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Stable Mercury Isotopes in Polished Rice (*Oryza sativa* L.) and Hair from Rice Consumers

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Supporting Information

ABSTRACT: Mercury (Hg) isotopic signatures were characterized in polished rice samples from China, U.S., and Indonesia (n = 45). Hg isotopes were also analyzed in paired hair samples for participants from China (n = 21). For the latter, we also quantified the proportion of methylmercury intake through rice (range: 31−100%), and the weekly servings of fish meals (range: 0−5.6 servings/weekly). For these participants, 29% (n = 6) never ingested fish, 52% (n = 11) ingested fish < twice/weekly, and 19% (n = 4) ingested fish ≥ twice/weekly. In rice and hair, both mass-dependent fractionation (MDF, reported as δ^{202}Hg) and mass-independent fractionation (MIF, reported as Δ^{199}Hg) of Hg isotopes were observed. Compared to rice, hair δ^{202}Hg values were enriched on average (±1 standard deviation) by 1.9 ± 0.61‰, although the range was wide (range: 0.45‰, 3.0‰). Hair Δ^{199}Hg was significantly inversely associated with %methylmercury intake from rice (Spearman's rho = −0.61, p < 0.01, n = 21), i.e., as the proportion of methylmercury intake from rice increased, MIF decreased. Additionally, hair Δ^{199}Hg was significantly higher for participants ingesting fish ≥ twice/weekly compared to those who did not ingest fish or ingested fish < twice/weekly (ANOVA, p < 0.05, n = 21); Overall, results suggest that Hg isotopes (especially MIF) in human hair can be used to distinguish methylmercury intake from rice versus fish.

1. INTRODUCTION

Mercury (Hg) is a global pollutant and potent neurotoxin.[1] In the environment and during metabolism, Hg undergoes transformations that modify its toxicity.[1] Hg is comprised of seven stable isotopes (196−204 amu), which can be used to elucidate processes governing Hg transformations.[2,3] All Hg isotopes are subject to mass-dependent fractionation (MDF, reported as δ^{202}Hg), while the highest degree of mass-independent fractionation (MIF) occurs for two odd-isotopes (reported as Δ^{199}Hg and Δ^{201}Hg). MDF has been observed for various abiotic/biotic transformations.[4−6] In environmental samples, the MIF isotopic signature is most likely obtained during methylmercury (MeHg) photodegradation or Hg(II) photoreduction.[2,3,7]

Hg isotopes have been used to track MeHg trophic transfer in aquatic food webs,[8−16] and in human fish-eating populations.[17−21] Among fish consumers, hair δ^{202}Hg was enriched by ∼2‰ compared to seafood, suggesting MDF occurred during MeHg metabolism.[17−21] However, no significant MIF was observed during trophic transfer because photochemical reactions are the primary cause for MIF, as noted above.[2,3,7] The absence of MIF during metabolic processes suggests MIF may be used as a tool to trace MeHg sources in food webs.

Fish ingestion is considered the primary exposure pathway for MeHg; however, rice ingestion is also an important dietary source of MeHg.[22−26] To the best of our knowledge, just two studies reported Hg stable isotopes in rice, and both were for rice samples from Wanshan, China.[27,28] Compared to fish tissue, rice δ^{202}Hg and Δ^{199}Hg values were more negative, as follows. The

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maximum Δ^{199}Hg value reported for rice was +0.06‰,[27] compared to +5.73‰ for freshwater fish,[12] while the maximum value for rice δ^{202}Hg was −0.48‰, compared to approximately +1.5‰ for freshwater fish.[3] Higher δ^{202}Hg and Δ^{199}Hg values for fish are attributed to enhanced photochemical degradation of Hg in the water column, which differs from flooded paddy soil.[2,3,27,28]

In the present study, we characterized Hg isotopes in polished rice samples from three countries, including China, U.S., and three artisanal and small-scale gold mining (ASGM) locations in Indonesia. In addition, Hg isotopes are reported for hair samples from 21 pregnant mothers in China, who also donated rice samples from home, i.e., hair/rice samples were paired. For the entire cohort of pregnant mothers, we previously reported rice ingestion comprised on average 79% (median: 88%) of dietary MeHg intake, while fish ingestion comprised on average 21% (median: 12%) of MeHg intake (n = 398 mothers).[23] Total Hg (THg) and/or MeHg concentrations for rice were previously reported for locations in China[23,29] and the U.S..[30]

For Hg isotopes, we hypothesize that rice Hg MDF will differ between ASGM and non-ASGM sites due to differences in the environmental Hg sources. For hair and rice MDF, we hypothesize that trophic transfer may differ from other studies among seafood consumers[17−21] due to potential differences in Hg speciation in rice and seafood, and/or differences in the metabolism of rice and seafood.[24] Lastly, we hypothesize that hair MIF may distinguish the proportion of MeHg intake from rice versus fish.

2. MATERIALS AND METHODS

2.1. Rice Collection and Polishing. Rice samples were from Daxin, China (n = 21),[23] Wanshan, China (n = 8),[29] Arkansas, U.S. (n = 3),[30] and three ASGM (n = 13) locations in Indonesia (n = 45 total rice samples). Daxin is located in Guangxi province, and this area is considered noncontaminated for Hg.[23] Wanshan is located in Guizhou province, China, the site of the former Wanshan Hg mine, which officially closed in 2002.[29] Rice was also cultivated at the University of Arkansas Rice Research and Extension Center.[30] There were no local Hg point sources; rice samples for this analysis were harvested from rice fields, which were continuously flooded (n = 2) or drained one time early in the rice cultivation season (n = 1).[30] Thirteen rice samples from Indonesia were harvested from villages located in Bombana (n = 1), Cisitu (n = 6), and Pangkal Jaya Village (n = 6). ASGM has been documented in all three sites, including the use of ball-mills, where liquid Hg(0) is mixed with crushed ores to recover gold.[31] In two Indonesian sites (Bombana and Cisitu), rice grain was collected from households located approximately 5−7 km from ASGM activities, and these sites are hereafter referred to as the Indonesian background sites. In Pangkal Jaya Village, rice grain was collected directly from paddy fields located next to ASGM activities; hereafter referred to as the Indonesian ASGM site. In all sites, rice samples were retained in this analysis if the rice THg concentration was >10 ng/g, measured using ^{202}Hg isotopic signals (see Section 2.4).

Rice samples from Arkansas and the ASGM site in Indonesia were dehulled and polished as previously described,[30] using different polishing discs for high- and low-Hg rice. Rice samples from China and the Indonesian background sites were already hulled and polished. All rice samples were ground to a powder, using two different coffee grinders for high- and low-Hg rice. In addition, the polisher and grinders were cleaned between samples with ethanol to prevent carry-over of Hg.

2.2. Hair Collection and Washing. In Daxin, China, hair samples were paired with rice samples collected from the same participants (n = 21) (see section 2.1). The following protocols were reviewed and approved by the Institutional Review Boards at the University of South Carolina and XinHua Hospital (China). Pregnant women were recruited at parturition at the Maternal and Child Health Hospital in Daxin county, China. After providing informed consent, mothers donated a hair sample, and a family member brought a rice sample from home. The hair sample was cut from the occipital region using stainless steel scissors, the proximal end was tied with dental floss, and the sample was stored in a plastic bag at room temperature. Rice samples were stored frozen (−26 °C), and then at −80 °C. For the present study, the portion of hair corresponding to the second trimester was analyzed.[32] There was insufficient volume of hair to analyze MeHg.

Prior to Hg analysis, hair samples were washed to remove exogenous Hg, using methods previously described.[23] Briefly, porcelain dishes were soaked overnight in 1.2 N hydrochloric acid (HCl), then triple-rinsed in double-distilled H_2O (DDI-H_2O) (>18.0 MΩ cm^{-1}). Hair samples were weighed into acid-washed porcelain dishes, 50 mL of 0.1% (v/v) 2-mercaptoethanol were added, samples were gently shaken for 1 h, triple-rinsed using DDI-H_2O, air-dried overnight in a biosafety cabinet equipped with a HEPA (high efficiency particulate air) filter (Baker Company, Sanford, ME), and then double-bagged to prevent further Hg contamination.

2.3. Food Frequencies and Dietary MeHg Intake. During their hospital stay, mothers filled out a 102-item semiquantitative food frequency questionnaire including categories for rice, pork, other meats, eggs, fruits, vegetables, and seven varieties of fish (ocean fish, freshwater fish, shrimp, eel, other shellfish, snails, and crab), reflecting food intake during the third trimester.[33] Mothers chose from eight options ranging from "never" to "≥ twice per day", which were converted to servings/day, as previously described.[23] Mothers selected the portion size (g/serving) for rice from three pictures containing known quantities of rice and/or actual bowls. The portion size for ocean fish and freshwater fish was 170 g/serving, while the portion size for other fish/shellfish varieties was 100 g/serving.[23] Rice MeHg concentrations were determined (see section 2.4). THg concentrations were quantified for freshwater fish tissue purchased in Daxin markets (n = 13) (see section 2.4), and fish tissue THg concentrations were determined for the other six varieties of fish/shellfish from a comprehensive literature search (SI Table S1).[23] Rice MeHg intake and fish MeHg intake were calculated using the following equations; for eq 2, we assumed fish tissue THg was approximately equivalent to fish tissue MeHg.[1]

$$\text{rice MeHg intake}(\mu\text{g/day}) = \text{servings/day} \times \text{g/serving} \times \text{rice MeHg}(\mu\text{g/g}) \quad (1)$$

$$\text{fish MeHg intake}(\mu\text{g/day}) = \text{servings/day} \times \text{g/serving} \times \text{fish THg}(\mu\text{g/g}) \quad (2)$$

Total dietary MeHg intake (μg/day) was determined by adding eqs 1 and (2); the proportion of dietary MeHg intake attributed to rice or fish was also obtained.

2.4. Hg Analyses. *THg and MeHg.* THg concentrations for fish, hair, and most rice samples (n = 34/45) were measured using U.S. Environmental Protection Agency (EPA) Method

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Table 1. Summary Statistics for Mercury Concentrations in Polished Rice (n = 45) and Hair (n = 21)[a]

		sample size (n)	mercury sources	THg (ng/g) Mean ±1 SD (range)	MeHg (ng/g) Mean ±1 SD (range)	%MeHg (of THg) Mean ±1 SD (range)
rice	all	45	NA	32 ± 46 (8.2−200)	8.6 ± 4.5 (1.8−22)	49 ± 28 (4.8−96)
rice	Daxin, China[b]	21	background	14 ± 2.8 (9.5−21)	8.4 ± 2.7 (5.3−15)	64 ± 21 (28−96)
rice	Wanshan, China[b]	8	former Hg mine	20 ± 9.5 (10−38)	8.0 ± 3.6 (2.9−13)	42 ± 13 (23−60)
rice	Bombana and Cisitu, Indonesia	7	background	15 ± 5.6 (8.9−26)	4.6 ± 3.8 (1.8−12)	28 ± 14 (13−46)
rice	Pangkal Jaya Village, Indonesia	6	ASGM	140 ± 44 (100−200)	11 ± 5.2 (5.7−18)	8.1 ± 4.7 (4.8−17)
rice	Arkansas, U.S.[b]	3	background	18 ± 8.8 (8.2−25)	16 ± 7.2 (7.8−22)	91 ± 5.3 (86−96)
hair (trimester 2)	Daxin, China	21	background	1500 ± 570 (1030−3050)	NA	NA

[a]ASGM (artisanal and small-scale gold mining), Hg (mercury), MeHg (methylmercury), NA (not applicable), SD (standard deviation), THg (total mercury). [b]References: Daxin (MeHg),[23] Wanshan (THg and MeHg),[29] and Arkansas, USA (THg and MeHg).[30]

7473,[34] including thermal decomposition, amalgamation, and quantification by atomic absorption spectrophotometry (Lumex Model RA-915+/PYRO-915+, St. Petersburg, Russia). A subset of rice samples (n = 11) were measured using cold acid digestion (EPA 1631), as follows.[35] Rice samples (0.5 g) were digested overnight in 40 mL borosilicate glass bottles with Teflon-lined lids using 2.5 mL of HCl and nitric acid (HNO_3) (4:1 HCL:HNO_3 v/v). Then samples were oxidized overnight using 0.2 N bromine monochloride (BrCl) (0.5%). The following day, hydroxylamine hydrochloride (0.050 mL) was added to neutralize BrCl, then Hg(II) was reduced to Hg(0) using stannous chloride, and Hg was purged onto gold traps. THg concentrations were quantified using the Merx-T and cold vapor atomic fluorescence spectrometry (CVAFS) (Brooks Rand Instruments, Seattle, WA).[35]

Rice MeHg was extracted using methods from Liang et al.[36] and extracts were analyzed using EPA Method 1630.[37] Briefly, ~0.5 g rice were weighed into a 50 mL polypropylene vial and digested in 2 mL of 25% (w/v) potassium hydroxide-methanol in a 75 °C oven. Then 6 mL of dichloromethane and 1.5 mL of HCl were added, samples were shaken, centrifuged (4000 rpm = 3000g, 30 min), and phases were separated. The organic layer was transferred to a preweighed 50 mL polypropylene vial, then 35 mL of DDI-H_2O were added, and vials were heated for 1.5 h at 60−70 °C to remove dichloromethane. MeHg extracts were analyzed following ethylation with sodium tetraethylborate, purge and trap onto Tenax traps, and quantification by gas chromatography-CVAFS (Brooks Rand Instruments, Seattle, WA).[37]

For quality assurance/quality control for all data sets, see SI Table S2. For THg, recovery of five certified reference materials averaged 74−110% (n = 35) and the relative percent difference between replicates averaged 8.5% (n = 87). For MeHg, the average recovery of matrix spikes and two certified reference materials ranged from 69 to 96% (n = 103), and the relative percent between replicates averaged 13% (n = 74). The minimum detection levels were 0.0095 μg/g for hair THg, 0.002 ng/g for rice MeHg, 0.5 ng/g for rice THg (using EPA 7473), and 0.01 ng/g for rice THg (using EPA 1631). All results exceeded the detection levels.

Hg Isotopes. Rice (~0.4 g) and hair samples (~0.015 g) were digested in aqua regia [rice: 5 mL HNO_3, hair: 1.5 mL of HNO_3:HCl (3:1 v/v)] in a water bath at 85−95 °C for 1.5−2.5 h,[17,28] then 0.25 mL of 0.2 N BrCl were added at least 12 h before analysis to convert all Hg to Hg(II). Rice and hair digests were diluted using DDI-H_2O to a final concentration of 0.3−1.0 ng/mL and ~1 ng/mL, respectively, with 5−20% acid concentration. Just before analysis, hydroxylamine hydrochloride (0.05 mL) was added to remove excessive BrCl. Standard reference materials [IAEA-086 (human hair) and TORT-2 (lobster)] were prepared and analyzed using appropriate protocols, described above. THg concentrations were monitored by multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) using ^{202}Hg signals, which yielded mean recoveries for rice, hair, and standard reference materials of 100% (median: 98%, range: 77−134%, n = 73). The sensitivity for ^{202}Hg was 0.5−0.6 V per ng/mL Hg, and the ^{202}Hg signals were <0.04 V for blank solutions.

Hg isotopes were analyzed using a Neptune Plus MC-ICP-MS, as described by Yin et al.[38] Briefly, Hg(II) extracts were continuously mixed and reduced to Hg(0) with 3% tin chloride using a cold vapor generator, and volatile Hg(0) was separated by a frosted glass phase separator and introduced to the MC-ICP-MS with argon gas. Instrumental mass bias was corrected using an internal thallium (Tl) standard (NIST SRM 997, 20 ng/g Tl in 3% HCl) and sample-standard bracketing. The Hg concentrations and acid matrices of the bracketing standard (NIST SRM 3133) differed by <10% compared to the neighboring samples. MDF is expressed using the δ^{202}Hg notation (eq 3), while MIF is expressed as the difference between the measured δ^{xxx}Hg value, the value predicted based on MDF, and the δ^{202}Hg value (eqs 4-6).[39]

$$\delta^{202}\mathrm{Hg} = [{}^{202/198}\mathrm{Hg}_{\mathrm{sample}} / {}^{202/198}\mathrm{Hg}_{\mathrm{NIST3133}} - 1] \times 10^3‰ \quad (3)$$

$$\Delta^{199}\mathrm{Hg} \approx \delta^{199}\mathrm{Hg} - (\mathrm{d}^{202}\mathrm{Hg} \times 0.2520) \quad (4)$$

$$\Delta^{200}\mathrm{Hg} \approx \delta^{200}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times 0.5024) \quad (5)$$

$$\Delta^{201}\mathrm{Hg} \approx \delta^{201}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times 0.7520) \quad (6)$$

The UM-Almadén secondary standard solutions with similar Hg concentrations (0.3, 0.5, and 1.0 ng/mL) and acid matrices (10%) were measured once every 10 samples. Data uncertainties

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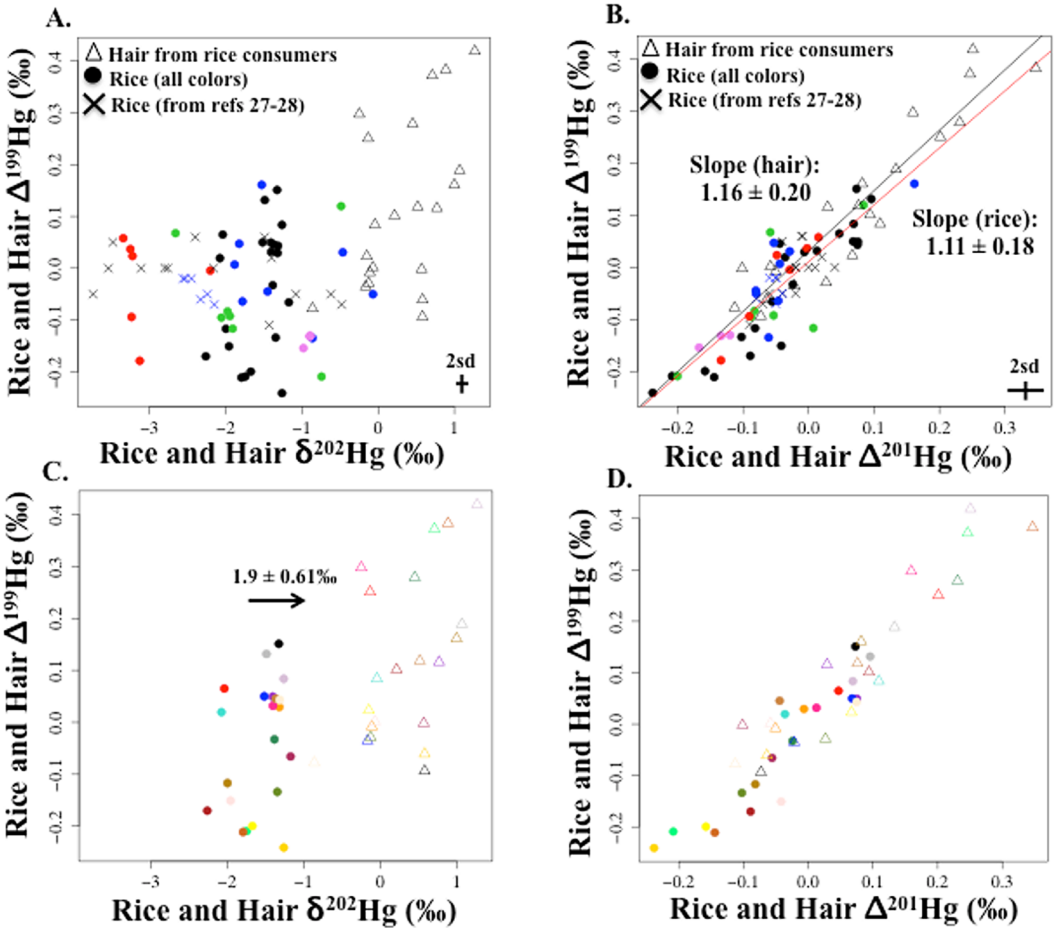

Figure 1. For rice and hair samples: (a) Δ^{199}Hg versus δ^{202}Hg and (b) Δ^{199}Hg versus Δ^{201}Hg, and simple linear regression (slope ±2 standard error) for rice (red line) and hair (black line) from this study. In graphs (a) and (b), rice from this study = closed circles (n = 45) and hair from this study = open triangles (n = 21). Legend for closed circles: black (Daxin, China), blue (Wanshan, China), green (Indonesian background sites), red (Indonesian artisanal and small scale gold mining sites), and pink (Arkansas, U.S.). Rice Hg isotope values from other studies include black ×'s[27] and blue ×'s.[28] Representative values for 2 standard deviations (sd) of analytical uncertainty measured for this study are shown in (a) and (b). Figures (c) and (d) are for the same parameters as in (a) and (b), respectively, including just the paired rice samples (closed circles) and hair samples (open triangles) from Daxin, China (n = 21 pairs) with corresponding colors for each pair. Figure 1c includes the difference between hair and rice δ^{202}Hg (average ±1 SD = 1.9 ± 0.61‰).

reflect the larger values of either the external precision of the replication of the UM-Almadén standard or the measurement uncertainty of standard reference materials. For UM-Almadén (n = 18) and standard reference materials, the overall average and uncertainty (±2 SD) agreed with previously reported results.[15,17,18,20,39] See SI Table S3 for all Hg isotope data.

Rice and hair THg and MeHg analyses were completed at the University of South Carolina, fish tissue THg was analyzed at Beijing Lumex Analytical Co. Ltd., China, and stable Hg isotopes were analyzed at the University of Wisconsin-Madison's State Laboratory of Hygiene.

2.5. Statistics. Bivariate associations between continuous variables were determined using Spearman's correlation or Pearson's correlation; for the latter, a $\log_{10}$-transformation was applied if the data elements were right-skewed. Differences between groups were compared using the Kruskal–Wallis test (for skewed variables) or one-way analysis of variance (ANOVA) (for normally distributed variables). Following ANOVA, pairwise differences were assessed using Sidak's test for multiple comparisons, and these p-values were reported in the text. Simple linear regression was used to assess the strength of the relationship between Δ^{199}Hg and Δ^{201}Hg.[2] An alpha-level of 0.05 was chosen as guide for significance. Stata 9.2 (College Station, Texas) and the R-platform were used for all statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Rice Hg. Concentrations of rice THg, rice MeHg, and rice percent MeHg (of THg) are reported in Table 1 and SI Table S3. Rice THg concentrations in the Indonesian ASGM site averaged 6.1–10 times higher compared to the four other sites, including two Indonesian background sites (Kruskal–Wallis, p < 0.01). Although ASGM was practiced at all three Indonesian sites, THg concentrations were elevated for rice harvested from paddies next to ASGM activities, and not from paddies located approximately 5–7 km away, suggesting contamination of rice paddies was somewhat localized. Rice MeHg concentrations averaged 1.5–3.5 times higher in Arkansas, compared to the other four sites (Kruskal–Wallis, p < 0.05). In addition, rice %MeHg (of THg) averaged 1.2–9.7 times higher in Arkansas compared to the other four sites (Kruskal–Wallis, p < 0.001). Although flooding-reflooding may result in higher soil MeHg,[24] the Arkansas rice samples were from fields that were continuously flooded or drained one time early in the season

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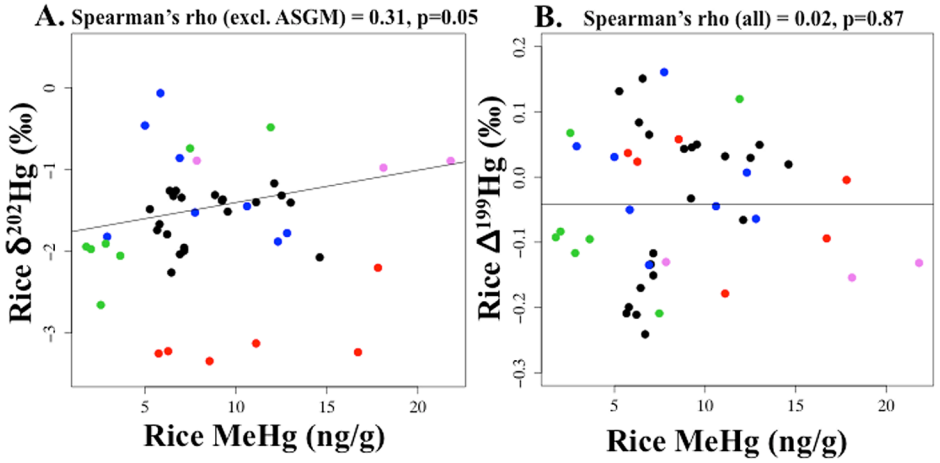

Figure 2. (a) Rice δ^{202}Hg versus rice methylmercury (MeHg) and (b) Rice Δ^{199}Hg versus rice methylmercury (MeHg). For rice: black (Daxin, China), blue (Wanshan, China), green (background sites in Indonesia), red [artisanal and small scale gold mining (ASGM) sites in Indonesia], and pink (Arkansas, U.S.). In Figure 2a, when rice from ASGM sites were included, Spearman's rho = 0.16, p = 0.30, n = 45.

(Section 2.1), which was similar to the hydrology used in China and Indonesia. Instead, higher rice MeHg in Arkansas possibly reflected differences in soil organic content, iron content, or other environmental factors that influenced microbial Hg methylation.[24] Rice THg and MeHg were positively correlated using Spearman's correlation (Spearman's rho = 0.41, $p < 0.01$, n = 45), and using Pearson's correlation, when variables were $\log_{10}$-transformed (Pearson's rho = 0.37, p = 0.02, n = 45).

Excluding the Indonesian ASGM site, all rice THg concentrations were within the range reported for global nonpolluted sites (range: 1.0−45 ng/g),[24] including rice samples from Wanshan, China (Table 1). Many studies from Wanshan reported higher rice THg concentrations; however, most rice samples were collected from paddies near the former Hg mine or near active Hg smelters.[24] Rice samples included in this analysis were originally from a feasibility pilot among pregnant women, who lived throughout Wanshan District, including less-contaminated areas.[29] For all rice samples, 24% (=11/45) of the rice MeHg concentrations were within the range reported for global nonpolluted sites (range: 0.86−5.8 ng/g),[24] including one sample from the Indonesian ASGM site.

3.2. Rice MDF and MIF. Rice δ^{202}Hg averaged (±1 SD) −1.69 ± 0.54‰ (range: −3.3‰, −0.07‰, n = 45) (Figure 1ac, SI Table S3). Rice Δ^{199}Hg and Δ^{201}Hg averaged (±1 SD) −0.04 ± 0.11‰ and −0.05 ± 0.09‰, respectively (range for both: −0.24‰, 0.16‰, n = 45) (Figure 1bd). The range for MIF (0.40‰) was narrow compared to the range for δ^{202}Hg (3.23‰). No significant MIF of Δ^{200}Hg was observed for rice (average ±1 SD: 0.00 ± 0.05‰). Rice Hg isotopes from this study were comparable to values for rice from Feng et al.[27] and Yin et al.,[28] which were included in Figures 1ab.

Rice samples from the Indonesian ASGM site had significantly lower δ^{202}Hg values (mean ±1SD: −3.1 ± 0.43‰, range: −3.3‰, −2.2‰, n = 6), compared to the other four sites, including the Indonesian background sites (mean ±1SD: −1.5 ± 0.54‰, range: −2.7‰, −0.07‰, n = 39) (ANOVA, $p < 0.0001$ for all pairwise associations), while no significant differences were observed between sites for both Δ^{199}Hg and Δ^{201}Hg (ANOVA, p = 0.42−1.0 for all pairwise associations). δ^{202}Hg values observed in this study for the Indonesian ASGM site were similar to δ^{202}Hg values for rice samples from two active Hg mining sites in Wanshan, China.[27]

MDF occurs during incorporation of Hg by rice;[28] however, uptake of Hg was not expected to differ between these locations. Instead, significantly lower δ^{202}Hg values in the Indonesian ASGM rice samples suggested higher incorporation of Hg(0) by two potential pathways, i.e., through the atmosphere or through the soil, as follows. ASGM miners use liquid Hg(0) to amalgamate gold particles, and the Hg-gold amalgamate is heated at a high temperature to release Hg(0). Rice paddies located next to ASGM activities were also possibly irrigated with Hg-laden runoff. Estrade et al.[4] reported heating of liquid Hg(0) volatilized the lighter Hg isotopes, i.e., for vapor collected following evaporation of liquid Hg(0), δ^{202}Hg values averaged (±2 SE) −6.65 ± 0.28‰ at 22 °C, while at 100 °C δ^{202}Hg values for vapor averaged (±2 SE) −0.79 ± 0.22‰. Significantly lower rice δ^{202}Hg values in the ASGM sites compared to the other four locations suggested higher incorporation of liquid Hg(0), which was not likely incinerated, and instead, was accumulated from the paddy soil. Alternatively, significantly lower δ^{202}Hg values possibly reflected higher incorporation of atmospheric Hg. Lower δ^{202}Hg values were reported in precipitation collected near coal-fired power plants compared to distant sites,[40] suggesting more negative δ^{202}Hg values in polluted air. Most Hg (~80%) in rice seeds is accumulated from the soil, while a smaller fraction of Hg originates from the atmosphere.[28] Both pathways possibly contributed to significantly lower δ^{202}Hg values in the ASGM site compared to the other locations.

Rice δ^{202}Hg was positively correlated with rice MeHg, excluding rice from the ASGM Indonesian site (Figure 2a, n = 39). When the ASGM site was included, Spearman's correlation was attenuated from 0.31 (p = 0.05, n = 39) to 0.16 (p = 0.30, n = 45). This positive association (excluding the ASGM site) was possibly due to fractionation of δ^{202}Hg during microbial Hg(II) methylation, reflecting preferential microbial methylation of lighter isotopes.[6,41,42] Conversely, rice Δ^{199}Hg was not correlated with rice MeHg (Figure 2b). This was not surprising because MIF is not produced by biotransformations, including microbial methylation/demethylation.[5,13,41] Using rice %MeHg (of THg) instead of rice MeHg, a positive correlation was observed between rice δ^{202}Hg and rice %MeHg (of THg) (when all data were included), while there was no correlation between Δ^{199}Hg and rice %MeHg (of THg) (SI Figure S1), similar to Feng et al.[27]

In environmental samples, the MIF isotopic signature is mainly obtained through MeHg photodegradation or Hg(II)

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photoreduction.[2] The strength of the relationship between ^{199}Hg and ^{201}Hg is used to distinguish between the two mechanisms.[2] In the present study, the rice Δ^{199}Hg:Δ^{201}Hg slope (±2 SE) was 1.11 ± 0.18 (r-squared = 0.78, n = 45) (Figure 1b), which was similar to the slope reported for Hg(II) photoreduction experiments (mean ±2 SE: 1.00 ± 0.02).[2] This differs from the slope for experimental MeHg photodegradation (1.36 ± 0.02‰, 2 SE),[2] which was also observed in fish tissue and biota (Δ^{199}Hg:Δ^{201}Hg slope: 1.26−1.32).[2,8,15,16] Results suggested that Hg accumulated in rice grain had undergone Hg(II) photoreduction rather than MeHg photodegradation, which was also reported for rice paddy soil, rice roots, leaves, stems, and seeds (Δ^{199}Hg:Δ^{201}Hg mean: ∼ 1.0) by Yin et al.[28]

3.3. Hair THg. For 21 pregnant mothers in Daxin, hair THg (trimester 2) averaged (±1SD) 1.5 ± 0.57 μg/g (range: 1.03 μg/g, 3.05 μg/g), which was higher than hair THg (trimester 3) reported for the entire cohort (0.48 ± 0.26 μg/g, range: 0.08 μg/g, 1.70 μg/g, n = 398).[23] For this analysis, we included rice samples with THg concentrations >10 ng/g, and therefore retained participants with higher hair THg. Hair THg concentrations for the second and third trimesters were significantly positively correlated, when $\log_{10}$-transformed (Pearson's rho = 0.47, p < 0.05, n = 21). Using Spearman's correlation, hair THg concentrations were positively correlated, but not significantly (Spearman's rho = 0.40, p = 0.08, n = 21).

3.4. Rice and Fish MeHg Intake. For these 21 mothers from Daxin, rice was the main but not exclusive dietary source for MeHg. Most mothers (86%) ate rice daily, averaging 1.8 meals/daily (median: 2.5 servings/daily, range: 0.08−2.5 servings/daily), while mothers ingested on average 1.0 fish meal/weekly (median: 0.21 meals/weekly, range: 0−5.6 meals/weekly), including six mothers (29%) who never ate fish, 11 mothers (52%) who ingested fish < twice/weekly, and four mothers (19%) who ingested fish ≥ twice/weekly (SI Table S3). In this rural inland region, freshwater fish, and shrimp were ingested most frequently (weekly ingestion by 13 and five mothers, respectively), while ocean fish, crab, and snails were ingested weekly by one mother each, and eel was never consumed. Using eqs 1 and (2), the average %MeHg intake from rice was 80% (median: 87%, range: 31−100%), while the average %MeHg intake from fish was 20% (median: 13%, range: 0−69%).

3.5. Hair MDF and MIF. Hair δ^{202}Hg averaged (±1 SD) 0.32 ± 0.54‰ (range: −0.86‰, 1.27‰) (Figure 1a, SI Table S3). Hair Δ^{199}Hg and Δ^{201}Hg averaged (±1 SD) 0.12 ± 0.16‰ (range: −0.09‰, 0.42‰) and 0.07 ± 0.13‰ (range: −0.11‰, 0.35‰), respectively (see Figure 1 for hair Δ^{199}Hg). The range for hair δ^{202}Hg was 2.13‰, while the range for hair Δ^{199}Hg and Δ^{201}Hg was narrow (0.51‰ and 0.46‰, respectively). No significant MIF of ^{200}Hg was observed for hair samples (average ±1 SD: 0.00 ± 0.05‰).

In previous studies among fish consumers, researchers utilized δ^{202}Hg in human biomarkers to investigate biotransformation and accumulation of MeHg.[17−21] For δ^{202}Hg, approximately +2‰ increase was reported in hair δ^{202}Hg relative to the dominant seafood for several cohorts, including Bolivian Esse Ejjas native people (offset: 2.0 ± 0.2‰),[17] a French cohort (2.2 ± 0.8‰),[18] U.S. dentists (∼2‰),[20] Faroese whalers (1.75‰),[19] and Gulf of Mexico anglers who predominantly consumed ocean fish (offset: 1.98−2.30‰).[19] In the latter study, the δ^{202}Hg offset varied from 1.4 to 3.2‰ for consumers of coastal fish, freshwater fish, and shellfish; the authors suggested this range of values potentially reflected differences in MeHg metabolism, discrepancies is dietary recall, or lower %MeHg (of THg) for these varieties of seafood compared to ocean fish or pilot whale.[19]

In the present study, the mean difference (±1 SD) in δ^{202}Hg values between paired hair and rice samples was 1.9 ± 0.61‰ (range: 0.45‰, 3.0‰) (Figure 1c). The offset range was wider than observed for most previous studies among seafood consumers,[17−20] which possibly reflected ingestion of fish, in addition to rice. However, for mothers reporting no fish consumption (n = 6), the δ^{202}Hg offset averaged 1.7 ± 0.91% and the range did not change (range: 0.45‰, 3.0‰). Variability in the δ^{202}Hg offset possibly reflected differences in rice %MeHg (of THg), which ranged from 28 to 96% for Daxin (SI Table S3), as previously suggested.[19] From Figure 2a, δ^{202}Hg increased as rice MeHg increased; therefore higher rice %MeHg (of THg) would likely be more enriched δ^{202}Hg. We found the δ^{202}Hg offset and rice %MeHg (of THg) were significantly inversely correlated (Spearman's rho = −0.56, p < 0.01, n = 21) (Figure 3).

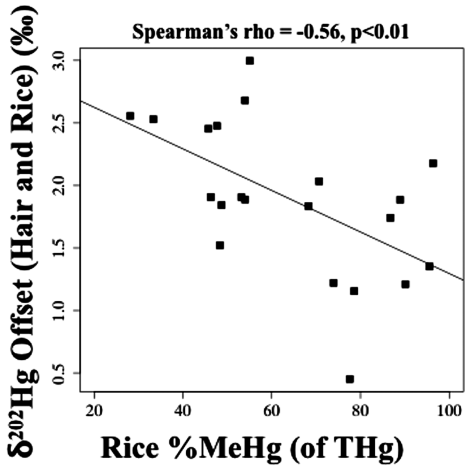

Figure 3. δ^{202}Hg offset (=hair δ^{202}Hg - rice δ^{202}Hg) versus rice % methylmercury (MeHg) [of total mercury (THg)] (n = 21).

This was consistent with the premise that MeHg is more enriched in δ^{202}Hg compared to inorganic Hg. Similar results were reported for tissues in whales and seals,[13] and for invertebrates in upland forest sites.[43] Preferential uptake of MeHg and excretion of inorganic Hg combined with in vivo demethylation of MeHg in the human body may lead to the larger offset in δ^{202}Hg values observed here among consumers that have higher inorganic Hg in their rice.

For Δ^{199}Hg (as well as Δ^{201}Hg), researchers reported no significant differences between the MIF signature of hair and the dominant seafood, and suggested the MIF isotopic signature was conserved during trophic transfer between seafood and seafood consumers.[17−21] However, from Figure 1c, six participants had higher Δ^{199}Hg values for hair compared to rice. Of the six participants, four ingested fish ≥ twice/weekly, one ingested fish < twice/weekly, and one did not ingest fish. We did not retain fish tissue for measurement of Hg isotopes; however, as noted in the Introduction, the magnitude for Δ^{199}Hg values in fish tissue is much higher compared to rice (maximum Δ^{199}Hg for rice: + 0.16‰ from this study, SI Table S3; maximum for freshwater fish: + 5.73‰[12]). Using the proportion of MeHg intake from rice, we found that hair Δ^{199}Hg was significantly inversely correlated with the %MeHg intake from rice (Spearman rho = −0.61, p < 0.01, n = 21) (Figure 4a). Using the number of fish meals, we also found participants consuming fish ≥ twice/weekly

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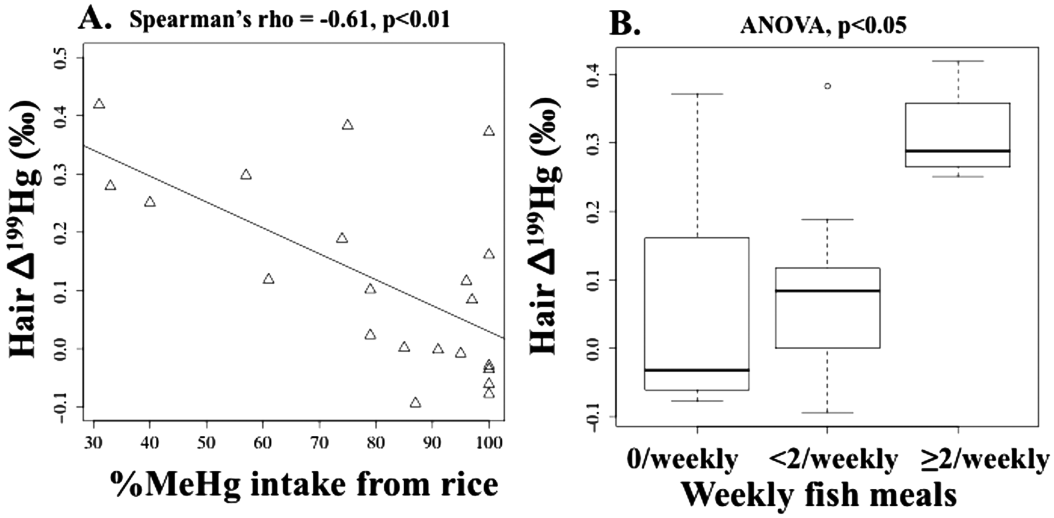

Figure 4. (a) Hair Δ^{199}Hg versus %methylmercury (MeHg) [of total mercury (THg)] intake from rice, and (b) Hair Δ^{199}Hg versus number of fish meals/weekly (0/weekly, < 2/weekly, ≥ 2/weekly).

had hair Δ^{199}Hg values that were significantly higher compared to most mothers who did not consume fish or ingested fish less often (ANOVA, $p < 0.05$ for both, $n = 21$) (Figure 4b). To interpret, as the proportion of dietary MeHg intake from rice increased, hair MIF decreased; similarly, for mothers ingesting fish ≥ twice/weekly, hair MIF increased compared to mothers ingesting less fish or no fish. Results suggest that Hg isotopes (especially MIF) in human hair can be used to distinguish MeHg intake from rice versus fish. We also considered whether the variability in hair Δ^{199}Hg was due to differences in rice %MeHg (of THg); however, this bivariate association was inverse but nonsignificant (Spearman's rho = −0.30, $p = 0.19$, $n = 21$).

In conclusion, stable Hg isotopes were measured in rice and hair samples. Although rice MeHg concentrations are lower compared to fish, rice ingestion is an important dietary source of MeHg.[24] The Hg isotopic composition in rice differs from fish, reflecting different Hg accumulation pathways.[28] In this study, rice δ^{202}Hg values were significantly lower for rice from the Indonesian ASGM site compared to other locations, including other Indonesian sites 5−7 km away, potentially reflecting uptake of Hg(0) through the soil or atmosphere. For rice consumers, the average offset (1.9‰) between rice and hair δ^{202}Hg was similar to other studies among seafood consumers.[17−20] However, the offset range (range: 0.45‰, 3.0‰) was wider in our study, which was likely due in part to the range of values for rice %MeHg (of THg) (range: 28−96%). In addition, Δ^{199}Hg was inversely correlated with the %MeHg intake from rice, and significantly higher for participants ingesting fish meals ≥ twice/weekly, compared to participants who did not ingest fish or ingested fish less frequently, suggesting the MIF isotopic signature was conserved, which was also reported for seafood consumers.[17−20] Therefore, the Hg MIF isotopic signature may be used to distinguish between these two dietary sources of MeHg (rice and fish). These results may be useful for future studies concerning MeHg exposure among rice consumers.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b01039.

Fish tissue THg (Table S1), quality assurance for THg and MeHg measurements (Table S2), Hg isotopes for rice and hair (Table S3), and bivariate scatterplots for Hg isotopes (rice δ^{202}Hg and rice Δ^{199}Hg) versus rice %MeHg (of THg) (Figure S1) (PDF)

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The authors declare no competing financial interest.

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