (OR) = 4.0, 95% CI 1.6-10.3). Althouth speculative, it is conceivable that the maternal antibody pool that is transfered to the fetus during pregancy and through breast milk in the first months of an infant’s life may already be altered by Hg exposure; however further data are needed to understand this possibility and its impact on childhood immune system function.

The development of respiratory infections, symptoms and allergies reflect complex integration and potential dysregulation of multiple immune functions (as well as exposure to infectious agents) and therefore may be more informative outcome measures than specific antibody or cellular targets. Although the literature and our findings are mixed regarding associations of Hg with atopic disorders (e.g. asthma, eczema or allergies), this is one of the few, if only, prospective studies of infection and *in utero* Hg exposure. In this context, our findings of relatively consistent associations of Hg exposure measures (both toenail concentrations and presence of silver-Hg amalgam) with infant respiratory infections are notable.

4.1 Strengths and Limitations

Our study benefits from prospectively collected data and availability of key potentially confounding or modifying factors. We stratified our analysis based on fish and seafood consumption not only to distinguish between elemental and MeHg exposures, but to take into account the potential for negative confounding due to the beneficial effects of nutrients in fish and seafood such as n-3 fatty acids. Additionally, we were able to assess outcome measures (allergies, upper respiratory infection) that reflect clinical manifestations of altered immune function with important public health implications. The incidence of childhood allergic diseases is increasing [49,50] and these disorders have substantial associated morbidities and medical/social costs. Food allergies were predomanantly reported in our cohort, which also have been found to be associated with the development of allergic asthma and allergic rhinitis [51]. Respiratory illnesses, including infections in children, also have substantial associated morbidities and costs [52,53].

Limitations of our study must also be considered. Our study population was composed predominantly of Caucasians, so the generalizability of these results to other races/ethnicities may be limited. Our study lacked conventional biomarkers of Hg exposure (for example, hair or blood levels for MeHg [54]). However, toenail Hg has the advantage of being stable overtime [29,55] and, furthermore, our toenail Hg levels correlated with fish/seafood consumption from a validated food frequency questionnaire [30] but did not correlate with having silver-Hg amalgam fillings supporting the likely utility of toenail Hg levels as a proxy for MeHg exposure.

Similarly, we collected information on elemental Hg exposure by assessing the presence of silver-Hg amalgam dental fillings, not through the more traditional biomarker, urinary Hg [38,56]. However, by restricting this analysis to non-fish eaters, we enhanced the likelihood that dental amalgams reflected inorganic Hg exposure. Additionally, our study may be limited due to having only one time point for Hg exposure assessment; we lacked information on postdelivery exposure to Hg which may also contribute to immune health outcomes in the study infants. Hg levels in breast milk are generally higher in populations with high fish consumption, than among those with low fish consumption[57], so it is possible that postnatal Hg exposure in our population varied by infant feeding and maternal fish consumption. However, we accounted for both of these as covariates in our analyses. Infant foods have been shown to contain MeHg especially when they are rice-based [58], and this represents another potential source of postnatal exposure that we were not able to account for in our analyses. That said, unless infant intake of solid foods is correlated with prenatal Hg exposure, it is unlikely that postnatal exposure from this source would confound our results. Information about respiratory infections, symptoms and allergies were obtained from parent/caregiver telephone interviews. The clinical determination of allergies in young infants is very difficult and so potential outcome misclassification is a limitation of our study which, if nondifferential, decreases our power. In general, our analyses of outcomes with very low prevalence likely suffer from statistical imprecision. This is particularly true in the stratified analyses (i.e., by sex and delivery mode) and will be important to reassess in future studies of our growing cohort.

5. Conclusions

Our findings, along with others, suggest that *in utero* exposure to both MeHg and elemental Hg could increase the risk of respiratory infections and respiratory symptoms in the first year of life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work is supported in part by US National Institutes of Health, National Institute of Environmental Health Sciences grant (1P20ES018175; ES022832) and US Environmental Protection Agency grant (RD83459901).

References

1. Mercury and health. 2013 at http://www.who.int/mediacentre/factsheets/fs361/en/.)
2. Kamps LR, Carr R, Miller H. Total mercury-monomethylmercury content of several species of fish. Bull Environ Contam Toxicol 1972;8:273–9. [PubMed: 4644493]
3. Sunderland EM, Amirbahman A, Burgess NM, et al. Mercury sources and fate in the Gulf of Maine. Environ Res 2012;119:27–41. [PubMed: 22572623]
4. Spry DJ, Wiener JG. Metal bioavailability and toxicity to fish in low-alkalinity lakes: A critical review. Environ Pollut 1991;71:243–304. [PubMed: 15092121]
5. Counter SA, Buchanan LH. Mercury exposure in children: a review. Toxicology and Applied Pharmacology 2004;198:209–30. [PubMed: 15236954]
6. Mottet NK, Shaw CM, Burbacher TM. Health risks from increases in methylmercury exposure. Environ Health Perspect 1985;63:133–40. [PubMed: 3908085]
7. Reynolds WA, Pitkin RM. Transplacental passage of methylmercury and its uptake by primate fetal tissues (38576). Proc Soc Exp Biol Med 1975;148:523–6. [PubMed: 1121504]
8. Lawrence DA, McCabe MJ Jr. Immunomodulation by metals. Int Immunopharmacol 2002;2:293–302. [PubMed: 11811932]
9. Grandjean P, Herz KT. Trace elements as paradigms of developmental neurotoxicants: Lead, methylmercury and arsenic. J Trace Elem Med Biol 2015;31:130–4. [PubMed: 25175507]
10. Heo Y, Lee WT, Lawrence DA. In vivo the environmental pollutants lead and mercury induce oligoclonal T cell responses skewed toward type-2 reactivities. Cell Immunol 1997;179:185–95. [PubMed: 9268502]
11. Zhang Y, Bolivar VJ, Lawrence DA. Maternal Exposure to Mercury Chloride During Pregnancy and Lactation Affects the Immunity and Social Behavior of Offspring. Toxicological Sciences 2013;133:101–11. [PubMed: 23392568]
12. Farina M, Rocha JBT, Aschner M. Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. Life sciences 2011;89:555–63. [PubMed: 21683713]
13. Giordano G, Costa LG. Developmental Neurotoxicity: Some Old and New Issues. ISRN Toxicology 2012;2012:814795. [PubMed: 23724296]
14. Schoeters GE, Den Hond E, Koppen G, et al. Biomonitoring and biomarkers to unravel the risks from prenatal environmental exposures for later health outcomes. Am J Clin Nutr 2011;94:1964S–9S. [PubMed: 21543535]
15. Karagas MR, Choi AL, Oken E, et al. Evidence on the human health effects of low-level methylmercury exposure. Environ Health Perspect 2012;120:799–806. [PubMed: 22275730]

16. Nyland JF, Fillion M, Barbosa F Jr., et al. Biomarkers of methylmercury exposure immunotoxicity among fish consumers in Amazonian Brazil. Environ Health Perspect 2011;119:1733–8. [PubMed: 21868305]
17. Belles-Isles M, Ayotte P, Dewailly E, Weber JP, Roy R. Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury. J Toxicol Environ Health A 2002;65:165–82. [PubMed: 11820504]
18. Osuna CE, Grandjean P, Weihe P, El-Fawal HA. Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicol Sci 2014;142:158–66. [PubMed: 25124724]
19. Grandjean P, Poulsen LK, Heilmann C, Steuerwald U, Weihe P. Allergy and sensitization during childhood associated with prenatal and lactational exposure to marine pollutants. Environ Health Perspect 2010;118:1429–33. [PubMed: 20562055]
20. Maserejian NN, Shrader P, Brown OA, et al. Dental sealants and composite restorations and longitudinal changes in immune function markers in children. Int J Paediatr Dent 2014;24:215–25. [PubMed: 24033362]
21. Weidinger S, Kramer U, Dunemann L, Mohrenschlager M, Ring J, Behrendt H. Body burden of mercury is associated with acute atopic eczema and total IgE in children from southern Germany. J Allergy Clin Immunol 2004;114:457–9. [PubMed: 15341030]
22. Jedrychowski W, Perera F, Maugeri U, et al. Intrauterine exposure to lead may enhance sensitization to common inhalant allergens in early childhood. A prospective prebirth cohort study Environmental research 2011;111:119–24. [PubMed: 21094490]
23. Shaheen SO, Newson RB, Henderson AJ, Emmett PM, Sherriff A, Cooke M. Umbilical cord trace elements and minerals and risk of early childhood wheezing and eczema. European Respiratory Journal 2004;24:292–7. [PubMed: 15332400]
24. Miyake Y, Tanaka K, Yasutake A, Sasaki S, Hirota Y. Lack of association of mercury with risk of wheeze and eczema in Japanese children: The Osaka Maternal and Child Health Study. Environmental Research 2011;111:1180–4. [PubMed: 21807364]
25. Farzan SF, Korrick S, Li Z, et al. In utero arsenic exposure and infant infection in a United States cohort: a prospective study. Environ Res 2013;126:24–30. [PubMed: 23769261]
26. Gilbert-Diamond D, Cottingham KL, Gruber JF, et al. Rice consumption contributes to arsenic exposure in US women. ProcNatl Acad Sci U S A 2011;108:20656–60.
27. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol 1989;18:858–67. [PubMed: 2621022]
28. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65. [PubMed: 4014201]
29. Garland M, Morris JS, Rosner BA, et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev 1993;2:493–7. [PubMed: 8220096]
30. Rees JR, Sturup S, Chen C, Folt C, Karagas MR. Toenail mercury and dietary fish consumption. J Expo Sci Environ Epidemiol 2007;17:25–30. [PubMed: 16912698]
31. Collaboration A, Aad G, Abajyan T, et al. The differential production cross section of the [Formula: see text](1020) meson in [Formula: see text] = 7 TeV [Formula: see text] collisions measured with the ATLAS detector. Eur Phys J C Part Fields 2014;74:2895. [PubMed: 25814898]
32. Gold DR, Willwerth BM, Tantisira KG, et al. Associations of cord blood fatty acids with lymphocyte proliferation, IL-13, and IFN-γ. The Journal of allergy and clinical immunology 2006;117:931–8. [PubMed: 16630954]
33. Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood. Cochrane Database Syst Rev 2015;7:CD010085.
34. Mahaffey KR, Sunderland EM, Chan HM, et al. Balancing the benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from fish consumption. Nutrition reviews 2011;69:493–508. [PubMed: 21884130]

35. Oken E, Rifas-Shiman SL, Amarasiriwardena C, et al. Maternal prenatal fish consumption and cognition in mid childhood: Mercury, fatty acids, and selenium. Neurotoxicol Teratol 2016;57:71–8. [PubMed: 27381635]
36. Zou G A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702–6. [PubMed: 15033648]
37. Liang KY, Zeger SL. Longitudinal Data-Analysis Using Generalized Linear-Models. Biometrika 1986;73:13–22.
38. Gul N, Khan S, Khan A, Nawab J, Shamshad I, Yu X. Quantification of Hg excretion and distribution in biological samples of mercury-dental-amalgam users and its correlation with biological variables. Environmental Science and Pollution Research 2016;23:20580–90. [PubMed: 27464660]
39. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142:1009–18. [PubMed: 22513989]
40. Madan JC, Hoen AG, Lundgren SN, et al. Association of cesarean delivery and formula supplementation with the intestinal microbiome of 6-week-old infants. JAMA Pediatrics 2016:1–8.
41. Sakamoto M, Tatsuta N, Izumo K, et al. Health Impacts and Biomarkers of Prenatal Exposure to Methylmercury: Lessons from Minamata, Japan. Toxics 2018;6:45.
42. Kim K-N. Low-level Mercury Exposure and Risk of Asthma in School-age Children. Epidemiology (Cambridge, Mass);26:733–9.
43. Croes K, De Coster S, De Galan S, et al. Health effects in the Flemish population in relation to low levels of mercury exposure: from organ to transcriptome level. Int J Hyg Environ Health 2014;217:239–47. [PubMed: 23920476]
44. Heinrich J, Guo F, Trepka MJ. Brief Report: Low-level Mercury Exposure and Risk of Asthma in School-age Children. Epidemiology 2017;28:116–8. [PubMed: 27755278]
45. Cardenas A, Koestler DC, Houseman EA, et al. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. Epigenetics 2015;10:508–15. [PubMed: 25923418]
46. Perricone C, De Carolis C, Perricone R. Glutathione: a key player in autoimmunity. Autoimmun Rev 2009;8:697–701. [PubMed: 19393193]
47. Gardner RM, Nyland JF, Evans SL, et al. Mercury Induces an Unopposed Inflammatory Response in Human Peripheral Blood Mononuclear Cells in Vitro. Environmental Health Perspectives 2009;117:1932–8. [PubMed: 20049214]
48. Christensen MM, Ellermann-Eriksen S, Rungby J, Mogensen SC. Comparison of the interaction of methyl mercury and mercuric chloride with murine macrophages. Archives of Toxicology 1993;67:205–11. [PubMed: 7684221]
49. Gupta RS, Springston EE, Warrier MR, et al. The Prevalence, Severity, and Distribution of Childhood Food Allergy in the United States. Pediatrics 2011;128:e9–e17. [PubMed: 21690110]
50. Akinbami LJ, Simon AE, Schoendorf KC. Trends in allergy prevalence among children aged 0-17 years by asthma status, United States, 2001-2013. J Asthma 2016;53:356–62. [PubMed: 26666655]
51. Hill DA, Grundmeier RW, Ram G, Spergel JM. The epidemiologic characteristics of healthcare provider-diagnosed eczema, asthma, allergic rhinitis, and food allergy in children: a retrospective cohort study. BMC Pediatrics 2016;16:133. [PubMed: 27542726]
52. Akinbami LJ, Moorman JE, Bailey C, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001-2010. NCHS Data Brief 2012:1–8.
53. Bahadori K, Doyle-Waters MM, Marra C, et al. Economic burden of asthma: a systematic review. BMC Pulm Med 2009;9:24. [PubMed: 19454036]
54. Somers EC, Ganser MA, Warren JS, et al. Mercury Exposure and Antinuclear Antibodies among Females of Reproductive Age in the United States: NHANES. Environ Health Perspect 2015;123:792–8. [PubMed: 25665152]
55. MacIntosh DL, Williams PL, Hunter DJ, et al. Evaluation of a food frequency questionnaire-food composition approach for estimating dietary intake of inorganic arsenic and methylmercury. Cancer Epidemiol Biomarkers Prev 1997;6:1043–50. [PubMed: 9419401]

56. Maserejian NN, Trachtenberg FL, Assmann SF, Barregard L. Dental amalgam exposure and urinary mercury levels in children: the New England Children's Amalgam Trial. Environ Health Perspect 2008;116:256–62. [PubMed: 18288327]

57. Rebelo FM, Caldas ED. Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. Environmental Research 2016;151:671–88. [PubMed: 27619212]

58. Cui W, Liu G, Bezerra M, Lagos DA, Li Y, Cai Y. Occurrence of Methylmercury in Rice-Based Infant Cereals and Estimation of Daily Dietary Intake of Methylmercury for Infants. Journal of Agricultural and Food Chemistry 2017;65:9569–78. [PubMed: 29067797]

Highlights:

- Risks of allergy, respiratory infections and symptoms, and atopic dermatitis (eczema) among infants in the New Hampshire Birth Cohort Study were investigated in relation to mothers' presence of silver-mercury dental amalgams and toenail mercury concentrations during pregnancy
- Among women who consumed fish, higher maternal toenail mercury concentrations were associated with an increased risk of lower respiratory infections and respiratory symptoms requiring a doctor visit among infants in their first year of life
- Presence of maternal dental amalgams (in the absence of prenatal fish consumption) was associated with elevated risk of upper respiratory infections
- Our findings raise the possibility of a public health impact of mercury exposure during pregnancy on infant immune outcomes.

Table 1.

Selected characteristics for mothers and infants participating in the New Hampshire Birth Cohort Study (n=1,321)

Maternal Sociodemographic covariates	**Mean (range) or n (%)**
Age at enrollment, years	31.4 (18.4-46.2)
<20	14 (1%)
20-29	496 (38%)
30-35	515 (39%)
>35	296 (22%)
Highest Educational Attainment [a]	
Less than 11th grade	14 (1%)
High school graduate, GED	137 (10%)
Junior college, some college, technical school	251(19%)
College graduate	514 (39%)
Postgraduate school	396 (30%)
Alternative Healthy Eating Index-2010 score	56.1 (22.9-92.0)
Relationship status[a]	
Single	137 (10%)
Married	1139 (86%)
Separated, divorced	36 (3%)
Smoked cigarettes during pregnancy[a]	
Yes	84 (6%)
No	1237 (94%)
Pre-pregnancy BMI (kg/m^2)[a]	25.9 (16.6-51.5)
Parity	
Nulliparous	568 (43%)
1-2	665 (50%)
≥3	88 (7%)
Delivery Type[a]	
Vaginal (spontaneous or induced)	912 (69%)
Caesarean	409 (31%)
Race	
White	1318 (100%)
Other	3 (0%)
Maternal Exposures	
Prenatal Toenail Total Hg (geometric mean (95%CI), range) μg/g	0.02 (0.01, 0.02), 0.00007-1.19
Have amalgam fillings[a]	
Yes	616 (47%)
No	680 (51%)
Fish/Seafood Consumption (servings per day)	0.2 (0-1.5)

Maternal Sociodemographic covariates	**Mean (range) or n (%)**
Never or < 1/month	298 (22.6)
1-3/month	276 (20.9)
≥ 1/week	747 (56.6)
Infant Characteristics	
Sex[a]	
Male	671 (51%)
Female	650 (49%)
Birth weight[a], grams	3453 (1080-5400)
Low Birth weight (<2500g)	50 (4%)
Gestational age, weeks	39.4 (25.3-44.9)
Premature (<37 weeks)	85 (6%)
Ever in day care [a]	
Yes at 4 months	307 (23%)
Yes at 8 months	393 (30%)
Yes at 12 months	419 (32%)
Infant Feeding (breast milk, formula, both)[a]	
4 months	511 (39%), 43 (3%), 453(34%)
8 months	371 (28%), 43 (3%), 633 (48%)
12 months	255 (17%), 53(4%), 769 (58%)

[a]n=25 missing dental amalgam fillings, n=9 missing education level, n= 9 missing relationship status, n= 6 missing maternal BMI, n=38 missing birth weight, n=4 missing delivery mode n=314, 274 and 274 missing infant feeding at 4, 8 and 12 months respectively, n=354 and 350 and 370 missing day care attendance at 4 and 8 months respectively.

Table 2.

Adjusted relative risks (RR) and 95% confidence intervals (CI) of infant respiratory infections, respiratory symptoms and eczema requiring a doctor visit, and allergies confirmed by a doctor in the first year of life in relation to a doubling of prenatal maternal toenail Hg concentrations among mothers who consumed fish or seafood.

	Infant Age											
	0-4 months (n=639)			5-8 months (n=645)			9-12 months (n=632)			Over the first year (n=706)		
Infant Outcome	**No. of events**	**RR**	**95% CI**	**No. of events**	**RR**	**95% CI**	**No. of events**	**RR**	**95% CI**	**No. of events**	**RR**	**95% CI**
Upper Respiratory Infection	158	1.0	(0.9-1.1)	305	1.0	(0.9-1.1)	380	1.0	(0.9-1.1)	843	1.00	(0.9-1.1)
Lower Respiratory Infection	30	**0.7**	**(0.5-1.0)** *	59	0.9	(0.7-1.1)	24	**1.4**	**(1.1-1.9)** **	113	0.90	(0.8-1.1)
Respiratory Symptoms	93	0.9	(0.8-1.1)	178	1.0	(0.9-1.2)	145	**1.2**	**(1.0-1.4)** *	416	1.06	(1.0-1.2)
Wheeze	19	0.8	(0.6-1.2)	33	1.0	(0.7-1.2)	26	1.1	(0.8-1.6)	78	0.97	(0.8-1.1)
Eczema	15	1.0	(0.7-1.6)	42	1.0	(0.8-1.3)	37	1.0	(0.7-1.3)	82	0.96	(0.8-1.2)
Allergy	6	1.1	(0.6-2.4)	17	1.1	(0.8-1.7)	20	1.0	(0.7-1.4)	43	1.05	(0.8-1.4)

* $p < 0.05$,

** $p < 0.01$

Models were adjusted for maternal age, parity, smoking, education, dietary index and fish intake, infant sex, gestational age, birthweight, feeding at month 4, 8, 12 and day care attendance. Sample sizes for eczema were n=609 for 0-4 months, n= 640 for 5-8 months, n=592 for 9-12 months and n=703 over the total year due to missing data.

Table 3.

Adjusted relative risks (RR) and 95% confidence intervals (CI) of infant respiratory infections, respiratory symptoms and eczema requiring a doctor visit, and any allergies confirmed by a doctor in the first year of life in relation to presence of maternal silver-mercury amalgam fillings during pregnancy, among those who did not consume fish or seafood.

	Total (n=218)			Girls (n=417)			Boys (n==101)		
Infant Outcome	**Events (n)**	**RR**	**95%CI**	**Events (n)**	**RR**	**95%CI**	**Events (n)**	**RR**	**95%CI**
Upper Respiratory Infection	227	**1.5**	**(1.1-2.1)** **	123	**1.5**	**(1.0-2.3)** *	104	**1.5**	**(1.0-2.3)** *
Lower Respiratory Infection	24	1.9	(0.9-4.0)	14	2.1	(0.7-7.0)	10	1.7	(0.4-6.2)
Respiratory Symptoms	130	1.4	(0.7-2.5)	69	**2.3**	**(1.3-4.3)** **	61	0.9	(0.5-1.7)
Wheeze	22	1.5	(0.5-4.4)	10	2.7	(0.9-8.4)	12	1.0	(0.4-2.6)
Eczema#	21	0.5	(0.2-1.4)	9	**0.2**	**(0.1-0.8)** *	12	0.8	(0.2-3.3)
Allergy	19	2.7	(0.8-8.8)	12	**5.5**	**(1.7-17.9)** **	7	1.2	(0.2-7.0)

* $p < 0.05$,

** $p < 0.01$

Models were adjusted for maternal age, parity, smoking, education, dietary index and fish intake, infant sex, gestational age, birthweight, feeding at month 4, 8, 12 and day care attendance. Sample sizes in the total sample for eczema were n=211 and for allergies confirmed by a doctor n=216 due to missing data. The distributions for girls/boys were 110/101 for eczema, 117/100 for allergy and 116/100 for allergies confirmed by a physician. # In girls, the eczema model was run without daycare and feeding covariates in order to achieve model convergence.