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Mercury and selenium co-ingestion assessment via rice consumption using an in-vitro method: Bioaccessibility and interactions

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ABSTRACT

Mercury (Hg) was reported to accumulate in rice grains, and, together with the selenium (Se) was found in rice, the co-exposure of Hg-Se via rice consumption may present significant health effects to human. This research collected rice samples containing high Hg:high Se and high Se:low Hg concentrations from high Hg and high Se background areas. The physiologically based extraction test (PBET) in vitro digestion model was utilized to obtain bioaccessibility data from samples. The results showed relatively low bioaccessible for Hg (<60%) and Se (<25%) in both rice sample groups, and no statistically significant antagonism was identified. However, the correlations of Hg and Se bioaccessibility showed an inverse pattern for the two sample groups. A negative correlation was detected in the high Se background rice group and a positive correlation in the high Hg background group, suggesting various micro forms of Hg and Se in rice from different planting locations. In addition, when the benefit-risk value (BRV) was calculated, some "fake" positive results showed while Hg and Se concentrations were directly used, which indicated that bioaccessibility should not be neglected in benefit-risk assessment.

1. Introduction

The interactions between mercury (Hg) and selenium (Se) have been studied since the 1970s. Pařízek and Ošťádalová (1967) first reported the protective effect of Se on Hg toxicity in laboratory mice. Also, a molar ratio of 1:1 between Hg and Se was found in marine mammals and in organs of human who exposed to high levels of inorganic Hg (Koeman, Peeters, Koudstaal-Hol, Tjioe, & De Goeij, 1973; Kosta, Byrne, & Zelenko, 1975). After that, numerous studies were conducted to explore the relationship and effects of Hg and Se, and many observations in animals supported the protective effect of Se on both inorganic Hg (IHg) and methylmercury (MeHg) (Khan & Wang, 2009). Selenium is still considered an effective method to detoxify the side effects of Hg ingestion; thus, Se and Hg levels in food and their relationship were investigated to assess their possible health effects on humans. As an essential protein resource, fish was focused because high Hg concentration was found since Minamata disease. Cabañero et al. (2004, 2007) considered that Hg toxicity from ingestion was correlated to the molar ratio between bioaccessible Se and Hg (Se:Hg), and that a high Se:Hg has an adequate protection effects from consuming sardine, swordfish and tuna. Similar results were also reported in other fish and shell fish species (Calatayud et al., 2012; Burger and Gochfeld. 2011, 2012; Afonso et al., 2015), widely sold in Europe and the US. However, for some fish species, the Se:Hg was reported <1 (e.g., blue shark), and a cautionary recommendation for consumption was suggested (Matos et al., 2015). In order to assess the potential health effect for Hg and Se ingestion by consuming fish, Ralston and Raymond (2013) conducted Se-Health Benefit Value (HBV_{Se}) to calculate the health risk. Zhang, Feng, Chan, and Larssen (2014) considered the essential Se ingestion amount and proposed Benefit-risk value (BRV) to evaluate the Hg and Se co-exposure. Based on the vast amount of fish consumption and the outcomes of the health risk assessments, fish-eating recommendations were provided by certain government agencies as a means to protect the human health (EPA, 2021).

While fish received plenty of attention in aquatic environment, rice, as an important food group, was found to concentrate Hg in terrestrial environment. Up to 214.7 mg/kg, rice samples collected in Wanshan district, eastern Guizhou province, has been reported to contain Hg

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concentration that cannot be ignored, because Wanshan is reported as a typical Hg polluted area with a long history of Hg mining activities (Feng et al., 2008; Li, Feng, Qiu, Shang, & Wang, 2008). Thus rice has been considered as an important pathway of Hg exposure, especially for the residents of inland China (Feng & Qiu, 2008; Zhang, Feng, Larssen, Qiu, & Vogt, 2010). In addition, Se concentrations in rice ranging from 0.025 to 1.88 mg/kg were reported in China, with a relatively high Se concentration found in certain high Se background areas, e.g., Enshi, which located in Hubei province and human Se poisoning occurred in the 1960s (Huang et al., 2013; Qin, Zhu, Liang, Wang, & Su, 2013; Dinh et al., 2018), and also in Se-enriched rice cultivars (Chen et al., 2002; Williams et al., 2009; Chang et al., 2019; Jiao et al., 2022). Due to the reports mentioned above, health risk assessments were conducted by some researchers. However, the health risks caused by Hg exposure via rice consumption are limited for commercial rice purchased in China and Sri Lanka (Wang et al., 2017; Xu et al., 2020a,2020b). When bioaccessibility was considered, the health risks were further reduced. The THg bioaccessibility in rice samples was reported <50% in China (Wu et al., 2018) and with a range of 12.6 ± 17.2–44.6 ± 5.0% in Canada (Lin, Santa-Rios, Barst, Basu, & Bayen, 2019), and the MeHg bioaccessibility was reported with a range of 40.5 ± 9.4% in China (Gong et al., 2018). The Se bioaccessibility was reported with a range of 71.5 ± 11.2% in rice samples collected in northeast China, which is relatively higher than the MeHg bioaccessibility in rice (Wang et al., 2021). Based on the research results mentioned above, the limited health risks reported can be related to the low Hg concentrations; most rice samples determined in these studies were collected in non-Hg contaminated areas. Thus, the attentions should be paid on the high Hg background areas.

Furthermore, Se and Hg levels have been reported in rice, and combined with the large amounts of rice consumed, the co-exposure of both Hg and Se may cause different health effects due to the negative health impacts of Hg and narrow safe-ingestion amount of Se, e.g., Table S1 showed the National Health Commission of People's Republic of China (NHC) advices of an estimated average requirement (EAR), recommended nutrient intake (RNI), and tolerable upper intake level (UL) of Se for residents of different sex, ages, and health conditions in the national standard (NHC, 2017). Similar recommendations were also shown in Table S1, which provided by the US Institute of Medicine for the EAR and recommended dietary allowance (RDA) of Se (Institute of Medicine, 2000). In the case where the ingested amount of Se exceeds the UL or less than the EAR, Se is considered to cause negative health impacts. To limit Hg ingestion, the WHO (WHO, 2010) has set provisional tolerable weekly intake (PTWI) of THg and MeHg respectively (Table S1). If co-ingested at relatively high Hg levels, the individual's health may seriously be affected by the synergistic effects of these two elements, which deserves careful investigation on extreme cases of Hg-Se co-exposure caused by rice consumption.

In this research, an in vitro digestion experiment was applied to determine the THg, MeHg and Se bioaccessibility of rice samples from high Hg and Se background areas. Most importantly, the bioaccessibility data were applied to investigate further (1) the interactions between Hg and Se in rice (2) the potential antagonism between Hg and Se. The health effects from Hg and Se co-exposure and the potential health benefit-risks from rice consumption (under the high Hg and Se concentrations) were discussed under the different Hg-Se interactions.

2. Material and method

2.1. Study area and sample collection

The rice samples were collected from Enshi and Wanshan areas which were introduced above. A total of 23 rice samples were collected from rice paddies, of which 11 were from Enshi (ES1-11) and 12 were from Wanshan (WS1-12). The two sampling locations were shown in Fig. S1.

Rice grains were used in this study. The collected rice grains were dried at room temperature, treated into polished rice, then milled into rice powder and stored in Ziploc polyethylene bags until analysis.

2.2. In vitro experiment

The physiologically based extraction test (PBET) in vitro digestion model was used in this study because our previous work has proved that this method is relatively more stable and accurate (Wu et al., 2018). The ingredients and parameters of PBET are listed in Table S2, which was firstly developed by Ruby, Davis, Schoof, Eberle, and Sellstone (1996) and rearranged by Ng, Juhasz, Smith, and Naidu (2015).

To simulate the food condition, each rice powder sample was weighed to 1 g into a 50 mL centrifuge tube, and deionized water was added to 1 mL. The tube was capped and placed into a 100 °C water bath for 30 min to simulate the cooking procedure. The cooked samples were used in the in vitro digestion experiment, and the simulated gastric fluid was added into centrifuge tubes to 50 mL. The tubes were shaken at 37 °C, 120 rpm for the time listed in Table 1, then centrifuged at 3000 rpm for 20 min and the supernatants were collected. The simulated intestinal juice was added into the residues, also shaken at 37 °C, 120 rpm, then centrifuged as described above and the supernatants were collected. The collected supernatant samples were frozen at −20 °C until further analysis.

2.3. Hg and Se concentration determination

To quantify the Hg levels in samples, each rice samples was weighed to 1 g to correspond to the in vitro experiment, and the supernatants were quantified as 10 mL into 15 mL centrifuge tubes. Two different digestion procedures were applied: (1) for the THg analysis, 5 mL of HNO_3 was added to each sample, and tubes were placed in a 95 °C water bath for 3 h; (2) for the MeHg analysis, 5 mL of KOH- CH_3OH solution (25% w/v) was added to each sample and placed into a 75 °C water bath for 4 h. After either procedure, each sample was cooled to room temperature and topped to 15 mL with deionized water. To determine the MeHg and THg concentrations, the procedures of the United States Environmental Protection Agency method 1630 (USEPA, 1998) and 1631 (USEPA, 2002) were followed, respectively. Cold vapor atomic fluorescence system (CVAFS) was applied for THg determination and gas chromatography (GC)-CVAFS for MeHg (Model III detector, Brooks Rand Instruments, the USA), which followed the USEPA method 1630 and 1631. The limits of detection (LODs) for THg and MeHg were 0.03 ng/L and 0.02 ng/L, respectively.

For Se detection, each rice sample was weighed to 0.2 g, and supernatants were quantified as 5 mL into 10 mL Teflon tubes. We followed the preprocessing method was introduced by Zhang et al. (2015). In brief, analytical grade concentrated HNO_3 (3 mL) was added and then the tubes were capped in a fume hood. After 3 h, the closed tubes were heated for 8 h at 155 °C in a blast drying oven (BPG-9240A, Yiheng Science Instrument Ltd., China). After cooling, 2 mL of 30% H_2O_2 was added, and the solution was recapped and heated for 45 min at 90 °C. Each digestion solution was then transferred into a 15 mL polytetrafluoroethylene (PFA) container and evaporated on a hot plate at 90 °C to near dryness. Selenium in each digestion solution was transformed into Se (IV) by adding 2.4 mL of 5 mol/L HCl (ultrapure grade), followed by incubation at 95–100 °C for 45 min. Each solution was finally diluted to 16 mL with deionized water. The total Se concentration (TSe) of the digestion solutions was quantified by the method introduced by Zhu, Li, Qin, and Li (2008), which using an atomic fluorescence spectrometer

Table 1
The reference value and measured value of Hg and Se in CRM.

CRM	THg (μg/kg)			MeHg (μg/kg)			TSe(μg/kg)		
	reference value	measured value	Recovery (%)	reference value	measured value	Recovery (%)	reference value	measured value	Recover (%)
GBW-10020	150 ± 20	145 ± 6	98 ± 9	—	—		170 ± 30	152 ± 11	91 ± 9
TORT-2	—	—		152 ± 13	140 ± 5	93 ± 5	—	—	

(AFS) equipped with a sequential injection hydride generation (HG) sampling system (Beijing Jitan Instrumentals Co. Ltd., Beijing, China) and the LOD was 0.2 μg /L.

2.4. Data analysis

To calculate the bioaccessible fraction, the Eq. (1) was applied:

$$Bioaccessibile\ fraction(ng) = C_{ext} \times V_{ext} \tag{1}$$

where C_{ext} (ng/mL) is the Hg concentration of the simulated digestion fluids, and V_{ext} (mL) is the volume of simulated digestion fluids;

The bioaccessibility of Hg in rice was calculated by the Eq. (2) introduced by the USEPA (USEPA, 2012).

$$Bioaccessibility(\%) = \frac{Bioaccessible\ fraction}{C_{sample} \cdot M_{sample}} \cdot 100 \tag{2}$$

where C_{sample} (ng/g) is the Hg concentration in the sample and M_{sample} (g) is the mass of the sample.

To describe the interaction of Se and Hg, the molar ratio (MR) was applied, which was calculated by the Eq. (3):

$$MR = \frac{Se}{Se\ molar\ weight} \Big/ \frac{Hg}{Hg\ molar\ weight} \tag{3}$$

In this equation, the molar weight of Hg, Se and MeHg was 200.6, 78.9 and 215, respectively.

To assess the possible health impacts of Se-Hg co-exposure on human due to rice consumption, the BRV introduced by Zhang et al. (2014) was utilized Eqs. (4) and (5):

$$BRV = PDI_{Se} - \Delta Se - PDI_{Hg} \tag{4}$$

$$PDI = (C \times IR)/bw \tag{5}$$

where PDI represents probable daily intake and is expressed as μg/kg bw/day, the parameter C is the concentration of contaminants in food and expressed as μg/kg, which were calculated with the bioaccessibility data in this research, and IR is the food ingestion rate (kg/d). ΔSe describes the minimal Se amount required for normal biological function when Hg exposure is zero (Zhang et al., 2014), the EAR values (NHC, 2017) were chosen as ΔSe in this research. The BRV was measured in nmol/kg bw/d.

All statistical analyses were performed with SPSS software (IBM Corporation, v26.0) for parametric or non-parametric test methods, based on the outcome of normality testing. Two-way analysis of variance (ANOVA) and multivariate analysis of variance were also applied to determine the effects of different factors on the concentration and bioaccessibility of Hg.

2.5. Quality assurance and quality control

The range of relative standard deviation (RSD) values ranged from 2.3% to 7.8% for duplicate samples. The Hg and Se concentrations in certified reference material (CRM) were determined from three duplicate samples listed in Table 1.

3. Results

3.1. Hg and Se concentrations

Based on the Chinese national standard of contaminants in food (GB2762-2017), the THg concentration in cereals is regulated to 20 μg/kg. As shown in Table 2 and Fig. 1A, all rice samples of ES met the standard, indicating that Hg contamination in the non-mercury contaminated area was limited and that the THg concentrations in the rice of these area were relatively low. However, for the WS rice samples, the THg concentration exceeded the standard in 7 samples. More than half of the samples in this group contained a high THg concentration which may cause adverse health effects through rice consumption. Also, WS sample group has relatively higher MeHg/THg ratio than the ES sample group. Previous research showed that MeHg in rice paddy is important source for rice (Meng et al., 2010), WS is Hg contaminated area thus it contains relatively high Hg concentration in rice paddy, which may cause the high MeHg concentration in rice. For Se concentrations, the Chinese national standard recommends a value ranging between 40 and 300 μg/kg for Se-enriched rice (GB/T22499-2008). Three of the WS samples exceeded the upper limit of the Se standard; however, this standard is not mandatory but more like a recommendation. Exceeding the standard does not imply definite adverse health effects. Nonetheless, the exceeding of both Hg and Se standard existed; thus, the health effects deserve attention.

Compared with the previous rice data also collected in these areas, THg concentrations in WS rice were relatively low, possibly related to the recent pollution control actions implemented by the local government. However, MeHg concentrations in WS rice were similar to previously reported data, implying that the MeHg concentration did not reduce with the THg concentration. A recent study in Wanshan showed that IHg absorbed by rice leaves could not be methylated into MeHg in

Table 2
Hg and Se concentrations in rice samples (μg/kg).

Location	N	THg		MeHg		MeHg/THg (%)	Se		Ref.
		mean ± SD	range	mean ± SD	range		mean ± SD	range	
ES	11	5.33 ± 0.35	3.49–7.27	1.62 ± 0.30	0.58–6.86	30 ± 5	68.8 ± 19.9	15.1–246.8	this research
WS	12	35.96 ± 7.50	6.92–82.03	10.12 ± 1.27	3.30–17.60	42 ± 8	276.4 ± 93.8	14.3–1127.9	this research
Enshi	\	\	\	\	\	\	590	90–1880	Chang et al., 2019
Wanshan	\		\	\	\	\	40 ± 6	17–130	Zhang et al., 2015
Wanshan	\		3.2–214	\	\	\	\		Feng et al., 2008
Wanshan	\	\	6.0–113	\	\	\	\	\	Li et al., 2008
Wanshan	\	\	\	7.0 ± 3.2	3.8–18	\	\	\	Meng et al., 2010
Wanshan	\	\	\		3.1–13.4	\		\	Li et al., 2008

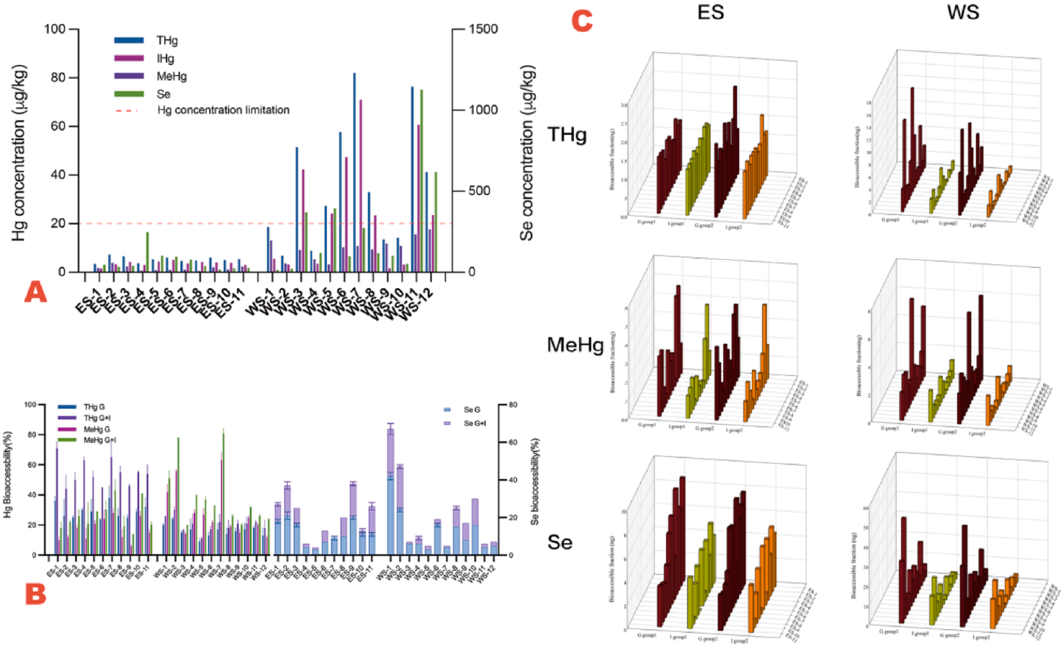

Fig. 1. The concentration, bioaccessible fraction and bioaccessibility of Hg and Se in rice samples, in Fig. 2C, group1 and group2 represent two parallel tests; G and I represent gastric and intestinal digestion stage, respectively.

above-ground parts (Liu, Meng, Poulain, Meng, & Feng, 2021). Due to the pollution control, the IHg deposition from the atmosphere was lower in Wanshan. Thus, the decrease in THg concentration is possibly related to the reduced absorption of IHg by leaves. However, the absorption of MeHg by roots from the rice paddy was not significantly affected. Moreover, Se concentrations in rice of ES were also lower than previously reported data because the farmlands containing high Se concentration were mainly converted to plant crops with high economic value.

3.2. Hg and Se bioaccessible fractions and bioaccessibility

As introduced by Ruby et al. (1996), oral stage was not included in PBET in vitro method, which were explained as relatively short time of oral contact (Ng et al., 2015). In addition, Hg and Se in rice were mainly bound to cysteine (Meng et al., 2014; Sun, Liu, Williams, & Zhu, 2010), thus the amylase in saliva may have limited effect on the bioaccessibility of Hg and Se in rice. Consequently, only gastrointestinal contact was included in this research. The bioaccessible fraction of Hg and Se were determined to represent the total mass of Hg and Se dissolved into simulated digestion fluid (Fig. 1C). In the gastric digestion stage, the THg and MeHg bioaccessible fractions for ES ranged from 1.13 ± 0.04 to 1.89 ± 0.80 ng and from 0.07 ± 0.03 to 0.45 ± 0.06 ng, respectively. For Se, the bioaccessible fractions ranged from 3.0 ± 0.1 to 8.7 ± 0.4 ng. In contrast, the THg, MeHg and Se bioaccessible fraction for WS ranged from 1.54 ± 0.62 to 14.05 ± 3.59 ng, from 0.88 ± 0.13 to 6.87 ± 0.49 ng, and from 5.2 ± 0.2 to 49.9 ± 1.4 ng, respectively. Overall, the bioaccessible fractions of WS samples in the gastric fluid were higher than those of the ES samples. Similar results were also obtained for the intestinal stage. The THg, MeHg and Se bioaccessible fractions for the ES samples were 1.22 ± 0.04 to 1.65 ± 0.29 ng, 0.04 ± 0.01 to 0.41 ± 0.01 ng, and 0.2 ± 0.3 to 5.4 ± 0.0 ng respectively. The THg, MeHg and Se bioaccessible fractions for the WS samples were 0.31 ± 0.07 to 3.74 ± 0.35 ng, 0.34 ± 0.06 to 2.18 ± 0.08 ng and 2.1 ± 0.1 to 22.1 ± 0.3 ng, respectively.

The THg, MeHg and Se bioaccessibility were shown in Fig. 1B. Se bioaccessibility was overall lower than 25% except in the WS-1 rice sample. After the intestinal digestion stage, Se bioaccessibility was elevated but lower than 50%, except for WS-1. The Se bioaccessibility between the two sample groups could not be distinguished superficially, and the mean values were 20 ± 4% and 20 ± 6% for the ES sample group and the WS sample group, respectively. However, the Hg bioaccessibility demonstrated a discernable pattern between the two sample groups. In the ES sample group, the THg bioaccessibility was higher than MeHg bioaccessibility, with a mean value of 54 ± 3% and 26 ± 3%, respectively. In contrast, the WS sample group showed an opposite trend, with mean value of 20 ± 1% and 40 ± 6% for THg and MeHg bioaccessibility, respectively. In general, the data of THg and MeHg bioaccessibility in the ES and WS sample groups showed an apparent difference, which will be tested by statistical methods in next part. Based on the reported bioaccessibility data (Table S3), MeHg bioaccessibility in the ES sample group and THg bioaccessibility in the WS sample group were consistent. However, the THg bioaccessibility in the ES sample group and MeHg bioaccessibility in the WS sample group were elevated. In addition, Se bioaccessibility in both sample groups were primarily lower than reported data, most likely because the samples used in those studies were purchased from markets. Due to the food safety regulation, commercial rice was mainly tested to meet the standard; thus, the Hg and Se concentrations were lower than the samples of our research samples. While the Se bioaccessibility of both sample groups was lower than the reported data, the THg and MeHg bioaccessibility showed a different trend for the two sample locations; thus, the relationship between Hg and Se should be discussed.

4. Discussion

4.1. Variation in Hg and Se bioaccessibility with different impact factors

As shown in Fig. 2A, combined with the results of the independent sample nonparametric test (Mann-Whitney *U* test), the p-value for the THg and MeHg were all <0.001, for the Se was 0.03 ($p < 0.05$). Consequently, the two sampling locations showed significant differences in Hg and Se concentrations. As mentioned above, Hg and Se bioaccessibility showed an apparent difference between the ES and WS groups (Fig. 2B), and the Mann-Whitney *U* test (Fig. 2B) verified the THg bioaccessibility to be statistically different between the two sample groups ($p < 0.05$). The mean value of THg bioaccessibility in the ES group was higher than that of the WS group but was statistically insignificant for MeHg bioaccessibility ($p = 0.22$) and for Se bioaccessibility

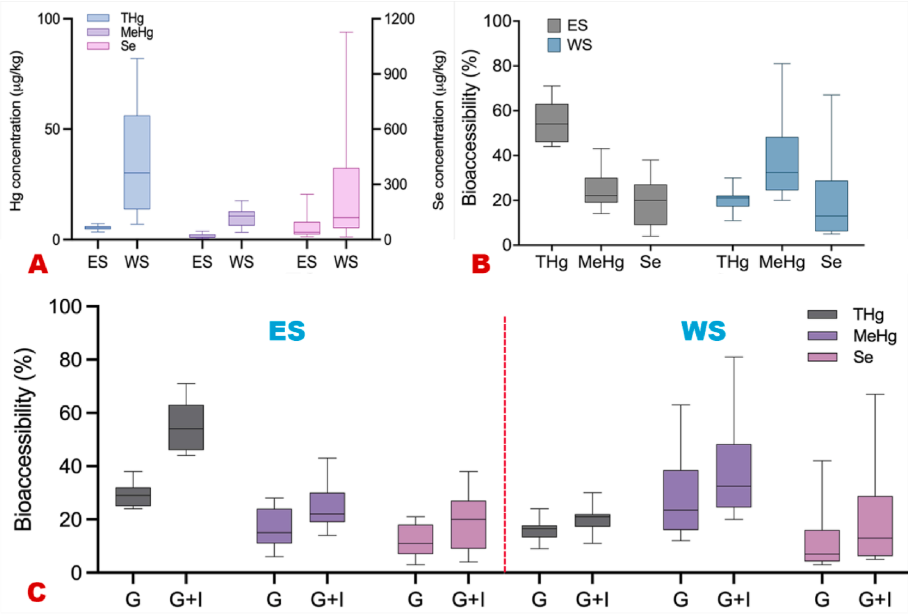

Fig. 2. The variations of Hg and Se in (A) concentrations between two sampling locations, (B) bioaccessibilities between two sampling locations and (C) bioaccessibilities between two digestion stages.

($p = 0.23$). From the mean value of THg, MeHg and Se concentration, WS samples showed higher Hg and Se concentrations than ES samples. In contrast, the mean values of THg bioaccessibility in both digestion stages of ES group were higher than those of the WS group.

Paired sample *t*-test was utilized to examine the differences in the two digestion stages (Fig. 2C). In both sample groups, significant p-values (<0.05) for THg, MeHg and Se bioaccesibility were obtained, and the average of D-value between gastric and gastrointestinal stage was negative. This suggested that the bioaccessibility of the gastrointestinal phase was significantly higher than the gastric phase. An independent sample nonparametric test was used to test for the difference between the two locations. According to the results of the Mann-Whitney *U* test and Kolmogorov-Smirnov test, only THg bioaccessibility of gastric and gastrointestinal phase for the two sampling locations showed a statistically significant difference ($p < 0.001$).

Results from our previous study showed that in non-mercury contaminated areas, planting locations have a limited effect on the Hg concentration in rice (Wu, Li, & Feng, 2022). However, in Xunyang County, which is also a Hg mining area, Liu et al. (2021) reported that the soil and atmosphere were both a source of IHg while the soil was the only source of MeHg in rice plants. In recent years, most Hg mining activities have been sealed in Wanshan due to pollution control; thus, atmospheric Hg concentration has decreased, and atmospheric deposition has been limited. The higher Hg concentration in the soil was likely the dominant impact factor for both IHg and MeHg concentrations in the WS sample group. Chang et al. (2019) showed that the decomposition of organic matter in rice paddies could promote the Se bioavailability and accumulation in rice; therefore, the soil was also the primary source of rice Se. Consequently, the significant difference in Hg and Se concentrations of the two sample groups might have been affected by higher Hg and Se concentrations in the soil of selected sampling sites.

Nevertheless, when analyzing the results of the two sampling locations and two digestion phases, the MeHg and Se bioaccessibility did not show significant differences. However, only the THg bioaccessibility showed a statistical difference between the two sampling sites. The IHg form was assumed to be relatively stable in rice because IHg cannot be methylated in rice plants (Liu et al., 2021). Our previous research also suggested that Hg forms can strongly affect the bioaccessibility (Wu et al., 2022). Combined with the much higher THg concentration but lower bioaccessibility in the WS samples, this research suggests that IHg bioaccessibility may be played an important role in the difference of THg bioaccessibility. The effect of Hg and Se antagonism was also speculated to be able to affect the Hg and Se bioaccessibility, which will be discussed in the following section.

4.2. Interaction and possible antagonism between Hg and Se bioaccessibility

Linear correlation was applied to determine the relationship between Hg and Se bioaccessibility (Fig. 3 and Table 3). The calculated correlations presented an inverse trend for the two sample groups. In the ES sample group, both THg and MeHg bioaccessibility showed a negative correlation with Se bioaccessibility, while the WS sample group showed a positive correlation. According to the results of linear correlation analysis results (Table 3), the correlation of MeHg and Se bioaccessibiliy showed a significant relationship ($p < 0.05$) in the ES sample group. In contrast, the THg bioaccessibility had a significant relationship ($p < 0.05$) with both MeHg and Se bioaccessibility in the WS sample group. Compared with the data of rice sample containing relatively low Hg and Se concentrations (Gong et al., 2018; Wang et al., 2021), the MeHg concentrations of the ES samples were similar to the data but Se concentration were relatively higher, and the MeHg and Se bioaccessibility was lower. Rice samples from WS contain much higher Hg and Se concentrations but relatively low THg and Se bioaccessibility, although the MeHg bioaccessibility was consistent with those reported previously. Gong et al. (2018) suggested that Se may not be the dominant factor of MeHg bioaccessibility due to the non-significant relationship of MeHg bioaccessibility with the Se concentration. This suggestion may be applied to the condition of rice with relatively low Hg and Se concentrations. However, due to the distinct Hg and Se geological background in this study, the ES samples contained low Hg concentrations and high Se concentrations. The THg bioaccessibility did not show a significant relationship with Se bioaccessibility but had a significant negative relationship with MeHg bioaccessibility, suggesting antagonism between Se and MeHg in ES samples.

The WS samples contained both high Hg and Se concentrations, and MeHg bioaccessibility was not affected by Se bioaccessibility. However, THg bioaccessibility was much lower than the ES sample group and the previously reported data (Table S3). Due to the high IHg fraction in the THg concentration for these samples, it could be speculated that the IHg

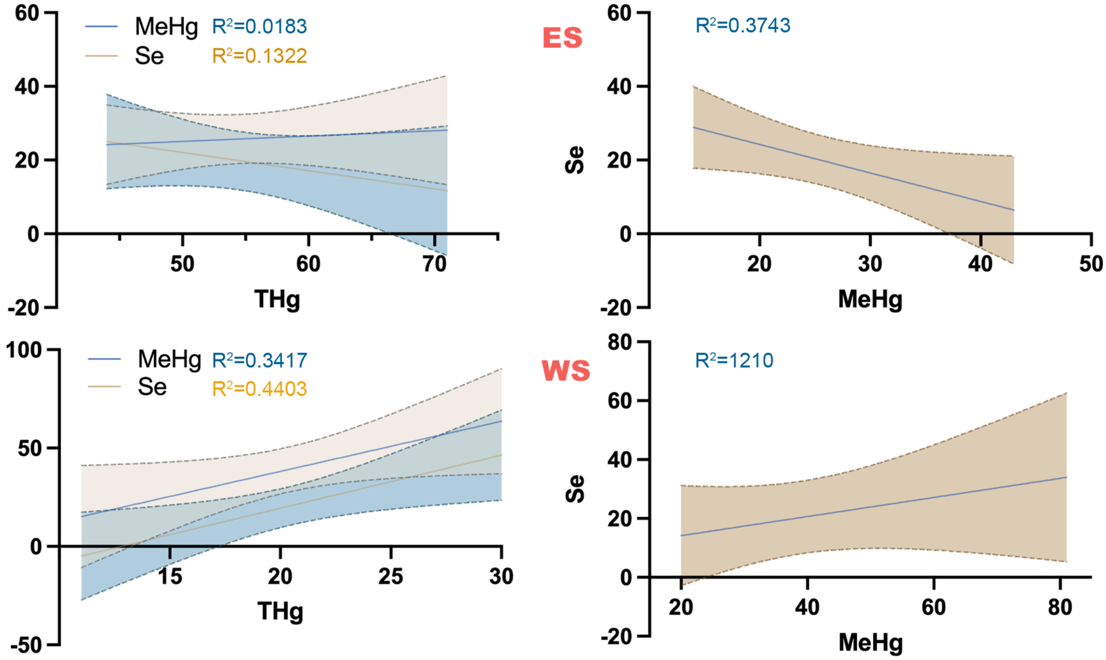

Fig. 3. The correllations of THg, MeHg and Se bioaccessibility in two sampling locations.

Table 3
The equation of correlation curve and the goodness of fit of Hg and Se bioaccessibility.

		THg bioaccessbility	MeHg bioaccessibility
ES	MeHg bioaccessibility	$Y = 0.1453 \bullet X + 17.80$ $R^2 = 0.0183$ $p = 0.69$	—
	Se bioaccessibility	$Y = -0.4941 \bullet X + 46.77$ $R^2 = 0.1322$ $p = 0.27$	$Y = -0.7734 \bullet X + 39.72$ $R^2 = 0.3743$ $p = 0.04$
WS	MeHg bioaccessibility	$Y = 2.549 \bullet X - 12.83$ $R^2 = 0.3417$ $p = 0.04$	—
	Se bioaccessibility	$Y = 2.698 \bullet X - 34.52$ $R^2 = 0.4403$ $p = 0.02$	$Y = 0.3243 \bullet X + 7.685$ $R^2 = 0.1210$ $p = 0.26$

bioaccessibility would be low. Combined with the relatively low Se bioaccessibility, it can be further hypothesized that Se in the WS samples mainly affected the IHg bioaccessibility. Mercury and Se were recently reported to bind in various micro forms in different tissues of rice, e.g., selenomethionine (SeMet) and selenomethylcysteine (SeMeSeCys) were easily transported to rice grain than inorganic forms such as selenite and selenate (Carey et al., 2012), and MeHg was mostly presented as MeHg-cysteine complex (MeHg-Cys) in rice grain (Meng et al., 2014), the previous results showed that various Hg and Se micro forms presents different behavior in rice plant, thus the content ratio of Hg and Se micro forms may lead to the differences in their bioaccessibility. Consequently, the possible reason of Se interacted with IHg in WS samples but with MeHg in ES samples was likely relate to the various micro forms of IHg and MeHg in rice samples.

Furthermore, the molar ratio (MR) value for Se:Hg to review the co-exposure of Hg and Se from fish, indicating that the a MR of Se:Hg >1 represents a potential detoxification function of Se on Hg exposure (Cabañero, Madrid, & Cámara, 2007; Burger & Gochfeld, 2012; Calatayud et al., 2012; Burger, Jeitner, Donio, Pittfield, & Gochfeld, 2013). This method was also utilized in this research to explore the interaction between Hg and Se in rice. The MR was calculated for each individual rice sample, and the average and the mean value were determined per group. The initial Hg and Se concentrations and bioaccessible fractions were used to determine the different MR values, represented as MR and MR_{bio} respectively (Table 4). All the MR values for both sample groups were higher than 1 but with considerable variation; both the MR values (mean value) of Se:THg and Se:MeHg for the ES sample group were higher than the WS group, although not statistically significant ($p > 0.05$, Mann-Whitney *U* test). When the bioaccessible fraction was considered, the MR_{bio} were statistically significantly lower ($p < 0.01$, paired sample *t*-test) than MR in both sample groups. However, as shown in Table 4, the MR_{bio} showed a different pattern compared to the MR values. The mean MR_{bio} of Se:THg in WS group was higher than the ES group, but with no statistically significant difference ($p > 0.05$, Mann-Whitney *U* test). Yet the mean MR_{bio} of Se:MeHg showed the opposite trend and with a statistically significance ($p < 0.01$, Mann-Whitney *U* test). These results provided evidence that combination of Se and Hg varied in two sample groups. Together with the differences in the Se and Hg correlation as discussed above, our results suggest a significantly different interaction mode of Se and Hg in rice samples, which may be related to differences in their content and concentration of micro-forms. The latter phenomenon deserves to be further investigated in future studies.

4.3. Potential health impact of human

As discussed in section 4.3, the Se and Hg interaction were varied in the two sampling groups, which may have caused a variation in the Se-Hg co-exposure rates from rice consumption by residents in the selected sampling locations. The reference intake values (Table S1) will be applied in health impact evaluation. The average body weight for male and female adults in China is 69.6 kg and 59 kg, respectively (SCIO, 2020). The data on rice consumption is based on the cereal ingestion recommendation of the Chinese Nutrition Society (CNS) as 0.2–0.3 kg/d for adults (CNS, 2022), where an upper ingestion rate of 0.3 kg/d was used in the assessment. The PDI and BRV were calculated using the concentration and the bioaccessible fraction of Se and Hg in rice samples for the two sampling locations. The PDI_{bio} and BRV_{bio} represented the value considered the bioaccessibility. The PTWI for THg and MeHg were converted into daily intake limitation of 0.72 μg/kg bw/d and 0.23 μg/kg bw/d, respectively. The EAR to UL of Se were also converted to an intake ranging between 0.72 and 5.75 μg/kg bw/d. The PDI and BRV were

Table 4
Description of MR of Se and Hg in two rice sample groups.

	Se:THg				Se:MeHg			
	MR		MR_{bio}		MR		MR_{bio}	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
ES	37.9 ± 13.8	6.3–168	8.1 ± 1.1	3.3–14.7	203.5 ± 85	20.5–989	87.7 ± 22.8	20.8–256
WS	18.8 ± 4.1	1.9–38.1	11.0 ± 1.4	1.9–16.8	81.3 ± 27.4	3.0–326	22.9 ± 4.6	3.9–49.3

expressed as μg/kg bw/d and μmol/kg bw/d as suggested by Zhang et al. (2014).

The PDI results showed that both Hg and Se ingestion were lower than the recommendation values. When bioaccessibility was considered, the PDI_{bio} was further reduced and much lower than the standard. Because the samples in this study were obtained from the high Hg and Se background areas, the Hg and Se concentrations in rice were relatively high. However, even in such an extreme situation, the rice consumption still showed a limited risk of causing Hg or Se poisoning. Furthermore, as shown in Table 5, BRV calculated with Hg and Se concentrations showed a noteworthy phenomenon; the mean BRV for ES samples was negative but for WS samples was positive. Based on the theory of BRV, Hg and Se concentrations in ES samples showed possible health risks ($BRV < 0$) but potential health benefits ($BRV > 0$) for WS samples. However, when bioaccessibility was considered, BRV_{bio} in both the ES and WS groups showed negative results, suggesting possible health risks in both sample groups. Due to the BRV considers the minimal Se requirement for human (Eq.4); thus, in this research, rice samples from ES contained relatively high Se but low Hg concentrations; the Se concentration did not fulfill the necessary standard, and the risk of Se insufficiency may have been overestimated. On the contrary, WS rice samples contained high Se and Hg concentrations, and the Se concentrations were even higher than the ES samples. Therefore, the risk of Hg ingestion was covered by the sufficient Se ingestion. Consequently, health risk assessment using the Hg and Se concentrations in rice may produce misleading results. When bioaccessibility was considered, the miscalculation could be corrected by the amount released into the digestion fluid. Overall, even though the BRV_{bio} showed negative health impacts, the values were small, indicating limited health risks by rice consumption. Combined with the results of PDI_{bio}, the risk of Hg poisoning caused by rice consumption was limited. The results of commercial rice samples and rice samples from non-mercury contaminated areas (Gong et al., 2018; Xu et al., 2020a; Wu et al., 2022) indicate that most of the rice with regulated Hg and Se concentrations may not be able to cause alarming health risks with Hg ingestion. However, for the residents of high Hg and Se background areas, the risk of chronic co-exposure to Hg and Se should not be neglected, and a more accurate assessment method for chronic exposure is required.

5. Conclusion

It is known that Hg can be accumulated in rice grain, and the health risk caused by rice consumption is a concern. The rice samples collected from the high Hg and Se background areas were selected to explore the extreme Hg and Se concentration conditions on rice. The bioaccessibility was investigated to determine the interaction between Hg and Se in simulated human digestion system. Based on our results, the THg concentration of WS rice samples were exceeded the Chinese food limitation, and the Se concentration in rice sample from both groups (ES and WS) were relatively higher. The PBET in vitro digestion model was applied to access the bioaccessibility fractions, showing relatively low bioaccessible percentages for Hg and Se in the both rice sample groups. None of the rice sample groups showed a statistically significant correlation between bioaccessible Hg and Se. However, inverse trends were observed. A possible reason for the latter may be the difference in micro-forms of Hg and Se in rice samples from the different planting locations. Consequently, the interaction between Hg and Se might have been affected by the micro forms and then showed in bioaccessibility. Thus, the micro-forms of bioaccessible Hg and Se in rice are important to bioaccessibility and deserve a further investigation.

For the benefit-risk assessment, the MR_{bio} of Se:Hg in rice was higher than 1, whereas the PDI_{bio} was lower than the recommended ingestion limitation. The BRV_{bio} was negative but relatively low, indicating that the risk of Hg exposure via rice consumption might be limited.

Table 5
PDI and BRV of rice consumption in two sampling locations (nmol/ kg bw/day).

		ES		WS		THg limitation	MeHg limitation	Se intake range
		Mean ± SD	Range	Mean ± SD	Range			
Male								
	PDI_{THg}	0.023 ± 0.005	0.015–0.031	0.115 ± 0.112	0.059–0.354	0.72	0.23	0.72–5.75
	PDI_{MeHg}	0.007 ± 0.004	0.003–0.017	0.044 ± 0.019	0.014–0.076			
	PDI_{Se}	0.297 ± 0.284	0.065–1.064	1.19 ± 1.40	0.062–4.862			
	$PDI_{bio\ THg}$	0.012 ± 0.001	0.010–0.014	0.030 ± 0.024	0.009–0.077			
	$PDI_{bio\ MeHg}$	0.002 ± 0.001	0.001–0.004	0.016 ± 0.009	0.005–0.029			
	$PDI_{bio\ Se}$	0.038 ± 0.015	0.016–0.059	0.104 ± 0.079	0.041–0.310			
	BRV_{THg}	−0.005 ± 0.004	−0.008–0.004	0.005 ± 0.017	−0.009–0.051			
	BRV_{MeHg}	−0.005 ± 0.004	−0.008–0.004	0.006 ± 0.018	−0.009–0.052			
	$BRV_{bio\ THg}$	−0.009 ± 0.001	−0.009–0.008	−0.008 ± 0.001	−0.009–0.006			
	$BRV_{bio\ MeHg}$	−0.009 ± 0.001	−0.009–0.008	−0.008 ± 0.001	−0.009–0.005			
Female								
	PDI_{THg}	0.027 ± 0.006	0.018–0.037	0.183 ± 0.132	0.035–0.388			0.85–6.78
	PDI_{MeHg}	0.008 ± 0.005	0.003–0.020	0.051 ± 0.022	0.017–0.089			
	PDI_{Se}	0.350 ± 0.335	0.077–1.255	1.41 ± 1.65	0.073–5.735			
	$PDI_{bio\ THg}$	0.014 ± 0.001	0.012–0.017	0.036 ± 0.028	0.009–0.090			
	$PDI_{bio\ MeHg}$	0.002 ± 0.001	0.001–0.004	0.018 ± 0.011	0.006–0.045			
	$PDI_{bio\ Se}$	0.045 ± 0.018	0.018–0.069	0.123 ± 0.093	0.049–0.366			
	BRV_{THg}	−0.007 ± 0.004	−0.010–0.003	0.004 ± 0.017	−0.010–0.049			
	BRV_{MeHg}	−0.007 ± 0.004	−0.010–0.003	0.004 ± 0.018	−0.010–0.051			
	$BRV_{bio\ THg}$	−0.010 ± 0.001	−0.011–0.01	−0.010 ± 0.001	−0.010–0.007			
	$BRV_{bio\ MeHg}$	−0.010 ± 0.001	−0.011–0.01	−0.010 ± 0.001	−0.010–0.007			

Consequently, future studies should focus more attention on the long-term health impacts of chronic co-exposure to Hg and Se on the residents in high Hg and Se background areas. However, it should be noted that the Hg and Se concentrations directly used to calculate the BRV showed “fake” positive results in some samples, while bioaccessibility data were used to calculate the $\mathrm{BRV}_{\mathrm{bio}}$ showed negative results. This implies that the concentrations of pollutants used in risk assessment calculation may lead to misestimation and that bioaccessibility should not be neglected.

CRediT authorship contribution statement

Ze Wu: Conceptualization, Resources, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Ping Li:** Supervision, Writing – original draft, Writing – review & editing. **Hui Dong:** Resources, Formal analysis, Investigation, Data curation. **Xinbin Feng:** Supervision, Writing – review & editing, Project administration.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113027.

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