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TITLE

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ABSTRACT

Methylmercury-associated effects on the cardiovascular system have been documented though discrepancies exist, and most studied populations experience elevated methylmercury exposures. No paper has investigated the impact of low-level elemental (inorganic) mercury exposure on cardiovascular risk in humans. The purpose of this study was to increase understanding of the association between mercury exposure (methylmercury and elemental mercury) and blood pressure measures in a cohort of dental professionals that experience background exposures to both mercury forms. Dental professionals were recruited during the 2010 Michigan Dental Association Annual Convention. Mercury levels in hair and urine samples were analyzed as biomarkers of methylmercury and elemental mercury exposure, respectively. Blood pressure (systolic, diastolic) was measured using an automated device. Distribution of mercury in hair (mean, range: 0.45, 0.02-5.18 μg/g) and urine (0.94, 0.03-5.54 μg/L) correspond well with the US National Health and Nutrition Examination Survey. Linear regression models revealed significant associations between diastolic blood pressure (adjusted for blood pressure medication use) and hair mercury (n=262, p=0.02). Urine mercury results opposed hair mercury in many ways. Notably, elemental mercury exposure was associated with a significant systolic blood pressure decrease (n=262, p=0.04) that was driven by the male population. Associations between blood pressure and two forms of mercury were found at exposure levels relevant to the general population, and associations varied according to type of mercury exposure and gender.

KEYWORDS mercury, blood pressure, epidemiology, gender difference, environmental exposure

INTRODUCTION[1]

Mercury is ranked a top three priority pollutant by the U.S. Environmental Protection Agency (EPA; US EPA, 1997) and the Centers for Disease Control (ATSDR, 2007). The chemical speciation of mercury is complex and dictates its environmental fate, human exposure pathways, and toxic impacts (Clarkson and Magos, 2006). The general population is largely exposed to methylmercury ($MeHg^{+}$) through fish consumption and to elemental mercury (Hg^{0}) through dental amalgams. Approximately 6,600 tons of mercury is released into the atmosphere annually and concentrations continue to rise in many regions of the world (Swain et al., 2007). Accordingly, mercury will remain of public health concern for the foreseeable future.

Health concerns associated with methylmercury and elemental mercury exposure are primarily focused on the nervous system (Clarkson and Magos, 2006; US EPA, 1997). However, in recent years epidemiological studies have suggested a negative impact of methylmercury on the cardiovascular system. Methylmercury exposure has been linked to acute myocardial infarction, and a multi-disciplinary research committee deemed this evidence compelling to include this outcome in the regulatory risk assessment of mercury (Roman et al., 2011). Though discrepancies exist, many studies have also found methylmercury-associated increases in diastolic (DBP) and systolic blood pressure (SBP). In a study of 251 fish-consumers in the Brazilian Amazon, Fillion et al. (2006) found that participants with higher hair mercury, a biomarker for methylmercury exposure, had an increased risk of elevated SBP. In a study of 42 male Faroese whalers, Choi et al. (2009) found a positive association between blood total

[1] Abbreviations: CRM (certified reference material); DBP (diastolic blood pressure); MDA (Michigan Dental Association); NHANES (National Health and Nutrition Examination Survey); SBP (systolic blood pressure)

mercury levels, also reflective primarily of methylmercury exposure, and both SBP and DBP. In another study from the Faroe Islands, Sørensen et al. (1999) found increased SBP and DBP in 7-year-old children in relation to prenatal methylmercury exposure, though this association was not observed when children were re-evaluated at 14 years old (Grandjean et al., 2004). Likewise, Valera et al. (2009) found a positive association with blood mercury and SBP in an Inuit population. From the 1999-2000 US National Health and Nutrition Examination Survey (NHANES), Vupputuri et al. (2005) found a negative association between blood total mercury and SBP, but only in women that did not consume fish. Dórea et al. (2005) did not observe positive associations between blood pressure and hair mercury levels in two Amazonian populations with heavy fish consumption.

The notion that methylmercury may be associated with increased risk of hypertension poses several health dilemmas. Hypertension may affect one billion people worldwide (including 65 million in the US) and rates continue to rise (Egan et al., 2010; Lawes et al., 2008). Methylmercury is mainly derived from fish consumption, but fish are promoted as an excellent source of nutrients (e.g. omega-3 fatty acids) and protein. Some scientific reviews have concluded that the heart-protective benefits of fish consumption outweigh health risks (Mozaffarian and Rimm, 2006), but when faced with the decision many consumers chose to avoid consuming fish (Oken et al., 2003).

In addition to methylmercury exposure, the general public is exposed to elemental mercury largely through dental amalgams. Though several animal studies have documented that elemental mercury may decrease myocardial mechanical activity, depress heart rate, promote

heart arrhythmias, and cause hypotension (Massaroni et al., 1995; Rhee and Choi, 1989; Rossoni et al., 1999), to our knowledge these relationships have not been investigated in an epidemiological study. Accordingly, the goal of this study was to increase understanding of the association between mercury exposure (both methylmercury and elemental mercury) and blood pressure in a cohort of dental professionals. This work extends upon previous studies that focused solely on methylmercury exposure by also considering exposures to elemental mercury. Further, mercury exposures in this study are more relevant to the general population than the aforementioned studies focused on susceptible groups (e.g., indigenous peoples, fish-consumers) with moderate to high methylmercury intakes.

MATERIALS AND METHODS

Study Population

A convenience sample of 284 dental professionals (dentists, hygienists, dental assistants) was recruited during the 2010 Michigan Dental Association (MDA) Annual Convention as part of a larger cohort designed to study the influence of genetic variability on mercury body burden (Goodrich et al., 2011; Wang et al., 2012). Institutional Review Board (IRB) approval for this work was obtained from the University of Michigan (HUM00027621). A self-administered survey was used to collect information on demographics (e.g., age, height), occupational practices, medical history, and alcohol consumption. Subjects also provided detailed information on fish consumption patterns (e.g., portion size, frequency of consumption of 28 fish species) which was used to calculate a mercury intake value (μg mercury/kg body weight/day) as described previously (Wang et al., 2012) based on the most recent mercury levels measured in common fish species in the US (Bahnick et al., 1994; Mierzykowski et al., 2001; US FDA). Total polyunsaturated fatty acid (PUFA; mg/kg body weight/day) and selenium (μg/kg body weight/day) intake values from species-specific fish consumption were also calculated using the US Department of Agriculture Nutrient Database. Subjects reported the number of mercury-containing dental amalgams in their own mouths along with the average number per week that they remove and place in their dental practice (amalgams handled). Subjects with missing data points (e.g. urine mercury, SBP, age) were excluded. Four additional subjects reporting kidney disease were excluded due to the potential effects on mercury excretion, resulting in a sample size of 262.

126 Mercury Exposure Assessment

127 Urine is used to assess elemental mercury exposure and hair is used to assess
128 methylmercury exposure (Berglund et al., 2005; Clarkson and Magos, 2006). From each
129 participant, spot urine samples (~30-50mL) were collected and stored frozen. Hair was collected
130 by cutting 20-50 strands from the occipital region of the head as close to the scalp as possible,
131 wrapping in paper, and then storing at room temperature.

132

133 Total mercury levels were measured using a direct mercury analyzer (DMA-80,
134 Milestone Inc., CT) according to US EPA Method 7473. Briefly, 800 μL of urine or 4-9 mg of
135 hair from the two cm closest to the scalp were analyzed according to methods we have
136 previously described (Basu et al., 2010; Goodrich et al., 2011; Paruchuri et al., 2010). In every
137 batch of 10-15 samples, one blank, one replicate sample, and a certified reference material (hair:
138 NIES Japan CRM #13; urine: Institut National de Sante Publique Quebec standard
139 QMEQAS08U-01; dogfish liver: DOLT4, National Research Council Canada) were included.
140 Specific gravity was measured using a refractometer (PAL-10S, Atago U.S.A., Inc., WA). Urine
141 mercury levels were adjusted to reflect the average specific gravity in all samples (1.017)
142 according to the method of Levine and Fahy (1945) as this has been shown to reduce variability
143 in metal analysis of spot urine samples (Lee et al., 1996; Mason and Calder, 1994). All final
144 values reported here are unadjusted.

145

146 The average theoretical method detection limit (3x standard deviation of blanks) was
147 0.003 μg/g mercury for hair and 0.014 μg/L mercury for urine. The average recovery of mercury
148 was 88.9±1.1% for the hair CRM, 71.5±3.9% for the mean urine CRM value, and 91.8±6.6% for

DOLT4. The mercury value in the urine CRM has a range of expected values, and our percent recovery was judged according to the reported mean. Machine accuracy is deemed high given that recovery of other reference materials (e.g., DOLT4) measured alongside the urine CRM had excellent recovery (>90%). Within-day (0.7% for hair, 4.2% for urine, 2.8% for DOLT4) and between day (1.0% for hair, 5.4% for urine, 6.1% for DOLT4) variability of CRMs were calculated, and these values corresponded well to replicate analysis of actual samples provided by participants (data not shown).

Blood Pressure and Pulse Assessment

Participants were seated for at least five minutes before blood pressure was measured. A commercially available blood pressure device (Omron HEM 432-C) was placed over the right brachial artery and used to measure SBP, DBP, and pulse. From each participant, three readings were averaged. Variability within replicates of individuals averaged 4.2% (SBP), 4.8% (DBP), and 3.3% (pulse).

Statistical Analyses

All statistical operations were performed using PASW® Statistics Software (v. 18; Chicago, IL). Preliminary data analysis included tabulation of descriptive statistics for all measurements. Bivariate (Pearson correlations) and multivariate analyses were performed to identify factors that influenced SBP and DBP. Blood pressure measurements of individuals using hypertension controlling medications were imputed 15 mmHg higher (SBP) and 10 mmHg higher (DBP) before linear regression as this has been shown to reduce bias and improve statistical power (Tobin et al., 2005). All bivariate and multivariate analyses were performed

with adjusted and unadjusted SBP and DBP; analyses with the latter excluded subjects using anti-hypertensive medication (n=39).

The backward elimination method was used to determine predictors of SBP and DBP (adjusted for medication use) with an initial cut-off significance value of $p>0.10$. Variables considered in the multivariate models were age, BMI, gender, race, occupation (dentist vs. non-dentist), alcohol (drinks/day), fish nutrients/toxicants (PUFA, selenium, mercury), personal amalgams, and occupational exposures (hours worked/week, categorical variable for number of amalgams handled/week). The final model for SBP included the only significant predictors ($p<0.05$): BMI, age, and gender. Significant predictors of DBP were BMI and age, though gender was also included in the final model to control for gender differences observed in our population. Hair and urine mercury (together and in separate models, with unadjusted or specific gravity adjusted urine mercury) were added into SBP and DBP base models to assess the association between mercury biomarkers and blood pressure after controlling for confounders. Multivariable linear regression models were run for the total population and for subgroups (males, females, dentists, non-dentists). Potentially influential subjects were identified using statistical diagnostics (e.g. Cook's distance, dfbeta) on total population models, and removed individually to assess the impact of the subject on the relationships between mercury biomarkers and blood pressure.

RESULTS

Table 1 outlines demographics, cardiovascular parameters, and major sources of mercury exposure in study participants, and is stratified according to gender, occupation (dentists versus non-dentists), and anti-hypertensive medication usage. Of all participants, 38% were males and 44% were dentists. Overall, males were significantly older, had greater BMIs and alcohol consumption compared with females while also having higher blood pressure and lower pulse. Dentists, of which 80% are males, likewise had similar differences compared to non-dentists (dental hygienists, dental assistants and other professionals, of whom 94% were female). A significantly larger proportion of individuals taking blood pressure medication were males and dentists (χ^2 test, p-value <0.05, data not shown). The influence of race-ethnicity on blood pressure could not be adequately assessed in this population as 92% of the subjects identified as non-Hispanic and Caucasian.

Table 2 reports total mercury levels in hair and urine. In this population, estimated mercury intake from fish consumption was the best predictor of hair mercury levels in linear regression modeling, though personal dental amalgams contributed to a lesser extent. Occupation and amalgams (personal and handled in the dental practice) were the predictors of urine mercury levels (data not shown) indicating hair and urine as biomarkers of primarily methylmercury and elemental mercury, respectively, as others have previously established (Berglund et al., 2005; Clarkson and Magos, 2006). All subjects had mercury levels above the method detection limit. Mean hair mercury (± standard deviation) was 0.45±0.53 μg/g (range: 0.02-5.18) and mean urine mercury was 0.94±0.99 μg/L (range: 0.03-5.54). While median hair and urine mercury values were 47% and 31% higher than U.S. population medians reported by NHANES (CDC, 2009;

McDowell et al., 2004), there is considerable overlap of the distributions for both biomarkers between the dental cohort and NHANES (Table 2). Mean hair and urine mercury levels were significantly higher in males and dentists, the latter of which correspond with greater occupational exposure to amalgams (ANOVA $p<0.05$).

Seventy-three participants (28% of study population) displayed hypertension (SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg as defined by the U.S. Department of Health and Human Services, 2004) and/or were using blood pressure medication at the time of measurement. Blood pressure measurements performed by us were in the hypertension range for 47 individuals (18%). Several significant correlations were found between hair mercury levels and blood pressure outcomes ($p<0.05$). Bivariate analyses estimated that SBP and DBP (adjusted for anti-hypertensive medication use) were significantly correlated with hair mercury levels ($r=0.22$, 0.19, respectively). There were no significant bivariate correlations between urine mercury and adjusted SBP ($r=0.05$) or DBP ($r=0.06$). BMI and age were significantly positively correlated with adjusted SBP ($r=0.33$, 0.58, respectively) and DBP (0.38, 0.31). Hair and urine biomarker measurements were also significantly correlated with one another ($r=0.29$).

Multivariate linear regression modeling of SBP and DBP was used to assess associations with urine or hair mercury levels after adjusting for BMI, age, and gender. Parameter estimates for total, gender stratified, and dentist-only populations in models of SBP and DBP (values first adjusted for hypertension-controlling medication use according to the method of Tobin et al., 2005 and referred to as "adjusted SBP/DBP") are reported in Table 3. In the majority of models, BMI, age, and gender were significant predictors of these outcomes. There was a trend towards

positive association with hair mercury and SBP and DBP in all models, though this association was only significant when modeling adjusted DBP (β=2.76 mmHg DBP increase per 1 μg/g Hg in hair, p=0.02. Further, the parameter estimates were consistently larger in males versus females. While a significant association was observed between hair mercury and DBP in the male-only model (β=2.94 mmHg, p=0.03), this model did not capture most of the variability in DBP among males (adjusted r^2=0.06). Results should be interpreted with caution. Alcohol consumption (drinks/day) and dental amalgams were near significant predictors (p<0.10) of adjusted DBP. However, inclusion of these parameters in the DBP model did not change parameter estimates (significance, magnitude) of mercury biomarkers (data not shown).

The urine mercury and blood pressure relationship differed from hair mercury results. Urine mercury levels were associated with decreased SBP (in total population model: β= -1.8 mmHg SBP per 1 μg/L Hg in urine), though this was only significant in models adjusting for anti-hypertensive medication use and appeared to be driven by the males and the dentists. Urine mercury was not associated with DBP, though negative trends were also observed among males and dentists. Even though several model parameters were significantly correlated with one another (e.g. BMI and age, hair and urine mercury), multicollinearity is not expected to be problematic as variance inflation factors were less than 1.5 for all aforementioned regression models.

The significance levels of parameter estimates for mercury biomarkers in blood pressure models were sensitive to several influential subjects discovered via standard diagnostic tests. The exclusion of one subject partially diminished the association between hair mercury and adjusted

DBP (β=2.29 mmHg, p=0.07). The magnitude and significance of the association between urine mercury and decreased SBP were slightly diminished when excluding several influential subjects, most of whom had urine mercury levels above the 95^{th} percentile ($0.06<p<0.13$ for new parameter estimates). Adjusting urine mercury for specific gravity altered its significance in the total population model of SBP (β= -1.75 mmHg, p=0.13) and the r-square of the model (adj r^2= 0.421, 1% decrease). Specific gravity-adjusted urine mercury remained significant in models of SBP with males or dentists alone.

DISCUSSION

There are a growing number of studies documenting an association between methylmercury exposure and elevated blood pressure but discrepancies exist. Despite the fact that our cohort was not initially designed to study cardiovascular effects of mercury exposure and lacks information on one important confounder- smoking status, our study contributes to data on mercury exposure and blood pressure in several ways. Here we report that exposures relevant to the general population to both elemental mercury and methylmercury may be associated with altered blood pressure measures, though the significance of these results is partially dependent on several subjects with higher exposure (>95th percentile). Interestingly, divergent blood pressure results were found for mercury type and may be influenced by gender. Hair mercury levels were associated with increased DBP (after adjustment for anti-hypertensive medication use according to the method of Tobin et al., 2005). For urine mercury, the results from linear regression models suggest that elemental mercury exposure is associated with decreased SBP in the total population, and this appears to be driven by the male subgroup. While Kobal et al. (2004) previously found an association between extremely high past exposures to elemental mercury (>800 μg/L urinary mercury) and increased SBP, to our knowledge this is the first human study to investigate elemental mercury exposures relevant to the general population in relation to blood pressure.

Previous studies have reported an association between methylmercury exposure and increased blood pressure (Choi et al., 2009; Fillion et al., 2006; Sørensen et al., 1999; Valera et al., 2009) but these have largely been conducted in populations of subsistence fish consumers

294 that experience moderate to high methylmercury exposures. Here, we find a similar trend
295 between elevated blood pressure and hair mercury levels in a population that is exposed to
296 methylmercury at concentrations that better reflect exposures of the general US population
297 (McDowell et al., 2004) and other countries (Díez et al., 2008; Gundacker et al., 2007). As
298 expected, the male gender, age, and BMI were significant predictors of increased SBP. Likewise,
299 age and BMI predicted DBP in multivariate linear regression, factors which are often associated
300 with increased risk for hypertension (Greenlund et al., 2009; Kim et al., 2007). In addition, we
301 found a trend towards a methylmercury exposure dependent increase in SBP and DBP across all
302 sub-groups in our study (e.g., males, females, dentists, excluding medication users), though this
303 relationship only attained statistical significance in models of adjusted DBP and was partially
304 dependent on one influential subject.
305
306 The prevalence of hypertension in our study population (28% of total had SBP $\geq$ 140
307 mmHg, DBP $\geq$ 90 mmHg, and/or reported using anti-hypertensive medication) is similar to the
308 U.S. average of 28.9% which continues to increase (Cutler et al., 2008). The fact that we found a
309 weak association between "background" methylmercury exposure and increased DBP within this
310 cohort suggests that the threshold of effect may be low, if a threshold exists, and the burden of
311 impact could be greater in populations with higher methylmercury exposure (e.g., subsistence
312 fish eating populations). These findings are of public health concern given that nearly 30% of
313 adults in the U.S. and ~ 1 billion worldwide may suffer from hypertension, and that elevated
314 blood pressure accounts for 54% of strokes, 47% of heart disease, and 14% of all deaths (Lawes
315 et al., 2008).
316

This is the first study, to our knowledge, to directly assess the relationship between relevant elemental mercury exposure and blood pressure outcomes in a human population that experiences background exposures. Dental amalgams typically consist of 50% mercury by weight (Clarkson and Magos, 2006). Accordingly, urine mercury levels among dental practitioners are strongly predicted by the number of amalgams they remove or place (Martin et al., 1995). In the 1970s and 1980s, urine mercury levels in dentists regularly exceeded 10 µg/L but values have dropped significantly in recent years owing to educational campaigns and a shift towards composite resin fillings (Eklund, 2010; Shapiro et al., 1982). This decrease is supported by the current study where urine biomarkers of elemental mercury exposure among dental professionals in Michigan mirrored the general US population (CDC, 2009; Table 2), suggesting that our findings may have broad relevance to public health. Despite low-level elemental mercury exposure (maximum=5.5 µg/L), associations with SBP were found. Unlike the hair mercury associations, a urine mercury-associated decrease in adjusted SBP was observed in the total population and was driven by the males and dentists. The significance of this association was influenced by several subjects with higher urine mercury levels, and as such this relationship should be further explored in a population with a wider range of exposure (maximum >10 µg/L). These findings suggest that levels of urine mercury found in the general adult US population, which average 20-100 times less than exposure limits set by the World Health Organization (50 µg/L), may be associated with alterations in blood pressure and that these may be gender-specific. While elemental mercury exposures of the general population are low, certain groups still remain at great risk of elemental mercury exposure, such as small-scale gold miners (Paruchuri et al., 2010).

For hair and urine mercury, gender influenced the observed trends. At this moment it is not clear why elemental mercury-associated decreases in SBP are observed in males only, or why methylmercury-associated increases in blood pressure are stronger in males, though increasing toxicological and epidemiological studies are stressing the importance of considering gender-specific differences in chemical exposures, toxicokinetics, and health impacts (Institute of Medicine, 2001; Vahter et al., 2007). Experimental rodent studies have documented gender differences in the distribution, metabolism, and elimination of methylmercury and inorganic mercury (Ekstrand et al., 2010; Thomas et al., 1986, 1987). With respect to hypertension, gender-specific differences have been reported in women in terms of age-related onset and metal sensitivity (Reckelhoff, 2001; Vahter et al., 2007). The differences observed in this study may reflect true gender differences in the relationship between mercury and blood pressure, or they may have resulted from random variation due to small sample sizes.

In addition to disparate gender results, elemental mercury results differed from the methylmercury results in many cases. The effect of elemental mercury on cardiovascular function in humans is not well characterized, but there are laboratory animal studies that may shed light on our findings. The general trends observed in our elemental mercury-exposed male population are consistent with animal studies that have reported that high doses of inorganic mercury cause depressed arterial systolic pressure (Massaroni et al., 1995; Rhee and Choi, 1989; Rossoni et al., 1999). Differences between elemental mercury and methylmercury effects may be realized at the cellular level. One purported mechanism by which mercury affects blood pressure is through disruption of calcium homeostasis, and there are reported differences among methylmercury and elemental mercury in terms of potency, sensitivity towards certain calcium

channel subtypes, the nature of inhibition, and alteration of channel function (Atchinson, 2003; Sakamoto et al., 1996). Evidence in animals and humans suggests that methylmercury-induced oxidative stress can inhibit production of nitric oxide, a vasodilator, and lead to vascular endothelial dysfunction, mechanisms related to hypertension (Dharmashankar and Widlansky, 2010; de Marco et al., 2009; Grotto et al., 2009; Mazerik et al., 2007). Several differential mechanisms may underlie the opposite association observed between elemental mercury and SBP. Massaroni et al. (1995) found mercuric chloride increased autonomic neurotransmitter release in rats experiencing hypotension following treatment. Inorganic mercury may furthermore impact blood pressure indirectly via interaction with the kidney, an organ specifically targeted by inorganic species of mercury (Clarkson and Magos, 2006). Mercurial drugs such as calomel inhibit sodium and chloride reabsorption in the kidney and were formerly prescribed as diuretics and anti-hypertensive medication until the mid-1900s (Norn et al., 2008; Wolf et al., 1966). Interactions between elemental mercury, kidneys and decreased SBP merit further exploration.

Even though this study had several limitations, associations were found between low-level mercury exposures and blood pressure alterations. Associations and trends observed here corroborate several epidemiological (for methylmercury) and animal (for elemental mercury) studies, and thus minimize concern of chance-related significant outcomes stemming from multiple statistical tests. While subjects did not know their urine or hair mercury levels before participating in the study, dental professionals are cognizant of mercury as a public health issue and likely were aware of occupational exposures and possibly environmental exposures they may have experienced. Since we observed mercury distributions that overlapped with biomarker

levels measured in NHANES participants, it is possible that dental professionals with lower than average occupational exposures self-selected to volunteer for this study. . If this negative selection bias did occur, it is not expected to significantly impact the results reported here as we were still able to explore relationships between a range of mercury biomarker levels, and SBP/DBP. Due to the cross-sectional design, we were unable to assess the impact of past exposures or lifestyle changes on blood pressure. Gender stratification was performed on all analyses due to the age, BMI, mercury exposure and occupational differences observed between our male and female participants, but this may have limited our power due to smaller sample size. Significant associations between hair mercury and DBP and urine mercury and SBP were still observed in the male population even with the decreased statistical power.

Our analyses did not include one major potential confounder- smoking status- as this information was not collected from our subjects. While smoking is often considered a risk factor for hypertension and has been shown to influence cadmium and lead biomarker levels, smoking has not been shown to affect mercury biomarker levels in most studies (Dewailly et al., 2001; Levy et al., 2007), with exceptions (Freire et al., 2010). Another limitation of this study may be the lack of mercury speciation in biomarker samples. While hair and urine are typically deemed biomarkers of methylmercury and inorganic mercury exposure, respectively, (Berglund et al., 2005), evidence in occupational cohorts with exposure to elemental mercury suggests that a fraction of hair mercury may reflect inorganic mercury exposure (Morton et al., 2004; Wranová et al., 2008). In the MDA cohort, amalgams were weakly associated with hair mercury even though fish consumption was the main predictor. However, mercury speciation of the MDA biomarker samples would be predicted to increase the significance of the relationships observed

409 (elemental mercury with decreased SBP and methylmercury with increased DBP) if the two
410 mercury forms truly have opposing associations with blood pressure.
411
412 This study reports significant, albeit borderline significant ($0.01<p<0.05$) and partially
413 outlier influenced, associations between elevated DBP and hair mercury and between decreased
414 SBP and urine mercury at exposure levels relevant to the general population. Even though these
415 differential relationships were observed in face of many study limitations, comparable significant
416 associations were observed (blood mercury with increased DBP, $p<0.05$, and urine mercury with
417 decreased SBP, $p<0.0001$) using NHANES data ($n>4,000$) after controlling for seven
418 confounders including smoking status and race (data not published). As such, future work on
419 mercury and cardiovascular health should consider both elemental mercury and methylmercury
420 at wide ranges of exposure in males and females to gain a better understanding of how these
421 toxicants influence blood pressure and ultimately cardiovascular disease.

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Table 1. Characteristics of total and stratified study population (mean ± SD).

	Total Population	Gender		Occupation		Blood Pressure Medication	
		Males	Females	Dentists	Non-Dentists	No	Yes
n	262	99	163	114	148	223	39
BMI (kg/m^2)	26.4 (4.5)	27.2 (3.7)	25.9 (4.9)[c]	26.6 (3.9)	26.2 (4.9)	26.2 (4.5)	27.8 (4.3)[c]
Age (years)	52.3 (12.3)	60.2 (10.8)	47.5 (10.6)[e]	57.8 (11.4)	48.0 (11.3)[e]	50.7 (12.0)	61.4 (9.8)[e]
SBP (mm Hg)	124 (15.3)	133 (13.3)	119 (14.1)[e]	130 (15.2)	120 (13.9)[e]	123 (14.6)	135 (15.1)[e]
DBP (mm Hg)	73.5 (9.3)	75.9 (8.2)	72.0 (9.7)[d]	75.0 (8.7)	72.3 (9.7)[c]	72.9 (9.2)	76.8 (9.6)[c]
Pulse (beats/min)	72.7 (11.8)	69.1 (12.9)	74.9 (10.5)[e]	69.8 (12.6)	75.0 (10.6)[e]	73.3 (11.5)	69.4 (13.1)
Alcohol (drinks/day)	0.42 (0.55)	0.54 (0.65)	0.34 (0.47)[d]	0.55 (0.66)	0.31 (0.43)[d]	0.38 (0.52)	0.64 (0.65)[d]
Amalgam[a]	3.58 (3.42)	4.01 (3.44)	3.33 (3.39)	4.15 (3.59)	3.15 (3.24)[c]	3.25 (3.12)	5.49 (4.40)[e]
Amalgams handled[a]	27.9 (47.3)	43.6 (57.1)	18.4 (37.4)[e]	48.0 (57.1)	12.4 (30.2)[e]	26.9 (46.9)	33.2 (50.0)
Hg intake[b] (µg/kg bw/day)	0.08 (0.12)	0.09 (0.13)	0.07 (0.12)	0.09 (0.13)	0.07 (0.12)	0.07 (0.12)	0.10 (0.14)

[a]Amalgam is the number of mercury-containing dental amalgams in the subject's mouth while amalgams handled is the sum of dental amalgams removed and placed per week in occupational practice.

[b]Hg intake estimated from reported fish consumption (type, portion size, consumption frequency).

[c] $p<0.05$, ANOVA tests comparing paired categories (male vs. female, dentists vs. non-dentists, blood pressure medication users vs. non-users)

[d] $p<0.01$

[e] $p<0.001$

Table 2. Mercury biomarker levels in total and stratified population.

		n	Mean	St dev	25th %	50th %	75th %	90th %	95th %
HAIR MERCURY (µg/g)									
	Total	262	0.45	0.53	0.14	0.28	0.55	1.06	1.31
	NHANES[a]	1726	0.47		0.09	0.19	0.42	1.11	1.73
Gender	Males	99	0.65	0.71	0.24	0.50	0.83	1.33	1.43
	Females	163	0.33[c]	0.34	0.11	0.21	0.43	0.82	1.06
Occupation	Dentists	114	0.64	0.69	0.25	0.48	0.83	1.31	1.69
	Non-Dentists	148	0.30[c]	0.29	0.11	0.19	0.39	0.72	1.00
Medication	No BP Meds	223	0.41	0.51	0.13	0.26	0.51	0.95	1.31
	BP Meds	39	0.66[b]	0.63	0.21	0.56	0.90	1.22	1.39
URINE MERCURY (µg/L)									
	Total	262	0.94	0.99	0.31	0.63	1.18	2.09	2.76
	NHANES[a]	1529				0.48	1.12	2.20	3.33
Gender	Males	99	1.27	1.22	0.51	0.85	1.50	2.66	4.87
	Females	163	0.74[c]	0.75	0.26	0.47	0.98	1.76	2.20
Occupation	Dentists	114	1.26	1.19	0.49	0.85	1.53	2.56	4.47
	Non-Dentists	148	0.69[c]	0.70	0.25	0.44	0.92	1.61	2.00
Medication	No BP Meds	223	0.93	1.02	0.29	0.60	1.13	1.94	3.54
	BP Meds	39	1.01	0.79	0.38	0.66	1.60	2.35	2.46

[a]NHANES- National Health and Nutrition Examination Survey. Urine data from CDC 2009; hair data from McDowell et al. 2004.

[b] $p<0.01$ and [c] $p<0.001$ for ANOVA comparing natural log-transformed values for paired categories (male vs. female, dentists vs. non-dentists, BP medication users vs. non-users).

Table 3. Parameter estimates for linear regression models (with p-values in parentheses below the β estimates). Blood pressure measurements of individuals using hypertension controlling medications were imputed 15 mmHg higher (SBP) and 10 mmHg higher (DBP) according to the method of Tobin et al. (2005).

Dependent Variable	**Population**	**n**	**Adj. R^2**	**BMI**	**Age**	**Female**	**Hair Hg**	**Urine Hg**
SBP (mmHg)	Total	262	0.43	0.97 (<0.001)	0.67 (<0.001)	-5.74 (0.005)	2.67 (0.11)	-1.80 (0.04)
	Males	99	0.24	1.17 (0.004)	0.59 (<0.001)		3.15 (0.13)	-3.26 (0.009)
	Females	163	0.35	0.86 (<0.001)	0.74 (<0.001)		1.54 (0.63)	0.71 (0.60)
	Dentists	114	0.44	1.15 (0.001)	0.72 (<0.001)	-7.89 (0.03)	2.07 (0.27)	-3.35 (0.003)
DBP (mmHg)	Total	262	0.22	0.83 (<0.001)	0.18 (0.002)	-1.26 (0.37)	2.76 (0.02)	-0.32 (0.61)
	Males	99	0.06	0.54 (0.04)	0.05 (0.59)		2.94 (0.03)	-1.13 (0.16)
	Females	163	0.26	0.89 (<0.001)	0.27 (<0.001)		1.87 (0.42)	1.10 (0.26)
	Dentists	114	0.15	0.71 (0.003)	0.12 (0.16)	-3.31 (0.17)	2.11 (0.10)	-0.88 (0.25)