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Determination of Total Mercury in Sushi Samples Employing Direct Mercury Analyzer

Marcelo A. Morgano · Raquel F. Milani · Adriana A. M. Perrone

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Abstract In this study a simple, fast, and low-chemical-consuming method was evaluated for the determination of total mercury in sushi samples by thermal decomposition amalgamation atomic absorption spectrometry (TDA-AAS) using a direct mercury analyzer (DMA). A small portion of fresh sushi sample (60 mg) is combusted in nickel or quartz containers, and the released mercury is trapped in a gold amalgamator, being desorbed at high temperatures and measured in an atomic absorption cell. The method accuracy was evaluated using certified reference materials (CRM) rice flour (NIST SRM 1568b), fish protein (NRC DORM-4), peach leaves (NIST SRM 1547), and oyster tissue (NIST SRM 1566b), and the method accuracy varied between 94 and 112 % for all the CRM. Analytical curves were prepared using 0.5 % nitric acid solutions (*v*/*v*) for low (0.5 to 50 μg kg^{-1}) and high concentrations (100 to 1000 μg kg^{-1}) and the correlation coefficients were above 0.999. The detection and quantification limits were 0.4 and 1.4 μg kg^{-1}, respectively. The method was applied to 30 sushi samples of tuna, salmon, and kani from Campinas City, São Paulo State, Brazil, and the results ranged from 6.2 to 761, 1.5 to 4.0, and <1.4 to 13.5 μg kg^{-1}, respectively. High amounts of total mercury were found in samples of tuna sushi, and tuna was the ingredient that most contributed to dietary Hg exposure.

Keywords Sushi · Mercury · Direct mercury analyser (DMA) · Dietary exposure

M. A. Morgano (✉) · R. F. Milani · A. A. M. Perrone
Institute of Food Technology, 2880 Brasil Av., PO Box 139, 13070-178 Campinas, SP, Brazil
e-mail: morgano@ital.sp.gov.br

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Introduction

Recently, an increase in consumption of Japanese cuisine has been observed, especially of dishes prepared with raw fish such as sushi and sashimi (Feng 2012; Moura Filho et al. 2007). Sushi is one of the most important Japanese foods and is based on an old technique for the conservation of fish muscle using vinegary rice. It is traditionally flavored with sugar, salt, and vinegar sauce and is combined with fish or seafood, seaweed, or even vegetables, spices, fruits, and egg (Burger et al. 2013). Fish is a low-fat source of protein and contains omega-3 fatty acids which reduce the cholesterol levels, the risk of some cancers, and the incidence of heart disease and high blood pressure (Ramel et al. 2010; Wu et al. 2012).

Tuna is one of the main ingredients used in sushi and may contain high levels of inorganic contaminants, since this species is at the top of the food chain (Nancy 2003). The seaweed (*nori*) may accumulate toxic elements and constitutes another important source of contaminants in sushi (Vidotti and Rollemberg 2004). A high exposure to mercury by humans may result in several health disorders including neurodevelopment deficit (JECFA 2006), low cognitive performance (Freire et al. 2010), an increase in cardiovascular diseases (Choi et al. 2009), and neurologic and locomotor disabilities (Hites et al. 2004). Many of these effects are probably due to toxic organic forms of mercury (IARC 1993).

Burger et al. (2013) carried out a study with 1289 fish consumers from New Jersey and verified that 77 % of them consumed sushi. Caucasians and Asians ate more sushi meals per month and more sushi pieces per meal than other ethnicities, with East Asians eating more than South Asians. In the same study, a consumption of more than 40 sushi units per month was estimated for different ethnic groups, and the authors also reported a great variability in mercury levels, since these foods are prepared using different fishes and seafood.

For the consumption of sushi and sashimi, the estimated intake of methyl mercury was higher than the provisional tolerable weekly intake (PTWI) of 1.6 μg kg^{-1} body weight or >0.3 μg kg^{-1} day^{-1}.

Lowenstein et al. (2010), using cold vapor atomic absorption spectrometry (CV-AAS), also found high levels of total mercury in tuna sushi samples from restaurants and supermarkets in New York, New Jersey, and Colorado. Mercury values were higher than the maximum limits established by Canada, The European Union, and The World Health Organization (WHO), with bigeye tuna (*Thunnus obesus*) being the species with the highest Hg levels (0.65 and 2.254 mg kg^{-1}). The authors recommended an effective quality control of the products used in sushi by the regulatory agencies.

Another study evaluated the total mercury content in rice cultivated in different locations in China and established a relationship between the presence of Hg in contaminated soils and high levels of this contaminant in the rice samples collected. The average concentrations of total Hg in rice seed (brown rice) were higher than the maximum permissible limit in China of 20 μg kg^{-1} for crops (Meng et al. 2014). Silva et al. (2012) studied total mercury in rice samples from Brazil and content varied from 4.10 to 13.72 ng g^{-1}. Kwon et al. (2009) also studied exposition to Hg from the consumption of seaweed and verified that this product may contribute to a daily intake of up to 0.022 μg kg^{-1} of total mercury.

A method for total Hg determination in fish tissue using cold vapor capacitively coupled plasma microtorch optical emission spectrometry (CV-μCCP-OES) was compared with direct mercury determination by TDA-AAS. It was demonstrated that the CV-μCCP-OES method ensures analytical performance similar to that of the TDA-AAS method used for Hg determination (Frentiu et al. 2014). Wang et al. (2013) compared a high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry method (HPLC-ICP-MS) with isotope dilution gas chromatography mass spectrometry method (ID-GC-MS) for the analysis of methylmercury in fish samples. HPLC-ICP-MS method offered advantages over ID-GC-MS method due to the determination of total mercury and methylmercury in a single chromatography run with shorter time analysis.

Despite these studies, the evaluation of mercury content in Japanese cuisine remains limited, particularly for sushi samples. Considering the above, the objectives of this study were to evaluate and validate a fast, simple, low-cost, and low-chemical-consuming method to determine the total mercury content in sushi samples using thermal decomposition amalgamation atomic absorption spectrometry (TDA-AAS) and evaluate the dietary exposure from the consumption of sushi.

Materials and Methods

Apparatus

The total mercury content was quantified by thermal decomposition amalgamation atomic absorption spectrometry using a Direct Mercury Analyzer (DMA-80, Milestone, Sorisole (BG), Italy). Using this technique, the samples are heated in a quartz or nickel container using compressed air as the oxidant gas. The Hg vapors pass through a catalyst, and the products of combustion are then removed and trapped in a gold amalgamator. High temperatures (850 °C) are applied for desorption, and the Hg content quantified by determining the absorption at 253.7 nm. The optimized conditions for drying and decomposition (*pyrolysis*) using 60 mg of sample were 200 °C for 60 s and 600 °C for 180 s, respectively.

Reagents

All the reagents used were of analytical grade or higher. Water (18.2 MΩ cm) was purified using a reverse osmosis system (Gehaka, São Paulo, Brazil), and the nitric acid was purified using a sub-boiling distiller (Distillacid, Berghof, Eningen, Germany).

Calibration curves were prepared by successive dilutions of a certified standard solution at 1000 mg L^{-1} of Hg (Fluka, Buchs, Switzerland) in 0.5 % (*v/v*) HNO_3. The instrument provide two ranges for Hg determination: high sensitivity cell, 0.5 to 50 μg kg^{-1}, and low sensitivity cell, 100 to 1000 μg kg^{-1} and a blank solution using the 0.5 % (*v/v*) solution.

Several certified reference materials were analyzed to verify the accuracy of the method: NIST SRM 1568b rice flour, NIST SRM 1547 peach leaves, NIST SRM 1566b oyster tissue, and NCR DORM-4 fish protein.

Method Evaluation

The optimized conditions for sample mass, decomposition temperature, and time were evaluated using a 2^3 full experimental design with center points. The optimized method was validated according to INMETRO (2011) guidelines for the following parameters: sensitivity (detection and quantification limits), precision, accuracy, and robustness. The statistical analysis and data evaluation were carried out using the software *Statistica* 5.5 (Stat Soft Inc., Tulsa, OK, USA).

Samples and Analytical Procedure

The total Hg levels of thirty sushi samples from Japanese restaurants and supermarkets in Campinas, SP, Brazil, collected between February and April of 2014, were determined

using the optimized method. The most consumed types of sushi: kani (n=10), salmon (n=10), and tuna (n=10) were triturated using domestic processors to obtain a homogenized mass. These homogenized samples were weighed directly into nickel containers (60 mg) and analyzed in the DMA-80 equipment.

Results and Discussion

Method Development and Evaluation

Evaluation of the Influence of Sample Mass on the Quantification of Total Hg

In order to evaluate the influence of sample mass on the quantification of total Hg, two samples were used: a certified reference material (NRC DORM-4: fish protein, Hg=410± 55 μg kg^{-1}) and a commercial sample of tuna sushi. Sample mass was evaluated in a 40 to 100 mg range, and the analyses were carried out in duplicate. Figure 1 shows the results obtained.

National Research Council Canada recommended a sample mass of 250 mg for NRC DORM-4. However, in this study lower values were evaluated, from 40 to 100 mg, which presented well agreement with the certified value, ranging between 365 and 400 μg kg^{-1}. The lowest recovery value was near to 90 % (using a 40 mg sample mass), and the nearest value to the NRC DORM-4 certified Hg value was obtained using a sample mass of 60 mg (Hg=400±2 μg kg^{-1}, recovery=98 %). A similar behavior for the effect of sample mass on the variation between the certified reference material and the tuna sushi samples can be observed in Fig. 1.

Design of Experiments

A 2^3 full design with a center point was used to determine the optimal conditions for the study of total Hg in sushi samples. The design considered the most important parameters that could influence the response of the mercury analyzer: sample mass (as observed in "Evaluation of the Influence of Sample Mass on the Quantification of Total Hg"), time, and temperature of sample decomposition. The experiments were carried out in duplicate with three center points, and the response monitored was the CRM recovery (a relationship in percentage between the value measured and the NRC DORM-4 certified value for Hg). The design parameters are summarized in Table 1.

As observed in Table 1, no significant effects, interactions, or curvature were observed in this model at a 95 % confidence level. This conclusion was provided by the acceptance of the null hypothesis (no significant effect), since all the intervals contained the zero value.

The experimental conditions were selected as follows. A decomposition time of 180 s was chosen due to its positive effect on the experimental design, which reported an increase in decomposition time with increase in Hg recovery. Although the main effects were also positive, the level −1 condition was used for the other factors. This choice was made considering the evaluation of sample mass, which demonstrated that 60 mg was adequate for both the certified material and the sushi sample (Fig. 1). A mild condition (600 °C instead of 650 °C) was used as the decomposition temperature in order to reduce the damage to the container used for sample decomposition.

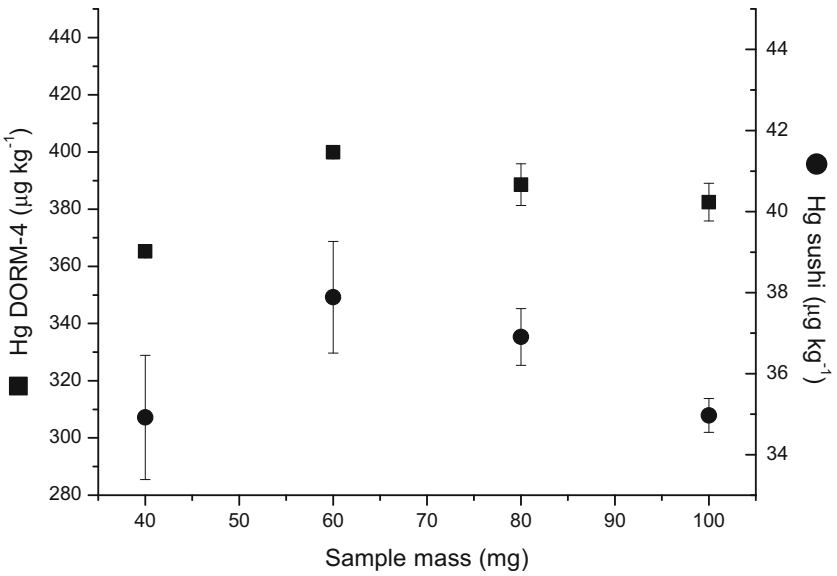

Fig. 1 Evaluation of the influence of sample mass on the determination of Hg by TDA-AAS

Table 1 Parameters of 2^3 full design with center points and the obtained responses

Factors/level	Sample mass (1)		$T_{decomposition}$ (2)	$t_{decomposition}$ (3)
−1	60 mg		600 °C	120 s
+1	70 mg		650 °C	180 s
0	65 mg		625 °C	150 s
	Effect	$t(10)$	Confidence limit (−95 %)	Confidence limit (+95 %)
Factor 1	1.400	2.186	−0.027	2.827
Factor 2	0.175	0.273	−1.252	1.602
Factor 3	1.425	2.225	−0.002	2.852
Interaction 12	−0.850	−1.327	−2.277	0.577
Interaction 13	−0.600	−0.937	−2.027	0.827
Interaction 23	0.025	0.039	−1.402	1.452
Interaction 123	0.450	0.703	−0.977	1.877
Curvature	−0.475	−0.295	−4.067	3.117

Method Validation

The analytical method was validated according to INMETRO (2011), the parameters evaluated being the correlation coefficient, accuracy using certified reference materials, the limits of detection and quantification, and the precision and robustness using a commercial sushi sample.

The equipment was calibrated for its two cells: one being used for sub-trace quantification (ppt to ppb) and the other for trace quantification (ppb to ppm), using analytical curves prepared by successive dilutions of a standard Hg solution in 0.5 % HNO_3 (*v*/*v*). For both curves, external calibration provided correlation coefficients over 0.999 (sub-trace, 0.9995 and trace, 0.9997) considering the range 0.5 to 50 μg kg^{-1} ($A=2.97\times10^{-3}+5.11\times10^{-2}$ Hg) and 100 to 1000 μg kg^{-1} ($A=9.65\times10^{-4}+8.91\times10^{-4}$ Hg) for sub-trace and trace quantification, respectively.

The limits of detection (LOD) and quantification (LOQ) were estimated by measuring ten analytical replicates using 60 mg of a sushi sample with a low Hg level. The estimated values were: LOD (3 s)=0.4 μg kg^{-1} and LOQ (10 s)=1.4 μg kg^{-1}. The sensitivity provided by the method was adequate to study total Hg in food samples, since the LOD was approximately ten times below the maximum limit allowed by the Brazilian regulation in any food (10 μg kg^{-1}), approximately 500× below the maximum limit allowed by the Brazilian and European regulations in non-predatory fishes (500 μg kg^{-1}) and approximately 1000× below that for predatory fishes (1000 μg kg^{-1}) (Brasil 1965, 2013; Commission Regulation 2008).

Method accuracy was evaluated using four certified reference materials with compositions similar to those of the sushi samples (seaweed, rice, and fish): NIST SRM 1568b *Rice flour*; NIST SRM 1547 *Peach leaves*; NIST SRM 1566b *Oyster tissue*; and NCR DORM-4 *Fish protein*. The experimental results can be seen in Table 2.

The method accuracy was considered satisfactory since the recovery values ranged between 94 and 112 %, and the *z* score values were below 1.10. The method precision was verified from the coefficient of variation of 18 replicates of a commercial sample (nine repetitions on two different days). The value obtained, 5.5 %, was considered adequate for this study.

Robustness was verified using the Youden test, which allows one to evaluate the influence of each parameter on the final result. A fractional factorial design (2^{7-4}_{III}) was used for this evaluation, considering seven factors that could be varied in the method procedure. The experiments were carried out in duplicate, and the design parameters are summarized in Table 3.

As observed in Table 3, none of these factors presented a significant effect on the model at a 95 % confidence level. This conclusion was provided by the acceptance of the null hypothesis, since all the intervals contained the zero value, and therefore the method was considered robust for these parameters.

Determination of Total Mercury in the Sushi Samples

Thirty sushi samples were acquired from five restaurants and supermarkets in the city of Campinas between the months of

Table 2 Evaluation of the certified reference materials using TDA-AAS

Reference material	Certified value (μg kg^{-1})	Experimental value (μg kg^{-1})	Recovery (%)	*z* score
Fish protein (*n*=11)	410±55	405±13	99±3	−0.14
Peach leaves (*n*=3)	31±7	34.6±0.7	112±2	0.51
Oyster tissue (*n*=3)	37.1±1.3	35.9±0.3	97±1	0.92
Rice flour (*n*=4)	5.91±0.36	5.53±0.23	94±4	1.06

Table 3 Parameters used in robustness evaluation and the obtained responses

Factor	Nominal	Variation	Effect	t(8)	Confidence limit (−95 %)	Confidence limit (+95 %)
Sample mass	60 mg	70 mg	7.5812	0.6497	−19.3262	34.4885
Drying time	60 s	40 s	5.6603	0.4851	−21.2471	32.5677
Drying temperature	200 °C	180 °C	−10.7680	−0.9228	−37.6754	16.1393
Decomposition time	180 s	120 s	−3.2783	−0.2810	−30.1856	23.6291
Decomposition temperature	600 °C	650 °C	4.9047	0.4203	−22.0027	31.8120
Sample container material	Nickel	Quartz	8.5472	0.7325	−18.3601	35.4546
Time between sample weighing and Hg quantification	0 min	30 min	17.0515	1.4613	−9.8559	43.9589

February and April of 2014. For the total Hg determination, the whole sushi (seaweed, rice, and seafood) was considered, and Table 2 shows the results obtained. Tuna sushi presented the highest Hg level, ranging between 6 and 761 μg kg^{-1}, followed by kani sushi (not detected and 13.5 μg kg^{-1}) and salmon sushi (1.5 and 4.0 μg kg^{-1}).

Since the mercury found in seafood is predominately in its organic form (Hight and Cheng 2006; Kuballa et al. 2011), in some studies the organic forms of mercury were also determined. Kuballa et al. (2011) carried out a study with 536 samples of marine foods from southwestern Germany and concluded that approximately 70 % of the total mercury in marine fish was found as methyl mercury. Kannan et al. (1998) carried out a study with fish muscle from South Florida (USA) and verified that methyl mercury contributed with 83 % of the total mercury level.

Since mercury is considered to be an inorganic contaminant with accumulative properties, and presents several toxic effects in the human organism (Martins et al. 2013; Melendez-Perez and Fostier 2013; Burger et al. 2013; Choi et al. 2009; JECFA 2006; Hites et al. 2004), the Joint FAO/OMS Expert Committee on Food Additives established a provisional tolerable weekly intake (PTWI) for inorganic mercury of 4 μg kg^{-1} body weight (bw) and of 1.6 μg kg^{-1} bw for methyl mercury (FAO/WHO 2011). In line with JECFA, the European Food Safety Authority and the Panel on Contaminants in the Food Chain (EFSA 2012) established a tolerable weekly intake (TWI) for inorganic mercury of 4 μg kg^{-1} bw, expressed as mercury, and 1.3 μg kg^{-1} bw for methyl mercury, expressed as mercury. The contribution from each type of sushi to the estimated weekly intake of total Hg was calculated considering an adult (60 kg) consuming either one or seven weekly portions of sushi (150 g/day). In Brazil, a portion of these varieties generally contains from six to nine pieces of sushi and the mean portion size was determined experimentally.

Sushi is generally constituted of 65 % rice, 5 % seaweed, and 30 % seafood (experimental data presented in "Evaluation of the Total Hg Level in the Sushi Fractions"), and with respect to the level of Hg, the major component is seafood. Considering a proportion of 70 % of the total mercury found in fish being methyl mercury (Kuballa et al. 2011), the total Hg levels obtained in the sushi samples examined in this study were calculated as MeHg (70 % of the total Hg) and inorganic Hg (30 % of the total Hg). The estimated intake was compared with the values established by JECFA and EFSA, and the results are presented in Table 4.

Table 4 shows the estimations for a low and high consumption of sushi during one week. A low consumption was considered to be that of a 60 kg adult, where 100 % of the MeHg TWI was obtained consuming one portion of tuna sushi a week. On the other hand, the consumption of seven portions of kani or salmon sushi/week (high consumption) contributed less than 14 % of the MeHg TWI.

Evaluation of the Total Hg Level in the Sushi Fractions

Some studies have verified the incidence of mercury in the tuna and salmon present in the fish and kani used in Japanese cuisine (Burger et al. 2013; Morgano et al. 2011, 2014). To verify the contribution of each sushi component (seaweed, rice, sesame, kani, and/or fish) to the total Hg content, the sushi samples were fractioned and their components individually analyzed. The results were then compared with the mean value obtained in the analysis of the homogenized sample ("Determination of Total Mercury in the Sushi Samples") and the data presented in Table 5.

Even though tuna was not the major component of the sushi, the greatest contribution was provided by this fish (204.2 μg kg^{-1} of a total of 210.7 μg kg^{-1}), whereas rice, which represented nearly 60 % of the sushi, was the ingredient contributing the lowest total Hg level. This evaluation also demonstrated good concordance between the value obtained for the homogenized tuna, salmon, and kani sushi samples (Hg=196.9 μg kg^{-1}; 3.76 μg kg^{-1}, and 8.0 μg kg^{-1}) and the sum of the constituents, 107, 91, and 78 %, respectively.

Table 4 Mercury level (mean and range) in sushi samples commercialized in Campinas, SP, Brazil and estimate of Hg intake considering an adult (60 kg) who consumes one and seven portions of sushi in a week (150 g/day)

Sushi	Unit	Tuna	Kani	Salmon
Total Hg	μg kg^{-1}	279 (6.2–761)	7.2 (<1.4–13.5)	2.90 (1.5–4.0)
MeHg	μg kg^{-1}	195 (4.34–533)	5.04 (ND–9.45)	2.03 (1.05–2.80)
Inorganic Hg (i-Hg)	μg kg^{-1}	84 (1.86–228)	2.16 (ND–4.05)	0.87 (0.45–1.20)
1 portion sushi/week				
MeHg	μg/150 g bw	0.49 (0.01–1.33)	0.013 (ND–0.024)	0.005 (0.003–0.007)
PTWI (MeHg)[a]	%	31 (0.7–83)	0.8 (0–1.5)	0.31 (0.19–0.44)
TWI (MeHg)[b]	%	38 (0.8–102)	1.0 (0–1.8)	0.38 (0.23–0.54)
Inorganic Hg	μg/150 g bw	0.21 (0.005–0.57)	0.005 (ND–0.010)	0.002 (0.001–0.003)
PTWI (i-Hg)[a, b]	%	5.3 (0.1–14)	0.1 (0–0.3)	0.05 (0.03–0.08)
7 portion sushi/week				
MeHg	μg/150 g bw	3.4 (0.08–9.3)	0.09 (0–0.17)	0.04 (0.02–0.05)
PTWI (MeHg)[a]	%	213 (5.0–581)	6 (0–11)	2.5 (1.3–3.1)
TWI (MeHg)[b]	%	263 (6.2–715)	7 (0–13)	3.1 (1.5–3.8)
Inorganic Hg	μg/150 g bw	1.5 (0.03–4.0)	0.04 (0–0.07)	0.01 (0.01–0.02)
PTWI (i-Hg)[a, b]	%	37 (0.8–100)	1.0 (0–1.8)	0.3 (0.3–0.5)

MeHg methyl mercury (70 % total Hg (according to Kuballa et al. 2011)), *i-Hg* inorganic mercury,

[a] Provisional tolerable weekly intake (PTWI) for 150 g/day of sushi by 60 kg adult (inorganic Hg=4 μg/kg bw and MeHg=1.6 μg/kg bw)

[b] Tolerable weekly intake (*TWI*) for 150 g/day of sushi by 60 kg adult (inorganic Hg=4 μg/kg bw and MeHg=1.3 μg/kg bw)

Conclusions

The proposed method for the analysis of total mercury in sushi by thermal decomposition amalgamation atomic absorption spectrometry, presented good sensitivity as shown by its low limits of detection and quantification, and satisfactory precision and accuracy. Other advantages are the lack of a sampling digestion step, contributing to a low chemical consumption, adequate sensitivity to quantify Hg in sushi samples, and a short analysis time, usually less than 6 min, faster than the time required by other classical techniques such as cold vapor atomic absorption spectrometry (CV-AAS) and inductively coupled plasma spectrometry using hydride generation (HG-ICP-OES).

The samples were analyzed using the proposed method and the results showed that an intake of one portion of tuna sushi may present mercury levels above 100 % of the TWI. Although seaweed and tuna are not the major components in this food, they were the main contributors to the total mercury intake.

Table 5 Evaluation of Hg contribution of sushi components

Sushi	Component	Hg (μg kg^{-1})	% sushi (m/m)	Hg contribution (μg kg^{-1})
Tuna (Hg=196.7 μg kg^{-1})	Rice	3.1	61	1.9
	Seaweed	57.3	6	3.4
	Sesame	62.7	2	1.2
	Tuna	658.8	31	204.2
	Sum	–	100 %	210.7 (107 %)[a]
Kani (Hg=8.0 μg kg^{-1})	Kani	19.6	30	5.9
	Rice	<1.4	65	0
	Seaweed	5.2	5	0.3
	Sum	–	100 %	6.2 (78 %)[a]
Salmon (Hg=3.76 μg kg^{-1})	Rice	<1.4	30	0
	Salmon	11.0	65	3.29
	Seaweed	2.5	5	0.13
	Sum	–	100 %	3.42 (91 %)[a]

[a] Relation between sum and Hg level analyzed in homogenized sample

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Conflict of Interest Marcelo A. Morgano declares that he has no conflict of interest. Raquel F. Milani declares that she has no conflict of interest. Adriana A.M. Perrone declares that she has no conflict of interest. This article does not contain any studies with human or animal subjects.

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