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 Δ²⁰⁵Hg) and mass-independent fractionation (MIF, reported as Δ¹⁹⁹Hg) of Hg isotopes were observed. Compared to rice, hair δ²⁰⁵Hg values were enriched on average ±1 standard deviation) by 1.9 ± 0.61‰, although the range was wide (range: 0.45‰–3.0‰). Hair Δ¹⁹⁹Hg 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 Δ¹⁹⁹Hg 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 Δ²⁰⁵Hg), while the highest degree of mass-independent fractionation (MIF) occurs for two odd-isotopes (reported as Δ¹⁹⁹Hg and Δ²⁰¹Hg). MDF has been observed for various abiotic/biotic transformations.¹⁴–¹⁶ 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 δ²⁰⁵Hg 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 δ²⁰⁵Hg and Δ¹⁹⁹Hg values were more negative, as follows. The
maximum $\Delta^{199}\text{Hg}$ value reported for rice was $+0.06\%_{e}$,27 compared to $+5.73\%_{e}$ for freshwater fish,12 while the maximum value for rice $\delta^{199}\text{Hg}$ was $-0.48\%_{e}$, compared to approximately $+1.5\%_{e}$ for freshwater fish.5 Higher $\delta^{199}\text{Hg}$ and $\Delta^{199}\text{Hg}$ 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 China2,29 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 consumers17–21 due to potential differences in Hg speciation in rice and seafood, and/or differences in the metabolism of rice and seafood.22 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 $^{203}\text{Hg}$ 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 ($-26\degree C$), and then at $-80\degree C$. 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.25 Briefly, porcelain dishes were soaked overnight in 1.2 N hydrochloric acid (HCl), then triple-rinsed in double-distilled H$_2$O (DDI-H$_2$O) ($>18.0$ 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$_2$O, 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.33 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.34 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).34 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.$^3$

\[
\text{rice MeHg intake (μg/day)} = \text{servings/day} \times \text{g/serving} \times \text{rice MeHg(μg/g)}
\]

(1)

\[
\text{fish MeHg intake (μg/day)} = \text{servings/day} \times \text{g/serving} \times \text{fish THg(μg/g)}
\]

(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
Article

7473, 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.37 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 (HNO₃) (4:1 HCl:HNO₃ 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 0.002 ng/g for rice MeHg, 0.5 ng/g for rice THg (using EPA Method 1630). Brieﬂy, rice samples (0.5 g) were digested using the Merx-T and cold vapor atomic ﬂuorescence spectrometry (CVAFS) (Brooks Rand Instruments, Seattle, WA).35

Table 1. Summary Statistics for Mercury Concentrations in Polished Rice (n = 45) and Hair (n = 21)4

| Sample | 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    | NA              | 32 ± 46                       | 8.6 ± 4.5                     | 49 ± 28                          |
|        | (8.2–200)       | (1.8–22)                      | (4.8–96)                      |                                  |
| Daxin, China | background | 14 ± 2.8                      | 8.4 ± 2.7                     | 64 ± 21                          |
| Wanshan, China | background | 15 ± 5.6                      | 4.6 ± 3.8                     | 28 ± 14                          |
| Bombana and Cisitu, Indonesia | background | 20 ± 9.5                      | 8.0 ± 3.6                     | 42 ± 13                          |
| Pangkal Jaya Village, Indonesia | background | 10–38                         | 2.9–13                        | 23–60                            |
| Arkansas, U.S. | background | 15 ± 5.6                      | 4.6 ± 3.8                     | 28 ± 14                          |
| Hair   |                 | 140 ± 44                      | 11 ± 5.2                      | 8.1 ± 4.7                        |

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

4682

δ²⁰²Hg = [δ²⁰²Hg\text{sample}/δ²⁰²Hg\text{NIST3133} − 1] × 10³‰

Δ²⁰⁹⁹Hg \approx δ²⁰⁹⁹Hg − (d²⁰²Hg × 0.2520)

Δ²⁰⁰⁹⁹Hg \approx δ²⁰⁰⁹⁹Hg − (d²⁰²Hg × 0.5024)

Δ²⁰¹⁰¹Hg \approx δ²⁰¹⁰¹Hg − (d²⁰²Hg × 0.7520)

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...
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 log10-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 Δ199Hg and Δ201Hg.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.
(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.25 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 log10-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 rice samples from the Indonesian ASGM site.

### 3.2. Rice MDF and MIF

Rice \( ^{202}\text{Hg} \) averaged (±1 SD) −1.69 ± 0.54‰ (range: −3.3‰, −0.07‰, \( n = 45 \)) (Figure 1ac, SI Table S3). Rice \( ^{198}\text{Hg} \) and \( ^{202}\text{Hg} \) 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 \( ^{202}\text{Hg} \) (3.23‰). No significant MIF of \( ^{200}\text{Hg} \) 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.,34 which were included in Figures 1ab.

Rice samples from the Indonesian ASGM site had significantly lower \( ^{202}\text{Hg} \) 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‰, −1.07‰, \( n = 39 \)) (ANOVA, \( p < 0.0001 \) for all pairwise associations), while no significant differences were observed between sites for both \( ^{198}\text{Hg} \) and \( ^{202}\text{Hg} \) (ANOVA, \( p = 0.42 \)−1.0 for all pairwise associations). \( ^{202}\text{Hg} \) values observed in this study for the Indonesian ASGM site were similar to \( ^{202}\text{Hg} \) values for rice samples from two active Hg mining sites in Wanshan, China.27

MDF occurs during incorporation of Hg by rice;26 however, uptake of Hg was not expected to differ between these locations. Instead, significantly lower \( ^{202}\text{Hg} \) 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), \( ^{202}\text{Hg} \) values averaged (±2 SE) −6.65 ± 0.28‰ at 22 °C, while at 100 °C \( ^{202}\text{Hg} \) values for vapor averaged (±2 SE) −0.79 ± 0.22‰. Significantly lower rice \( ^{202}\text{Hg} \) 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 \( ^{202}\text{Hg} \) values possibly reflected higher incorporation of atmospheric Hg. Lower \( ^{202}\text{Hg} \) values were reported in precipitation collected near coal-fired power plants compared to distant sites,40 suggesting more negative \( ^{202}\text{Hg} \) 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 \( ^{202}\text{Hg} \) values in the ASGM site compared to the other locations.

Rice \( ^{202}\text{Hg} \) 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 \( ^{202}\text{Hg} \) during microbial Hg(II) methylation, reflecting preferential microbial methylation of lighter isotopes.6,41,42 Conversely, rice \( ^{199}\text{Hg} \) 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 \( ^{202}\text{Hg} \) and rice %MeHg (of THg) (when all data were included), while there was no correlation between \( ^{198}\text{Hg} \) 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)
photoreduction. The strength of the relationship between $^{199}\text{Hg}$ and $^{202}\text{Hg}$ is used to distinguish between the two mechanisms.2 In the present study, the rice $\Delta^{199}\text{Hg}:\Delta^{202}\text{Hg}$ slope ($\pm 2 \text{ SE}$) was $1.11 \pm 0.18$ ($r$-squared = 0.78, $n = 45$) (Figure 1b), which was similar to the slope reported for Hg(II) photoreduction experiments ($\pm 2 \text{ SE} = 1.00 \pm 0.02$).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 ($\Delta^{199}\text{Hg}:\Delta^{202}\text{Hg}$ 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 ($\Delta^{199}\text{Hg}:\Delta^{201}\text{Hg}$ mean = $\pm 1.0$) by Yin et al.28

### 3.3. Hair THg

For 21 pregnant mothers in Daxin, hair THg (trimester 2) averaged ($\pm 1 \text{ SD}$) 1.5 ± 0.57 µg/g (range: 1.03–6.25 µg/g, 3.05 ± 0.35 µg/g), which was higher than hair THg (trimester 3) reported for the entire cohort (0.48 ± 0.26 µg/g, range: 0.08–1.53 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 $r = 0.47, p < 0.05, n = 21$). Using Spearman correlation, hair THg concentrations were positively correlated, but not significantly (Spearman’s $r = 0.40, p = 0.08, n = 21$).

### 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/day (median: 2.5 servings/daily, range: 0.8–2.5 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 $< 2$ times/weekly, and four mothers (19%) who ingested fish $\geq 2$ times/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 $\delta^{202}\text{Hg}$ averaged ($\pm 1 \text{ SD}$) 0.32 ± 0.54‰ (range: $-0.86$‰ to 1.27‰) (Figure 1a, SI Table S3). Hair $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ averaged ($\pm 1 \text{ SD}$) 0.12 ± 0.16‰ (range: $-0.09$‰ to 0.42‰) and 0.07 ± 0.13‰ (range: $-0.11$‰ to 0.35‰), respectively (see Figure 1a for hair $\Delta^{199}\text{Hg}$). The range for hair $\delta^{202}\text{Hg}$ was 2.13‰, while the range for hair $\Delta^{199}\text{Hg}$ and $\Delta^{201}\text{Hg}$ was narrow (0.51‰ and 0.46‰, respectively). No significant MIF of $^{202}\text{Hg}$ was observed for hair samples (average $\pm 1 \text{ SD}$: 0.00 ± 0.05‰).

In previous studies among fish consumers, researchers utilized $\delta^{202}\text{Hg}$ in human biomarkers to investigate biotransformation and accumulation of MeHg.17–21 For $\delta^{202}\text{Hg}$, approximately +2‰ increase was reported in hair $\delta^{202}\text{Hg}$ relative to the dominant seafood for several cohorts, including Bolivian Esse Ejas native people (offset: $2.0 \pm 0.2$‰),17 a French cohort (2.2 ± 0.8‰,15,16 U.S. dentists (∼2‰),17 Faroese walruses (1.75‰),19 and Gulf of Mexico anglers who predominantly consumed ocean fish (offset: $1.98 \pm 2.3$‰).19 In the latter study, the $\delta^{202}\text{Hg}$ 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 ($\pm 1 \text{ SD}$) in $\delta^{202}\text{Hg}$ values between paired hair and rice samples was $1.9 \pm 0.61$‰ (range: 0.45‰ to 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 $\delta^{202}\text{Hg}$ offset averaged 1.7 ± 0.91‰ and the range did not change (range: 0.45‰ to 3.0‰). Variability in the $\delta^{202}\text{Hg}$ 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, $\delta^{202}\text{Hg}$ increased as rice MeHg increased; therefore higher rice %MeHg (of THg) would likely be more enriched $\delta^{202}\text{Hg}$. We found the $\delta^{202}\text{Hg}$ offset and rice %MeHg (of THg) were significantly inversely correlated (Spearman’s $r = -0.56, p < 0.01, n = 21$ (Figure 3).

This was consistent with the premise that MeHg is more enriched in $\delta^{202}\text{Hg}$ compared to inorganic Hg. Similar results were reported for tissues in whales and seals,16 and for invertebrates in upland forest sites.18 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 $\delta^{202}\text{Hg}$ values observed here among consumers that have higher inorganic Hg in their rice.

For $\Delta^{199}\text{Hg}$ (as well as $\Delta^{201}\text{Hg}$), 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 $\Delta^{199}\text{Hg}$ values for hair compared to rice. Of the six participants, four ingested fish $\geq 2$ times/weekly, one ingested fish $< 2$ times/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 $\Delta^{199}\text{Hg}$ values in fish tissue is much higher compared to rice (maximum $\Delta^{199}\text{Hg}$ for rice: +0.16‰ from this study, SI Table S3; maximum for freshwater fish: +5.73‰). Using the proportion of MeHg intake from rice, we found that hair $\Delta^{199}\text{Hg}$ 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 $\geq 2$ times/weekly
had hair $\Delta^{199}\text{Hg}$ 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 $\geq$ 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 $\Delta^{199}\text{Hg}$ 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. The Hg isotopic composition in rice differs from fish, reflecting different Hg accumulation pathways. In this study, rice $\delta^{202}\text{Hg}$ 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 $\delta^{202}\text{Hg}$ was similar to other studies among seafood consumers. 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, $\Delta^{199}\text{Hg}$ was inversely correlated with the %MeHg intake from rice, and significantly higher for participants ingesting fish meals $\geq$ 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. 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.

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**Notes**
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