Assessment of influences of cooking on cadmium and arsenic bioaccessibility in rice using in vitro physiologically based extraction test

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Abstract

The health risks associated with the consumption of rice may decrease if consumers use cooking practices which can reduce the concentrations of metal(loid)s and their bioaccessibility. The effects of cooking on the bioaccessibility of Cd and As in three contamination levels of rice were studied. The results indicated that cooking reduced bioaccessibility of Cd and As in rice. Cooking resulted in a significant increase in Cd and As concentrations in the residual fraction. Low volume cooking rice to dryness remove total Cd by about 10% for rice A and B, while middle or high volume water had no effect on Cd bioaccessibility in all rice types. In contrast, low volume cooking did not remove As, but a significant decrease was observed when cooking with middle or high volume water. This study provides information for a better understanding of more realistic estimation of metal(loid)s exposure from rice and the possible health risks.

Full Text

Assessment of Influences of Cooking on Cadmium and Arsenic Bioaccessibility in Rice Using In Vitro Physiologically Based Extraction Test

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ABSTRACT

The health risks associated with rice consumption may decrease if consumers adopt cooking practices that reduce metal(loid) concentrations and their bioaccessibility. This study investigated the effects of cooking on cadmium (Cd) and arsenic (As) bioaccessibility in rice at three contamination levels. The results indicated that cooking reduced the bioaccessibility of both Cd and As in rice, while causing a significant increase in their concentrations in the residual fraction. Low-volume cooking (rice cooked to dryness with a 2:1 water-to-rice ratio) removed approximately 10% of total Cd from rice A and B, whereas medium- or high-volume water ratios had no effect on Cd bioaccessibility across all rice types. In contrast, low-volume cooking did not remove As, but a significant decrease was observed when cooking with medium or high water volumes. This study provides valuable information for more realistic estimation of metal(loid) exposure from rice and associated health risks.

Keywords: Human exposure; Risk assessment; PBET; In vitro method; Established daily intake (EDI); Target hazard quotient (THQ)

1. Introduction

Dietary intake represents a major route of human exposure to metal(loid)s, raising growing concerns about contamination in rice (Sohn, 2014; Zhuang, Lu, Li, Zou, & McBride, 2014). As a staple food for half of the world's population, rice exhibits higher efficiency in cadmium (Cd) and arsenic (As) accumulation and soil-to-grain transfer compared to other cereal crops. Rice has been identified as a significant contributor to dietary Cd and As intake across numerous geographical regions, including China, Japan, Bangladesh, and India (Bae et al., 2002; Sun, de Wiele, Alava, Tack, & Laing, 2012a). The most notorious case of Cd poisoning through food consumption is itai-itai disease in Japan during the 1960s, which resulted from consumption of Cd-contaminated rice (Kobayashi, 1978). Contaminated rice is not unique to Asia; high As levels were also found in rice sold in the United States in 2012 (CR, 2012).

Total heavy metal concentrations in rice are commonly used to assess contamination in food matrices (Zhuang, McBride, Xia, Li, & Li, 2009). However, this metric does not always reflect the actual amount available to consumers.

Horiguchi et al. (2004) suggested that ingested heavy metal doses do not equal absorbed pollutant doses in reality, as a fraction of ingested metals may be excreted while the remainder accumulates in body tissues, affecting human health. Understanding heavy metal bioavailability in rice helps estimate the amount that can be absorbed by the human body, necessitating determination of oral bioaccessibility. Oral bioaccessibility is defined as the fraction of contaminant released from the food matrix into digestive juice chyme and becomes available for absorption—i.e., enters the bloodstream (Oomen et al., 2002)—and serves as an indicator of maximal oral bioavailability of contaminants in food (Versantvoort, Oomen, Kamp, Rompelberg, & Sips, 2005). In recent years, several in vitro digestion models have been proposed and extensively used to study bioaccessibility and risk assessment of contaminants (including heavy metals, organic pollutants, and mycotoxins) from food, soils, toys, and herbal medicine (Oomen et al., 2002; Rompelberg, & Sips, 2005; Versantvoort, Oomen, Kamp, Wang, Duan, & Teng, 2014).

Various static in vitro models have been employed to determine As bioaccessibility or bioavailability from rice (Laparra, Vélez, Barberá, Farré, & Montoro, 2005; Signes-Pastor, Al-Rmalli, Jenkins, Carbonell-Barrachina, & Haris, 2012), mushrooms (Llorente-Mirandes Llorens-Muñoz, Funes-Collado, Sahuquillo, & López-Sánchez, 2016; Sun, Liu, Yang, & Zhuang, 2012b), and seafood. For Cd bioaccessibility, limited studies have focused on vegetables (Intawongse, & Dean, et al., 2008; Pelfrêne et al., 2015) and seafood products (Houlbrèque et al., 2011). Data on Cd bioaccessibility in rice remain scarce, with only a few studies (Aziz et al., 2015; Yang et al., et al., 2014) based on in vitro digestion, highlighting the importance of conducting more bioaccessibility studies of Cd and As in rice to improve risk assessment.

Food is generally cooked before consumption to increase palatability. Compared to raw rice, cooked rice is more appropriate for human health risk studies since samples must reflect what consumers actually ingest for risk assessment to reflect real exposure scenarios (Devesa, Velez, & Montoro, 2008). Numerous studies have examined effects of common food processing procedures on heavy metal levels in food. Cooking processes may, under certain conditions, alter contaminant concentration or speciation in food matrices (Wang, Duan, & Teng, 2014), including seafood (Houlbrèque et al., 2011), vegetables (Pelfrêne et al., 2015), rice (Naseri, Rahmanikhah, Beiygloo, & Ranjbar, 2014), and mushrooms (Llorente-Mirandes Llorens-Muñoz, Funes-Collado, Sahuquillo, & López-Sánchez, 2016). These changes depend on cooking conditions (time, temperature, and medium). Little is known about how rice cooking affects Cd bioaccessibility and associated human health risks. Information on how rice processing or preparation methods influence contaminants, particularly Cd, remains scarce. There is an urgent need to consider cooking effects when evaluating risks associated with rice consumption based on Cd and As bioaccessibility.

The main objectives of this study were: (1) to measure Cd and As bioaccessibility concentrations in raw rice samples at three contamination levels using in vitro digestion and determine the relationship between Cd and As bioaccessibility and total concentration; (2) to investigate how cooking influences Cd and As bioaccessibility and the extent of Cd and As removal when rice is cooked with different water volumes; and (3) to assess human health risks based on bioaccessible Cd and As concentrations from different contamination levels of cooked rice via the ingestion pathway.

2. Materials and Methods

2.1. Sampling and Cooking Methods

The long-grain rice used in this study is widely consumed in southern China. Three contamination levels were selected: 0–0.2 mg Cd per kg (rice A; low Cd level, purchased from public supermarkets in southern China), 0.2–1.5 mg Cd per kg (rice B; medium Cd level, purchased from local markets near a mining area), and 1.5–5 mg Cd per kg (rice C; high Cd level, grown in a laboratory). For laboratory-grown rice, soil was spiked with a considerable amount of Cd, and rice plants were grown for 120 days before harvest. All rice samples were washed three times with double-distilled deionized (Milli-Q) water at room temperature. Cooking experiments used 100 g samples. Washed rice was cooked in acid-washed beakers for 30 minutes with 200 mL double-distilled deionized water using a 2:1 (low volume) water-to-rice weight ratio until no water remained. To examine cooking water effects, washed rice samples were also cooked using 4:1 (medium volume) and 6:1 (high volume) water-to-rice ratios until the rice reached an edible texture, with remaining water (gruel) discarded. Portions of gruel and cooked rice were freeze-dried for Cd and As determination.

Cooked rice samples were dried to constant weight, milled to fine powder, and stored at 4 °C until analysis. Cadmium and As concentrations in raw/cooked rice and cooking water were also determined.

2.2. In Vitro Evaluation of Bioaccessibility

The physiologically based extraction test (PBET) method was modified from previously described protocols (Intawongse, & Dean, 2008; Ruby et al., 1993). The gastric stage used 0.5 g raw or cooked rice samples in 100 mL screw-cap Sarstedt tubes with 50 mL freshly prepared gastric solution. The gastric solution contained 1.25 g L^{-1} pepsin, 0.50 g L^{-1} citric acid, 0.50 g L^{-1} maleic acid, 420 l L^{-1} DL-lactic acid, and 500 l L^{-1} acetic acid dissolved in water, with pH adjusted to 1.5 using HCl. The mixture was incubated at 37 °C with orbital-horizontal shaking at 150 rpm for 60 minutes, then centrifuged at 3000 rpm for 10 minutes. A 5 mL aliquot was collected and filtered through a 0.45 µm filter disk for analysis. Five millilitres of original gastric solution was back-flushed through the filter into the sample tube to maintain the original solid:solution ratio. For the gastrointestinal stage, 52.5 mg bile salts and 15 mg pancreatin were added to the sample tube, and mixture pH was raised to 7.0 with saturated $NaHCO_3$.

Samples were incubated at 150 rpm in a thermostatic bath at 37 °C for an additional 2 hours, then a second 5.0 mL aliquot was collected and filtered. Extracts were stored at 4 °C until analysis. The resultant sample residue (residual fraction) was further digested with aqua regia as described by Intawongse and Dean (2008).

2.3. Determination of Cd and As

Rice samples (0.5 g) were predigested overnight with 5 mL nitric acid in 50 mL centrifuge tubes at room temperature, then heated in a microwave oven (Anton-Paar PE Multiwave 3000). Duplicate analyses were performed for quality control. Reagent blanks and standard reference rice samples (GBW10010 (GSB-1) and GBW10045 (GSB-3)) were included in each batch. Heavy metal recovery rates in standard reference rice samples ranged from 93% to 108%. Heavy metal concentrations in rice samples and extracts were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7700x, Agilent Scientific Technology Ltd., USA). Blank and drift standards were run after every three determinations to calibrate the instrument.

2.4. Data Analysis

Bioaccessibility (%) of Cd and As was calculated as a percentage per digestion using the following equation (Oomen et al., 2002):

Bioaccessibility (%) = $\times$ 100

To evaluate potential hazardous exposure to Cd and As via rice consumption (single or long-term), established daily intake (EDI) and target hazard quotient (THQ) were calculated based on bioaccessibility data (Zhuang, McBride, Xia, Li, & Li, 2009) using the following equations:

EDI = THQ = $\times 10^{-3}$

where RC is daily rice consumption (g person^{-1} d^{-1}), BC is bioaccessible metal concentration, ED represents exposure duration (70 years), EF is exposure frequency (365 days per year), AT is average time for noncarcinogens (365 days year^{-1} × number of exposure years, assuming 70 years), 10^{-3} is the unit conversion factor, and RfD represents oral reference dose (1 and 0.3 µg kg^{-1} day^{-1} for Cd and As, respectively) as suggested by USEPA (2010). Rice consumption rates of 389 g d^{-1} for adults and 277 g d^{-1} for children were obtained from Wang (2005).

2.5. Statistical Analyses

All statistical analyses were performed using SPSS software (Ver 18.0; SPSS, Chicago, IL, USA) and Excel 2013. Data were reported as mean or mean with standard deviation (SD) from multiple samples of each rice type. Means were considered significantly different if p values were < 0.05.

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3. Results and Discussion

3.1. Concentrations of Cd and As in Raw and Cooked Rice

Total Cd and As concentrations for the three rice types at different contamination levels are listed in Table 1 . All rice samples from the mining area and laboratory were contaminated with Cd, exceeding China's maximum allowable concentration of 0.2 mg kg^{-1} Cd in rice (MHPRC, 2012). For raw rice A purchased from southern China markets, approximately 30% of samples exceeded the maximum allowable value, suggesting this rice type could contribute to dietary Cd exposure in populations with high rice intake. Average As values in raw rice A and B were below the maximum allowable value of 0.2 mg kg^{-1} established by both the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2014) and China (MHPRC, 2012). Compared to values reported by Fang et al. (2014), Cd and As concentrations in market rice from this study fall within the range found in rice sampled from public markets in southern China.

The effects of traditional Chinese cooking (water:rice 2:1) on Cd and As concentrations were evaluated across three contamination levels, with results shown in Table 1. Cooking caused significant Cd concentration changes in rice A and B, decreasing by approximately 10% compared to raw rice. However, for highly contaminated rice C, low-volume cooking showed no significant difference. Naseri, Rahmanikhah, Beiygloo, & Ranjbar (2014) reported that cooking can reduce Cd concentration in rice grains, while Wang, Duan, & Teng (2014) found no statistical differences between microwave-cooked and raw rice. Our results align with studies showing cooking decreased Cd concentration in *Agaricus blazei* Murill (Sun, Liu, Yang, & Zhuang, 2012b) and seafood (Atta et al., 1997). Conversely, another study reported increased total Cd concentration in Chilean mussels after cooking (Houlbrèque et al., 2011). The Cd decrease with cooking may relate to Cd solubilization in leaching water, as Cd is typically protein-bound and thermal treatment can enhance protein degradation, releasing Cd as free salts, soluble amino acids, and uncoagulated protein (Perelló, Martí-Cid, Llobet, & Domingo, 2008).

Cooking slightly decreased As concentration by about 3.5–6% across all rice types compared to raw rice. These results align with previous studies (Laparra, Vélez, Barberá, Farré, & Montoro, 2005; Sengupta et al., 2006; Sun, de Wiele, Alava, Tack, & Laing, 2012a) reporting that cooking rice to dryness cannot remove As. Regarding As in other foods, studies suggest high percentages of As release into cooking water from mushrooms (Llorente-Mirandes Llorens-Muñoz, Funes-Collado, Sahuquillo, & López-Sánchez, 2016) and mussels (Houlbrèque et al., 2011), while some observed increased total As concentrations in products cooked with contaminated water (Signes-Pastor, Al-Rmalli, Jenkins, Carbonell-

Barrachina, & Haris, 2012). These studies demonstrate that heavy metal(loid) reduction through cooking depends on cooking conditions (time, medium, temperature, etc.).

3.2. Bioaccessibility of Cd and As in Rice

3.2.1. Bioaccessibility of Cd and As in Raw Rice Oral bioaccessible concentrations of Cd and As measured in gastric and gastrointestinal fractions for raw rice using the in vitro PBET method are presented in Fig. 1 [Figure 1: see original paper]. Significant differences ($p < 0.05$) were found in Cd and As concentrations in gastric and gastrointestinal extracts depending on rice type and contamination level.

Cadmium bioaccessibility varied between 68% and 87% in the gastric fraction, contrasting with the 16.9% Cd bioaccessibility from uncooked rice reported by Yang et al. (2012). Compared to values recorded in raw vegetables by Pelfrêne et al. (2015), our bioaccessibility values for gastric and gastrointestinal fractions were lower or similar, with Cd bioaccessibility ranging from 81–89% in the gastric phase and 63–72% in the gastrointestinal phase. Amiard et al. (2008) found median Cd bioaccessibility in commercial shellfish was 54%. High bioaccessibility percentages during in vitro digestion may be explained by most Cd accumulating in plant cell vacuoles, except what is absorbed by cell walls, making Cd easily released from plant tissues (Fu, & Cui, 2013; Hall, 2002). In the gastric phase, most Cd was dissolved by enzymes, though a portion remained absorbed in plant tissues (Hur, Lim, Decker, & McClements, 2011). In raw rice, Cd bioaccessibility in gastrointestinal fractions was significantly lower than in gastric fractions, consistent with literature (Fu, & Cui, 2013; Intawongse and Dean, 2008; Pelfrêne et al., 2015). This may be attributed to pH increase and addition of organic components like bile extract and pancreatin in the gastrointestinal phase. At typical gastrointestinal pH (increased from 1.5 in gastric phase to 7.0), precipitation and/or resorption of solubilized Cd may occur (Mounicou, Szpunar, Andrey, Blake, & Lobinski, 2002), or Cd may form insoluble complexes with dietary phytate (Versantvoort, Oomen, Kamp, Rompelberg, & Sips, 2005). When Cd concentration in rice increased, bioaccessibility in raw rice changed, but no difference was observed between medium (rice B) and high (rice C) levels. This suggests Cd bioaccessibility in rice is highly sample-dependent, though excessive Cd could not be fully released from the rice matrix or was converted to insoluble forms. Specifically, the Cd percentage remaining in the residual fraction was 14–16% of the total in rice A, B, and C, consistent with values from several vegetables measured by Intawongse and Dean (2008).

Arsenic bioaccessibility in the gastric phase was 62%, 84%, and 93% for raw rice A, B, and C, respectively (Fig. 1 [Figure 1: see original paper]), with significant differences among rice types. Literature reports As bioaccessibility values ranging from approximately 50% to 100% for rice (Signes-Pastor, Al-Rmalli, Jenkins, Carbonell-Barrachina, & Haris, 2012; Sun, de Wiele, Alava, Tack, & Laing, 2012a) and 63–99% for inorganic As in rice (Laparra, Vélez, Barberá, Farré, & Montoro, 2005; Signes-Pastor, Al-Rmalli, Jenkins, Carbonell-Barrachina, & Haris, 2012). In raw rice, As bioaccessibility increased when comparing gastric to gastrointestinal fractions. This increase was statistically significant ($p < 0.05$) for rice A and B, but not for rice C ($p > 0.05$). During 2 hours of intestinal digestion, the bioaccessible As fraction increased significantly to 75% for rice A, 92% for rice B, and 96% for rice C (Fig. 1). Pancreatic enzymes and bile in simulated intestinal juices likely break down polysaccharides into monosaccharides and cleave denatured proteins into free amino acids and small peptides (2–6 amino acid residues), making these compounds more amenable to intestinal absorption (Sun, de Wiele, Alava, Tack, & Laing, 2012a). These processes could further release protein-bound As, thereby increasing As bioaccessibility (Sun, de Wiele, Alava, Tack, & Laing, 2012a). Notably, the percentage of As in the gastrointestinal phase was much higher than that of Cd, indicating the gastrointestinal phase plays an important role in As solubilization during ingestion. The small As concentration remaining in the residual fraction was 3–14% of total As in raw rice, highest in rice B.

3.2.2. Bioaccessibility of Cd and As in Cooked Rice Oral bioaccessible concentrations of Cd and As measured in gastric and gastrointestinal fractions for cooked rice using the in vitro PBET method are shown in Fig. 2 [Figure 2: see original paper]. Compared to raw rice, cooking significantly decreased Cd bioaccessibility in rice B and C (high Cd levels) in both gastric and gastrointestinal phases, while showing no or slight effect on rice A (low Cd level). This aligns with previous studies showing cooking significantly decreased Cd bioaccessibility in various food matrices (Wang, Duan, & Teng, 2014), shellfish (Amiard et al., 2008), *Agaricus blazei* murill (Sun, Liu, Yang, & Zhuang, 2012b), and Chilean mussels (Houlbrèque et al., 2011). This may be explained by the heating process thoroughly destroying tissues, leading to more adsorption during ingestion and lower metal bioaccessibility. Our results showed cooking treatment widened the gap between gastric and intestinal phase bioaccessibility (Fig. 2), possibly because heating reduces plant digestibility (Savoie, Charbonneau, & Parent, 1989) and some functional groups (lysine, methionine, phenylalanine, histidine, and cystine) have affinity for metal ions (Chou, & Shen, 2007). Cadmium bioaccessibility in cooked rice varied from 70–74% in gastric extraction and 41–46% in gastrointestinal phase (Fig. 2), similar to the 74% reported by Wang, Duan, & Teng (2014). Although total Cd concentration varied drastically among rice types, Cd bioaccessibility percentage remained relatively constant (70–74%).

Arsenic bioaccessibility in three cooked rice types varied from 38–67% in gastric fraction and 72–80% in gastrointestinal fraction (Fig. 2). Average As bioaccessibility (ranging between 55% and 71% across both phases) in our three cooked rice types was similar to values reported by Sun, de Wiele, Alava, Tack, & Laing (2012a) but lower than those reported by Laparra, Vélez, Barberá, Farré, & Montoro (2005) and Signes-Pastor, Al-Rmalli, Jenkins, Carbonell-Barrachina, & Haris (2012). However, those studies focused on As bioaccessibility from rice cooked with As-contaminated water. Trenary et al. (2012) reported mean total As bioaccessibility for 17 rice samples was 61% (range 45–79%) using an in vitro synthetic gastrointestinal extraction protocol, though some studies reported that cooking long-grain white rice does not affect As bioaccessibility (Horner, & Beauchemin, 2013).

In the residual fraction, cooking substantially increased Cd and As bioaccessibility: 22%, 29%, and 41% for Cd and 40%, 47%, and 38% for As were recovered in rice A, B, and C, respectively (Fig. 2). This likely occurs because cooking reduces Cd and As bioaccessibility by binding metals to other compounds and forming non-bioaccessible complexes in the residual fraction. To date, few studies have examined the residual fraction during in vitro digestion (Intawongse, & Dean, 2008), finding Cd concentration in the residual phase of several raw vegetables ranged between 16% and 38%. Mounicou, Szpunar, Andrey, Blake, & Lobinski (2002) further extracted insoluble residue from cocoa samples after the gastrointestinal phase, recovering an additional 20% or 30% of Cd using phytase and cellulose, respectively. Interestingly, after cooking, bioaccessible As concentration in the residual fraction of rice C was significantly higher ($p < 0.05$) than in both gastric and gastrointestinal phases. This insoluble Cd or As in the residual fraction might be bound to dietary fiber constituents such as crystalline cellulose microfibers that are difficult to destroy (Mounicou, Szpunar, Andrey, Blake, & Lobinski, 2002). We hypothesize that excessive Cd could not be completely extracted by simulated gastric and intestinal juices in this in vitro method, possibly binding to components forming strong insoluble complexes in the gastrointestinal lumen. Clearly, more research on the residual fraction is needed to better understand metal distribution during digestion.

Food matrix contamination level is considered a factor affecting bioaccessibility, with a dose-proportional relationship between contamination level and bioaccessibility/bioavailability often assumed in risk assessment. This study observed a positive relation ($p < 0.05$) between Cd bioaccessibility in both gastric and gastrointestinal phases and increasing total Cd concentration across three raw rice types (Fig. 3 [Figure 3: see original paper]). High contamination levels in raw rice explained a large proportion of variance in predicted bioaccessibility levels ($R^2 = 0.98$). Similar relationships were found between contamination level and Cd bioaccessibility in raw rice (R^2 ranging 0.91–0.95 in bioaccessible fraction) by Yang et al. (2012) and in vegetables ($R^2 = 0.99$ in gastric and gastrointestinal phases) by Pelfrêne et al. (2015). Linear regression in Fig. 3 showed a statistically significant relationship ($p < 0.01$) between rice total As concentration and percent bioaccessible As following gastric and gastrointestinal phases in raw rice, implying As bioaccessibility is concentration-dependent.

3.3. Bioaccessibility of Cd and As in Cooked Rice with Different Water-to-Rice Ratios

Little is known about Cd and As leaching from cooked rice prior to consumption, particularly for Cd. Two general rice preparation methods exist: (1) cooking with excessive water and discarding the remaining water; and (2) cooking with measured water amounts that are fully absorbed. Bioaccessibility of Cd and As in cooked rice with three water-to-rice ratios (2:1, 4:1, and 6:1) is presented in Fig. 4 [Figure 4: see original paper].

No significant differences were observed in Cd bioaccessibility across all cooked rice types. Cadmium bioaccessibility was reduced to a mean of 46–61% of total Cd in all cooked rice levels (Fig. 4). Variation among low-, medium-, and high-volume cooking was $< 5\%$ in Cd bioaccessibility for the three rice types, indicating very low Cd leaching into boiling water during cooking and that abundant cooking water did not change bioaccessible Cd across contamination levels. To date, no previous data on Cd bioaccessibility reduction in different contamination levels of rice subjected to various cooking treatments have been reported, preventing comparison with literature.

This study found that cooking rice in large water volumes (water:rice 6:1) had the greatest effect in reducing As levels. Average As bioaccessibility trends in cooked rice A, B, and C followed the order: $2:1 > 4:1 > 6:1$. Cooking rice to dryness at a 2:1 water-to-rice ratio resulted in the least Cd and As loss compared to larger cooking water volumes. Several studies noted that low-volume cooking (no water discarded) did not significantly change As concentration in cooked rice relative to raw rice (Natio, Matsumoto, Shindoh, & Nishimura, 2015; Raab, Baskaran, Feldmann, & Meharg, 2009; Sengupta et al., 2006). Laparra, Vélez, Barberá, Farré, & Montoro (2005) reported similar results, finding that no washing and medium-volume cooking (water:rice 4:1) until no water remained did not remove total As from brown and white rice. High-volume cooking (water:rice 6:1) followed by discarding excess water reduced As bioaccessibility to a mean of 37–58% of total As across all rice levels (Fig. 4). Significant differences ($p < 0.05$) were found among As bioaccessibility at 2:1, 4:1, and 6:1 water-to-rice ratios for all rice types. Compared to Raab, Baskaran, Feldmann, & Meharg (2009), who reported high-volume cooking (water:rice 6:1) effectively removed 65% of total As (ranging 55–72%) from raw rice, and Sengupta et al. (2006), who found approximately 57% of total As removal at a 6:1 water-to-rice ratio, our results are consistent. Since metal(loid)s are not evaporated or broken down into safer compounds during boiling, frying, or other cooking processes, they only transfer from the food matrix to frying oil, boiling water, or cooking stocks. Therefore, large cooking water volumes are effective for reducing As concentration in cooked rice (Raab, Baskaran, Feldmann, & Meharg, 2009).

3.4. Health Risk Assessment of Cd and As in Rice

Average bioaccessible EDIs and THQs for Cd and As in adults and children via consumption of rice A and B are presented in Table 2 . Based on bioaccessibility data, EDI values for Cd and As from rice A for adults and children were 0.34 and 0.53 µg kg^{-1} day^{-1}, and 0.44 and 0.70 µg kg^{-1} day^{-1}, respectively—well below provisional tolerable daily intakes (PTDI: 0.83 µg day^{-1} kg^{-1} BW for Cd and 2.1 µg day^{-1} kg^{-1} BW for As, JECFA). For daily average consumption of rice B, Cd

EDIs for adults and children were 212% and 280% of PTDI values, respectively, while As EDIs were below recommended PTDI. These results indicate long-term, large consumption of rice B from mining areas would result in high Cd exposure. The fact that cooked rice showed higher Cd and As bioaccessibility is particularly concerning since this rice type is most commonly consumed in China.

As shown in Table 2, except for Cd THQs from rice A, bioaccessible THQs for Cd and As from rice A and B all exceeded 1, indicating relatively high health risks for inhabitants near mining areas. In China, "cadmium rice" or "arsenic rice" produced from contaminated soils near mining areas sold in markets poses great health risks to consumers (Sun, de Wiele, Alava, Tack, & Laing, 2012a; Zhuang, McBride, Xia, Li, & Li, 2009). EDI and THQ values for Cd and As were higher for children than adults, raising concerns about high metal(loid) exposure in children through rice consumption. Given contaminated rice situations, health risks from Cd and As poisoning are greatest for people eating rice several times daily, though eating less rice is not an option in many world regions where it is an irreplaceable cultural, dietary, and lifestyle staple.

It should be noted that Cd and As absorption during ingestion would be highly variable in human populations because rice metal(loid) bioaccessibility is influenced by many factors (Intawongse, & Dean, 2008; Mounicou, Szpunar, Andrey, Blake, & Lobinski, 2002; Sun, de Wiele, Alava, Tack, & Laing, 2012a), including nutritional characteristics, gastrointestinal tract contents, crystalline cellulase microfibers and phytates, microbial processes, metal species and speciation, and food processing methods. Consequently, further in-depth studies on effective methods for reducing Cd and As bioaccessibility in rice are required for more accurate risk assessment.

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Conflict of Interest Statement

The authors declare no conflict of interest affecting this work.

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Figure 1. Bioaccessibility of Cd and As (%, in gastric, gastrointestinal and residual phases) in three contamination levels of raw rice.

Figure 2. Bioaccessibility of Cd and As (%, in gastric, gastrointestinal and residual phases) in three contamination levels of cooked rice.

Figure 3. Relationship between oral bioaccessibility and total concentrations (mg kg^{-1}) of Cd and As in selected rice samples.

Figure 4. The average bioaccessibility of Cd and As in the ingestion phases (mean and SD expressed as a % of total Cd concentration) in cooked rice. Low, middle and high volume cooking are water:rice 2:1, 4:1 and 6:1, respectively. Different letters indicate significant differences at $p < 0.05$ as calculated by the least significant difference (LSD) test.

Note: Figure translations are in progress. See original paper for figures.