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Kinetics and metabolism of mercury in rats fed with mercury contaminated rice using mass balance and mercury isotope approach

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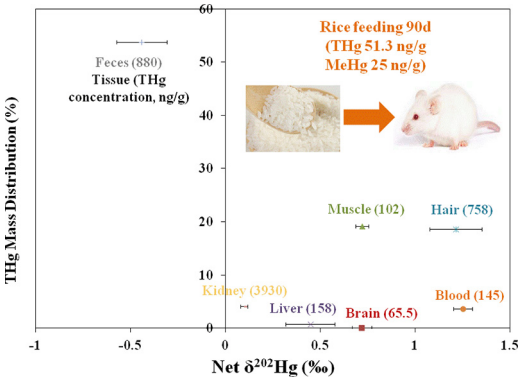
HIGHLIGHTS

- A mass balance and isotope model in rats was established to investigate mercury (Hg) toxicokinetics.
- Overall 80% of feeding total Hg were recovered and only 32% of feeding methyl Hg were recovered in rats.
- Positive net fractionations of δ^{202}Hg were observed in hair and blood samples and negative fractionations in feces (−0.44‰).
- Demethylation of methyl Hg in the intestine was the important detoxification process in rat body.

GRAPHICAL ABSTRACT



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ABSTRACT

Consumption of mercury (Hg) contaminated rice can be a major environmental health issue but the toxicokinetics is not well known. Hg isotopes have been shown to be good tracers in studying Hg exposure and metabolic processes. We established a Hg mass balance and Hg isotope model in rats fed with Hg contaminated rice (THg 51.3 ng/g; MeHg 25 ng/g) for 90 days to investigate Hg toxicokinetics. Overall 80% of feeding THg was recovered in rat body and excrement, while the excrement accounted for 55% of total observed THg in rats. Feces were the main route of Hg elimination in rats, while urinary excretion was negligible. However, only 32% of utilized MeHg was recovered in rats, indicating significant demethylation of MeHg in rat body. Positive net fractionations of δ^{202}Hg (relative to the feeding rice) were observed in hair and blood samples (1.21‰ and 1.25‰, respectively), which have similar trend with the results obtained in human hair study, exhibiting higher δ^{202}Hg values (2‰– 3‰) than consumed fish and rice. Most importantly, we observed negative net fractionations in feces (−0.44‰), which confirmed the missed Hg with negative δ^{202}Hg signal. We concluded that mass balance and Hg isotope are useful tools for quantifying toxicokinetics of Hg. Demethylation of MeHg in the intestine were the important detoxification process in rat body characterizing with negative net Hg fractionations in feces.

1. Introduction

Gaseous elemental mercury (Hg) is the dominant form of Hg in the atmosphere and exhibits a long residence time, which can predispose it to

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long-range transport (Gustin et al., 2015). Therefore, Hg is considered as a global pollutant (Si and Ariya, 2018). Methylmercury (MeHg) is the major organic form of Hg and it can be bio-accumulated and bio-magnified in the aquatic food chain and pose a serious environmental health concern (Knightes et al., 2009). Consumption of fish is generally regarded as the main exposure pathway of MeHg in humans (Ginsberg and Toal, 2009). However, recent studies have shown that rice grown in Hg polluted sites can accumulate high levels of MeHg and the MeHg contaminated rice can be an important source of MeHg for the local residents (Feng et al., 2008; Zhang et al., 2010; Li et al., 2012).

Hg toxicity depends on its chemical forms. MeHg is more toxic to humans than inorganic Hg (IHg). Approximately 95% of ingested MeHg can be absorbed into the bloodstream, where it enters red blood cells and binds to hemoglobin (NRC, 2000). MeHg can then be transported to various body tissues, and even cross the blood-brain and placental barriers via a MeHg-L-cysteine complex (Clarkson and Magos, 2006). MeHg demethylation in vivo takes place principally in the liver and intestines (Wang et al., 2013; Feng et al., 2015). The major routes of Hg excretion in humans are the feces and urine in the form of IHg (NRC, 2000).

Hg stable isotopes may provide new insights into the biogeochemical cycle of Hg in the environment (Blum et al., 2014). Mass dependent fractionation (MDF, denoted δ^{202}Hg) occurs during physical, chemical, and biological processes (Bergquist and Blum, 2007; Zheng and Hintelmann, 2009). In contrast, mass independent fractionation (MIF, denoted Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg) only occurs via specific pathways, such as photochemical processes (Blum et al., 2014). Hg isotopes, therefore, can provide information on the sources and biogeochemical fate of Hg in the environment (Blum et al., 2014), including insights into the trophic transfer of Hg in fish and mammals (Perrot et al., 2010, 2012; Kwon et al., 2012, 2014). Because MDF and MIF is limited during Hg trophic transfer in fish, Hg isotopes can be used as an direct source tracer of Hg in the aquatic food chain (Kwon et al., 2012). In fish consumers such as humans and whales, significant MDF has been widely observed relative to consumed fish, but MIF is not expected to occur. Mammalian body tissues (e.g., muscle, hair) have exhibited higher δ^{202}Hg values (2‰– 3‰) than dietary sources such as fish and rice, indicating that heavier Hg isotopes are preferentially enriched during Hg metabolism in mammals (Laffont et al., 2009, 2011; Li et al., 2014, 2017; Rothenberg et al., 2017; Du et al., 2018). However, the missed Hg with negative net δ^{202}Hg signal still remained unclear. Feces are another important route of Hg elimination, but the isotopic signature of Hg in feces still remains unclear. Investigation of Hg isotopic composition in feces may provide additional information on Hg metabolism in mammal bodies.

Our previous study showed that the main pathway of human MeHg exposure is rice consumption in Wanshan Hg mine (Feng et al., 2008). The MeHg toxicokinetics model based on fish consumption underestimated the human hair MeHg levels in rice consuming population (Li et al., 2015). However, this study did not include any direct tracers in the study design resulting in a high degree of uncertainty, particularly in the rate of metabolism and route of exposure. In this study, we performed a sub-chronic exposure experiment to elucidate the toxicokinetics of Hg in rats that were fed with MeHg contaminated rice for 90 days. Hg concentrations, speciation, and isotopic compositions in rice, rat tissues, and feces were measured, and a Hg isotope based mass balance model was developed. Our prediction is that the isotope fractionation method can effectively be used as tracer to show the toxicokinetics of Hg from ingestion of Hg contaminated rice.

2. Materials and methods

2.1. Animals and feeding

The experimental protocol was reviewed and approved by the Animal Research Ethics Committee of the Harbin Medical University (Harbin, China). Sprague-Dawley rats weighing 70– 80 g were purchased from Vital River Laboratories (Beijing, China) and housed individually at the Pathogen-Free Rat Breeding Unit at the Harbin Medical University. Temperature was maintained at 22 ± 2 °C with a 12-h light/dark cycle and rats have free access to food and water. Rats were acclimated for 1 week prior to the experiments. A total of 8 rats (4 male & 4 female) were fed with rice containing 25 ng/g MeHg (THg: 51.3 ng/g), which had been collected from the Wanshan Hg Mine in China in 2012. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for MeHg at 1.6 μg/kg/week (0.23 μg/kg/d, JECFA, 2011). For rural residents in Hg mining area in Guizhou Province, rice is the staple food and the daily rice ingestion rate was 600 g. Considering the body weight of 60 kg for adults, this guideline is corresponding to rice MeHg levels of 23 ng/g. Therefore, we set the rice MeHg level to test its toxicity to humans. The feed was composed of 77.5% rice powder, 14.0% casein, 4.0% soybean oil, 3.5% mineral mix (AIN-93 M-MX), and 1% vitamin mix (AIN-93-VX). Detailed information of the feeding experiment was reported in a companion paper (Li et al., 2018). In this study, we selected 2 rats for Hg isotope study (male rat: C4M; female rat: C4F).

2.2. Sample collection

After 90 days of treatment, the rats were sacrificed, and blood, brain, liver, kidney, muscle, and hair tissues were dissected to determine the total Hg (THg) and MeHg concentrations (Biewald and Billmeier, 1978). The wet and dry weights of the brain, kidney, and liver were measured. Blood volume was calculated from each rat's body weight using a ratio of 47 mL/kg body weight (Bencze, 1994). Total hair weight during the experiment was calculated from body surface area. Hair growth weight was given for one strand of hair as 0.07 mg/cm/month, which is equivalent to 0.002 mg daily growth (Qin et al., 2003). Total daily growth was calculated by 0.002 mg/day multiplied by hair density. Hair density was 223 strands of hair per surface area of cm^2 (Nobuo, 2000). Total body surface area was calculated by body weight using the Meeh-Rubner formula:

$$\text{Total body surface area } (cm^2) = 9.1 \times \sqrt[3]{(\text{body weight } [g])^2} \quad (1)$$

After excluding hair, brain, kidney, and liver weights, the remaining body weight was considered muscle (including bone), and water content was assumed to be 60% when converting into dry weight (Sahin et al., 2006).

During the 90-day treatment, the amounts of rice consumed by each rat were recorded daily and body weight was recorded weekly. Feces and urine samples were collected weekly for THg analysis. Feces weight and urine volume were recorded daily for establishment of mass balance model.

2.3. Hg speciation analysis

Feces and tissue samples were freeze-dried prior to chemical analysis. THg concentrations in solid samples (feces, brain, liver, kidney, muscle, and hair) were determined using an RA-915 Hg analyzer coupled with a PYRO-915 attachment (Lumex Instruments, Mission, BC, Canada). Whole blood and urine samples were digested with a mixture of nitric and sulfuric acid (v/v 4:1) at 95 °C for 3 h, and quantified using BrCl oxidation, $SnCl_2$ reduction, purge, gold trap, and cold vapor atomic fluorescence spectrometry (Li et al., 2015). For MeHg analysis, brain, liver, kidney, muscle, hair, and blood samples were digested using the KOH-methanol/solvent extraction technique and quantified using aqueous ethylation, purge, trap, and GC-CVAFS detection (MERX, Brooks Rand Instruments, Seattle, WA, USA) (Li et al., 2015). Since the major (>90%) form of Hg in feces and urine is IHg (Sherman et al.,

2013), MeHg concentrations were not measured in feces and urine in this study.

Quality control consisted of method blanks, certified reference materials, and blind duplicates. The limits of detection were 0.02 ng/g for THg in solid samples, 0.08 μg/L for THg in blood and urine samples, and 0.003 ng/g for MeHg, respectively. Recoveries of THg and MeHg concentrations in selected certified reference materials (NIES-13, TORT-2, ZK021-1, and GBW10020) ranged from 93.9% to 98.4% as shown in SI Table S1 (Li et al., 2018). The relative percentage difference was lower than 10% for Hg speciation analysis in duplicate samples.

2.4. Hg isotope analysis

Suitable amounts of rice, rat tissue, blood, and feces samples (depending on THg concentration) were digested in 5 mL HNO_3 at 120 °C for 6 h. The digest was diluted to 15%– 20% acid and THg concentrations of approximately 1 ng/g prior to Hg isotope analysis by a Plasma II MCICPMS (Nu Instruments, Wrexham, UK) at the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences (Yin et al., 2013). δ^{202}Hg, Δ^{199}Hg, Δ^{200}Hg, and Δ^{201}Hg were calculated relative to National Institute of Science and Technology certificated reference material 3133 Hg standard solution, following recommended nomenclature (Bergquist and Blum, 2007).

The mean δ^{202}Hg and Δ^{199}Hg values for UM-Almaden secondary solutions (n = 4) were −0.53‰ ± 0.09‰ (2 SD), and −0.02‰ ± 0.04‰ (2 SD), respectively, comparable with previous results (Bergquist and Blum, 2007). Certified reference materials TORT-2 (lobster hepatopancreas) and BCR482 (lichen) were prepared and measured in the same manner as the samples to evaluate the accuracy and precision of the method. The measured values for TORT-2 (δ^{202}Hg: −0.06 ± 0.12‰, Δ^{199}Hg: 0.75 ± 0.05‰, 2 SD, n = 4) and BCR482 (δ^{202}Hg: −1.49 ± 0.23‰, Δ^{199}Hg: −0.60 ± 0.09‰, 2 SD, n = 4) were in good agreement with previous results (Estrade et al., 2010; Tsui et al., 2012; Kwon et al., 2014; Yin et al., 2016). Uncertainties reflect the larger values of the uncertainties of either duplicate certificated reference materials or UM-Almaden.

2.5. Statistical analysis

All data were analyzed using SPSS 19.0 (IBM SPSS, Armonk, NY, USA). The Hg data in samples are generally described by giving the mean ± standard deviation (SD). In addition to descriptive statistics, Hg concentrations and Hg isotope data in rice, feces, and tissues were compared using ANOVA. Relationships between covariant sets of data were subjected to regression analysis. Differences are declared as significant in case that $p < 0.05$.

3. Results and discussion

3.1. Hg concentrations

Organ weights and cumulative weights of feces and urine are shown in Fig. S1. As reported in our companion paper (Li et al., 2018), the low MeHg dose used in our experiment had no observable effects on organ development in the rats. Fig. 1 shows the THg concentrations in different organs; the highest level were observed in the kidney, followed by hair, liver, blood, muscle, and brain. The Hg enrichment factor in tissues and excrement (i.e., $[Hg]_{tissue}/[Hg]_{diet}$) were calculated (Table 1).

Since MeHg is highly neurotoxic, the brain is expected to be a target of MeHg poisoning (Clarkson and Magos, 2006). However, the brain showed relative low enrichment factor (1.28) and the kidney exhibited the highest THg concentrations and enrichment factors. Feces samples had THg concentrations (dry weight) of 860 ± 76.7 ng/g (Mean ± SD, n = 11) and 902 ± 108 ng/g (n = 11) for C4M and C4F rats, respectively. The enrichment factor in feces was 17.2. Urine samples showed relatively low THg concentrations of 3.60 ± 0.19 ng/mL for C4M and 3.03 ± 0.89 ng/mL for C4F, respectively. Enrichment factors in urine averaged at 0.065, which was the lowest in all rat tissues and excrement.

MeHg bioaccumulation in different organs was shown in Fig. 2. Similar with THg, the highest level were also observed in the kidney, followed by hair, blood, liver, brain, and muscle. The muscle and brain showed relative low enrichment factor of MeHg (1.29 and 1.85, respectively). The kidney also exhibited the highest MeHg concentrations and enrichment factors.

3.2. Hg mass balance in rat

Daily rice intake is shown in Fig. S2. Multiplied by the THg concentration in rice (51.3 ng/g), the average total amount of consumed Hg was 131.0 μg for C4M and 80.1 μg for C4F. The sum of measured Hg in body tissues, feces, and urine was estimated to be 109.7 and 61.9 μg for C4M and C4F, respectively. Therefore, the overall recovery rate was 83.8% and 77.2% for male and female rats, respectively. Mass fractions of Hg in body tissues, feces, and urine are shown in Fig. S3. Excrement (feces and urine) accounted for 54.8% and 55.8% of total recovered Hg in C4M and C4F, respectively. Almost all the Hg was excreted via feces as feces contributed 95.3% and 92.5% of the recovered Hg in the excrement. Hg showed a differential accumulation in different organs, i.e. the amount of Hg accumulated is not dependent on the weight of organ. Liver and kidney contributed <10% and the brain accounted for only 0.15% of the Hg body burden. Hair and muscle, each accounted for 40% of the Hg body burden.

MeHg intake from feeding rice was 63.0 μg and 39.0 μg in C4M and C4F, respectively. However, the MeHg body burden was estimated to be 20.5 μg and 12.1 μg in C4M and C4F, respectively, implying that only 32% of MeHg in the feeding rice was recovered in rat bodies on average. As well, hair, muscle, and blood together accounted for 94% of the total MeHg burden in the rats.

3.3. Mass-dependent fractionation

The Hg isotopic compositions in rice, rat tissues, and feces are shown in Fig. 3. We did not measure the isotopic composition of urine because of the small sample volume and low THg concentrations. The average δ^{202}Hg and Δ^{199}Hg values in rice samples were −2.93‰ ± 0.12‰ (n = 3, SD) and 0.09‰ ± 0.05‰ (n = 3, SD), respectively. These δ^{202}Hg values are comparable with those (−2.59‰ ± 0.98‰, 2SD, n = 17) reported by Du et al. (2018), but lower than values reported by Li et al. (2017) (−1.63‰ ± 0.31‰, 1 SD, n = 7), Yin et al. (2013) (−2.38‰ ± 0.32‰, 2 SD, n = 6), Feng et al. (2016) (−1.60‰ ± 2.80‰, 2 SD, n = 14), and Rothenberg et al. (2017) (−1.23‰ ± 1.38‰, 2 SD, n = 8). The Δ^{199}Hg values in rice samples were comparable to those reported by Du et al. (2018) (0.03‰ ± 0.06‰, 2 SD, n = 15), but slightly higher than values published by Yin et al. (2013) (−0.06‰ ± 0.05‰), Li et al. (2017) (−0.01‰ ± 0.03‰), Feng et al.

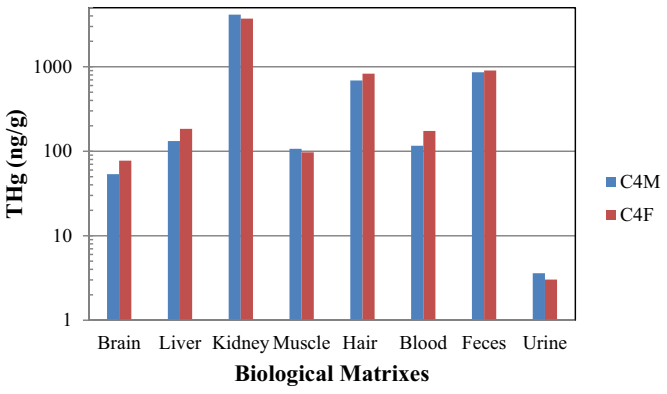

Fig. 1. THg concentrations in biological matrixes of the rats.

Table 1
Enrichment factor, Hg isotope, and net values in rice, rat tissues, and excrement.

	Enrichment factor (THg)	Enrichment factor (MeHg)	δ^{202}Hg (‰)	Net δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Net Δ^{199}Hg (‰)
Rice	–	–	−2.93 ± 0.12	0	0.09 ± 0.05	0
Brain	1.28	1.85	−2.21 ± 0.05	0.72	0.18 ± 0.002	0.09
Muscle	1.99	1.29	−2.21 ± 0.03	0.72	0.13 ± 0.015	0.04
Liver	3.08	1.90	−2.48 ± 0.13	0.45	0.06 ± 0.018	−0.03
Hair	14.8	14.6	−1.72 ± 0.14	1.21	0.17 ± 0.053	0.08
Blood	2.83	5.69	−1.68 ± 0.12	1.25	0.22 ± 0.042	0.13
Feces	17.2	–	−3.37 ± 0.13	−0.44	0.06 ± 0.019	−0.03
Kidney	76.6	37.0	−2.83 ± 0.02	0.10	0.14 ± 0.008	0.05
Urine	0.065	–	–	–	–	–

(2016) (−0.01‰ ± 0.05‰), and Rothenberg et al. (2017) (−0.04‰ ± 0.11‰).

Large variations in δ^{202}Hg (−3.37‰ to −1.59‰) were observed in rat tissues and feces (Fig. 3). Hair and blood samples showed the highest δ^{202}Hg values, averaging −1.72‰ ± 0.14‰ (n = 2) and −1.68‰ ± 0.12‰ (n = 2), respectively. Compared to the rice, positive net δ^{202}Hg (net $\delta^{202}Hg = \delta^{202}Hg_{tissue} - \delta^{202}Hg_{rice}$) of 1.21‰ and 1.25‰ were observed for hair and blood samples, respectively. A positive net δ^{202}Hg fractionation between dietary source and body tissues has been previously reported by many studies. The positive offsets of δ^{202}Hg from

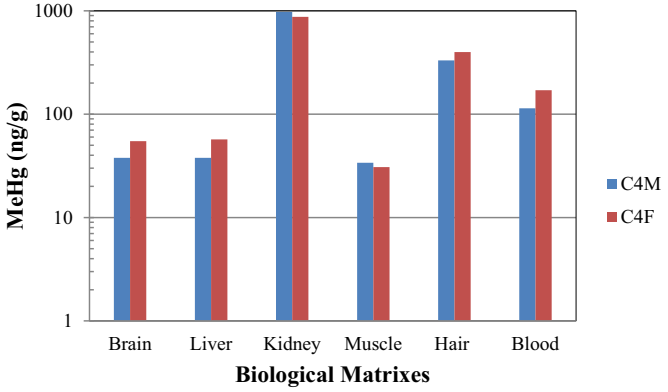

Fig. 2. MeHg concentrations in biological matrixes of the rats.

diet were observed in mink hair (0.30‰) and blood (0.38‰) (Ma et al., 2018). Positive shifts of approximately 2‰ in δ^{202}Hg were observed between diet (fish and rice) and human hair in populations from Bolivia (2.0‰ ± 0.2‰) (Laffont et al., 2009), the United States (2‰) (Sherman et al., 2013), France (2.2‰ ± 0.8‰) (Laffont et al., 2011), the Faroe Islands (1.75‰) (Li et al., 2014), the Gulf of Mexico (1.40‰– 2.35‰) (Li et al., 2014), and China (2.25‰– 2.7‰) (Li et al., 2017). These positive shifts can be explained by the preferential demethylation of isotopically lighter MeHg in the intestines (Sherman et al., 2013). Demethylation in the intestine was an important pathway for MeHg detoxification (Wang et al., 2017; Liao et al., 2019). Moreover, the gut microbiota was confirmed to be a potential factor causing individual variation in MeHg absorption, body burden, and potential toxicity (Rothenberg et al., 2015; Guo et al., 2018). However, the roles of gut microbes in the transformation of MeHg should be further studied.

Brain, muscle, liver, and kidney showed average δ^{202}Hg values of −2.21‰ ± 0.05‰, −2.21‰ ± 0.03‰, −2.48‰ ± 0.13‰, and −2.83‰ ± 0.02‰, respectively, corresponding to positive net δ^{202}Hg of 0.72‰, 0.72‰, 0.45‰, and 0.10‰, respectively, compared to the δ^{202}Hg in rice. Feces yielded the lowest δ^{202}Hg values of −3.37‰ ± 0.13‰. In comparison with rice, a negative net δ^{202}Hg fractionation of −0.44‰ was observed for feces, indicating that feces is an important route for the elimination of lighter Hg isotopes in rats. Urine has also been shown as an elimination route for lighter Hg isotopes, as MeHg enriched in lighter Hg isotopes is preferentially demethylated (Sherman et al., 2013). In this study, the isotopic composition of urine samples was not measured, but we conclude that feces play a greater part than urine in eliminating lighter Hg isotopes in rats (−0.44‰), because feces contributed 93% of total extraneous Hg.

3.4. Mass independent fractionation

Slightly positive mass independent fractionation signals were observed in rat tissues and feces, with Δ^{199}Hg values ranging from 0.06‰ to 0.22‰ (Fig. 3), and shifts of −0.03‰ and 0.13‰ from rice

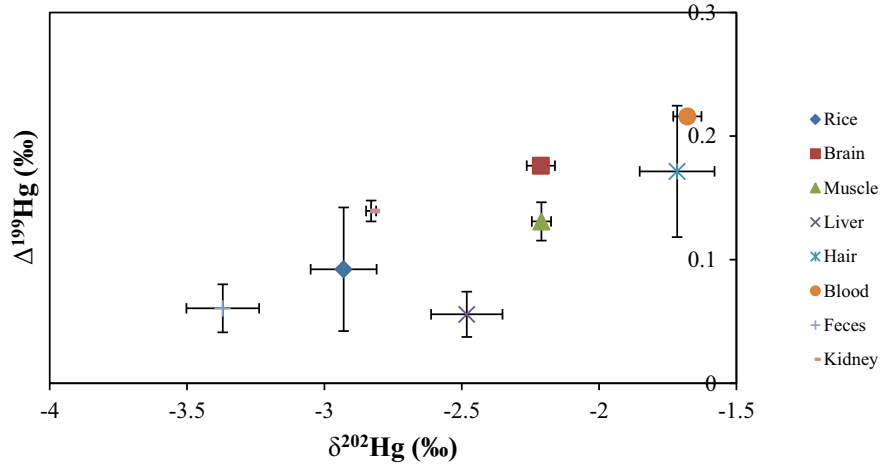

Fig. 3. δ^{202}Hg and Δ^{199}Hg values in rice, rats tissues, and feces.

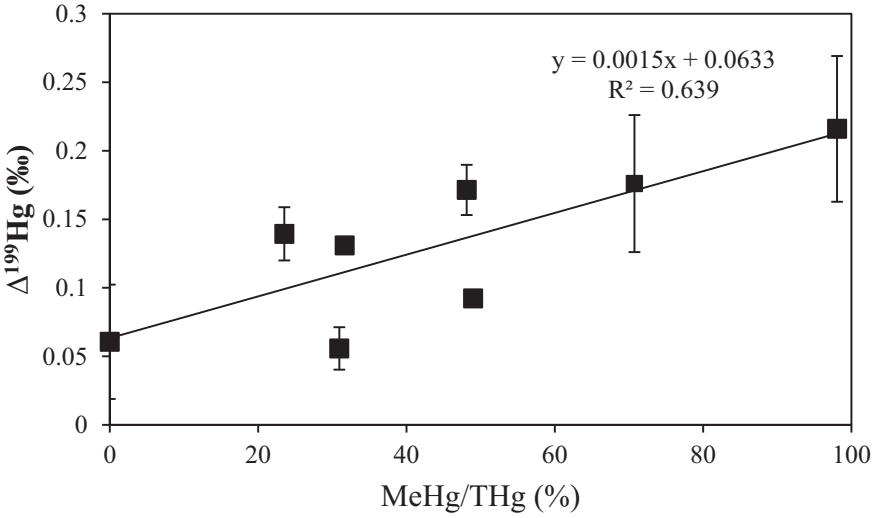

Fig. 4. Relationship between Δ^{199}Hg and %MeHg in rice, rats tissues, and feces.

Δ^{199}Hg (0.09‰ ± 0.05‰). In previous studies, limited mass independent fractionation of Hg isotopes was expected to occur during Hg metabolism and trophic transfer (Perrot et al., 2012; Kwon et al., 2012, 2014; Blum et al., 2013). The observed mass independent fractionation in organisms can be explained by aqueous Hg(II) photo-reduction and MeHg photo-degradation processes prior to bio-accumulation (Blum et al., 2013). In this study, a significant correlation between Δ^{199}Hg and MeHg percentage ($r^2 = 0.64$, $p < 0.05$) was observed in rice, rat tissues, and feces (Fig. 4), confirming the combination of IHg and MeHg fractions with distinct mass independent fractionation signals. According to this regression relationship, the Δ^{199}Hg signature in these samples was calculated to be 0.06‰ for IHg and 0.21‰ for MeHg, respectively. This finding was similar with previous study, which observed distinct higher Δ^{199}Hg values (0.16‰) in MeHg than IHg for rice samples using compound-specific stable isotope analysis (Li et al., 2017).

3.5. Hg isotope model simulation

Based on the mass balance model for the rats, the modeled Hg isotope values (δ^{202}Hg and Δ^{199}Hg) in the rice were calculated from Hg isotopes in rat tissues and feces. The formulae are:

$$\delta^{202}Hg_{rice-model} = \sum_{1}^{n}\left(fi \times \delta^{202}Hg_i\right) \quad (2)$$

$$\Delta^{199}Hg_{rice-model} = \sum_{1}^{n}\left(fi \times \Delta^{199}Hg_i\right) \quad (3)$$

where $\delta^{202}Hg_{rice\text{-}model}$ and $\Delta^{199}Hg_{rice\text{-}model}$ refer to the modeled δ^{202}Hg and Δ^{199}Hg values in the rice; fi is the Hg fraction in rat tissues and feces to total body burden; $\delta^{202}Hg_i$ and $\Delta^{199}Hg_i$ are the δ^{202}Hg and Δ^{199}Hg values in rat tissues and feces.

Using Eqs. (2) and (3), the modeled δ^{202}Hg and Δ^{199}Hg values in the rice were obtained. The modeled δ^{202}Hg value in the rice was −2.75‰, which was comparable to the determined value (−2.93 ± 0.12‰). The analytical uncertainty of δ^{202}Hg was considered to be 0.10‰ (2 SD). The modeled Δ^{199}Hg value in the rice was 0.10‰, in good agreement with the determined value (0.09‰ ± 0.05‰). The analytical uncertainty of Δ^{199}Hg was considered to be 0.04‰ (2 SD), and the modeled Δ^{199}Hg value was located within the range of analytical uncertainty. This indicated the successful of the rat model on Hg mass balance and Hg isotope. It also indicated that mass balance method and Hg isotopes are useful tools for understanding the metabolic processes of Hg.

4. Conclusions

In this study, we developed a rat model of Hg consumption to evaluate Hg toxicokinetics by the mass balance theory and Hg isotope approach. The highest accumulation of THg was observed in the kidney. About 80% of ingested Hg was recovered in rat body and excretion. Excretion accounted for 55% of the total recovered Hg, and feces were the principal route of elimination. However, only 32% of feeding MeHg was recovered, indicating significant demethylation of MeHg in rat body. We confirmed positive net fractionation of δ^{202}Hg in hair and blood samples, and negative net fractionation in feces. An offset of 2‰ in δ^{202}Hg values has been observed in the hair of humans who consumed Hg contaminated fish, but the missing negative signal is as yet unaccounted for. We found a negative net fractionation of δ^{202}Hg in feces, confirming it to be the principal route of Hg elimination. Our findings indicate that mass balance method and Hg isotopes are useful tools for understanding the metabolic processes of Hg. Demethylation of MeHg in intestine were the important detoxification process in rat body.

CRediT authorship contribution statement

Ping Li: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Writing - review & editing. **Runsheng Yin:** Supervision, Writing - review & editing. **Buyun Du:** Methodology, Project administration. **Chongyang Qin:** Methodology. **Baixiang Li:** Conceptualization, Methodology, Supervision. **Hing Man Chan:** Conceptualization, Methodology, Supervision. **Xinbin Feng:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139687.

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