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Assessment of chronic mercury exposure within the U.S. population, National Health and Nutrition Examination Survey, 1999–2006

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Abstract The purpose of this study was to assess chronic mercury exposure within the US population. Time trends were analyzed for blood inorganic mercury (I-Hg) levels in 6,174 women, ages 18–49, in the NHANES, 1999–2006 data sets. Multivariate logistic regression distinguished a significant, direct correlation within the US population between I-Hg detection and years since the start of the survey (OR = 1.49, $P < 0.001$). Within this population, I-Hg detection rose sharply from 2% in 1999–2000 to 30% in 2005–2006. In addition, the population averaged mean I-Hg concentration rose significantly over that same period from 0.33 to 0.39 μ/L (Anova, $P < 0.001$). In a separate analysis, multivariate logistic regression indicated that I-Hg detection was significantly associated with age (OR = 1.02, $P < 0.001$). Furthermore, multivariate logistic regression revealed significant associations of both I-Hg detection and mean concentration with biomarkers for the main targets of mercury deposition and effect: the liver, immune system, and pituitary. This study provides compelling evidence that I-Hg deposition within the human body is a cumulative process, increasing with age and in the population over time, since 1999, as a result of chronic mercury exposure. Furthermore, our results indicate that I-Hg deposition is associated with the significant biological markers for main targets of exposure, deposition, and effect. Accumulation of focal I-Hg deposits within the human body due to chronic mercury exposure provides a mechanism which suggests a time dependent rise in the population risks for associated disease.

Keywords Mercury · NHANES · Pituitary · Luteinizing hormone · Autism · Alzheimer's disease

Abbreviations

NHANES	National Health and Nutritional Survey
I-Hg	Blood inorganic mercury
CH_3Hg	Methyl mercury
T-Hg	Blood, total mercury
U-Hg	Urinary mercury
Hg++	Mercuric ions
Hg	Elemental mercury
LH	Luteinizing hormone
WBC	White blood cell count
AD	Alzheimer's disease
OR	Odds ratio
CI	Confidence interval
P(I-Hg detect)	Probability of I-Hg detection

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Background

Chronic mercury exposure is associated with elevated risks for autism (Bernard et al. 2001, 2002; Adams et al. 2007; Geier and Geier 2006, 2007; Nataf et al. 2006; Windham et al. 2006; Palmer et al. 2006, 2008), mental impairment (Davidson et al. 2006; Axelrad et al. 2007), and neurodegenerative disease (Thompson et al. 1988; Hock et al. 1998; Yu et al. 2001; Khan et al. 2005). Results from a previous analysis of NHANES estimated that, as a result of chronic, organic mercury exposure, 300,000–600,000 American children were born with elevated risks (>5.8 μ CH_3Hg/L blood) of neuro-developmental disorders during 1999–2000 (Mahaffey et al. 2004). A consensus of scientists have recently determined that both the rate of mercury emissions from coal burning power plants and the rate of mercury deposition from the atmosphere are increasing with time (Anonymous 2007). The incidence of associated neuro-developmental disorders may be rising in tandem (Chen et al. 2007).

Scientists have declared that, "health effects of methylmercury should focus on long-term exposure" (Rice 1989; Rice et al. 1989). To assess time trends of risks for disease associated with mercury exposure, it is necessary to determine whether chronic mercury exposure within the human population is increasing over time. Hair mercury concentrations are not the best bioindicator for chronic mercury exposure as there is variability in hair treatment and growth rate, and hair mercury levels do not correlate with mercury body burden, tissue, and organ levels (Budtz-Jorgensen et al. 2004). Blood mercury concentration is widely considered the appropriate indicator of absorbed dose that corresponds to deposition within the human body (Magos and Clarkson 1972). While humans face chronic exposure from both the organic mercury form (due to consumption of fish) and the elemental form (due to inhalation from air, and dental amalgams), there is strong evidence that blood, inorganic mercury concentration is the most fitting bioindicator of chronic exposure to both organic and elemental mercury forms. Organic mercury is a poor indicator of exposure as it has a short half life within the human body, on the order of several months (Berlin 1986). In chronic exposure trials on monkeys, organic mercury has been shown to demethylate and form long lasting inorganic mercury deposits in the brain which persist for years (Vahter et al. 1994, 1995). The enzyme catalase oxidizes the elemental form of mercury into inorganic mercury ions within the human body (Clarkson 2002). From a longitudinal trial of chronic mercury exposure on a cohort of industrial laborers, it has been shown that chronic mercury exposure reduces the elimination rate of mercury from the blood (Sallsten et al. 1993). These authors concluded that a change in elimination rates "probably reflects higher accumulation of Hg in tissues with a slow Hg turnover". In other words, due to the deposition of I-Hg in organs of the human body, there is an accumulation of I-Hg due to chronic exposure which is detectable in the blood. Therefore, due to the biotransformations of mercury forms (both elemental and organic) within the human body, blood I-Hg may be the most fitting bioindicator of chronic mercury exposure, organ deposition, and toxic effect.

The purpose of this study was to assess chronic mercury exposure within the US population. To this end, we used I-Hg as the bioindicator of chronic mercury exposure. Chronic mercury exposure and inorganic mercury deposition has been shown to target the pituitary (Vahter et al. 1995; Cornett et al. 1998), immune system (Gallagher et al. 1995; Hemdan et al. 2007), and liver (Clarkson et al. 2003; Berlin 1986). To estimate links with neurodegenerative disease, this study quantified associations between measurements for chronic mercury exposure and targets of mercury deposition and effect within the human body: the pituitary, immune system, and liver. The bioindicators chosen were luteinizing hormone (LH) (pituitary), white blood cell count (immune system), bilirubin and albumin (liver). Here, we report that there is a trend of increasing I-Hg within the US population over time. Furthermore, chronic mercury exposure and resultant I-Hg deposition was significantly associated with each of the biochemical profile markers for the main targets of mercury deposition and toxic effect.

Materials and methods

NHANES data

The stated purposes of the NHANES include estimating the percent of persons in the US population

that possess certain risk factors for disease. To this end, we used NHANES, blood I-Hg measurements to estimate the population risks of chronic mercury exposure and associated diseases. The NHANES target population is the civilian, non-institutionalized US population. NHANES includes over-sampling of low-income persons, African Americans, and Mexican Americans. In addition to analyzing the NHANES population as a raw population of individuals, this study employed recommended survey analysis to reflect the complex survey design and sample weighting methodology of the NHANES datasets (as described in the Analytic and Reporting Guidelines, Sept., 2006 version, and the NHANES Public Data Release File Documentation). In this study, only associations that were significant in the raw population were re-analyzed as a survey population. Survey weighted population design and analysis lends external validity to this study and extends inferences based on these data to reflect the US population.

The NHANES is a continuous survey beginning in 1999 and data are released in 2 year increments. There were no reported changes in the methodology for measuring mercury detection or biochemical profiles between the four survey groups. However, I-Hg (NHANES code: lbxihg) values below the limit of detection (0.4 μ/L) were assigned estimate values that varied from 1999–2000 (0.3 μ/L = assigned estimate for values below LOD), 2001–2002 (0.28 μ/L = assigned estimate for values below LOD), 2003–2004 (0.30 μ/L), and 2005–2006 (0.25 μ/L). These unexplained and unnecessary changes in estimate values given to measurements below the limit of detection may have artificially stabilized the Mean I-Hg blood concentrations between survey groups. In our study, we compensate for this by calibrating all measurements below the limit of detection with the same estimate value of 0.30 μ/L. Separate analysis of data with this calibration and without resulted in no fundamental changes to our results or conclusions.

Due to the complex survey design and sampling methodology, each measurement has its own weighting, primary sampling unit (PSU), and stratum. The sampling weights are used to produce unbiased national estimates and reflect the unequal probabilities of selection, non-response adjustments, and adjustments to independent population controls. The PSU's generally represent single counties in America although some small counties are combined. The appropriate weight variable for our analysis in the combined years 1999–2006 is described on the NHANES website. WT99–06 = (1/2) × WT99–02 + (1/4) × WT03–04 + (1/4) × WT05–06, where WT99–02 is the variable WTMEC4YR from the NHANES 2001–2002 demographic file dataset, WT03–04 is the variable WTMEC2YR from the NHANES 2003–2004 demographic file dataset, and WT05–06 is the variable WTMEC2YR from the NHANES 2005–2006 demographic file dataset. The stratum variable is SDMVSTRA and the PSU variable is SDMVPSU. The PSU and stratum help estimate variances that would have been estimated using the true design structure. To estimate sampling error, a Taylor series linearization method was employed. In STATA the 1999–2006 NHANES data set is processed by: svyset[pw = WT99–06], psu(sdmvpsu) strata(sdmvstra), where WT99–06 is the combined weight described above. For analysis of LH, as it was only measured in as subset of years 1999–2002, wtmec 4 year was used as the appropriate weighting.

This study analyzed the NHANES, 1999–2000, 2001–2002, 2003–2004, and 2005–2006 data sets after converting SAS files to STATA format, and using STATA 8.0 (StataCorp) software to perform the analysis. The population of women ages 18–49 years was chosen for the time trend analysis due to the availability of data on an adult population, as no men were tested for mercury levels during the first four survey years (1999–2002). Children were excluded from our time trend analysis to focus on a uniform, adult population. The survey weighted population for the time trend analysis was weighted to extend inference for a US population of over 62 million people.

Total mercury in whole blood was measured by flow injection cold vapor atomic absorption analysis with on-line microwave digestion. Inorganic mercury in whole blood was measured by using stannous chloride as reductant without employing microwave digestion system. Mercury vapor (reduced from inorganic mercury compounds) was measured via a quartz cell at 253.7 nm. The difference in the total reduced mercury (by sodium tetrahydroborate) and inorganic reduced mercury (by stannous chloride) was taken to represent organic mercury in whole blood.

Statistical analysis

Statistical analysis was performed on both raw and survey weighted populations with both naïve and robust estimates of variance in the raw populations. We used multivariate logistic regression to adjust for both age and race in our analysis. We used both logistic regression and linear regression to determine time trends of blood inorganic mercury detection and mean concentration within the population as a function of years since the survey began. Associations reported in this study are rigorous and persisted in raw, adjusted, and survey populations with both naïve and robust models. Associations were considered significant if P values were less than or equal to 0.05.

Many sources of variation exist in the measurement of inorganic mercury including temperature, storage time, and experimental error (Christopher et al. 2001; Bornhorst et al. 2005). As storage time increases before measurement, so does sample variation around the mean. Previous studies clearly and consistently demonstrate that proper methods for mercury detection limit storage time to 2 or 3 days before sampling. Long periods of storage time before sampling is a common routine that increases the variance between measurements and underestimates the original mercury sample concentrations. Inorganic mercury detection is particularly susceptible to sample loss over time. In NHANES data sets, the overwhelming majority of the population (70–95%) had undetectable levels of inorganic mercury [below the limit of detection, 0.4 μ/L (LOD)]. Due to indeterminate storage times for up to a year, the measurement of inorganic mercury was subject to great measurement error and variance. The population below the LOD was all given standard estimate values by NHANES. This resulted in the vast majority of the population consisting of identical values for I-Hg. Because of these aforementioned factors in NHANES measurement of I-Hg, the I-Hg variable may not be considered a continuous variable. For the purposes of this study, I-Hg was transformed into I-Hg detection, a binary variable: 0 for non-detect (below the LOD), 1 for detect (above the LOD). To identify associations between I-Hg detection and the biochemical profile markers, Anova, K-Wallis test, and logistic regression analysis were performed. In addition, I-Hg mean concentration was also analyzed as a continuous variable using multivariate linear regression, adjusting for both age and race. The results for I-Hg detection and I-Hg mean concentration are comparable and consistent.

Three biochemical profile markers were chosen to represent the main targets for mercury deposition and effect: bilirubin (liver), LH (pituitary), and white blood cell count (immune system). Blood inorganic mercury was compared with these selected biochemical profile markers in the full population of women (ages 0–85). Data were analyzed as raw populations for internal validity and again as survey weighted populations for external validity. To be rigorous, only those associations which were significant in the raw populations were then analyzed in the survey weighted population and presented as significant results in this study.

Results

For the time trend analysis, the combined NHANES 1999–2006, female population consisted of 6,168 women, divided in four, balanced survey groups of 2 year increments: 1999–2000 ($n = 1{,}455$), 2001–2002 ($n = 1{,}635$), 2003–2004 ($n = 1{,}462$), 2005–2006 ($n = 1{,}622$). The survey weighting design extends inferences based on this data with external validity to 62 million American women. In the third and fourth survey groups (2003–2006), mercury measurements for men were added to the survey design. In order to maintain a uniform population, analysis of these males whose mercury data only began collection in 2003 was not reported in this study. However, the correlations presented in our results did persist in this full population of both men and women. Therefore, it is likely that inferences based on our results can be extended to the full US population of both men and women.

The analysis of age and I-Hg was done on the full population of women, ages 0–85, $n = 12{,}770$, with a survey weighted population that extends inference to 102 million American women. Our analysis of biochemical profile markers, the biomarkers for targets of mercury effect, was also performed on the full population of women (ages 0–85). This included, I-Hg as a function of albumin ($n = 8{,}221$, survey population = 73 million women), bilirubin ($n = 10{,}100$, survey population = 90 million), and

white blood cell count ($n = 12{,}749$, survey population = 102 million). The biochemical profile marker, LH, was measured only in a subsample of this population; 1,133 Women, restricted to the ages 35–49 years. Due to the biological changes in LH brought about during peri-menopause (above the age of 40), we analyzed LH in the surveyed population as well as a subpopulation restricted to women ages 35–40 ($n = 485$, survey population = 12 million).

The raw data and survey weighted data provide evidence of an increase in the mean proportion of I-Hg detection within the survey population as a function of years since the baseline, 1999–2000 survey population (Fig. 1a). From the marginal model of our multivariate logistic regression, there is evidence of a significant increase in the proportion

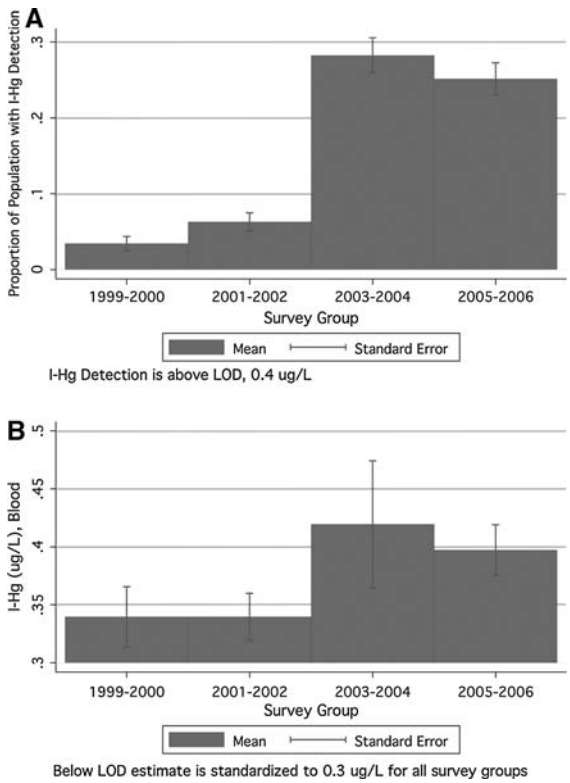

Fig. 1 Increase of I-Hg within the US population over time. Histograms of I-Hg measurement variables for each survey group, in the 1999–2006 NHANES, female, raw population, (Ages 18–49, $n = 6{,}168$). **a** Mean proportion of population with blood I-Hg detection (above lod, >0.4 μ/L). **b** Population, mean concentration of blood I-Hg, μ/L. I-Hg concentrations for all measures below LOD (0.4 μ/L) were standardized to the estimate value of 0.3 μ/L for all survey groups

of the population with detectable blood inorganic mercury (I-Hg) over the years 1999–2006. Figure 1a reflects the raw population; in the survey weighted population, time trends are even more striking. In the first survey group (1999–2000), the survey weighted mean proportion of the population with detectable levels of I-Hg was only 2.3%. This measure rose to 6.9% in the second survey population (2001–2002), then jumped to 30% in the third population (2003–2004), and finally settled at 27% for the final survey population (2005–2006). Every succeeding year, survey participants were 49% more likely to have detectable levels of inorganic mercury in their blood as compared to participants of the same race and age in the preceding survey year (OR = 1.49, $P < 0.001$) (Table 1). To ensure the validity of these results in assessing the risks of I-Hg detection between survey populations, the analysis was repeated with Anova, the non-parametric *K*-Wallis test, and multivariate linear regression using I-Hg concentration as a continuous variable. In the raw population, both naive and robust estimates were performed on the multivariate linear regression. This analysis was repeated on the survey weighted population. These tests confirmed that the mean concentration of blood inorganic mercury significantly increased, in a time dependent manner, over the years of the survey (Fig. 1b; Table 1). Figure 1b represents the raw population, not the survey weighted population. In fact, the survey weighted mean concentrations of I-Hg rose 24% from the first survey group, 1999–2000 (0.33 μ/L I-Hg, calibrated) to the last, 2005–2006 (0.41 μ/L, calibrated). These changes are even more drastic when one considers that the baseline concentration is not 0 but the estimated value, 0.3 μ/L, given to samples below the LOD. So, in fact, the percentage change was in actuality from 0.03 (0.33–0.30) to 0.11 μ/L (0.41–0.30) which is a 367% increase. No matter how you interpret the data, there is a significant increase in blood I-Hg over the years of the survey.

To determine if differences in I-Hg between survey populations were due to differences in exposure, we also analyzed time trends of organic mercury and urinary mercury. During the course of these surveys, 1999–2006, mean concentrations of organic mercury and urinary mercury did not significantly change between the survey weighted populations. This suggests that there was no change in either

Table 1 Correlations with Hg exposure, in women from NHANES 1999–2006

Population	Mercury species	Outcome	Test	Correlation
Ages 18–49	I-Hg Detection	Years since 1999	Logistic regression[a]	OR 1.49, CI (1.40–1.60), $P < 0.001$, $n = 6{,}174$
Ages 18–49	I-Hg Concentration	Survey year	Anova[b]	$P < 0.001$, $n = 6{,}174$
Ages 1–85	I-Hg Detection	Age (years)	Logistic regression[a]	OR 1.02, CI (1.01–1.02), $P < 0.001$, $n = 12{,}770$
Ages 1–85	I-Hg Concentration	Age (years)	Anova[b]	$P < 0.001$, $n = 6{,}174$
Ages 1–85	I-Hg Detection	Albumin	Logistic regression[a]	OR 0.940, CI (0.919–0.961), $P < 0.001$, $n = 8{,}221$
Ages 1–85	I-Hg Concentration	Albumin	Logistic regression[a]	Coefficient −0.0277, CI (−0.00297–−0.00258), $P = 0.004$, $n = 8{,}221$
Ages 1–85	I-Hg Detection	Bilirubin	Logistic regression[a]	OR 1.03, CI (1.01–1.05), $P < 0.001$, $n = 10{,}100$
Ages 1–85	I-Hg Concentration	Bilirubin	Logistic regression[a]	Coefficient 0.0007, CI (0.0006–0.0007), $P = 0.004$, $n = 10{,}100$
Ages 1–85	I-Hg Detection	WBC	Logistic regression[a]	OR 0.948, CI (0.921–0.975), $P < 0.001$, $n = 12{,}749$
Ages 1–85	I-Hg Concentration	WBC	Logistic regression[a]	Coefficient −0.00237, CI (−0.0027–−0.00204), $P = 0.006$, $n = 12{,}749$
Ages 35–49	I-Hg Concentration	LH	Logistic regression[a]	Coefficient −0.00031, CI (−00042–−0.00019), $P < 0.001$, $n = 1{,}133$
Ages 35–49	I-Hg Std. concentration[d]	LH	Logistic regression[a]	Coefficient −0.00033, CI (−0.00044–−0.00022), $P < 0.001$, $n = 1{,}133$
Ages 35–49	I-Hg Concentration	Ln(LH)	Logistic regression[a]	Coefficient −0.0035, CI (−0.0063–−0.0008), $P < 0.013$, $n = 1{,}133$
Ages 35–49	I-Hg Std. concentration[d]	Ln(LH)	Logistic regression[a]	Coefficient −0.0044, CI (−0.0071–−0.0016), $P = 0.003$, $n = 1{,}133$
Ages 35–40	I-Hg Detection	LH	Logistic regression[c]	OR 0.939, CI (0.904–0.976), $P = 0.056$, $n = 485$
Ages 35–40	I-Hg Concentration	LH	Logistic regression[a]	Coefficient −0.000969, CI (−0.00174–−0.000194), P=0.016, $n = 485$
Ages 35–40	I-Hg Std. concentration[d]	LH	Logistic regression[a]	Coefficient −0.00104, CI (−0.0018–−0.00027), $P = 0.010$, $n = 485$

[a] Controlled for age, race and survey weighted

[b] Confirmed by *K*-Wallis Test and multivariate linear regression, controlled for age, race, and survey weighted

[c] Controlled for race, and survey weighted

[d] I-Hg Concentration is standardized to a consistent estimate value of 0.3 μ/L Ln(LH) values are natural log transformed to normalize the distribution

organic or elemental mercury exposure between the survey groups.

In our marginal model of multivariate logistic regression, age in years, was found to be significantly, directly associated with I-Hg detection (OR = 1.02, $P < 0.001$) (Table 1; Fig. 2). Figure 2 illustrates that at age 20, there is a 15% mean proportion of the population with I-Hg detection, while at age 80, the figure doubles to 30% of the population. This direct association between age and I-Hg was confirmed with Anova, the non-parametric *K*-Wallis test, and multivariate linear regression using I-Hg concentration as a continuous variable, adjusted for the possible confounding effects of race (Table 1).

From logistic regression analysis, there is evidence that I-Hg detection was significantly, inversely associated with Albumin in the full, survey weighted population (OR = 0.94, $P < 0.001$) (Table 1; Fig. 3a). An Odds Ratio of 0.94 indicates that there was a 6% decrease in the population averaged risk of I-Hg detection associated with a one standard deviation rise in albumin, among people of the same race and age (Table 1). This association was

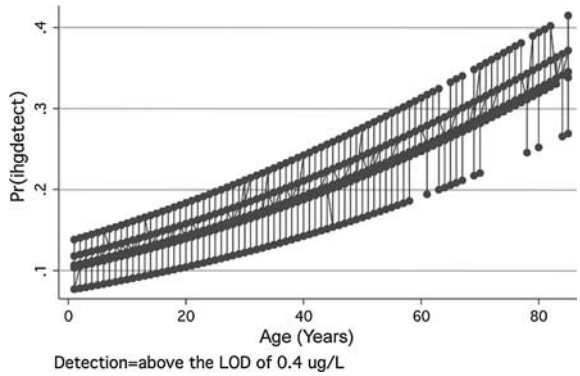

Fig. 2 Increase of I-Hg detection as a function of age. Probability of I-Hg detection [P(I-Hg detect)] as a function of age, in the 1999–2006 NHANES, female, survey weighted population (Ages 1–85, $n = 12{,}770$)

confirmed with Anova, the non-parametric *K*-Wallis test, and multivariate linear regression using I-Hg concentration as a continuous variable (Table 1). In a similar analysis, bilirubin was found to be significantly, directly associated with I-Hg detection (Fig. 3b) and I-Hg concentration (Table 1). An Odds Ratio of 1.03 indicates that there was a 3% increase in the population averaged risk of I-Hg detection associated with a one standard deviation rise in bilirubin, among people of the same race and age (Table 1).

White blood cell count was significantly, inversely associated with I-Hg detection (Fig. 3c) and I-Hg concentration (Table 1). An odds ratio of 0.95 indicates that there was a 5% decrease in the population averaged risk of I-Hg detection associated with a one standard deviation rise in white blood cell count, among people of the same race and age (Table 1).

Luteinizing hormone was inversely associated with both blood, I-Hg concentration ($P < 0.001$) and I-Hg standardized concentrations ($P < 0.001$) (I-Hg concentrations were standardized for values below the LOD of 0.4 μ/L with consistent estimate values of 0.3 μ/L) (Table 1). These relationships persisted after LH values were natural log transformed to normalize their distribution. LH was inversely correlated with I-Hg detection ($P = 0.056$) (Fig. 3d), I-Hg concentration ($P = 0.016$), and I-Hg standardized concentration ($P = 0.010$) (Table 1) in the survey weighted, sub-population of women ages 35–40. Although the *P*-value of 0.056 for I-Hg detection is marginal at best, it is included on the strength of the other, more significant correlations with parallel measures (I-Hg concentration and standardized concentration). An odds ratio of 0.94 indicates that there was a 6% decrease in the population averaged risk of I-Hg detection associated with a one standard deviation rise in LH, among people of the same race and age between the ages 35–40 years (Table 1). These associations were adjusted for age in years as a continuous variable.

The reported associations between I-Hg and biochemical profile markers had low correlation coefficients so they are considered significant but weak. These results were confirmed by *T*-Tests and give evidence to reject the null hypothesis that the mean levels of these biochemical profile markers were the same for both groups of I-Hg detection. Therefore, the alternative hypothesis, that mean values of these biochemical profile markers were different for the two groups of I-Hg detection is accepted.

Discussion

This study provides evidence that I-Hg deposition within the US population has increased over time as a result of chronic mercury exposure. Furthermore, this study demonstrates that I-Hg deposition is a cumulative process associated with age, and correlated with the main biological targets of mercury deposition and effect: pituitary, immune system, and liver.

Low correlation coefficients indicate that associations between I-Hg and biochemical profile markers were weak. Weak associations are often typical of biological systems. However, large sample comparisons may produce results with no biological significance. Yet, the fact that these associations persisted in smaller subpopulations restricted by age and year of survey, and within both raw and survey weighted populations, suggests that these results were not a statistical anomaly.

This study presents population averaged results and therefore, conclusions cannot be drawn on individuals. This makes it difficult to interpret associations but previous research offers likely interpretations. In the present study, we used blood, inorganic mercury as a measure of chronic mercury exposure. Within the US population, this measure of chronic mercury exposure distinguished significant,

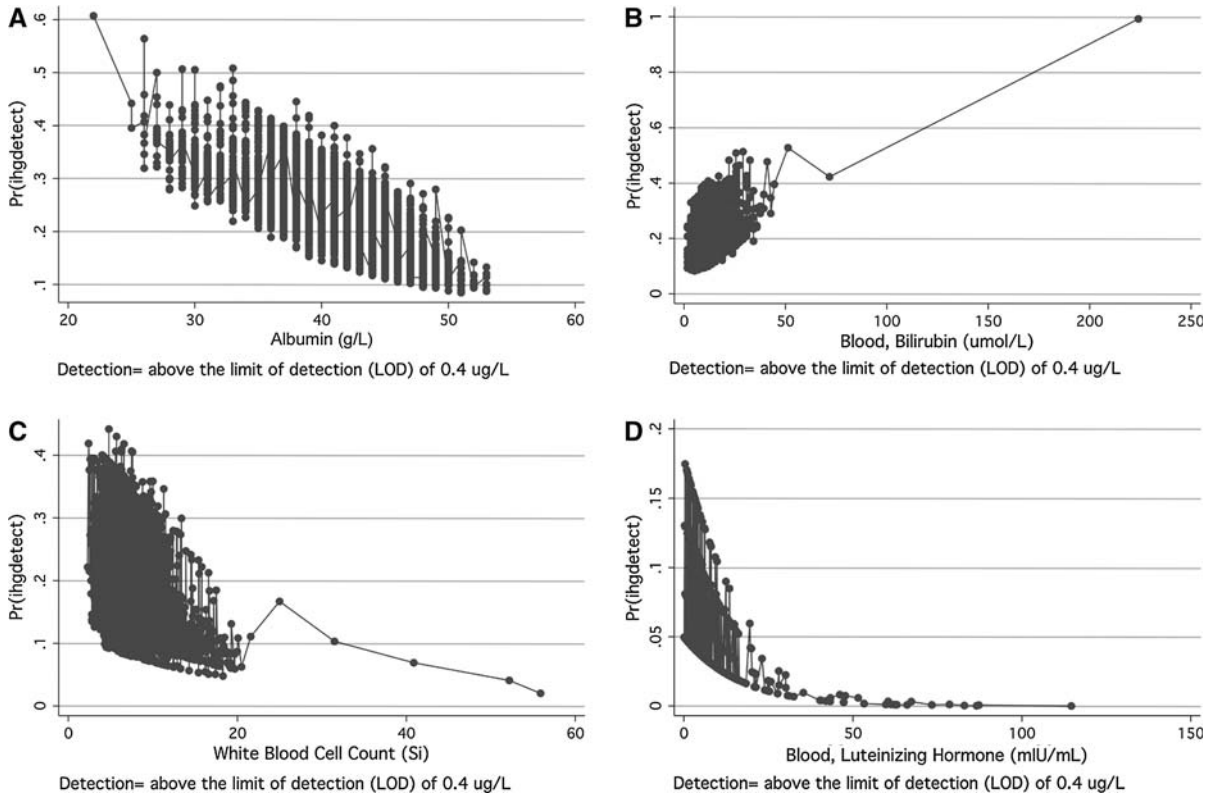

Fig. 3 Associations between I-Hg detection and biochemical profile markers for targets of mercury deposition and effect, in the 1999–2006 NHANES, female population. **a** P(I-Hg detect) as a function of blood albumin concentration (Ages 18–85, $n = 8{,}221$). **b** P(I-Hg detect) as a function of blood bilirubin concentration (Ages 18–85, $n = 10{,}100$). **c** P(I-Hg detect) as a function of white blood cell count (Ages 18–85, $n = 12{,}749$). **d** P(I-Hg detect) as a function of blood, luteinizing hormone (LH) concentration (Ages 35–40, $n = 485$)

biological correlations with the main targets of mercury deposition and effect. There is previously established evidence that chronic mercury exposure results in inorganic mercury deposition, and resultant toxic effect on three main targets in the body: the pituitary, liver, and immune system. Previous studies have shown that chronic, organic mercury exposure results in significant inorganic mercury deposits within the brain which persist for years (Vahter et al. 1995). After chronic, organic mercury exposure, the inorganic form of mercury was found to deposit in the brain for almost 2 years, whereas the organic form had a half life in the brain of only 1 month. The pituitary was the region with the greatest inorganic mercury deposition, at concentrations that were 200–300% higher than the overall, mean brain concentration. Our study confirmed this association by determining a significant correlation between I-Hg and LH within the US population. Similarly, mercury exposure within the body has been demonstrated to form lasting inorganic mercury deposits in the liver (Farris et al. 1993). Our study confirmed this association by distinguishing a significant correlation between I-Hg and both albumin and bilirubin. Other studies have shown that a direct interaction between the immune system and mercury exposure leads to the suppression of white blood cell activation (Gallagher et al. 1995). Even at sub acute, chronic mercury exposure levels, in vitro experiments demonstrate the immunomodulatory effects of mercury exposure (Hemdan et al. 2007). Our study confirmed this association by distinguishing a significant, inverse relationship between I-Hg and white blood cell count within the US population. The associations presented in this study between I-Hg and the biochemical profile markers for the main targets of I-Hg deposition, validate the approach taken in this study to use blood I-Hg as the most fitting

bioindicator of chronic mercury exposure. These associations confirm previous data that has demonstrated chronic mercury exposure forms inorganic mercury deposits in organs of the body which are detectable through a rise in blood I-Hg (Sallsten et al. 1993).

A recent study of the 1999–2004 NHANES survey groups reported a geographical variability in blood, organic mercury levels (Mahaffey et al. 2009). To our knowledge, this study did not control for the effects of time since the survey began. As geography and time both change between survey groups, it may be impossible to determine which factor is primarily responsible for changes over time; perhaps they both play a role. To address this issue, we asked whether the dramatic rise in I-Hg over time found in our study was the result of a difference in exposure levels between the survey groups. If there was a difference in organic mercury exposure due to differential fish consumption between survey populations, that should be reflected in blood, organic mercury concentrations. In a similar manner, if there was a difference in elemental mercury exposure due to differential dental amalgam number or atmospheric mercury levels between survey populations, it should be reflected in urinary mercury concentrations. In fact, neither blood organic mercury, nor urinary mercury significantly changed between survey populations. This led to the conclusion that mercury exposure did not change between survey groups, or that both urinary mercury and blood organic mercury levels were incapable of reflecting real changes in chronic exposure. This failure could be explained as they both reflect short term, recent levels of exposure, and they both reach steady state concentrations which limit their ability to gauge exposure levels. If the latter is true, that blood organic and urinary mercury levels are poor indicators of exposure, then that would reaffirm this study's assumption that I-Hg is the most fitting bioindicator of chronic mercury exposure. If the former is true, that exposure did not change between survey populations, then how could one explain the dramatic and significant rise in I-Hg levels over time? The answer to this question is consistently supported by the results of this study; chronic mercury exposure, beyond a certain level, results in a time dependent accumulation of I-Hg deposits within organs of the human body.

This study reported a significant, age dependent increase in the risk of I-Hg detection. This finding lends strong support to the theory that chronic mercury exposure results in the accumulation of I-Hg deposits within the organs of the human body. Our results indicate that as a population, it seems likely that chronic mercury exposure has reached a critical level where deposition of I-Hg within the human body is accumulating over time. The different forms of mercury have different toxico-kinetic properties (Clarkson et al. 2003; Clarkson 2002). However, inorganic mercury deposits have been shown to exhibit neurotoxic properties that are as severe as organic and elemental forms. In addition, the biotransformation of methylmercury into inorganic mercury deposits has been shown to be involved both in the persistence of mercury in the human brain and in causing brain damage after methylmercury poisoning (Davis et al. 1994). Therefore, if the US population is facing a time dependent process of accumulating I-Hg deposits, it is logical to assume that the risks of associated neuro-developmental and neurodegenerative diseases will rise as well.

Associations between chronic mercury exposure and bilirubin reflect a physiological connection between exposure and the rate of excretion. The bile is an important step for excretion of mercury from the liver. Impaired excretion of methyl mercury and increased enterohepatic circulation may elevate the rate of I-Hg deposition and the risks of associated neurodegenerative disease. Furthermore, changes in bilirubin and albumin are clinical symptoms of hypothyroidism and kidney disease. These diseases are also linked to mercury exposure and I-Hg deposition.

In our study, I-Hg was correlated to LH, a biomarker for the pituitary. Multivariate linear regression indicated that LH was inversely associated with blood I-Hg concentration in the population tested for LH, women ages 35–49 years. I-Hg concentration was also inversely associated with LH in the subpopulation of women, before peri-menopause (ages 35–40). Beginning at age 40, peri-menopause produces changes in LH and LH levels gradually increase while estrogen and progesterone levels decrease. The levels of each of these hormones are highly variable. It is likely that these peri-

menopause changes mask the association between I-Hg detection and LH in the population above 40 and this explains why the association between I-Hg detection and LH is more consistent within the subpopulation of women under 40.

Luteinizing hormone is a gonadotropin secreted by the anterior pituitary that is involved in androgen stimulation, mitogenisis, and immune regulation (Casadesus et al. 2005). Studies have demonstrated that Alzheimer's disease (AD) patients have elevated serum and neuronal levels of LH as compared to controls (Barron et al. 2006). Brain regions affected by AD show elevated expression of LH receptors (Casadesus et al. 2005). In cell cultures, LH stimulates amyloid beta production, a key element in oxidative stress leading to AD pathology. LH receptors are also found on immune cells where they are associated with neuroprotection and a role in the pro-inflammatory signaling process in the brain (Barron et al. 2006). Thus, the association between I-Hg deposition and LH provides a mechanistic link between chronic mercury exposure and associated disease.

The pituitary also secretes thyroid regulating hormones. Focal impairment of the pituitary due to I-Hg deposition may result in both hypothyroidism and immune system impairment. Mercury exposure has been shown to decrease both thyroid stimulating hormone (TSH), which is secreted by the pituitary (Nishida et al. 1989), and thyroid hormone, which it regulates (Kawada et al. 1980). This relationship between I-Hg deposition and hypothyroidism is linked to impaired migration of neurons, impaired development of the brain's cytoarchitecture, and associated neuro-developmental disease such as autism (Soldin et al. 2008).

The relationship between I-Hg and the pituitary provides a link between exposure and disease through a role in immune regulation and inflammation. "Disturbances at any level of the hypothalamic-pituitary-adrenal axis or glucocorticoid action lead to an imbalance of this system and enhanced susceptibility to infection and inflammatory or autoimmune disease," (Webster et al. 2002). Inorganic mercury (I-Hg) deposits are associated with neurotoxic and immune pathways implicated in neurodegeneration (Counter and Buchanan 2004). There is ample evidence for immunogenetic susceptibility to mercury exposure (Berlin 1986; Hultman and Hansson-Georgiadis 1999; Burbacher et al. 2005).

Focal deposition of I-Hg in the pituitary, liver, and immune system, provides a mechanism to link chronic mercury exposure with associated diseases in a causal manner. As both industrial emissions of mercury and atmospheric deposition of mercury are increasing, it is logical to assume that chronic mercury exposure is rising as well. Here, we present evidence that chronic mercury exposure has increased over time within the US population, from 1999–2006. A dose response relationship analysis of methyl mercury and cases of poisoning has previously demonstrated that a toxic threshold in blood mercury concentration triggers a disease response in acute exposure (Bakir et al. 1973). Due to the cumulative, focal deposition of inorganic mercury in target organs of the body, chronic mercury exposure may decrease the dose necessary to reach a toxic threshold for disease response.

Conclusion

This study is the first to report that there is a rise in the mean blood I-Hg detection and I-Hg concentration within the US population over time. The results of this study suggest that due to chronic mercury exposure, inorganic mercury deposits accumulate in organs of the human body, in a time dependent manner. Furthermore, this study indicates that I-Hg deposition within the human body is significantly associated with biomarkers for the main targets of chronic mercury exposure, deposition and effect: the liver, immune system, and pituitary. These correlations between chronic mercury exposure, I-Hg deposition, and biochemical profile markers for the targets of I-Hg deposition confirm strong links between exposure and associated disease. The evidence presented in this study indicates that effects of chronic mercury exposure within the US population may result in a significant rise over time in the population risks of associated neuro-developmental and neurodegenerative diseases.

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