



Occurrence and bioaccessibility of mercury in commercial rice samples in Montreal (Canada)



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ABSTRACT

The objective of this study was to increase the understanding of mercury exposure via rice commonly consumed in a major North American city. Rice samples were collected from Montreal markets ($n = 89$) between 2016 and 2017 and analyzed for total mercury (THg) content. THg content ranged from 0.7 ± 0.1 to $9.3 \pm 0.5 \text{ ng g}^{-1}$ dw. Significant differences ($p < 0.05$) were recorded among the various rice types and countries of origin. Overall, cooking had little effect on the THg concentrations in rice. Thiols play a major role in the fate of Hg, therefore thiol contents in rice were measured, and a weak but significant relationship between thiol and THg contents in rice ($p < 0.05$) was observed. An *in vitro* gastro-intestinal digestion method was used to assess the bioaccessibility rate of THg in cooked rice samples, and less than 44.5% of THg from the initial rice samples was bioaccessible after *in vitro* digestion. Dietary exposure to Hg through rice consumption was calculated for the typical Canadian population and values were all below current provisional tolerable weekly intake guidelines. This study improves our understanding of Hg exposures via rice in a large North American city.

1. Introduction

Mercury is a ubiquitous contaminant present in foods at varying concentrations. It has been widely studied as different species, including inorganic Hg and methylmercury (MeHg). For example, MeHg is of particular concern for its bioaccumulation and toxicity to humans (Eagles-Smith et al., 2018; Ha et al., 2017). Dietary sources are a major pathway of Hg exposure to humans (UNEP/WHO, 2008; Sheehan et al., 2014). To date, most studies have focused on fish and seafood as a major source of exposure to Hg. Recently, studies have reported that rice is an important bioaccumulator of Hg and could become an important dietary source of this toxicant, especially for populations that have high rice consumption rates (Rothenberg et al., 2014). Indeed, rice was assessed as a major source of Hg (including MeHg), in the diet of inhabitants of some Chinese inland cities, even rivaling that of seafood: a study from China indicated that, in areas with severe Hg pollution, rice consumption may account up to 94–96% of the probable daily intake of MeHg (Feng et al., 2008; Zhang et al., 2010a, 2010b). Given that rice serves as the main staple of more than half of the world's population and accounts for 20 percent of the total food energy intake of the world's population, there is a need to increase understanding of

Hg exposure from rice in other geographic regions (FAO, 2006). In North America, however, there is a limited amount of data on the occurrence of Hg in rice (Rothenberg et al., 2014). For example, in Canada, we found one study that reported upon total mercury (THg) levels in rice. In this study, THg contents were reported in two individual rice composites from a total diet study from Whitehorse in 1998 (THg: 0.57 ng g^{-1}) and Ottawa in 2000 (THg: 1.8 ng g^{-1}) (Dabeka et al., 2003).

Recent studies on a range of food items have suggested that only a fraction of the ingested mercury reaches the bloodstream, which is defined as the bioavailable fraction (Moreda-Pineiro et al., 2011; Bradley et al., 2017). Bioaccessibility relates to the soluble fraction of ingested contaminant at the end of the digestive processes and can be used as a conservative estimate for bioavailability. *In vitro* testing is one of the most common approaches for estimating Hg bioaccessibility from food (Ruby et al., 1999; Ouédraogo and Amyot, 2011; Siedlikowski et al., 2016), and aims at assessing the contaminant behavior under controlled conditions simulating the key processes and/or factors in gastrointestinal digestion, e.g. temperature, agitation, pH, enzyme and chemical composition (Jadan-Piedra et al., 2016). Based on *in vitro* studies, bioaccessibility of Hg to humans from fish consumption ranges

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from 2% to 100% for MeHg and from 0.2% to 94% for inorganic Hg (Bradley et al., 2017). To date, bioaccessibility and bioavailability of Hg species have been relatively well characterized for seafood and only recently, three studies have estimated the bioaccessibility of THg in rice powder samples in China (Liao et al., 2018; Wu et al., 2018; Gong et al., 2018). THg bioaccessibility ranged from 33.5% to 44.3% in cooked rice powder samples (Liao et al., 2018), and was lower than 50% in rice powder samples from background sites and a Hg mining area in China (Wu et al., 2018). Even though these studies help increase understanding of Hg bioaccessibility in rice samples, they are not necessarily relevant to the North American commercial rice products. In addition, the above two bioaccessibility studies were conducted on rice powder, and THg bioaccessibility from actual cooked rice grains is not known.

There is increasing evidence that multiple factors (sources, Hg speciation, cooking methods, nutrients) may affect Hg bioavailability in seafood (Bradley et al., 2017). Notably, higher cysteine concentrations, and thus stronger MeHg-protein binding, has been suggested to explain the lower MeHg bioaccessibility from crayfish (Peng et al., 2017). Phytochelators or their precursor, glutathione (GSH), play a major role in the defense mechanism of plants against metal contamination. For example, a severe depletion in GSH content was observed in Hg-treated rice roots under controlled conditions (Chen et al., 2012). Thus, thiol contents (including cysteine and glutathione) in rice may be related to THg levels and could serve as a marker of contamination.

The objectives of this research were to: (i) measure THg content in market rice samples in Montreal (Canada) and estimate the contribution of rice as a dietary source of THg in the Canadian population, (ii) to investigate the effect of cooking on THg content in rice samples (iii) to assess the bioaccessibility rate of THg in cooked rice samples collected in Canada and (iv) to explore the relationship between thiol and THg levels in rice.

2. Materials and methods

2.1. Chemicals

A mercury standard (1000 µg/mL) was purchased from VWR International (Radnor, PA, USA). Cysteine hydrochloride monohydrate, GSH, guanidine hydrochloride (UltraPure), tris(hydroxymethyl)aminomethane (Tris, ACS reagent ≥ 99.8%), glycine (≥ 98.5%), 5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB, BioReagent, ≥ 98%), ethylenediaminetetraacetic acid (EDTA, ≥ 98%), α-amylase (≥ 10 units/mg solid), porcine pepsin (≥ 400 units/mg), pancreatin from porcine (4 × ; USP specifications), bile salts, sodium carbonate (ACS reagent ≥ 99.8%), hydrochloric acid (36.5–38.0%), sodium chloride (≥ 99.999%), sodium hydroxide (≥ 99.99%) were purchased from Millipore-Sigma (Burlington, MA, USA). Ultrapure water (18.2 MΩ cm) was produced from a Milli-Q system (Millipore Corporation).

2.2. Sample collection

The expected variability of THg in rice was assessed from a preliminary pilot study (unpublished work), and it was estimated that > 61 samples would be needed to assess THg in rice with a relative error of 15%. Therefore, eighty-nine rice samples (73 white rice and 16 brown rice) were collected from grocery shops and supermarkets located on the Island of Montreal (QC, Canada), between April 2016 to March 2017. Rice samples (including organic rice samples) were classified according to six types: basmati rice, short and medium grain rice, long grain rice, jasmine rice, red & black rice (Fig. 1). The detailed profile of rice samples is presented in Table 1. These rice products were imported from eight countries: USA (48% of the samples), Thailand (17%), Pakistan (13%), India (8%), Italy (4%), Vietnam (3%), Argentina (2%), China (2%) and Spain (1%). This distribution somehow mirrored Canadian rice import figures (See Supplementary Fig. S1, Statistics Canada, 2015), and therefore rice consumption figures in

Canada (since rice is mostly imported).

Twenty to 50 g of rice were transferred into a polyethylene bag upon opening of the commercial products. In the laboratory, rice samples were washed three times with Milli-Q water (23 °C) directly in Ziploc bags and then dried in an oven at 50 °C overnight according to the reported procedures for rice (Al-Saleh and Shinwari, 2001; Horvat et al., 2003). In the present study, a preliminary test confirmed that washing/drying did not influence significantly the THg concentrations of rice samples ($n = 5$, $p > 0.05$). After drying, rice samples were ground into powder with a stainless-steel laboratory blender (Waring, Torrington, CT, USA). The moisture content of each dried rice sample was measured according to the methods modified from the previous literature (Chen, 2003).

2.3. Cooking procedures for rice samples

Eleven rice samples were randomly selected among the various rice types to investigate the effect of cooking. One gram of each type of rice was weighed in individual 8 mL vials and cooked with Milli-Q water. The ratio of water to rice powder was 2:1 for white rice as reported (Li et al., 2010). For brown rice, this ratio had to be increased to 2.5:1 to ensure the complete cooking of the rice powder, a ratio also reported for rice (Wang et al., 2014). All the vials were placed in a water bath (100 °C) for 25 min. The weight of vials (containing rice powder and water) was recorded before and after cooking, to ensure that no major water loss would occur in the vials during the cooking process. In addition, four rice samples were selected and cooked as whole grains. Around 10–15 g of whole grain rice samples were cooked in vials (rice: water: 1:2), in a water bath (100 °C) for 25 min.

2.4. THg analysis

THg content in rice was measured (in duplicate) by thermal decomposition, amalgamation, and atomic absorption spectrophotometry (absorbance: 253.7 nm) in a direct mercury analyzer (Nippon MA-3000, Japan), similar to previously described methods (Rutkiewicz and Basu, 2013; Basu et al., 2009). For each individual sample, 200 mg of dried and pulverized rice was transferred to a ceramic analytical vessel and loaded in the autosampler of the instrument. Controlled heating was used to liberate Hg from the matrix in the furnace inside the mercury analyzer. The temperature program was 180 °C for 120 s followed by 850 °C for 120 s. The limit of detection (LOD) of the method was estimated from the analysis of procedural blanks. Standard reference materials (SRM) NIST-SRM 1573 (tomato leaves, National Institute of Standards and Technology, Gaithersburg, MD, US; about 30 mg per analysis), DORM-4 and DOLT-5 (fish muscle protein and dogfish liver, National Research Council, Canada; about 400 mg per analysis) were used to assess the accuracy of the method. THg content in rice samples both as whole grain and as homogenized powder ($n = 5$ samples in each set) were measured to assess the variability of THg among rice grains.

2.5. Thiol content measurement

The thiol content of rice was measured with a method adapted from Gujral and Rosell (2004), relying on a chromogenic reaction of thiol groups with DTNB, generating 2-nitro-5-thiobenzoic acid (TNB), a product with a maximum absorbance at 412 nm. In this method, a tris-glycine (Tris-Gly) buffer was prepared by dissolving 10.4 g Tris, 6.9 g glycine and 1.2 g EDTA in 1 L of Milli-Q water and adjusting pH to 8.0 with HCl. A GdnHCl/Tris-Gly solution containing 5 M guanidine hydrochloride (GdnHCl, 0.4778 g mL⁻¹) was then prepared. The Ellman's reagent contained 4 mg of DTNB in 1 mL of Tris-Gly buffer (pH 8.0) and was prepared fresh daily. For white rice samples, rice flour (200 mg) was suspended in 1 mL of GdnHCl/Tris-Gly solution, vortexed for 10 min and centrifuged at 16000 × g (4 °C) for 5 min. GdnHCl/

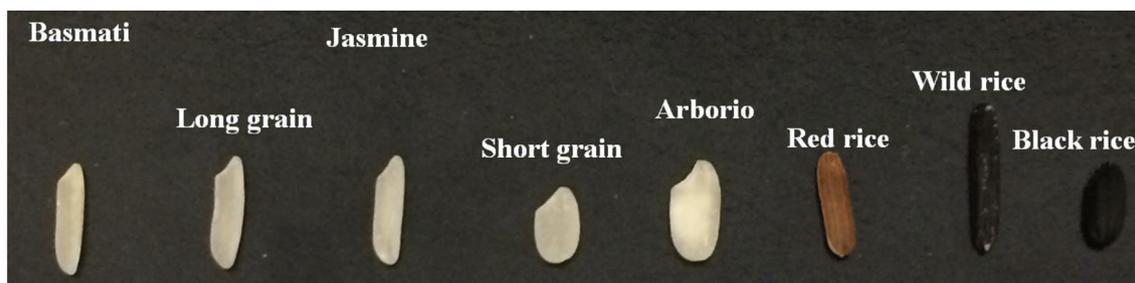


Fig. 1. Morphology of various types of rice samples studied in the current work.

Table 1
Types and origin of the rice samples.

Country of origin	Number of rice samples collected					
	basmati	short and medium grain	long grain	jasmine	red and black	total
USA	3/1*	14/3*	13/7*	1/0*	1	43
Thailand	0	1/1*	0	9/0*	4	15
Pakistan	11/1*	0	0	0	0	12
India	6/1*	0	0	0	0	7
Italy	0	4/0*	0	0	0	4
Vietnam	0	1/0*	0	2/0*	0	3
Argentina	0	0	1/1*	0	0	2
China	0	0	0	0	2	2
Spain	0	1/0*	0	0	0	1
Total per Type	23	25	22	12	7	89

Note: X/Y* indicates the number of white and brown rice samples collected respectively for the category. 0: no rice samples was collected in this category in the present study.

Tris–Gly solution (0.75 mL) and Ellman's reagent (0.05 mL) were added to 0.5 mL of the clear supernatant. After 20 min, the absorbance (412 nm) of the solution was recorded. The approach used for brown rice samples was similar except that the supernatant was filtered through a 0.22 μm filter before mixing with the GdnHCl/Tris–Gly buffer and the Ellman's reagent. The method was not suitable to detect thiol levels for black and red rice samples, because pigments interfere with TNB absorbance measurements (Wrolstad et al., 2005). As for parboiled rice, we observed that the extraction solution was absorbed quickly by most of the parboiled rice, resulting in little extraction of thiol groups. Therefore, thiol contents of parboiled rice samples were not measured. Cysteine hydrochloride monohydrate was used to build the standard calibration curve (0–0.15 mM) for thiol content. GSH (0.7 $\mu\text{mol/g}$ –3.4 $\mu\text{mol/g}$) was spiked in selected samples to assess the recovery of the method (Rahman et al., 2006).

2.6. Bioaccessibility of THg in rice

Human digestion can be reproduced in a simplified manner in the laboratory using a 3-phase *in vitro* digestion model, mimicking the oral, gastric and intestinal phases (Minekus et al., 2014; Siedlikowski et al., 2016). Simulated saliva fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared (See Supplementary Fig. S2, Tables S1 and S2) following the guidelines developed by Minekus et al. (2014). In short, 1.5 g cooked or raw rice was weighed in a 50 mL Erlenmeyer flask and digested with 1.5 mL of the SSF enzyme solution for 2 min (37 °C). Then, 4.5 mL of the SGF enzyme solution was added and the flask was incubated for 1 h (37 °C, 120 rpm). During this hour, the intestinal enzyme solution was prepared with pancreatin and bile salts. Once the gastric digestion was finished, the pH of all the samples was adjusted to 6.5–7.5 (using 6M HCl or 1M NaOH), and the SIF enzyme solution was added. The intestinal digestion lasted for 2 h (37 °C,

120 rpm). Samples were then centrifuged at 3000 g (4 °C, 15 min), and the weights of the supernatant and the particulate pellet were recorded. Both supernatants and pellets were stored at –20 °C until THg analysis. Each digestion test was completed in triplicates. The percentage of THg in the aqueous phases (supernatants) of this bioaccessibility assay was defined as “bioaccessible”. THg bioaccessibility (%) was calculated from the mass (ng) of THg measured in the initial rice samples and in the pellets after the *in vitro* gastrointestinal digestion as previously described (Ouedraogo and Amyot, 2011; Siedlikowski et al., 2016) using the following equation:

$$\text{THg bioaccessibility} = \frac{[\text{THg in initial cooked rice} - \text{THg in pellets}]}{\text{THg in initial cooked rice}} \times 100\% \quad (1)$$

2.7. Statistical analysis

Excel (2017, Microsoft) and SPSS (IBM) software were used for statistical analysis. Specific tests were used as described in the results section below. Kolmogorov–Smirnov test was used to test the normality of data. When the normality of samples was not verified, data were tested with non-parametric test (Kruskal–Wallis) for multiple comparisons. Statistical tests were performed using Excel, SPSS and SigmaPlot v13.0 (Systat Software Inc) to compare groups, with the significance level $p < 0.05$.

3. Results and discussion

3.1. THg in raw and cooked rice samples

The accuracy of the THg analysis was confirmed through the systematic analysis of SRMs. The mean recoveries averaged $89.8 \pm 7.9\%$ for NIST-SRM-1573 ($n = 68$), $100.4 \pm 2.6\%$ for DORM-4 ($n = 5$), and $87.7 \pm 1.4\%$ for DOLT-5 ($n = 3$). The method detection limit for THg was estimated to 0.4 ng g^{-1} , all the detected samples were above the limit of detection. For selected samples, THg was measured in both whole grains and as homogenized rice powder ($n = 5$ for each set) and the relative standard deviations (RSD) for the measurements are reported in Table S3. RSDs obtained for the homogenized rice powders ($< 11.4\%$) were 3–13 times lower compared to the whole grains (7.6–77.5%). Since this research explored small variations in the THg content during cooking or digestion, it was therefore necessary to perform the study on the homogenized rice powder as the variability amongst rice grains was too large. The RSD values of rice powder sample replicates averaged $7.5 \pm 7.7\%$.

The THg contents of different rice types are presented in Table 2. The individual THg content of each of the 89 samples are presented in the Supplementary Information (Table S4) and ranged from 0.7 ± 0.1 to $9.3 \pm 0.5 \text{ ng g}^{-1}$ (dry weight basis). The average concentration for the 89 samples was $2.8 \pm 1.8 \text{ ng g}^{-1}$, and there was an overall variation factor of 13 amongst these samples. The highest THg content ($9.3 \pm 0.5 \text{ ng g}^{-1}$) was recorded in Carnaroli rice (Italy, sample code *sho & med gr20*, See Supplementary Table S4), a type of short grain rice.

Table 2
Mean THg content (ng.g⁻¹, dry weight basis) in different types of rice available in the Canadian market.

Rice type	THg (ng.g ⁻¹ , dry weight basis)
Brown rice (n = 15)	3.5 ± 2.3
White rice (n = 67)	2.6 ± 1.6
Basmati rice (n = 23)	1.7 ± 0.7
Short and medium grain rice (n = 25)	2.0 ± 1.8
Long grain rice (n = 22)	4.1 ± 1.7
Jasmine (n = 12)	2.9 ± 1.3
Black and red rice (n = 7)	4.7 ± 1.2

Table 3
THg content (ng.g⁻¹) in market rice samples in various countries.

Countries	THg (ng.g ⁻¹)	References
Canada	2.8 ± 1.8	This study.
Canada	Ottawa: 0.57 Whitehorse: 1.8	Dabeka et al. (2003)
Brazil and Spain	Brazil: 3.1 (2.1–4.4) Spain: 2.1 (1.6–3.3)	da Silva et al. (2010)
China	5 ± 3	Huang et al. (2013)
Europe	3.04 ± 2.7	Brombach et al. (2017)
Kuwait	4–14	Jallad (2015)

These results are compared with the THg content in rice samples recorded in the literature (Table 3). The THg content in the present study are in line with the two values reported in two individual composite samples collected about 20 years ago in Canada cities including Ottawa (0.57 ng.g⁻¹) and Whitehorse (1.8 ng.g⁻¹) (Dabeka et al., 2003). The present mean concentration was also in line with other market basket surveys with a large sample size in China (5 ± 3 ng.g⁻¹, n = 224; Huang et al., 2013) and in Europe (3.04 ± 2.7 ng.g⁻¹, n = 87; Brombach et al., 2017).

THg concentrations in rice samples were not normally distributed ($p > 0.05$, Kolmogorov-Smirnov test and Shapiro-Wilk tests). Based on the non-parametric Wilcoxon-signed rank test, there was no difference ($p > 0.05$) between THg concentrations of brown rice (n = 15, 3.5 ± 2.3 ng.g⁻¹) and white rice samples (n = 61, 2.6 ± 1.6 ng.g⁻¹) from the market. This is consistent with the study by Brombach et al. (2017) which also demonstrated similar concentrations between the two types of rice. To date, the comparison among various types of commercial rice products has not been reported in Canada, though it had been explored in Europe and China (Brombach et al., 2017; Zhang et al., 2010a). In the present study, THg contents were compared across rice types subgroups (basmati rice, black and red rice, jasmine rice, long grain rice, short and medium grain rice, sweet rice). Black and red rice samples, as well as long grain rice, had higher mercury contents compared to sweet, short/medium grain and basmati rice (Kruskal-Wallis, $p < 0.05$). THg contents were measured in 11 selected rice samples before and after cooking (Fig. 2). There was a significant linear correlation ($R = 0.93$, $p < 0.0001$) between THg content in cooked rice (ng.g⁻¹, wet weight basis) and the levels in the initial raw rice (ng.g⁻¹ dw). When converted on a dry weight basis, THg concentrations did not vary significantly ($p > 0.05$, Wilcoxon-signed rank test) between raw and cooked rice samples. This result is in line with previous research aimed at addressing whether cooking alters Hg contents of fish samples, which also concluded culinary treatments had little effect on the THg concentrations in fish (Ouédraogo and Amyot, 2011).

3.2. Thiol content measurement

The analytical performances of the analysis of thiol content in rice were assessed, and the recoveries for spiked GSH ranged from 85 to 100% (n = 12). The limit of quantification (LOQ) was estimated as 0.4 μmol.g⁻¹ rice powder (dry weight basis). Cysteine hydrochloride

monohydrate (thiol standard). Most of detected values were above the LOD. The RSD of the measurements of real rice samples averaged 5.2 ± 4.0%. The average thiol content value (n = 74) is 0.86 ± 0.24 μmol.g⁻¹ rice (dry weight basis). The mean thiol contents of the various rice types are summarized in Table 4. No significant differences were noted among the various rice types (Kruskal-Wallis, $p > 0.05$, see Supplementary Fig. S3). Thiol content (μmol.g⁻¹ dry weight basis) was tested for correlation with THg concentration (Fig. 3), revealing a significant though weak linear correlation between the two parameters ($R = 0.29$; $p = 0.01$). Literature has indicated thiol groups playing a very important role in the detoxification of metals in plants, and some have observed relationships in plants exposed under controlled conditions to an excessive amount of Hg (Chen et al., 2012). Thiol content did appear to be a marker at some degree for THg in rice in the present study. Studying the synergetic effects of multiple heavy metals could also help explain the distribution of thiol contents: other toxic metals are also known to be bound with thiol groups in rice plants, such as arsenic, cadmium, selenium, etc (Kumar et al., 2016).

3.3. THg bioaccessibility in rice in Canada

Preliminary experiments were conducted to assess the recovery of Hg in the *in vitro* digestion models using raw and cooked rice. THg was measured in both pellet and supernatant fractions after digestion. THg recoveries averaged 97 ± 27% (n = 7), confirming no Hg loss in the models.

In the present study, THg bioaccessibility was assessed for cooked rice powder and cooked whole grain rice to investigate any potential "surface effect". For each experiment, THg levels before and in the particulate phase after digestion were compared using paired t-tests. For cooked rice powder samples, significant differences ($p < 0.05$) were found for two out of four rice samples (Table 5). Similarly, THg contents for cooked whole grain rice samples decreased significantly ($p < 0.05$) after the digestion for 3 out of 4 samples, which indicates that some THg became bioaccessible during the digestion. Bioaccessibility percentage ranged from 0 to 38.6% for cooked rice powder and from 12.5 to 44.6% for cooked rice grains (Table 5). In the case of the slightly negative bioaccessibility percentage, THg was considered as "not bioaccessible", taking the overall standard deviation into account. Overall, bioaccessibility percentage values in cooked rice powder were not significantly different compared to whole rice grains (one-way ANOVA, $p > 0.05$).

In 2014, Meng et al. had reported that some Hg species occur mostly at the surface of rice grains. The present result confirms that overall, this does not contribute to major differences in bioaccessibility between cooked rice powder and rice grains THg bioaccessibility was recently in commercial and environmental rice powder samples from China (Liao et al., 2018; Wu et al., 2018). In these studies, results showed that < 50% of the THg was bioaccessible in the studied rice powder samples, which is consistent with what we reported here on commercial rice from the Canadian market. To date, most of the Hg bioaccessibility studies have focused on fish (See Supplementary Table S5) and overall the THg bioaccessibility in rice (0–44.6%) is comparable to these studies (See Supplementary Table S5, Table S6). This may be somewhat surprising given that differences attributable to various factors could be expected. First, the food matrix is known to affect the digestion process and metal bioaccessibility (Minekus et al., 2014). Zhuang et al. (2016) reported that Cd bioaccessibility percentage values were greater in plant-based food compared to fish foods, possibly because Cd species accumulate in the vacuoles of plants and are more easily extracted during the digestion. The use of modified *in vitro* gastrointestinal digestion protocols in this research can also affect the bioaccessibility. For example, different "solid to liquid" ratio (solid:food volume, liquid:digestive fluids) have been reported in the literature on bioaccessibility. Ruby et al. (1999) showed that the solid to liquid ratio can affect metal diffusion mechanisms between the solid and liquid phases during the

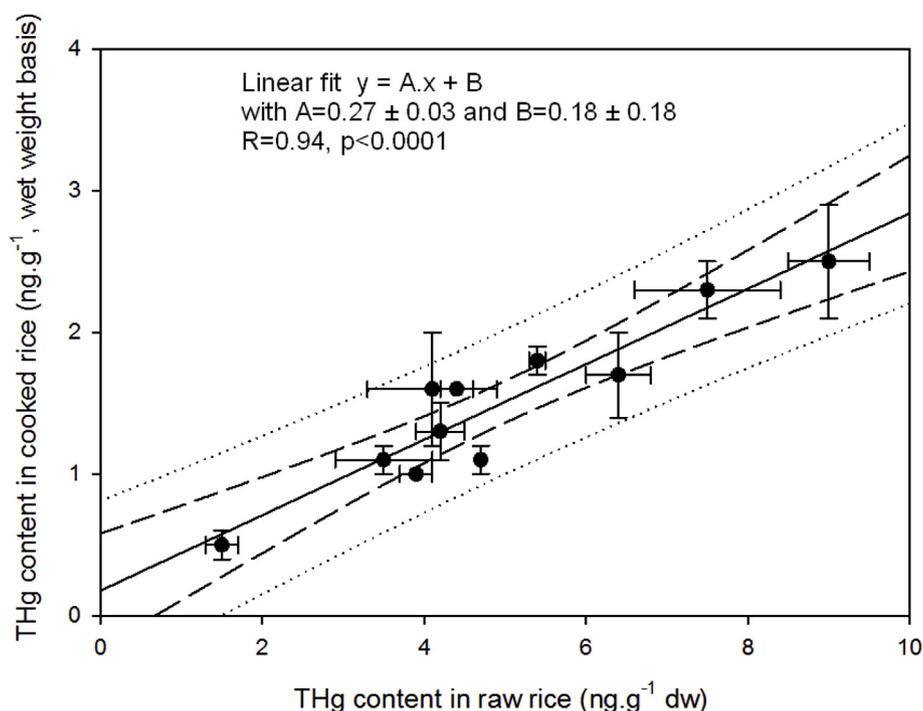


Fig. 2. THg content in cooked rice (ng.g^{-1} , wet weight basis) in selected rice samples compared to the levels in the initial raw rice (ng.g^{-1} dw). The plain line represents the linear fit. The dashed and the dotted lines represent the 95% confidence and prediction bands respectively.

Table 4

Thiol content values of various rice types.

Type of rice	Average thiol content ($\mu\text{mol/g dw}$)
Brown rice (n = 15)	0.85 ± 0.14
White rice (n = 47)	0.86 ± 0.25
Basmati rice (n = 23)	0.72 ± 0.24
Long grain rice (n = 17)	0.91 ± 0.21
Jasmine (n = 12)	0.96 ± 0.23
Short and medium grain rice (n = 24)	0.91 ± 0.21

digestion process.

3.4. Estimated dietary exposure to THg through rice consumption

The present data were used to estimate the dietary exposure to THg. In Canada, the mean daily intake of rice is about 18.6 g.day^{-1} , while the daily intake for a population with a rice-based diet (various countries in Asia) has been reported to reach 578 g.day^{-1} of raw rice (Statistics Canada, 2015; FAO, 2002). Provisional tolerable weekly intake (PTWI) values of 1.6 and $4 \mu\text{g kg}^{-1}$ bw (bodyweight) have been defined for MeHg and inorganic mercury respectively (JECFA, 2010). Rice consumption may differ for different Canadian subpopulations. Based on the mean intake of rice in Canada and the mean THg level recorded in the present study, the weekly dietary exposure would be estimated to $0.006 \mu\text{g kg}^{-1}$ bw for a 60-kg adult (details on the calculations are presented as supplementary material). In an extreme situation combining a high rice consumption (578 g.day^{-1}) and the maximum THg concentration of 9.3 ng g^{-1} found in one rice sample in this research, the weekly dietary exposure would reach $0.62 \mu\text{g kg}^{-1}$ bw, which would correspond to 15.5% or 38.8% of the PTWI for inorganic Hg and MeHg respectively. These estimates are estimated to be worst case scenarios especially since MeHg accounts for a fraction of THg, about $71 \pm 26\%$ in commercial rice samples from Europe according to Brombach et al. (2017). In addition, THg bioaccessibility values obtained from the present study were used to assess the literature reported

THg exposure in rice studies. The highest THg bioaccessibility in this study is around 45%. Considering the worst-case scenario, with the highest THg bioaccessibility values, dietary exposure values inclusive of the bioaccessibility percentage values would represent an even smaller fraction of the Hg PTWIs set by JECFA (2010).

A limitation of the present study is the focus on THg rather than targeting Hg species in rice samples. THg was measured in the rice samples rather than Hg species due to the ease and low cost of THg analyses. As species such as MeHg are relevant for risk assessment, future studies should assess to what extent MeHg varies among commercially-available types of rice.

4. Conclusions

Based on the present study, THg content ranged from 0.7 ± 0.1 to $9.3 \pm 0.5 \text{ ng g}^{-1}$ (dry weight basis), with a mean value of 2.8 ng g^{-1} (dry weight basis). Results were in line with the levels reported in other market basket surveys with a large sample size in China or in Europe. Significant differences were recorded among the various rice types and countries of origin. Overall, cooking had little effect on the THg concentrations in rice. There was a weak but significant relationship between thiol and THg contents in rice ($p < 0.05$). Since the amount of mercury ingested does not always reflect the amount that is available to the body, an *in vitro* gastro-intestinal digestion method was used to assess the bioaccessibility rate of THg in cooked rice samples. Results indicated that after *in vitro* digestion, less than 44.5% of THg from the initial rice samples was bioaccessible. Dietary exposure to Hg through rice consumption was calculated and compared with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional tolerable weekly intake (PTWI) (2010) values, which were all below the PTWI guidelines. This study improves our understanding of Hg exposures via rice in a large North American city.

Conflicts of interest

The authors declare that there are no conflicts of interest.

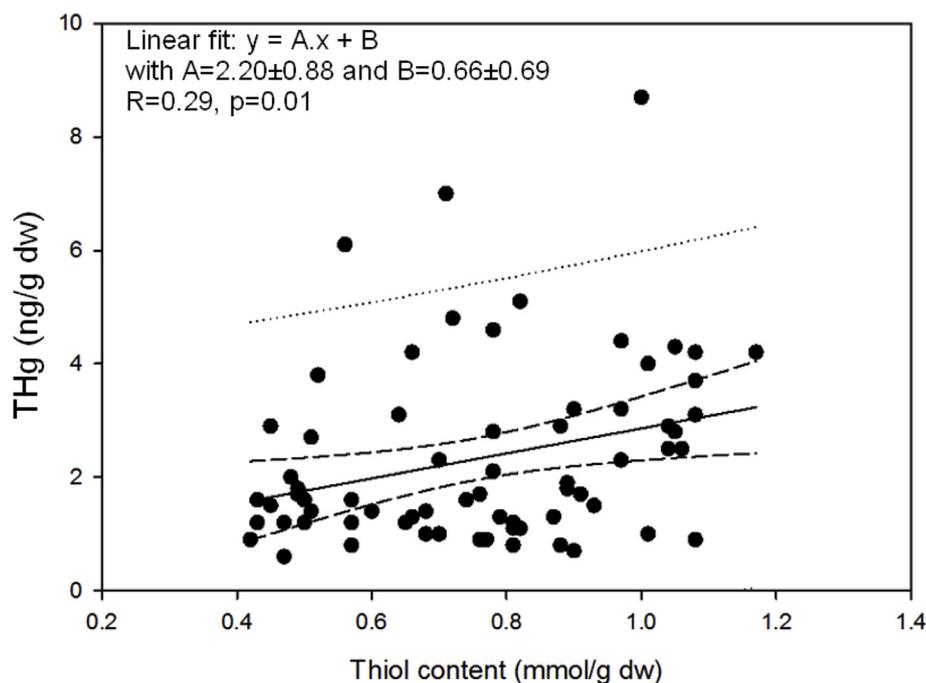


Fig. 3. Thiol content ($\mu\text{mol.g}^{-1}$) versus THg levels (ng.g^{-1}) in rice. The plain line represents the linear fit. The dashed and the dotted lines represent the 95% confidence and prediction bands respectively.

Table 5

THg bioaccessibility in various types of rice samples.

Rice sample type	Sample ID	Bioaccessibility (%)	
		Rice powder (n = 3)	Rice grains (n = 3)
Short & Medium grain/white	<i>sho & med gr20</i>	24.5 ± 31.5	44.6 ± 5.0**
Jasmine white rice	<i>jas6</i>	38.6 ± 1.5**	36.0 ± 6.0**
Long grain white rice	<i>lgr6</i>	−4.5 ± 16.5	35.0 ± 3.7**
Long grain brown rice	<i>lgr5 br</i>	37.3 ± 1.3**	12.6 ± 17.2

Significant differences between initial rice samples and after-digestion pellets are denoted by asterisks: ** $p < 0.05$. See Table S2 for a description of sample IDs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2019.02.006>.

Transparency document

Transparency document related to this article can be found online at <https://doi.org/10.1016/j.fct.2019.02.006>.

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