Environmental Science & Technology

Article

pubs.acs.org/est

New Insight into Biomarkers of Human Mercury Exposure Using Naturally Occurring Mercury Stable Isotopes

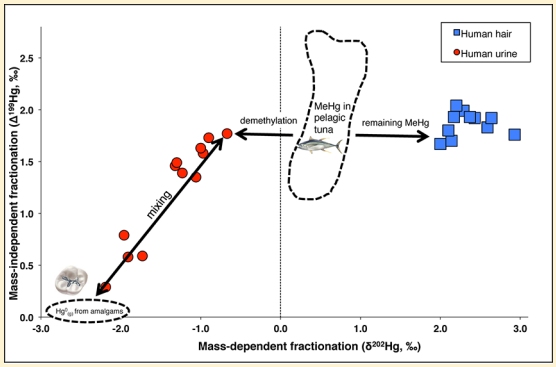
Laura S. Sherman,[†,*] Joel D. Blum,[†] Alfred Franzblau,[‡] and Niladri Basu[‡]

[†]Department of Earth and Environmental Sciences, University of Michigan, 1100 N. University Ave., Ann Arbor, Michigan 48109, United States

[‡]Department of Environmental Health Sciences, University of Michigan, 1415 Washington Heights, Ann Arbor, Michigan 48109, United States

Supporting Information

ABSTRACT: Human exposure to methylmercury (MeHg) and elemental mercury vapor ($Hg^0_{(g)}$) are often estimated using total Hg concentrations in hair and urine, respectively. We investigated whether Hg stable isotopes could be used to better distinguish between exposure to $Hg^0_{(g)}$ versus MeHg. We found that hair from North American dental professionals was characterized by high positive Δ^{199}Hg values (mean = 1.86‰, 1 SD = 0.12‰, n = 11). This confirms that among people who regularly consume fish, total Hg concentrations in hair reflect exposure to MeHg. In contrast, we found that urine from the same individuals was characterized by a range of Δ^{199}Hg values (0.29 to 1.77‰, 2 SD = 0.06‰, n = 12) that were significantly correlated to the number of dental amalgams in each individual's mouth. We hypothesize that fish-derived MeHg is demethylated within the body, causing mass-dependent fractionation and the excretion of inorganic Hg in urine. Mercury in urine therefore represents a mixture of demethylated fish-derived MeHg and amalgam-derived inorganic Hg. We estimate that the majority (>70%) of Hg in urine from individuals with <10 dental amalgams is derived from ingestion of MeHg in fish. These data suggest that within populations that consume fish, urine total Hg concentrations may overestimate Hg exposure from personal dental amalgams.



1. INTRODUCTION

Humans are regularly exposed to both methylmercury (MeHg) and inorganic mercury (Hg). Exposure to MeHg occurs primarily due to consumption of higher trophic level fish.[1−3] Once ingested, MeHg is rapidly absorbed into the bloodstream from the gastrointestinal tract and distributed throughout the body.[2,4,5] Because it can easily cross the blood-brain and placental barriers, MeHg can cause severe central nervous system damage and developmental delays, especially in fetuses and newborns.[2,3,6] Most of the Hg in hair is MeHg (usually >80%; ref 7 and 8) and MeHg is taken up by both hair follicles and brain cells as MeHg-cysteine complexes.[3,9] As a result, epidemiological studies often use total Hg concentrations in hair as biomarkers for MeHg exposure from fish consumption.

Inorganic Hg can also cause central nervous system and kidney damage.[2,10] Exposure to inorganic Hg occurs primarily through inhalation of elemental Hg vapor ($Hg^0_{(g)}$). This can occur occupationally among industrial workers, small-scale gold miners, and dental professionals who place Hg amalgam fillings.[3,11] However, as dentists have increasingly switched to resin-based composite restorations, occupational exposure of dentists to $Hg^0_{(g)}$ has decreased.[12] $Hg^0_{(g)}$ can also be emitted into the oral cavity from the surfaces of personal dental amalgams, but the magnitude of this exposure remains controversial.[5,13] The majority (~80%) of inhaled $Hg^0_{(g)}$ is absorbed into the bloodstream and rapidly oxidized within cells.[5] The resulting oxidized inorganic Hg is largely transported to the kidneys where it is excreted in urine.[5,14] Because Hg in urine is almost entirely inorganic Hg (>98%), total Hg concentrations in urine are commonly used as a biomarker for $Hg^0_{(g)}$ exposure.[7,8] However, some studies have observed correlations between urine total Hg concentrations and fish consumption,[15−17] suggesting that inorganic Hg derived from demethylation of ingested MeHg may also be excreted in urine.

It may be possible to use naturally occurring Hg stable isotope ratios in human hair and urine to more definitively distinguish between exposure to MeHg versus $Hg^0_{(g)}$. The seven stable isotopes of Hg (196 to 204 amu) can undergo mass-dependent fractionation (MDF) during a number of processes including methylation[18] and demethylation,[19,20] reduction,[20,21] and volatilization.[22,23] Mercury stable isotopes can also undergo mass-independent fractionation (MIF), which is reported as the deviation of a measured isotope ratio from

Received: December 21, 2012
Revised: February 25, 2013
Accepted: March 6, 2013
Published: March 6, 2013

the ratio theoretically predicted to result from MDF. Mercury stable isotope ratios are reported using delta notation as

$$\delta^{xxx}\mathrm{Hg}(‰) = ([(^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{sample}/(^{xxx}\mathrm{Hg}/^{198}\mathrm{Hg})_{SRM3133}] - 1) \times 1000 \quad (1)$$

where ^{xxx}Hg is an isotope of Hg and SRM 3133 is a National Institute of Standards and Technology (NIST) Standard Reference Material (SRM).[20] Mass-independent fractionation is reported using capital delta notation as

$$\Delta^{xxx}\mathrm{Hg} = \delta^{xxx}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times \beta) \quad (2)$$

where β is equal to 0.252 for ^{199}Hg, 0.502 for ^{200}Hg, and 0.752 for ^{201}Hg for kinetic fractionation reactions.[20] The greatest magnitude of MIF of Hg isotopes occurs during photochemical reactions that generate long-lived radical pairs.[20,24] During these reactions, because only the odd-mass-number isotopes of Hg have unpaired nuclear spin and nuclear magnetic moments, the odd- and even-mass-number isotopes of Hg react at slightly different rates. Several studies have observed large positive MIF of Hg isotopes in fish and hypothesized that photochemical demethylation of MeHg results in MIF such that the remaining aqueous MeHg that enters the food web displays positive Δ^{199}Hg values.[20,25−27] In contrast, because the Hg in geologic deposits that is mined for use in industrial processes, gold mining, and dental amalgams has not undergone photochemical cycling, it does not display significant MIF of Hg isotopes.[28−31] As Laffont et al. recently demonstrated, MIF of Hg isotopes in human hair can be used to distinguish between exposure to fish-derived MeHg and geologically derived $Hg^0_{(g)}$.[31,32]

We undertook this study to further investigate whether Hg stable isotope ratios in human hair and urine can be used to quantify exposure to $Hg^0_{(g)}$ and MeHg more accurately than measurement of total Hg concentrations. We measured Hg isotope ratios in hair and urine from a group of North American dental professionals. These individuals potentially inhale $Hg^0_{(g)}$ during placement of dental amalgams and due to volatilization from their personal amalgams.[12,13] They are also exposed to varying amounts of MeHg through the consumption of fish and other seafood.[33] Although Hg exposure has been studied among dental professionals,[34,35] Hg stable isotope ratios have not previously been employed to assess this exposure.

2. EXPERIMENTAL SECTION

2.1. Sample Collection. For a larger public health study, a convenience sample of 515 dental professionals (dentists and hygienists) was recruited at the 2009 and 2010 Michigan Dental Association (MDA) Annual Conventions.[34,35] Each participant completed a survey detailing demographic characteristics, occupational practices (e.g., number of amalgams handled per week), number of personal dental amalgams, and fish consumption patterns.[34,35] Daily intake of MeHg from fish was estimated based on species specific average Hg concentrations.[35,36] Spot urine samples (∼20−50 mL) were collected into Hg-free containers and stored frozen. Hair samples were cut close to the scalp and wrapped in paper.

2.2. Total Hg Concentration Analyses. Total Hg concentrations in urine and hair samples were measured using a Direct Mercury Analyzer 80 (Milestone Inc., CT) according to U.S. EPA Method 7473 as previously described.[34,35] One empty boat blank, one sample replicate, and at least one standard reference material were included in every set of 10−15 samples (see Supporting Information (SI)).

2.3. Sample Processing for Hg Isotope Ratio Analyses. Because accurate Hg isotope ratio measurements cannot be made on samples containing less than ∼8 ng of Hg, only a limited number of samples could be analyzed for Hg isotope ratios (hair: n = 11; urine: n = 12). The hair samples were not washed prior to analysis because no methods have been shown to quantitatively remove exogenous $Hg^0_{(g)}$ contamination from hair.[37,38] Depending on the measured total Hg concentration and amount of hair available, between ∼6 and ∼170 mg of hair per sample was weighed into a ceramic boat and covered with activated alumina and sodium carbonate powders (Nippon Instruments) that had previously been combusted at 800 °C for 8 h to remove any Hg. Urine samples were homogenized by vigorous shaking and then pipetted into ceramic boats in ∼4 mL aliquots for a total of 4 to 27 mL of urine per sample. Each aliquot was evaporated to dryness in an oven at 60 °C for 4−6 h. The dried material was then covered with the Hg-free powders. After the samples were prepared, the Hg was thermally released using previously published methods (see SI; ref 27). Human hair and urine procedural standards and blanks were processed according to the same methods (see SI).

2.4. Mercury Stable Isotope Analyses. Mercury isotope ratios were measured in samples and standards using multicollector inductively coupled plasma mass spectrometry according to previously published methods.[20] During each analytical session, the UM-Almadén secondary standard was analyzed five times. We estimate sample analytical uncertainty for each isotope ratio as the larger of two times the standard deviation of the same ratio measured either in the relevant procedural standards or the UM-Almadén secondary standard.

2.5. Statistical Analyses. Relationships between measured variables were characterized using linear regression. The statistical significance of each correlation was assessed using the SPSS Statistics program (v. 20.0.0, IBM). For all statistically significant relationships, the assumptions of linearity, independence of errors, homoscedasticity, and normality of the error distribution were assessed and validated. The relationships between hair and urine Δ^{199}Hg and Δ^{201}Hg values were analyzed using York regression, which incorporates error in both the dependent and independent variables.[39]

3. RESULTS AND DISCUSSION

3.1. Total Hg Concentrations. Total Hg concentrations measured in hair and urine samples are presented in the SI (Table SI−S1). Because we were only able to analyze high concentration samples for Hg isotope ratios, the total Hg concentrations of these samples do not represent the range or average concentrations in hair (0.49 ± 0.63 μg/g) or urine (1.04 ± 1.18 μg/L) from the larger dental professional cohort.[34] In addition, these analytical requirements and the limited size of the hair samples resulted in the inclusion in this study only of individuals who consumed fairly large amounts of fish each month (Table SI−S1). Here, the average hair total Hg concentration was 3.02 μg/g (1 SD = 1.62 μg/g, n = 12) and the average urine total Hg concentration was 2.54 μg/L (1 SD = 1.68 μg/L, n = 12). As observed in previous studies, these hair total Hg concentrations were nearly significantly correlated with MeHg intake from fish (p = 0.063, r^2 = 0.304; Figure SI−S1).[7,16,35] However, there were no significant relationships between urine total Hg concentrations and the number of personal amalgams (p = 0.375, r^2 = 0.080) or the number of

dx.doi.org/10.1021/es305250z | Environ. Sci. Technol. 2013, 47, 3403−3409

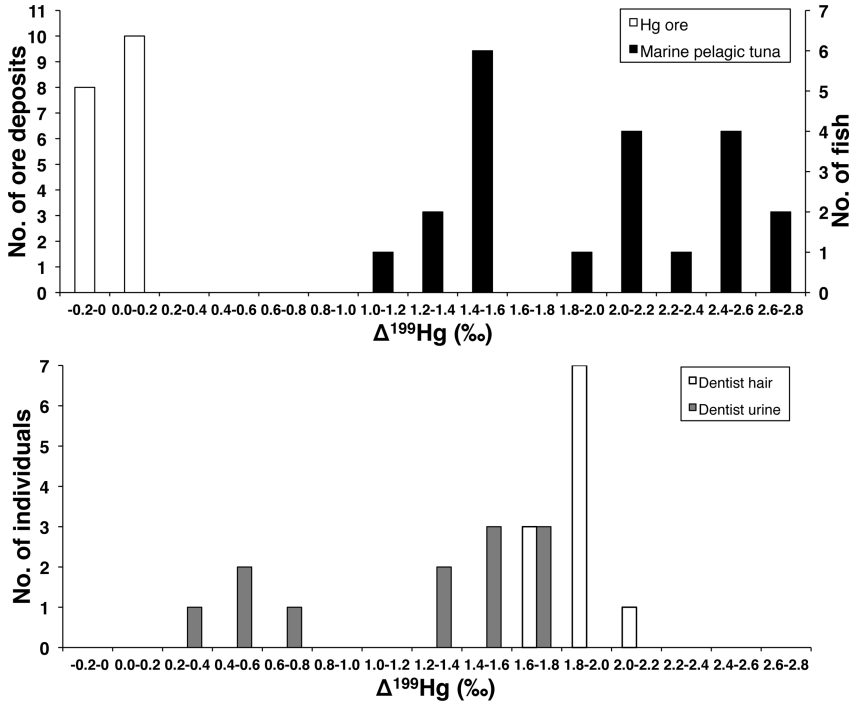


Figure 1. Mass-independent fractionation of Hg isotopes in tuna, Hg ores, and human hair and urine samples. Δ^{199}Hg values are binned into equal 0.20‰ intervals. Top panel shows previously measured Δ^{199}Hg values in marine pelagic tuna including the average value of the BCR CRM 464 tuna fish standard reference material across multiple studies[25−27,43,44] (black columns) and previously measured Δ^{199}Hg values in geologic Hg ore deposits[28,29,31] (hatched columns). Lower panel depicts Δ^{199}Hg values measured in hair samples (white columns) and urine samples (gray columns) from the dental professionals.

amalgams handled each week (p = 0.387, r^2 = 0.076). Increased care in handling of Hg and use of composite fillings has decreased dental professionals' occupational exposure to $Hg^0_{(g)}$.[12] In the larger study, number of amalgams handled each week was a less strong predictor of urine total Hg concentrations than number of personal amalgams.[34]

3.2. Mass-Independent Fractionation of Hg Isotopes. Complete Hg isotopic data are presented in the SI (Table SI−S1). Hair samples from the dental professionals exhibited positive Δ^{199}Hg values (mean = 1.86‰, 1 SD = 0.12‰, n = 11) that are similar to those measured previously in hair samples from Northern Europeans.[31] Although it has been suggested that MIF might occur due to metabolic processes within organisms,[40] no known biotic processes or trophic transfers have been shown to produce MIF of Hg isotopes[18,19,21,41] and abiotic dark equilibrium reactions only produce a small amount of MIF (<~0.3‰; refs 23 and 42). Instead, we hypothesize that the observed positive MIF in the hair samples is due to ingestion of MeHg that displays positive Δ^{199}Hg values. As shown in Figure 1, Δ^{199}Hg values in the hair samples are similar to those previously observed in marine pelagic tuna.[25−27,43,44] On average, 54 ± 17% by mass (1 SD, n = 12) of fish consumed by the dental professionals were marine pelagic species (including tuna, cod, halibut, swordfish, shark, and mackerel). Because these large, predatory fish often have higher concentrations of MeHg,[25] due to mass balance, it is likely that consumption of these species effectively controls the Δ^{199}Hg values observed in the hair samples. In addition, the ratio of Δ^{199}Hg to Δ^{201}Hg in the hair samples is 1.22 ± 0.22 (1SE, n = 11; Figure SI−S2). This is consistent with Δ^{199}Hg/Δ^{201}Hg ratios that have been observed in fish populations.[20,26,27,32,41,43,45−47] These data further support the hypothesis that among people who eat fish, hair total Hg concentrations reflect exposure to MeHg from fish consumption.

In contrast, urine samples from the dental professionals were characterized by a range of positive Δ^{199}Hg values (0.29‰ to 1.77‰; Figure 1). As described previously, Hg in dental amalgams is mined from geologic deposits, which do not display significant MIF (Figure 1, refs 28−30). Therefore, if the majority of the Hg in the urine samples resulted from exposure to inhaled $Hg^0_{(g)}$, we would expect to observe urine Δ^{199}Hg values close to 0‰. However, most of the urine samples (MDA-1 to 7, MDA-11; n = 8) displayed large positive Δ^{199}Hg values that were similar to those measured in the corresponding hair samples (mean urine Δ^{199}Hg = 1.55‰, 1 SD = 0.15‰, n = 8). These data suggest that a significant portion of the Hg in the urine samples is derived from fish consumption. Although several previous studies have suggested that Hg derived from fish and seafood can contribute to urine Hg levels, it was not previously possible to support or resolve this hypothesis analytically.[15−17] These Hg isotope results directly challenge the assumption that total Hg concentrations in urine dominantly reflect exposure to inhaled $Hg^0_{(g)}$.

We hypothesize that urine Δ^{199}Hg values are controlled by mixing of inorganic Hg derived from demethylation of ingested MeHg that displays positive Δ^{199}Hg values with inhaled $Hg^0_{(g)}$ from dental amalgams that displays no significant MIF. As depicted in Figure 1, four of the urine samples display lower Δ^{199}Hg values (MDA-8 to 10, MDA-12) than the hair samples. These variations in MIF are not due to differences in Hg intake via fish consumption (p = 0.148, r^2 = 0.197, n = 12) or the number of amalgams handled each week (p = 0.385, r^2 = 0.076, n = 12). However, as shown in Figure 2, the urine Δ^{199}Hg values are nearly significantly correlated with the number of personal amalgams in each individual's mouth (p = 0.057, r^2 =

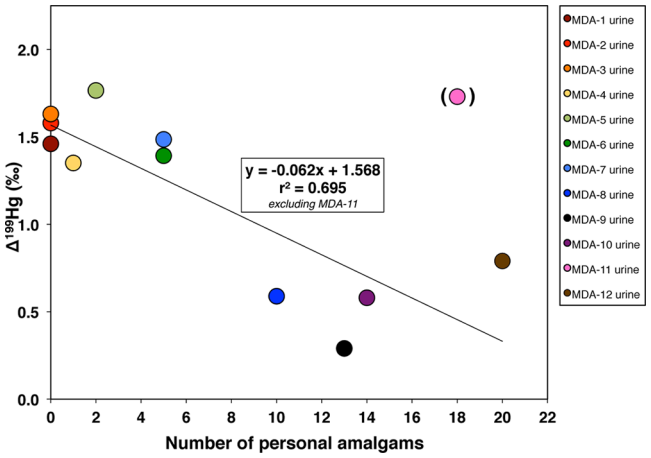


Figure 2. Number of personal dental amalgams versus urine Δ^{199}Hg values. Filled circles represent urine samples from individual dental professionals. Linear regression depicted as a solid line (p = 0.001, r^2 = 0.695, n = 11) does not include individual MDA-11 (pink circle bracketed by parentheses).

0.317, n = 12). As the number of personal amalgams increases, urine Δ^{199}Hg values decrease. In fact, the four dental professionals with lower urine Δ^{199}Hg values all have 10 or more personal dental amalgams (Table SI-S1). This suggests that exposure to inhaled inorganic $Hg^0_{(g)}$ increases as the number of dental amalgams in an individual's mouth increases. The urine sample from individual MDA-11 does not fit this trend. Although this individual has 18 personal amalgams, his urine sample displayed a similarly positive Δ^{199}Hg value (1.73 ± 0.06‰, 2 SD) to his hair sample (2.04 ± 0.06‰, 2 SD). If this individual is excluded, the correlation between urine Δ^{199}Hg values and personal dental amalgams is much stronger (p = 0.001, r^2 = 0.695, n = 11; Figure 2). It is possible that this individual over-reported the number of personal dental amalgams in his mouth, perhaps by counting each restored surface rather than individual Hg amalgams. It is also possible that individual MDA-11 may have consumed significantly more fish than reported in the dietary survey. Although he reported consuming 2640 g of fish per month (0.400 μg Hg/kg body weight/day; Table SI-S1), the total Hg concentration in his hair sample was much higher than expected based on this level of fish consumption (Figure SI-S1). Consumption of a large amount of fish by individual MDA-11 may have resulted in the observation of a higher than expected urine Δ^{199}Hg value.

If we assume that Hg in urine from the dental professionals represents a mixture of demethylated MeHg originally ingested in fish and inhaled $Hg^0_{(g)}$ from dental amalgams, we can use a simple two-end-member mixing model to estimate the relative exposure of each individual to these sources of Hg (see SI). To do this we assume that the positive MIF observed in the hair samples is due entirely to consumption of fish and that $Hg^0_{(g)}$ released from amalgams displays no significant MIF.[28−30] As shown in Table 1, we estimate that, on average, 83 ± 9% (1 SD, n = 8) of the Hg in urine from the eight individuals with higher urine Δ^{199}Hg values (MDA-1 to 7, MDA-11) was originally ingested as MeHg in fish. These results directly contradict the assumption that urine total Hg concentrations primarily reflect exposure to inhaled $Hg^0_{(g)}$. In contrast, a large percentage of the total Hg in urine from individuals with lower urine Δ^{199}Hg values (MDA-8 to 10, MDA-12) resulted from inhalation of $Hg^0_{(g)}$ (mean = 69 ± 10%, 1 SD, n = 4). In populations that eat

Table 1. Hg in Urine Resulting from Exposure to MeHg and $Hg^0_{(g)}$[a]

individual ID	no. of personal dental amalgams	hair Δ^{199}Hg (‰)	urine Δ^{199}Hg (‰)	% Hg in urine from fish MeHg	% Hg in urine from amalgam $Hg^0_{(g)}$
MDA-1	0	1.92	1.46	76.3	23.7
MDA-2	0	1.67	1.58	94.7	5.3
MDA-3	0	1.83	1.63	89.0	11.0
MDA-4	1	1.92	1.35	70.5	29.5
MDA-5	2	1.99	1.77	89.2	10.8
MDA-6	5	1.93	1.39	72.2	27.8
MDA-7	5	1.70	1.49	87.8	12.2
MDA-8	10	1.76	0.59	33.8	66.2
MDA-9	13	n.d.	0.29	16.0*	84.0*
MDA-10	14	1.80	0.58	32.6	67.4
MDA-11	18	2.04	1.73	84.9	15.1
MDA-12	20	1.93	0.79	41.2	58.8

[a]Individual dental professionals are labeled as "MDA-1", etc. Percent Hg in each urine sample derived from fish MeHg and inhaled $Hg^0_{(g)}$ were calculated using a two-end-member mixing model (see SI). Because it was not possible to analyze a hair sample from individual MDA-9 for Hg isotopes, for the purposes of this calculation we assume that the Δ^{199}Hg value of the hair sample from MDA-9 was equal to the average of the other hair samples (mean = 1.86‰).

fish and lack significant occupational exposure to inhaled $Hg^0_{(g)}$, total Hg concentrations in urine may overestimate exposure to $Hg^0_{(g)}$ from dental amalgams. However, if an individual has a large number of personal dental amalgams, exposure to inhaled $Hg^0_{(g)}$ can contribute significantly to Hg concentrations in urine.

3.3. Mass-Dependent Fractionation of Hg Isotopes.

Although it is unlikely that metabolic processes cause MIF, processes occurring within the human body may cause MDF of Hg isotopes. As shown in Figure 3, the hair and urine samples from the dental professionals displayed distinctly different δ^{202}Hg values. The hair samples exhibited positive δ^{202}Hg values (mean = 2.35‰, 1 SD = 0.28‰, n = 11) which were, on average, 1.9‰ higher (1 SD = 0.28‰, n = 11) than those of marine pelagic tuna (mean δ^{202}Hg = 0.52‰, 1 SD = 0.22‰, n = 21; refs 25−27, 43, 44). Laffont et al. similarly observed that δ^{202}Hg values in human hair were ∼2‰ higher than those measured in consumed fish.[31,32] In contrast, urine samples from the dental professionals displayed negative δ^{202}Hg values (mean = −1.35‰, 1 SD = 0.48‰, n = 12). We hypothesize that demethylation of MeHg within the body causes MDF and results in the observed hair and urine δ^{202}Hg values.

Demethylation of MeHg in the human body is relatively poorly understood, but it is known that MeHg can be demethylated both in the gastrointestinal tract by anaerobic microbes[5,48,49] and within other tissues such as red and white blood cells,[2,7,50] liver and kidney cells,[48,51] brain cells,[52] and hair follicles.[7] The produced inorganic Hg is eliminated from the body in feces or transported to the kidney and excreted in urine.[53−55] Environmental microorganisms and some abiotic processes are known to preferentially demethylate the lighter isotopes of Hg, which results in higher δ^{202}Hg values in remaining MeHg and lower δ^{202}Hg values in the produced inorganic Hg.[19,20] We hypothesize that in vivo demethylation of ingested MeHg may cause similar MDF in the human body, resulting in the accumulation of the lighter isotopes in the produced inorganic Hg that is excreted in urine and heavier

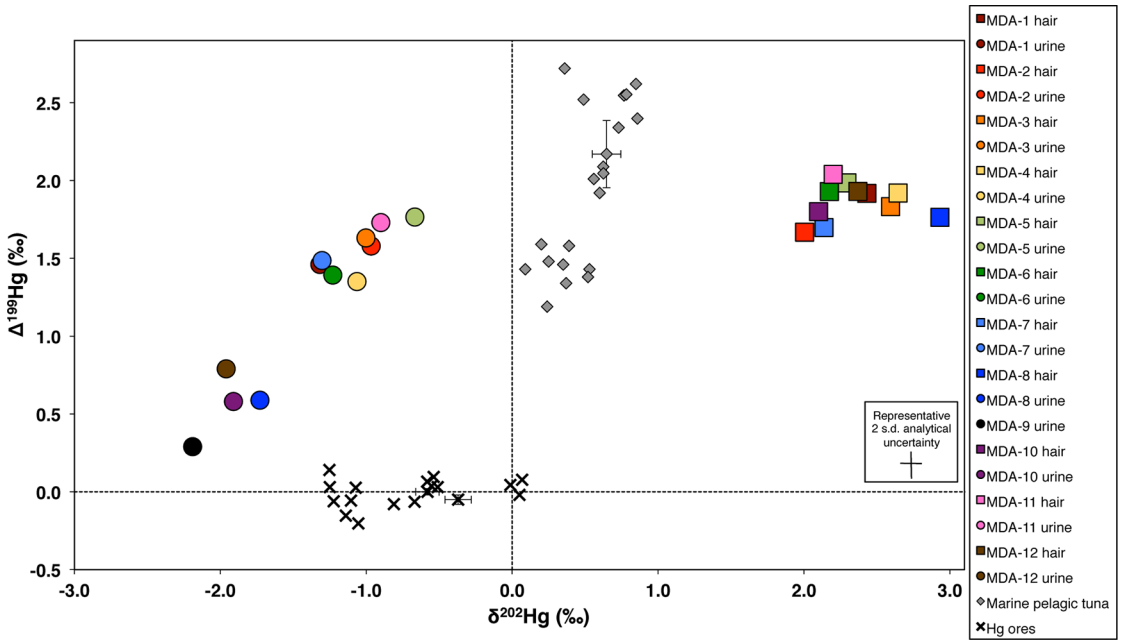


Figure 3. Isotopic compositions (δ^{202}Hg versus Δ^{199}Hg) of hair and urine samples. Filled square symbols represent hair samples from individual dental professionals; corresponding urine samples are shown as filled circles using the same colors. Representative 2 SD analytical uncertainty for the hair and urine samples is shown. Gray diamonds depict previously measured isotopic compositions of marine pelagic tuna and the average reported value for the BCR CRM 464 tuna fish standard reference material.[25−27,43,44] Error bars for the BCR CRM 464 tuna fish standard are 1 SD of the average of reported values. Black ×'s depict previously measured geologic Hg ore deposits.[28,29,31] Where more than one measurement was made per ore deposit, the mean value is presented and 1 SD error bars are shown.

isotopes of Hg in the remaining MeHg that is incorporated into hair. Based on mass balance and the average δ^{202}Hg values of marine pelagic tuna, hair samples, and urine samples that are composed primarily of demethylated MeHg (i.e., MDA-2, 3, 5; mean $\delta^{202}Hg_{demethylated\ MeHg}$ = −0.88‰), we estimate that ∼57% of fish-derived MeHg is demethylated within the body prior to uptake of the remaining MeHg into hair. Although this estimate is larger than that suggested by some previous pharmacokinetic models,[56,57] other models suggest that significant daily demethylation and loss of MeHg occurs within the human body (1.6% of body burden per day; ref 54). This estimate of demethylation also supports our conclusion, based on the urine Δ^{199}Hg values, that a significant portion of ingested MeHg is demethylated and excreted in urine.

In contrast to humans, it is interesting that MDF of Hg isotopes does not appear to occur in fish due to demethylation of ingested MeHg.[41] Although some studies suggest that demethylation of MeHg does not occur in fish,[58,59] other research indicates that demethylation may occur over time in fish livers.[60,61] However, no significant offsets in δ^{202}Hg values have been observed between consumed MeHg and fish muscle tissue.[41] Perrot et al. did observe significant MDF (∼1‰) between seals and their prey fish in Lake Baikal.[26] Data from this study further suggest that demethylation of MeHg occurs differently between fish and mammals. Whereas in vivo MeHg demethylation in mammals causes significant MDF of Hg isotopes, no such MDF appears to occur in fish organs. Future studies are needed to more fully explore and understand these differences.

We can also use the observed urine δ^{202}Hg values to estimate the δ^{202}Hg value of $Hg^0_{(g)}$ released from dental amalgams. As above, we assume that the δ^{202}Hg value of demethylated MeHg is most closely approximated by urine samples from individuals MDA-2, 3, and 5. Based on the relative proportion of Hg in each individual's urine from fish-derived MeHg versus inhaled $Hg^0_{(g)}$ (Table 1), we estimate that $Hg^0_{(g)}$ released from dental amalgams has an average δ^{202}Hg value of −2.1‰ (1 SD = 1.3‰, n = 12). This δ^{202}Hg value is lower than those measured in geologic ore deposits from which Hg used in amalgams is derived.[28−31] However, previous studies have shown that the lighter isotopes of Hg preferentially volatilize as $Hg^0_{(g)}$ from liquid Hg metal.[23,31] Similar MDF may occur during the volatilization of $Hg^0_{(g)}$ from dental amalgams into the oral cavity, resulting in the inhalation of $Hg^0_{(g)}$ with lower δ^{202}Hg values than that of the solid amalgams.

In summary, these data demonstrate that Hg isotope ratios in human hair and urine samples can be used to more accurately assess exposure to MeHg and $Hg^0_{(g)}$ than traditional measures of total Hg concentrations in the same media. Mass-independent fractionation of Hg isotopes can be used to differentiate between exposure to fish-derived MeHg (with high positive Δ^{199}Hg values) and geologically derived $Hg^0_{(g)}$ inhaled from dental amalgams (with no significant MIF). Mass-dependent fractionation of Hg isotopes can be used to better understand processes occurring in the human body such as demethylation of MeHg. As observed in previous studies, we found that the majority of Hg in hair samples from North American dental professionals results from ingestion of MeHg in fish.[34,35] However, we found that a significant percentage of the Hg in urine from many of these individuals also results from ingestion and demethylation of fish-derived MeHg. This is contrary to the widely cited assumption that Hg in urine is largely derived from inhalation of $Hg^0_{(g)}$. In fact, only urine from individuals who had more than 10 personal dental amalgams contained a large percentage of inorganic Hg resulting from exposure to $Hg^0_{(g)}$. It is important to note that these findings likely do not apply to individuals with high levels of occupational exposure to $Hg^0_{(g)}$ (e.g., small-scale gold

dx.doi.org/10.1021/es305250z | Environ. Sci. Technol. 2013, 47, 3403−3409

miners) or those who do not consume fish. However, among people who regularly consume fish, demethylation of fish-derived MeHg can contribute significantly to urine total Hg concentrations and result in the overestimation of $Hg^0_{(g)}$ exposure from dental amalgams.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental methods and supporting figures and data are presented. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 734-764-9314; fax: 734-763-4690; e-mail: lsaylors@umich.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funding was provided by the John D. MacArthur Professorship to J.D.B. and by a NIOSH Training Grant (No. T42 OH 008455-04) and Michigan Institute for Clinical and Health Research Grant (MICHR-UL1RR024986) to N.B. We are grateful to M. W. Johnson, Y. Wang, and J. Goodrich for assistance with sample collection and analyses. We thank three anonymous reviewers for comments that improved this manuscript.

■ REFERENCES

(1) Mergler, D.; Anderson, H. A.; Chan, L. H. M.; Mahaffey, K. R.; Murray, M.; Sakamoto, M.; Stern, A. H. Methylmercury exposure and health effects in humans: A worldwide concern. *Ambio* **2007**, *36* (1), 3−11.

(2) Clarkson, T. W. The three modern faces of mercury. *Environ. Health Perspect.* **2002**, *110* (Suppl. 1), 11−23.

(3) Clarkson, T. W.; Magos, L. The toxicology of mercury and its chemical compounds. *Crit. Rev. Toxicol.* **2006**, *36*, 609−662.

(4) Kershaw, T. G.; Clarkson, T.; Dhahir, P. H.; The relationship between blood levels and dose in methylmercury in man. *Arch. Environ. Health* **1980**, *35* (1).

(5) Clarkson, T. W.; Vyas, J. B.; Ballatori, N. Mechanisms of mercury disposition in the body. *Am. J. Ind. Med.* **2007**, *50* (10), 757−764.

(6) Grandjean, P.; Budtz-Jørgensen, E.; White, R. F.; Jørgensen, P. J.; Weihe, P.; Debes, F.; Keiding, N. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. *Am. J. Epidemiol.* **1999**, *150* (3), 301−305.

(7) Berglund, M.; Lind, B.; Björnberg, K. A.; Palm, B.; Einarsson, Ö.; Vahter, M.; Inter-individual variations of human mercury exposure biomarkers: A cross-sectional assessment. *Environ. Health: Global Access Sci. Source* **2005**, *4* (20); DOI: 10.1186/1476-069X-4-20.

(8) McDowell, M. A.; Dillon, C. F.; Osterloh, J.; Bolger, P. M.; Pellizzari, E.; Fernando, R.; Montes de Oca, R.; Schober, S. E.; Sinks, T.; Jones, R. L.; Mahaffey, K. R. Hair mercury levels in U.S. children and women of childbearing age: Reference range data from NHANES 1999−2000. *Environ. Health Perspect.* **2004**, *112* (11), 1165−1171.

(9) Cernichiari, E.; Brewer, R.; Myers, G. J.; Marsh, D. O.; Lapham, L. W.; Cox, C.; Shamlaye, C. F.; Berlin, M.; Davidon, P. W.; Clarkson, T. W. Monitoring methylmercury during pregnancy: Maternal hair predicts fetal brain exposure. *NeuroToxicology* **1995**, *16* (4), 705−710.

(10) Bårregard, L. Biological monitoring of exposure to mercury vapor. *Scand. J. Work, Environ. Health* **1993**, *19* (suppl 1), 45−49.

(11) Li, P.; Feng, X.; Qiu, G.; Shang, L.; Li, G. Human hair mercury levels in the Wanshan mercury mining area, Guizhou Province, China. *Environ. Geochem. Health* **2009**, *31*, 683−691.

(12) Eklund, S. A. Trends in dental treatment, 1992 to 2007. *J. Am. Dent. Assoc., JADA* **2010**, *141* (4), 391−399.

(13) Berglund, A. Estimation by a 24-h study of the daily dose of intra-oral mercury vapor inhaled after release from dental amalgam. *J. Dent. Res.* **1990**, *69*, 1646−1651.

(14) Suzuki, T.; Hongo, T.; Matsuo, N.; Imai, H.; Nakazawa, M.; Abe, T.; Yamamura, Y.; Yoshida, M.; Aoyama, H. An acute mercuric mercury poisoning: Chemical speciation of hair mercury shows a peak of inorganic mercury value. *Hum. Exp. Toxicol.* **1992**, *11*, 53−57.

(15) Martin, M. D.; Naleway, C.; Chou, H. N. Factors contributing to mercury exposure in dentists. *J. Am. Dent. Assoc., JADA* **1995**, *126* (11), 1502−1511.

(16) Ohno, T.; Sakamoto, M.; Kurosawa, R.; Dakeishi, M.; Iwata, T.; Murata, K. Total mercury levels in hair, toenail, and urine among women free from occupational exposure and their relations to renal tubular function. *Environ. Res.* **2007**, *103*, 191−197.

(17) Abe, T.; Akagi, H.; Akimichi, T.; Hongo, T.; Nakano, A.; Ohtsuka, R. High hair and urinary mercury levels of fish easters in the nonpolluted environment of Papua New Guinea. *Arch. Environ. Health* **1995**, *50* (5), 367−373.

(18) Rodriguez-Gonzalez, P.; Epov, V. N.; Bridou, R.; Tessier, E.; Guyoneaud, R.; Monperrus, M.; Amouroux, D. Species-specfic stable isotope fractionation of mercury during Hg(II) methylation by an anaerobic bacteria (*Desulfobulbus propionicus*) under dark conditions. *Environ. Sci. Technol.* **2009**, *43* (24), 9183−9188.

(19) Kritee, K.; Barkay, T.; Blum, J. D. Mass dependent stable isotope fractionation of mercury during *mer* mediated microbial degradation of monomethylmercury. *Geochim. Cosmochim. Acta* **2009**, *73*, 1285−1296.

(20) Bergquist, B. A.; Blum, J. D. Mass-dependent and mass-independent fractionation of Hg isotopes by photo-reduction in aquatic systems. *Science* **2007**, *318* (5849), 417−420.

(21) Kritee, K.; Blum, J. D.; Johnson, M. W.; Bergquist, B. A.; Barkay, T. Mercury stable isotope fractionation during reduction of Hg(II) to Hg(0) by mercury resistant microorganisms. *Environ. Sci. Technol.* **2007**, *41*, 1889−1895.

(22) Zheng, W.; Foucher, D.; Hintelmann, H. Mercury isotope fractionation during volatilization of Hg(0) from solution into the gas phase. *J. Anal. At. Spectrosc.* **2007**, *22*, 1097−1104.

(23) Estrade, N.; Carignan, J.; Sonke, J. E.; Donard, O. F. X.; Mercury isotope fractionation during liquid-vapor evaporation experiments. *Geochim. Cosmochim. Acta* **2009**, *73*, 2693−2711; DOI: 10.1016/j.gca.2009.01.024.

(24) Buchachenko, A. L. Magnetic isotope effect: Nuclear spin control of chemical reactions. *J. Phys. Chem.* **2001**, *105* (44), 9995−10011.

(25) Senn, D. B.; Chesney, E. J.; Blum, J. D.; Bank, M. S.; Maage, A.; Shine, J. P. Stable isotope (N, C, Hg) study of methylmercury sources and trophic transfer in the northern Gulf of Mexico. *Environ. Sci. Technol.* **2010**, *44* (5), 1630−1637.

(26) Perrot, V.; Pastukhov, M. V.; Epov, V. N.; Husted, S.; Donard, O. F. X.; Amouroux, D. Higher mass-independent isotope fractionation of methylmercury in the pelagic food web of Lake Baikal (Russia). *Environ. Sci. Technol.* **2012**, *46*, 5902−5911.

(27) Sherman, L. S.; Blum, J. D. Mercury stable isotopes in sediments and largemouth bass from Florida lakes, USA. *Sci. Total Environ.* **2012**, DOI: 10.1016/j.scitotenv.2012.09.038.

(28) Stetson, S. J.; Gray, J. E.; Wanty, R. B.; Macalady, D. L. Isotopic variability of mercury in ore, mine-waste calcine, and leachates of mine-waste calcine from areas mined for mercury. *Environ. Sci. Technol.* **2009**, *43*, 7331−7336.

(29) Hintelmann, H.; Lu, S. High precision isotope ratio measurements of mercury isotopes in cinnabar ores using multi-collector inductively coupled plasma mass spectrometry. *Analyst* **2003**, *128*, 635−639.

(30) Smith, C. N.; Kesler, S. E.; Blum, J. D.; Rytuba, J. J. Isotope geochemistry of mercury in source rocks, mineral deposits and spring deposits of the California Coast Ranges, USA. *Earth Planet. Sci. Lett.* **2008**, *269*, 399−407.

(31) Laffont, L.; Sonke, J. E.; Maurice, L.; Monrroy, S. L.; Chincheros, J.; Amouroux, D.; Behra, P.; Hg speciation and stable isotope signatures in human hair as a tracer for dietary and occupational exposure to mercury. *Environ. Sci. Technol.* **2011**, *45* (23), 9910−9916; DOI: 10.1021/es202353m.
(32) Laffont, L.; Sonke, J. E.; Maurice, L.; Hintelmann, H.; Pouilly, M.; Bacarreza, Y. S.; Perez, T.; Behra, P. Anomalous mercury isotopic compositions of fish and human hair in the Bolivian Amazon. *Environ. Sci. Technol.* **2009**, *43*, 8985−8990.
(33) Sunderland, E. M. Mercury exposure from domestic and imported estuarine and marine fish in the U.S. seafood market. *Environ. Health Perspect.* **2007**, *115* (2), 235−242.
(34) Goodrich, J. M.; Wang, Y.; Gillespie, B.; Werner, R.; Franzblau, A.; Basu, N. Glutathione enzyme and selenoprotein polymorphisms associate with mercury biomarker levels in Michigan dental professionals. *Toxicol. Appl. Pharmacol.* **2011**, *257*, 301−308.
(35) Goodrich, J. M.; Wang, Y.; Gillespie, B.; Werner, R.; Franzblau, A.; Basu, N. Methylmercury and elemental mercury differentially associate with blood pressure among dental professionals. *Int. J. Hyg. Environ. Health* **2012**, DOI: 10.1016/j.ijheh.2012.03.001.
(36) Wang, Y.; Goodrich, J. M.; Gillespie, B.; Werner, R.; Basu, N.; Franzblau, A. An investigation of modifying effects of metallothionein single-nucleotide polymorphisms on the association between mercury exposure and biomarker levels. *Environ. Health Perspect.* **2012**, *120* (4), 530−534.
(37) Morton, J.; Carolan, V. A.; Gariner, P. H. E. Removal of exogenously bound elements from human hair by various washing procedures and determination by inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* **2002**, *455*, 23−34.
(38) Li, Y.-F.; Chen, C.; Li, B.; Wang, J.; Gao, Y.; Zhao, Y.; Chai, Z. Scalp hair as a biomarker in environmental and occupational mercury exposed populations: Suitable or not? *Environ. Res.* **2008**, *107*, 39−44.
(39) York, D. Least-squares fitting of a straight line. *Can. J. Physi.* **1966**, *44*, 1076−1086.
(40) Das, R.; Salters, V. J. M.; Odem, A. L. A case for in vivo mass-independent fractionation of mercury isotopes in fish. *Geochem. Geophys. Geosystems* **2009**, *10*, Q11012.
(41) Kwon, S. Y.; Blum, J. D.; Carvan, M. J.; Basu, N.; Head, J. A.; Madenjian, C. P.; David, S. R. Absence of fractionation of mercury isotopes during trophic transfer of methylmercury to freshwater fish in captivity. *Environ. Sci. Technol.* **2012**, *46* (14), 7527−7534.
(42) Ghosh, S.; Schauble, E. A.; Couloume, G. L.; Blum, J. D.; Bergquist, B. A.; Estimation of nuclear volume dependent fractionation of mercury isotopes in equilibrium liquid-vapor evaporation experiments. *Chem. Geol.* **2013**, *336*, 5−12; DOI: 10.1016/j.chemgeo.2012.01.008.
(43) Perrot, V.; Epov, V. N.; Pastukhov, M. V.; Grebenshchikova, V. I.; Zouiten, C.; Sonke, J. E.; Husted, S.; Donard, O. F. X.; Amouroux, D.; Tracing sources and bioaccumulation of mercury in fish of Lake Baikal−Angara River using Hg isotopic composition. *Environ. Sci. Technol.* **2010**, *44* (21), 8030−8037; DOI: 10.1021/es101898e.
(44) Epov, V. N.; Rodriguez-Gonzalez, P.; Sonke, J. E.; Tessier, E.; Amouroux, D.; Maurice, L.; Donard, O. F. X. Simultaneous determination of species-specific isotopic composition of Hg by gas chromatography coupled to multicollector ICPMS. *Anal. Chem.* **2008**, *80* (10), 3530−3538.
(45) Gantner, N.; Hintelmann, H.; Zheng, W.; Muir, D. C. Variations in stable isotope fractionation of Hg in food webs of Arctic lakes. *Environ. Sci. Technol.* **2009**, *43*, 9148−9154.
(46) Gehrke, G. E.; Blum, J. D.; Slotton, D. G.; Greenfield, B. K.; Mercury isotopes link mercury in San Francisco Bay forage fish to surface sediments. *Environ. Sci. Technol.* **2011**, *45* (4), 1264−1270; DOI: 10.1021/es103053y.
(47) Point, D.; Sonke, J. E.; Day, R. D.; Roseneau, D. G.; Hobson, K. A.; Vander Pol, S. S.; Moors, A. J.; Pugh, R. S.; Donard, O. F. X.; Becker, P. R. Methylmercury photodegradation influenced by sea-ice cover in Arctic marine ecosystems. *Nat. Geosci.* **2011**, *4*, 188−194.
(48) Dock, L.; Rissanen, R.-L.; Vahter, M. Demethylation and placental transfer of methyl mercury in the pregnant hamster. *Toxicology* **1994**, *94*, 131−142.
(49) Rowland, I. R. Interactions of the gut microflora and the host in toxicology. *Toxicol. Pathol.* **1988**, *16* (2), 147−153.
(50) Young, J. F.; Wosilait, W. D.; Luecke, R. H.; Analysis of methylmercury disposition in humans utilizing a PBPK model and animal pharmacokinetic data. *J. Toxicol. Environ. Health, Part A* **2001**, *63* (1).
(51) Suda, I.; Hirayama, K. Degradation of methyl and ethyl mercury into inorganic mercury by hydroxyl radical produced from rat liver microsomes. *Arch. Toxicol.* **1992**, *66*, 398−402.
(52) Vahter, M. E.; Mottet, N. K.; Friberg, L. T.; Lind, S. B.; Charleston, J. S.; Burbacher, T. M. Demethylation of methyl mercury in different brain sites of *Macaca fascicularis* monkeys during long-term subclinical methyl mercury exposure. *Toxicol. Appl. Pharmacol.* **1995**, *134*, 273−284.
(53) Farris, F. F.; Dedrick, R. L.; Allen, P. V.; Smith, J. C. Physiological model for the pharmacokinetics of methyl mercury in the growing rat. *Toxicol. Appl. Pharmacol.* **1993**, *119*, 74−90.
(54) Smith, J. C.; Allen, P. V.; Turner, M. D.; Most, B.; Fisher, H. L.; Hall, L. L. The kinetics of intravenously administered methyl mercury in man. *Toxicol. Appl. Pharmacol.* **1994**, *128*, 251−256.
(55) Gearhart, J. M.; Clewell, H. J. I.; Crump, K. S.; Shipp, A. M.; Silvers, A. Pharmokinetic dose estimates of mercury in children and dose-response curves of performance tests in a large epidemiological study. *Water, Air, Soil Pollut.* **1995**, *80*, 49−58.
(56) Miettinen, J. K., Absorption and elimination of dietary (Hg++) and methyl mercury in man. In *Mercury, Mercurial, and Mercaptans*; Miller, M. W., Clarkson, T. W., Eds.; Charles C. Thomas: Springfield, IL, 1973; pp 233−243.
(57) Aberg, A.; Ekman, L.; Rolf, F.; Greitz, U.; Persson, G.; Snihs, J. Metabolism of methylmercury (^{203}Hg) compounds in man. *Arch. Environ. Occup. Health* **1969**, *19*, 478−484.
(58) Sandheinrich, M. B.; Wiener, J. G., Methylmercury in freshwater fish: Recent advances in assessing toxicity of environmentally relevant exposures. In *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*; Beyer, W. N., Meador, J. P., Eds.; CRC Press: Boca Raton, FL, 2011; Vol. 2, pp 169−190.
(59) Wiener, J. G.; Spry, D. J., Toxicological significance of mercury in freshwater fish. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; Lewis: Boca Raton, FL, 1996; pp 297−339.
(60) Gonzalez, P.; Dominique, Y.; Massabuau, J. C.; Boudou, A.; Bourdineaud, J. P. Comparative effects of dietary methylmercury on gene expression in liver, skeletal muscle, and brain of the zebrafish (*Danio rerio*). *Environ. Sci. Technol.* **2005**, *39*, 3972−3980.
(61) Barst, B. D.; Gevertz, A. K.; Chumchal, M. M.; Smith, J. D.; Rainwater, T. R.; Drevnick, P. E.; Hudelson, K. E.; Hart, A.; Verbeck, G. F.; Roberts, A. P. Laser ablation ICP-MS co-localization of mercury and immune response in fish. *Environ. Sci. Technol.* **2011**, *45*, 8982−8988.