Sushi commercialized in Brazil: Organic Hg levels and exposure intake evaluation

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ABSTRACT

The presence of organic mercury (methylmercury) in tuna, salmon and kani sushis marketed in restaurants specialising in Japanese foods (Campinas, São Paulo, Brazil), was investigated by atomic absorption spectrometer with thermal decomposition and amalgamation. Total mercury was analyzed directly, whilst organic mercury was quantified after a previous extraction with toluene in an acid solution, assisted by microwaves. Under these analytical conditions there was no interconversion between the inorganic and organic mercury. High sensitivity was observed for organic mercury, with limits of detection and quantification of 2.0 and 6.6 $\mu g \ kg^{-1}$. The organic mercury contents ranged from 12 to 583 $\mu g \ kg^{-1}$, 6.6 to 8.2 $\mu g \ kg^{-1}$ and no detected values, for the tuna, kani and salmon sushi, respectively. The mean proportion of organic Hg/total Hg for tuna sushi was 88%, indicating that the most toxic form of mercury, organic Hg, predominate in this food. The estimated exposure to methylmercury was made by taking into account the Provisional Tolerable Weekly Intake (PTWI 1.6 $\mu g/kg$) considering the daily consumption of 150 g and 20 g per adults (60 kg) and children (15 kg), respectively. Our results demonstrated that the consumption of tuna sushi may exceed 100% of PTWI.

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1. Introduction

Fish is recognized as an important source of many essential nutrients and its consumption is widely encouraged to prevent hypertension, cancer and coronary heart disease (Sioen, Henauw, Verdonck, Thuyne, & Camp, 2007). However, fish can contain toxic elements in their tissues, such as Hg, and consequently may represent a source of human exposure to such components (Burger, Stern, & Gochfeld, 2005; Dorea & Barbosa, 2005; Morgano, Rabonato, Milani, Miyagusku, & Quintaes, 2014).

The effects of high exposure to Hg in humans include neurodevelopmental deficits (JECFA, 2004; Steuerwald et al., 2000), poor cognitive performance (Freire et al., 2010; Oken et al., 2008), increased rates of cardiovascular disease (Choi et al., 2009), and neurological and locomotion deficits (Hightower & Moore, 2003; Hites, Carpenter, Hamilton, Knuth, & Schwager, 2004). The National Health and Nutrition Examination Survey estimates that

8–15% of fetuses in the USA have excessive exposure to Hg (Trasande, Landrigan, & Schechter, 2005). Recently, the FDA (US Food and Drug Administration) and EPA (US Environmental Protection Agency) have advised pregnant women, those who may become pregnant, breastfeeding mothers, and young children to broaden the variety of fish they eat and choose those lower in Hg, restricting fish consumption to 2 or 3 servings/week (Burger, Stern, & Gochfeld, 2005).

Mercury can be found in the environment in various chemical species. All Hg species are considered toxic, but organic species such as methylmercury ($MeHg^+$) and ethylmercury are considered more toxic than elemental Hg and its inorganic species. It is well recognized that the main pathway of human exposure to Hg is through eating fish containing $MeHg^+$, which is the most common Hg species found in fish. Due to biomagnification along the food chain, $MeHg^+$ reaches maximum levels in fish at the top of the food chain, and as a result, about 90% of the total Hg present in fish can be found as $MeHg^+$ (Horvat & Gibičar, 2005).

An accurate analytical method for the determination of organic Hg species is required to assess the real toxicity of the samples (Harrington, 2000). The analysis of organic Hg is generally carried

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out using chromatographic separation techniques coupled with different detectors (Zhang, Yang, Dong, & Xue, 2012). The chromatographic separation techniques include: gas chromatography (GC) (Barst et al., 2013; Kenšová, Kružíková, & Svobodová, 2012; Nevado, Martín-Doimeadios, Bernardo, Moreno, Ropero, & de Marcos Serrano, 2011), liquid chromatography (HPLC) (Batista, Rodrigues, De Souza, Oliveira Souza, & Barbosa, 2011; Chen et al., 2013) and ionic chromatography (IC) (Shade & Hudson, 2005). The most commonly used techniques are: inductively coupled plasma mass spectrometry (ICP-MS) (Batista et al., 2011; Clémens, Monperrus, Donard, Amouroux, & Guérin, 2011), atomic absorption spectroscopy (AAS) (Naozuka & Nomura, 2011; Sarıca & Türker, 2012), atomic fluorescence spectrometry (AFS) (Nevado et al., 2011; Zhang et al., 2012), electron capture detection (ECD) (Kehrig et al., 2009; Kenšová et al., 2012), microwave induced plasma-atomic emission spectrometry (MIP-AES) (Sanz, De Diego, Raposo, & Madariaga, 2003), atomic emission detection (Kuballa, Leonhardt, Schoeberl, & Lachenmeier, 2011) and isotope dilution mass spectrometry (IDMS) (Demuth & Heumann, 2001), and for the determination of total mercury, thermal decomposition amalgamation atomic absorption spectrometry (TDA AAS) (Morgano, Milani, & Perrone, 2015).

Japanese dishes usually include tuna of various species, salmon, eel, and many other fish, as well as shrimp and crab, which may be consumed in sushi dishes as well as vegetarian varieties (Burger, Gochfeld, Jeitner, Donio, & Pittfield, 2013).

Sushi, technically referring to fish and other items served with vinegar and sticky rice (Nibble, 2012), has become a generic term often encompassing sashimi (raw fish) and several varieties of fish surrounded by rice (maki rolls), and fish over rice (nigiri). The consumption of sushi and related dishes has recently increased greatly in Brazil and other countries, with these foods being available over lunch counters, grocery stores, especially restaurants and sushi bars (Martins, 2006). Although there is a growing trend for the consumption of sushi (Issenberg, 2007), there is very little quantitative data on either the consumption patterns of sushi or the contaminants in sushi (Lowenstein, Burger, Jeitner, Amato, Kolokotronis, & Gochfeld, 2010).

Regarding to the presence of methylmercury in sushi samples commercialized in Brazil Southwest, this work aims: i) to develop and validate a quick, simple, low cost method with minimal reagent consumption; ii) to quantify organic mercury (methylmercury) in sushi samples using the technique of thermal decomposition amalgamation atomic absorption spectrometry (TDA AAS); iii) to estimate the organic mercury intake from sushi consumption and iv) to delineate an organic extract stability study.

2. Materials and methods

2.1. Instrumentation

The technique of TDA AAS using a direct mercury analyzer (DMA-80, Dual Cell, Milestone, Sorisole, Italy) was used to quantify both the total and organic mercury content of sushi samples. The organic mercury extracts were obtained via microwave extraction (Start E, Milestone, Sorisole, Italy). The samples were heated in a nickel or quartz container, making use of compressed air as the oxidant gas. A catalyst removed the combustion products and the Hg vapors were trapped in a gold amalgamator. Temperatures around 850 °C were applied for desorption, and the Hg content was quantified by determining the absorption at 253.7 nm.

2.2. Reagents and standards

Only analytical grade reagents were used in this study. The

water (18.2 MΩ cm) was purified using a reverse osmosis system (Gehaka, São Paulo, Brazil) and the nitric acid using a sub-boiling distiller (Distillacid, Berghof, Eningen, Germany). Toluene (Synth, Diadema, Brazil) and a 30% HCl solution (Merck, Darmstadt, Germany) were used for the microwave extractions. A 2.5% L-cysteine solution (Sigma, Steinheim, Germany) was prepared to stabilize the organic mercury species. Certified standard solutions of mercury at 1000 mg l^{-1} (Fluka, Sigma Aldrich, Steinheim, Germany) were used to construct the analytical curves, together with a 0.5% (v/v) solution of HNO_3.

2.3. Samples

A total of 60 sushi samples were acquired from different Japanese restaurants and supermarkets located in Brazil Southwest (city of Campinas, São Paulo state), with 20 samples each of the most consumed types of sushi: 20 samples of Yellowfin tuna (*Thunnus albacares*), 20 of salmon (*Salmo salar*) and 20 of kani (a mix of fish species flavoring with crab meat). Yellowfin tuna came from the South and Southwest area of the coast of Brazil, which is included in FAO fishing area (Atlantic, Southwest). Salmon samples came from Chile coast, whilst kani were acquired from distribution centers located in the Southeast of Brazil.

The samples were separately triturated according to their specie, taking a complete dish with all ingredients, using a domestic processor to obtain a homogenized mass. The homogeneous mass samples were kept under freezing until analyses. Sample portions weight was determined experimentally as, approximately, 150 g (6 pieces of sushi).

The contribution of each sushi component (seaweed, rice, kani and/or fish) was determined in a previous work of our group. The obtained values were, in average: 65% of rice, 30% of fish and/or kani and 5% of seaweed (Morgano et al., 2015).

2.4. Determination of total and organic mercury in the sushi samples

2.4.1. Determination of total mercury

For the determination of total mercury, the homogenized samples were weighed directly into nickel containers and the value determined using TDA AAS. According to Morgano et al. (2015) the optimal conditions for the total mercury analysis were: drying process (200 °C for 60 s) and decomposition process (600 °C for 180 s), using a 60 mg sample.

2.4.2. Determination of organic mercury

The extraction method for the organic species present in sushi samples was developed using a certified reference material (CRM) with a certified MeHg$^+$ value. The following parameters were optimized: the extraction temperature employed in the system assisted by microwaves; the extraction time; the concentrations of the L-cysteine solution and the volume of organic solvent (toluene) (Carbonell, Bravo, Fernandez, & Tarazona, 2009; Huang, Pan, Han, Wu, Tang, & Tan, 2012; Maggi, Berducci, Bianchi, Giani, & Campanella, 2009 and Ruiz-de-Cenzano, Rochina-Marco, Cervera, & de laGuardia, 2014).

The samples were subjected to closed extraction assisted by microwaves using an organic solvent (toluene) in an acid solution. A PFA teflon extraction vessel was weighed on an analytical balance and a 1 g aliquot of sample introduced, to which was added: 8 mL of toluene pa, 1 mL of demineralized water and 0.75 mL of a 30% (v/v) HCl solution. The vessels were sealed and transferred to a 1000 W microwave extractor which was programmed as follows: (a) room temperature to 110 °C in 10 min; (b) maintain a constant temperature of 110 °C for 5 min. After cooling, the vessels were opened and

a 4 mL aliquot of the organic phase withdrawn and transferred to a centrifuge tube containing 2 mL of a 2.5% L-cysteine solution (w/v). After manual agitation, the solution was centrifuged for 6 min at 3500 rpm and the aqueous phase used for the analysis.

A DMA analyzer was used to determine the organic mercury fraction by measuring 100 mg of the aqueous phase containing L-cysteine organic mercury into a quartz vial. The optimal reading conditions in the mercury analyzer were: sample drying temperature = 120 °C for 60 s; decomposition temperature = 300 °C for 180 s; desorption temperature = 850 °C for 12 s; absorbance determined at 253.7 nm. The ranges for the two detection cells of the equipment were: $0.5–20$ µg kg^{-1} and $20–1000$ µg kg^{-1}.

2.5. Stability of the organic mercury extracts

The stability of the organic extract was tested under two conditions: storage in polypropylene tubes under ambient (22 °C) and refrigerated (4 °C) conditions; and also in glass tubes under ambient and refrigerated conditions. The determination continued for 22 days according to the amount of extract available.

2.6. Exposure risk assessment of methylmercury from the consumption of sushi

To estimate the exposure to organic mercury, the occasional consumption (1 portion/week) to the moderate consumption (7 portions/week) of sushi was considered:

Estimated exposure to methylmercury

$$= \left[\text{MeHg}^+\right] \times (\text{portion/body weight});$$

where: [MeHg$^+$] (average and range concentration, µg kg^{-1}); portion of 150 g and 20 g of sushi were considered for a 60 kg adult and a 15 kg child body weight (bw), respectively; the estimated exposure to methylmercury (µg kg^{-1} bw^{-1}).

The maximum number of tolerated weekly portions for adults and for children was indicated by values above 100% of PTWI:

$$\%PTWI = 100 \times (\text{Estimated exposure}$$
$$\text{to methylmercury})/\left(\text{MeHg}^+ \text{ PTWI}\right);$$

where MeHg$^+$ PTWI = 1.6 µg kg^{-1} bw (WHO, 2015).

3. Results and discussion

3.1. Validation of the method for the determination of total and organic mercury

The method was validated based on the INMETRO (2011) recommendations and consisted of evaluating the following: linearity of the analytical curves, accuracy (using certified reference materials and recovery tests), sensitivity (detection and quantification limits) and the precision.

The evaluation of linearity was assessed by the regression coefficients of the analytical curves obtained for the two mercury analyzer cells. For both concentration ranges (low: $0.5–20$ µg kg^{-1}, and high: $20–1000$ µg kg^{-1}), satisfactory values were observed, with r^2 > 0.999.

The sensitivity of the method was evaluated by analyzing a sushi sample with low mercury content, carrying out seven analytical repetitions. The values obtained for organic and total mercury were: LOD (3 s) of 2.0 µg kg^{-1} and 0.4 µg kg^{-1}; LOQ

(10 s) = 6.6 µg kg^{-1} and 1.4 µg kg^{-1}, respectively; "s" being the standard deviation value for the concentrations of seven replicates. These values were lower than those reported by Kuballa, Moellers, Schoeberl, and Lachenmeier (2011) who used the GC-MS method to study MeHg$^+$ levels in fish samples, obtaining values of 6.0 µg kg^{-1} and 20 µg kg^{-1} for LOD and LOQ, respectively. Cheng and Hight (2008) analyzed MeHg$^+$ using the HPLC-ICP-MS, and also observed higher values than those obtained in this study (3.8 µg kg^{-1} and 28 µg kg^{-1} for LOD and LOQ, respectively). Wang et al. (2013) comparing two methods (HPLC-ICP-MS and GC-MS using isotope dilution, ID-GC-MS) to study MeHg$^+$, obtained the following LOD and LOQ values: 5 ng g^{-1} and 15 ng g^{-1}; and 3.4 ng g^{-1} and 10.2 ng g^{-1}, respectively.

Regarding the determination of MeHg$^+$ in biological samples, Maggi et al. (2009) used an extraction solution containing a HBr/toluene and L-cysteine mixture, with detection by TDA AAS. Despite obtaining lower values for LOD and LOQ (1.5 ng g^{-1} and 2.5 ng g^{-1}, respectively), the sample preparation method was applied using two extraction stages and a large amount of chemical reagents: about 35 mL of toluene and 10 mL of concentrated HBr. In the method proposed here, smaller solvent volumes were used and only one extraction step.

The accuracy of the MeHg$^+$ method was assessed using certified reference materials (NRC TORT-2 lobster hepatopancreas and NCR DORM-4 fish protein) and recovery trials at three levels. In our previous study (Morgano et al., 2015), accuracy for total mercury method was evaluated using three certified reference materials with similar compositions to the sushi samples: NIST SRM 1568b rice flour, NIST SRM 1566b oyster tissue and NCR DORM-4 fish protein. Table 1 summarizes the obtained results.

As shown in Table 1, the recovery ranged between 94 and 111% for methylmercury, consistent with the recommendations of the AOAC (2013), where the values are between 75 and 120% for this level of concentration. In addition, the values for the z-scores were calculated from the experimental data, and both CRMs demonstrated satisfactory values (less than 2.0) according to those recommended by INMETRO (2011).

Recovery trials for organic Hg were also carried out. For this evaluation, a sushi sample with low organic mercury content was fortified at three different levels: 10, 100 and 1000 µg kg^{-1} of methylmercury, using solutions prepared from the salt of this specie (Sigma Aldrich, Steinheim, Germany). The values obtained in (µg kg^{-1}) were: 11.1 ± 0.2; 94 ± 4 and 964 ± 67 respectively; which correspond to recoveries of 111%, 94% and 96%. Thus the values observed ranged between 94 and 111%, consistent with the AOAC (2013).

In order to verify the absence of interconversion between inorganic and organic mercury, the sushi sample was fortified with 100 µg kg^{-1} of a standard solution containing only inorganic mercury (i-Hg). The result obtained was below the limit of quantification of the method and therefore it could be concluded that interconversion between the Hg species did not occur.

The precision of the method for MeHg$^+$ was evaluated using 16 analytical repetitions (8 repetitions/day) from a sample of tuna sushi (organic Hg = 192 ± 17 µg kg^{-1}). The value obtained for the coefficient of variation was 9.0%, which satisfies the condition recommended by the AOAC (2013), which is 16% in the concentration range studied. The total Hg coefficient of variation was 5.5% (Morgano et al., 2015).

3.2. Assessing the stability of the organic extract

According to data available in the literature, glass is the most suitable container for the storage of Hg (Houserova, Kuban, Spurny, & Habarta, 2006). In this study, the evaluation of the stability of

Table 1
Accuracy evaluation of the analytical methods using certified reference materials for $MeHg^+$ and total mercury (n = 3).

	Certified reference materials	Certified values ($\mu g\ kg^{-1}$)	Values obtained ($\mu g\ kg^{-1}$)	Recovery (%)	Z-score
Methylmercury	Fish protein	354 ± 31	333 ± 22	94 ± 6	−0.3
	Lobster hepatopancreas	152 ± 13	168 ± 3	111 ± 2	1.3
Total mercury[a]	Oyster tissue	37.9 ± 1.1	35.9 ± 0.3	97 ± 1	0.9
	Rice flour	5.89 ± 0.39	5.61 ± 0.23	95 ± 4	1.1
	Fish protein	412 ± 57	406 ± 15	97 ± 14	−0.2

[a] Morgano et al. (2015).

organic extract was performed considering both glass and polypropylene tubes. In Fig. 1 the observed results are presented.

Fig. 1 demonstrates that when the organic extract was stored in a glass tube and kept at ambient conditions, its stability is significant for up to 21 days. In the present study a gradual reduction of methylmercury was observed after 22 days of refrigerated storage in a brown glass bottle.

The extraction method used by Houserova et al. (2006) was also based on a high-pressure microwave system, making use of 6 mol/l HCl + 0.1 mol/l NaCl as the extraction agent. The researchers verified that the organic extracts remained stable for 3 days under ambient conditions, using 20 mL of solvent, repeating the extraction procedure twice, and storing in new polypropylene tubes with caps (Ruiz-de-Cenzano et al., 2014). The present results also showed that the extract kept in glass and stored under refrigeration showed similar stability to that at room temperature up to the 11th day, after which a drop of approximately 10% of the organic Hg concentration was observed. Therefore it was concluded that the longer the storage time of the organic extract, the greater the propensity of the mercury content to decrease in solution. In addition, wide variations between replicates were observed with increase in the storage time of the extract.

Similar assays were carried out with the storage of the organic extracts in polypropylene tubes (Corning, New York, USA - PP) under ambient and refrigerated conditions. The graph presented in Fig. 1 shows that refrigerated storage resulted in a higher relative stability profile, where the extract was stable for 22 days (the entire analytical period), whereas the extract maintained at room temperature proved to be as stable as the one submitted to refrigeration for 14 days, after which it showed a decline of approximately 7% in the organic Hg content, corroborating with studies on the possibility of the adsorption of Hg on plastic containers used for storage purposes (Houserova et al., 2006).

3.3. Results obtained for the samples

The Joint FAO/WHO (2011) Expert Consultation on the Risks and Benefits of Fish Consumption concluded that amongst women of childbearing age, pregnant women and nursing mothers, when considering the benefits of docosahexaenoic acid (DHA) versus the risks of methylmercury, fish consumption lowered the risk of suboptimal neurodevelopment in their offspring compared to not eating fish under most of the circumstances evaluated. More specifically it was concluded that even considering the highest estimate for the methylmercury risk, the neurodevelopmental risks of not eating fish exceeded the risks of eating fish for up to at least seven 100 g servings per week for all fish containing less than 0.5 $\mu g/g$ (mg/kg) methylmercury (WHO, 2015).

Table 2 shows the results obtained for total (tHg), inorganic (iHg) and organic (oHg) mercury present on the samples and the ratios of oHg/tHg and iHg/tHg. For organic mercury, the assessment was carried out for those samples with values greater than 6.6 $\mu g\ kg^{-1}$ of total mercury, which corresponds to the LOQ of the oHg method. Levels below the limit of quantification of the methods were not considered to the average calculation.

Of the 20 tuna sushi samples analyzed, a broad concentration range was observed for organic mercury: >6.6–583 $\mu g\ kg^{-1}$ (Table 2). This result was possibly due to the origin and age of the fish. In the study of Burger et al. (2013), sushi samples containing eel, salmon, crab and tuna were analyzed and significant differences in mercury levels amongst the types and components of the sushi were found, with tuna sushi having the highest levels and eel, crab, and salmon having lower levels. In this study, the levels in the tuna rolls averaged 470 $\mu g\ kg^{-1}$.

For what concerns tuna fish, literature reported similar
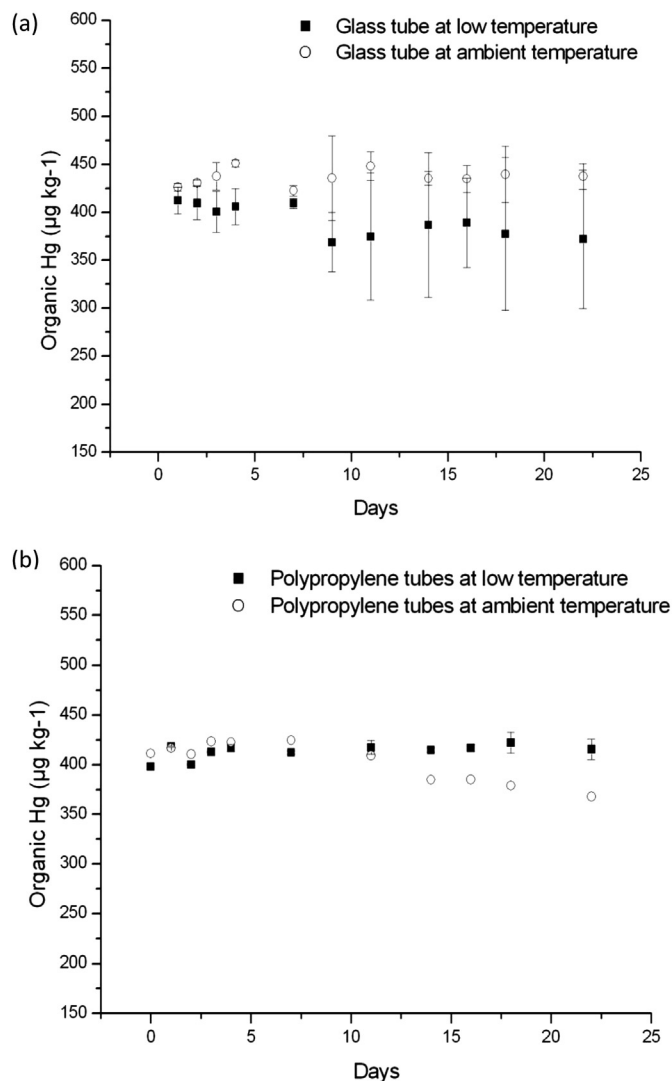
Fig. 1. Stability graph of $MeHg^+$ extracts stored in: (a) glass tubes (b) polypropylene tubes.

Table 2
Total (tHg), organic (oHg) and inorganic (iHg) mercury levels observed in the sushi samples analyzed.

Sample	tHg (µg kg^{-1})	oHg[a] (µg kg^{-1})	oHg/tHg (%)	iHg (µg kg^{-1})	iHg/tHg (%)
Tuna 1	11.8 ± 0.1	<6.6	–	–	–
Tuna 2	43 ± 1	41 ± 1	95	2	5
Tuna 3	197 ± 7	187 ± 14	95	10	5
Tuna 4	438 ± 10	413 ± 18	94	25	6
Tuna 5	63 ± 3	48 ± 2	76	15	24
Tuna 6	88 ± 6	75 ± 2	85	14	16
Tuna 7	761 ± 67	559 ± 2	73	202	27
Tuna 8	595 ± 51	533 ± 14	90	62	10
Tuna 9	587 ± 13	583 ± 6	99	4	1
Tuna 10	6.2 ± 0.4	<6.6	–	–	–
Tuna 11	30 ± 3	26 ± 1	87	4	13
Tuna 12	432 ± 33	392 ± 7	91	40	9
Tuna 13	58 ± 2	47 ± 3	81	11	19
Tuna 14	80.3 ± 0.2	69 ± 5	86	11	14
Tuna 15	292 ± 17	278 ± 15	95	14	5
Tuna 16	45 ± 3	31 ± 4	69	14	31
Tuna 17	144 ± 14	130 ± 8	90	14	10
Tuna 18	178 ± 1	169 ± 3	95	9	5
Tuna 19	159 ± 8	142 ± 3	89	17	11
Tuna 20	62 ± 2	57 ± 2	92	5	8
Kani1	12 ± 1	8 ± 1	67	4	33
Kani2	13.5 ± 0.8	7.8 ± 0.4	58	5.7	42
Kani 3	<1.4	–	–	–	–
Kani 4	<1.4	–	–	–	–
Kani 5	2.8 ± 0.8	–	–	–	–
Kani 6	2.3 ± 0.1	–	–	–	–
Kani 7	3.4 ± 0.4	–	–	–	–
Kani 8	9.6 ± 0.7	<6.6	–	–	–
Kani 9	9.4 ± 1.1	<6.6	–	–	–
Kani 10	5.5 ± 0.8	–	–	–	–
Kani 11	11.4 ± 1.2	<6.6	–	–	–
Kani 12	4.5 ± 0.3	–	–	–	–
Kani 13	7.9 ± 0.4	<6.6	–	–	–
Kani 14	6.6 ± 1.1	–	–	–	–
Kani 15	10.5 ± 0.9	<6.6	–	–	–
Kani 16	4.5 ± 0.2	–	–	–	–
Kani 17	5.8 ± 0.1	–	–	–	–
Kani 18	9.8 ± 1.2	<6.6	–	–	–
Kani 19	6.3 ± 1.3	–	–	–	–
Kani 20	3.4 ± 0.3	–	–	–	–
Salmon 1	3.2 ± 0.3	–	–	–	–
Salmon 2	3.1 ± 0.3	–	–	–	–
Salmon 3	4.6 ± 0.4	–	–	–	–
Salmon 4	3.1 ± 0.4	–	–	–	–
Salmon 5	2.7 ± 0.3	–	–	–	–
Salmon 6	1.4 ± 0.2	–	–	–	–
Salmon 7	3.8 ± 0.3	–	–	–	–
Salmon 8	3.1 ± 0.2	–	–	–	–
Salmon 9	3.0 ± 0.4	–	–	–	–
Salmon 10	1.9 ± 0.1	–	–	–	–
Salmon 11	2.6 ± 0.5	–	–	–	–
Salmon 12	3.1 ± 0.4	–	–	–	–
Salmon 13	1.7 ± 0.2	–	–	–	–
Salmon 14	1.5 ± 0.1	–	–	–	–
Salmon 15	4.1 ± 0.2	–	–	–	–
Salmon 16	3.7 ± 1.0	–	–	–	–
Salmon 17	2.8 ± 0.1	–	–	–	–
Salmon 18	<1.4	–	–	–	–
Salmon 19	<1.4	–	–	–	–
Salmon 20	3.1 ± 0.3	–	–	–	–

[a] oHg: analyzed in samples with total Hg > 6.6 µg kg^{-1}.

concentrations for total Hg: a mean of 390 µg kg^{-1} was found in a study of three important commercially marine fishes in Sri Lanka. The fish species analyzed were yellowfin tuna (*Thunnus albacores*), swordfish (*Xiphias gladius*) and red snapper (*Lutjanus* sp) (Jinadasa, Rameesha, Edirisinghe, & Rathnayake, 2010).

The groups of Ordiano-Flores, Galván-Magaña, and Rosiles-Martínez (2011) and Olmedo, Pla, Hernandez, Barbier, Ayouni, and Gil (2013) performed an Hg evaluation in yellowfin tuna (*T. albacares*) muscle in different sites of Pacific Ocean and Spain coasts. They obtained mean Hg values of 210 µg kg^{-1} and 470 µg kg^{-1}, respectively. Martorell, Perellò, Martì-Cid, Llobet, Castell, and Domingo (2011) studying the diet intake of Catalonia population (Spain) found Hg levels in tuna fish of 554 mg kg^{-1}.

A mean concentration of 350 µg kg^{-1} for Hg in tuna fish was also

observed by Galimberti, Corti, Cressoni, Moretti, Menotta, Galli, and Cambiaghi (2016), which developed an assessment elements (Hg, Cd, Pb) in fishery products and fish imported in North Italy, from extra-European Union Countries.

Sushi samples present different mercury levels: tuna > kani > salmon. These results are comparable with studies of other authors concerning to predatory fishes, such as tuna. These species are present in marine pelagic ecosystems at the top of food chain and they tend to accumulate great quantities of Hg. Regarding the ratio (in percent) between the organic and total mercury for tuna sushi, values of 69–99% were observed, with an average of 88%. This result is higher than those reported by Kuballa et al. (2011) who found a ratio of 70%, and it is consistent with the study of Burger et al. (2013) who presented a ratio of 90% between the organic and total mercury. Regarding the levels of methylmercury, Burger et al. (2013) found an average value of 600 $\mu g\ kg^{-1}$ in tuna sushi; Bosch, O'Neill, Sigge, Kerwarth, and Hoffman (2016) found values ranging from 230 to 1024 $\mu g\ kg^{-1}$ in tuna muscle, whereas in this work a mean of 560 $\mu g\ kg^{-1}$ was determined.

The inorganic mercury (i-Hg) values were calculated as the difference between the total and organic mercury levels, with values between 2 and 202 $\mu g\ kg^{-1}$ for tuna sushi and 4–5.7 $\mu g\ kg^{-1}$ for kani sushi in the present study. The ratios, in percent, between the total and inorganic levels ranged from 1 to 31% and 33–42% for the tuna and kani sushi, respectively. For the kani sushi samples, values near to 8 $\mu g\ kg^{-1}$ were also found for the organic mercury concentration, with a ratio corresponding to an average of 63% of the total mercury present in these samples (Fig. 2). There is no available data in the literature about mercury species in kani.
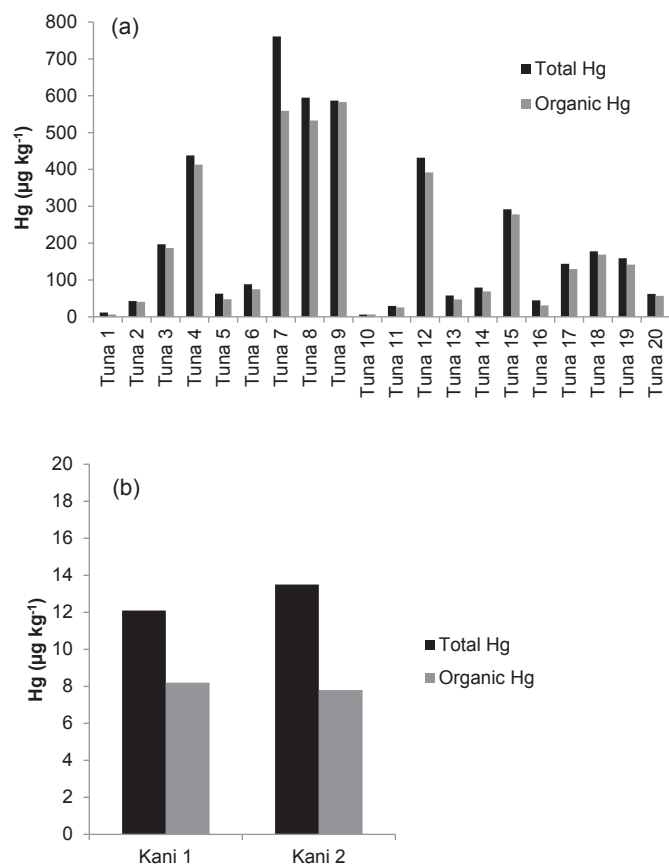

Fig. 2. Organic mercury levels observed in the samples of (a) Tuna sushi and (b) Kani sushi.

For salmon sushi, the inorganic mercury content was not calculated since the organic mercury levels were below the method limit of quantification (6.6 $\mu g\ kg^{-1}$). In our study, the mean value of total mercury was 2.9 $\mu g\ kg^{-1}$ for this sushi type. In literature are very few data regarding to sushi samples. Data for the presence of total Hg in pink and red canned salmon samples from the United States, showed levels of 22–79 and 15–67 $\mu g\ kg^{-1}$, respectively (Ikem & Egiebor, 2005). Low values were verified for Zhang, Naidu, Kelley, Jeewett, Dasher, and Duff (2001), which observed Hg values varying from 25 to 137 $\mu g\ kg^{-1}$ for salmon. In a previous study of our group (Morgano et al., 2014) performed in samples marketed in Brazil, total Hg levels in salmon sashimi (S. salar) ranging from 10 to 20 $\mu g\ kg^{-1}$. These values are higher than levels of this study: levels ranged from no detected to 2.9 $\mu g\ kg^{-1}$.

3.4. Estimation of the daily intake of methylmercury from the consumption of sushi and the exposure risk assessment

The estimated exposure to organic mercury was calculated considering a consumption of sushi: 150 g (for adults), value experimentally obtained by weighing the commercially available sushi and 20 g (for children), considering a minor portion. A number of sushi consumption situations were considered: occasional consumption (1 portion/week) ranging to moderate consumption (7 portions/week). In order to enable a comparison with the PTWI values established for $MeHg^+$, some considerations were admitted:

- As methylmercury ($MeHg^+$) is the main organic chemical specie in fish, the organic mercury content was considered as $MeHg^+$;
- For kani sushi samples with $MeHg^+$ <6.6 $\mu g\ kg^{-1}$ (LOQ method for organic mercury) the values for $MeHg^+$ were estimated based on the average proportion (63%) determined experimentally for kani sushi samples – "Kani 1" and "Kani 2" (see Table 2);
- For salmon sushi samples with $MeHg^+$ <6.6 $\mu g\ kg^{-1}$ (LOQ method for organic mercury), the $MeHg^+$ levels were estimated using reported data in literature (Zhang et al., 2001). In this study salmon muscles were analyzed and the average proportion obtained for $MeHg^+$ content was of 78%.

The values estimated for exposure are shown in Table 3, where it can be seen that the tuna sushi samples had significant $MeHg^+$ values: considering the weekly consumption of one portion of this sushi, average and maximum values of 0.50 $\mu g\ kg^{-1}$ bw (body weight) and 1.25 $\mu g\ kg^{-1}$ bw were estimated for adults, corresponding to 31% and 91% of the Provisional Tolerable Weekly Intake (PTWI), respectively. For the consumption of 4 servings, the average and maximum values exceeded 100% of the PTWI established for $MeHg^+$: 121% and 352%, respectively. Low values were estimated for the kani and salmon sushi varieties: a weekly consumption of 7 servings of these sushi could contribute up to 8.0% and 3.8%, respectively.

Values established by the World Health Organization for the PTWI (1.6 $\mu g\ kg^{-1}$) were used considering adults (60 kg) and children (15 kg; age 2–6 years), according to FAO/WHO (2011) recommendations. In the Table 3 a sushi portion of 150 g and 20 g was considered for adults and children, respectively. For children, the weekly consumption of seven potions of tuna sushi exceeds the PTWI for $MeHg^+$ by 100%.

Therefore, divulging of the data concerning the high methylmercury levels and its toxicity is extremely important with respect to tuna sushi consumption by children, since the habit of eating fish, especially raw fish, has increased significantly and is increasing

Table 3
Estimated exposure to methylmercury (average and range), assuming the consumption of 1−7 weekly sushi portions by a 60 kg adult and a 15 kg child.

Sushi	Portion/week	Adults[c]		Children[d]	
		MeHg^{+e} (µg kg^{-1} bw)	%PTWI	MeHg^{+e} (µg kg^{-1} bw)	%PTWI
Tuna	1	0.50 (<0.09−1.25)	31 (<6−91)	0.2 (<0.03−0.8)	17 (<1.9−47)
	2	0.91 (<0.19−2.50)	60 (<12−176)	0.5 (<0.06−1.6)	29 (<3.7−97)
	3	1.41 (<0.27−3.75)	92 (<18−264)	0.8 (<0.11−2.1)	44 (<5.6−146)
	4	1.91 (<0.36−5)	**121 (<25−352)**[e]	1.1 (<0.14−2.7)	58 (<7.5−194)
	5	2.32 (<0.47−6.25)	**157 (<30−477)**	1.3 (<0.17−3.3)	73 (<9.3−242)
	6	2.88 (<0.54−7.5)	**186 (<36−568)**	1.6 (<0.20−4.0)	87 (<11.2−291)
	7	3.33 (<0.66−8.75)	**216 (<41−657)**	1.9 (<0.23−4.8)	**102 (<13.1−340)**
Kani[a]	1	0.011 (<0.019−0.020)	0.71 (<0.1−1.1)	0.01 (<0.0001−0.003)	0.38 (<0.01−0.7)
	2	0.022 (<0.004−0.036)	1.42 (<0.2−2.3)	0.02 (<0.0003−0.005)	0.76 (<0.03−1.3)
	3	0.033 (<0.006−0.054)	2.13 (<0.3−3.3)	0.03 (<0.0004−0.008)	1.14 (<0.04−2.0)
	4	0.044 (<0.008−0.072)	2.84 (<0.4−4.2)	0.04 (<0.0005−0.010)	1.52 (<0.05−2.5)
	5	0.055 (<0.009−0.091)	3.55 (<0.6−5.7)	0.05 (<0.0007−0.013)	1.90 (<0.07−3.2)
	6	0.066 (<0.01−0.108)	4.26 (<0.7−6.8)	0.06 (<0.0008−0.015)	2.28 (<0.08−3.7)
	7	0.077 (<0.013−0.128)	4.97 (<0.8−8.0)	0.07 (<0.0009−0.018)	2.66 (<0.09−4.4)
Salmon[b]	1	0.006 (<0.001−0.015)	0,35 (<0.1−0.4)	0.003 (<0.0001−0.001)	0.19 (<0.01−0.5)
	2	0.012 (<0.007−0.021)	0,70 (<0.7−1.0)	0.009 (<0.0002−0.002)	0.38 (<0.04−1.0)
	3	0.018 (<0.013−0.027)	1.1 (<1.3−1.6)	0.015 (<0.0003−0.003)	0.57 (<0.05−1.5)
	4	0.024 (<0.019−0.033)	1.4 (<1.9−2.2)	0.021 (<0.0004−0.004)	0.76 (<0.08−2.0)
	5	0.030 (<0.025−0.039)	1.7 (<2.5−2.8)	0.027 (<0.0005−0.005)	0.95 (<0.09−2.5)
	6	0.036 (<0.031−0.045)	2.1 (<3.1−3.4)	0.033 (<0.0006−0.006)	1.14 (<0.11−3.0)
	7	0.042 (<0.037−0.051)	2.4 (<3.7−4.0)	0.039 (<0.0007−0.007)	1.33 (<0.12−3.5)

PTWI: provisional tolerable weekly intake for MeHg$^+$ = 1.6 µg kg^{-1} bw (body weight) (WHO, 2015).
[a] For non analyzed samples, values were estimated from the experimental data (63%).
[b] Estimated values based on literature available data (78%) (Zhang et al., 2001).
[c] Assuming a value of 60 kg for the weight of an adult (WHO, 2009).
[d] Assuming the value of 15 kg for the weight of a child (WHO, 2009).
[e] Values above 100% of the PTWI are highlighted in the table.

in popularity in other countries besides Japan (Yano, Yokoyama, Satomi, Oikawa, & Chen, 2004). Thus the contaminants in fish sushi, as well as the health benefits, need to be considered when examining the risks and benefits of fish consumption.

The work developed by Burger et al. (2013) used the common risk assessment default assumption of a 70 kg male for the EPA's default risk guidance. The mean daily intake assuming a 60 kg body weight (bw) varied around values of 0.34 µg/kg/day, which is greater than the threshold oral reference dose published by the USEPA (2015) of 0.1 µg/kg/day, based on the neurodevelopmental effects of methylmercury, and also that published by the Agency for Toxic Substances and Disease Research, who indicated a minimum risk level of 0.3 µg/kg/day (ATSDR, 1999). Nevertheless, the estimated exposure for children was not evaluated.

Estimating the mercury exposure from sushi is complex. This task is closely related to the eating habits of people from different regions, ethnicity, income, and also as a function of the number of fish-sushi meals per month, number of fish-sushi pieces per meal, and hence the number of fish-sushi pieces per month. An interview carried out by Burger et al. (2013) in a New Jersey university community regarding fish and sushi consumption, showed that 77% of the community consumed sushi (mean = 3.27 meals/ month). Caucasians and Asians ate more sushi meals/month, and more sushi pieces/meal than other ethnicities, with East Asians eating more than South Asians. In the same study it was reported that some people in all ethnic groups ate more than 40 fish-sushi pieces/month. This data suggests that East Asians and Caucasians are more at risk from mercury in fish than other ethnic groups because of their sushi consumption patterns. Despite most of Japanese meals are composed by numerous fish species, sushi is generally composed by just one. Therefore, it is important to aware regular consumers of this dish regarding to mercury exposure, especially categories considered "vulnerable", as young children, pregnant and breastfeeding women to include a wide range of fish species in their diet, as well to control the consumption of tuna sushi.

4. Conclusion

The method developed for organic mercury using a system assisted by microwaves and TDA AAS was successfully applied to the sushi samples, agreeing to the concepts of "green chemistry", with high extraction efficiency and a reduction in the risk of contamination and loss when compared with conventional methods in open systems. There was no interconversion between the inorganic and organic chemical species of mercury under the analytical conditions used. From the studies carried out to assess the organic extract stability, it could be seen that the organic fractions suffered no significant variation for three weeks when stored in glass tubes and held under either ambient or refrigerated conditions.

The study revealed that samples of salmon and kani sushi had low values of Hg whilst tuna sushi presented the highest overall levels of total and organic Hg, with an average proportion of organic Hg/total Hg of 88%, indicating that the most toxic form of mercury (organic) predominated in this food. The evaluation of exposure to MeHg$^+$ from sushi consumption was performed for adults and children: while for kani and salmon sushi the contribution for PTWI was insignificant; four portions (600 g) of tuna sushi could contribute 100% of the PTWI for MeHg$^+$ for adult and for children (one of the most susceptibility groups) seven portions (140 g) of tuna sushi was sufficient to exceed 100% of the PTWI.

The present results highlighted the importance of including sushi consumption in risk assessments for fish intake and mercury exposure. People who eat fish frequently (more than once a week), young children, pregnant and breastfeeding women, should choose wisely and include a wide range of fish species in their diet.

Acknowledgments

References

AOAC. (2013). *Association of official agricultural chemists. Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals.*

ATSDR — Agency for Toxic Substances and Disease Registry. (1999). Available from: http://app2.erg.com/registration/index.htm. Accessed 15.10.15.

Barst, B. D., Hammerschmidt, C. R., Chumchal, M. M., Muir, D. C., Smith, J. D., Roberts, A. P., et al. (2013). Determination of mercury speciation in fish tissue with a direct mercury analyzer. *Environmental Toxicology and Chemistry, 32*(6), 1237–1241.

Batista, B. L., Rodrigues, J. L., De Souza, S. S., Oliveira Souza, V. C., & Barbosa, F., Jr. (2011). Mercury speciation in seafood samples by LC-ICP-MS with a rapid ultrasound-assisted extraction procedure: application to the determination of mercury in Brazilian seafood samples. *Food Chemistry, 126*(4), 2000–2004.

Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., & Hoffman, L. C. (2016). Mercury accumulation in Yellowfin tuna (Thunnus albacares) with regards to muscle type, muscle position and fish size. *Food Chemistry, 190*, 351–356.

Burger, J., Gochfeld, M., Jeitner, C., Donio, M., & Pittfield, T. (2013). Sushi consumption rates and mercury levels in sushi: ethnic and demographic differences in exposure. *Journal of Risk Research, 16*(8), 1057–1075.

Burger, J., Stern, A. H., & Gochfeld, M. (2005). Mercury in commercial fish: optimizing individual choices to reduce risk. *Environmental Health Perspectives, 113*(3), 266–271.

Carbonell, G., Bravo, J. C., Fernandez, C., & Tarazona, J. V. (2009). A new method for total mercury and methylmercury analysis in muscle of seawater fish. *Bulletin of Environmental Contamination Toxicology, 83*, 210–213.

Chen, X., Han, C., Cheng, H., Wang, Y., Liu, J., Xu, Z., et al. (2013). Rapid speciation analysis of mercury in seawater and marine fish by cation exchange chromatography hyphenated with inductively coupled plasma mass spectrometry. *Journal of Chromatography A, 1314*, 86–96.

Cheng, J., & Hight, C. S. (2008). High performance liquid chromatographic-inductively coupled plasma-mass spectrometric determination of methylmercury and total mercury in seafood. *Elemental Analysis Manual for Food Related Products*, section 4.8, version 1. http://www.fda.gov/downloads/Food/FoodScienceResearch/LaboratoryMethods/UCM479981.pdf Accessed 25.04.16.

Choi, A. L., Budtz-Jorgensen, E., Jorgensen, P. J., Salonen, J. T., Tuomainen, T., Murata, K., et al. (2009). Methylmercury exposure and adverse cardiovascular effects in Faroese whaling men. *Environmental Health Perspectives, 117*, 367–372.

Clémens, S., Monperrus, M., Donard, O. X., Amouroux, D., & Guérin, T. (2011). Mercury speciation analysis in seafood by species-specific isotope dilution: method validation and occurrence data. *Analytical and Bioanalytical Chemistry, 401*(9), 2699–2711.

Demuth, N., & Heumann, K. G. (2001). Validation of methylmercury determinations in aquatic systems by alkyl derivatization methods for GC analysis using ICP-IDMS. *Analytical Chemistry, 73*(16), 4020–4027.

Dorea, J. G., & Barbosa, A. C. (2005). Fish consumption and blood mercury: proven health benefits or probable neurotoxic risk. *Regulatory Toxicology and Pharmacology, 42*(2), 249–250.

FAO/WHO. (2011). *Report of the joint expert consultation on the risks and benefits of fish consumption.* Rome: FAO Fishery and Aquaculture Report.

Freire, C., Ramos, R., Lopez-Expinosa, M., Diez, S., Vioque, J., Ballester, F., et al. (2010). Hair mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. *Environmental Research, 110*, 96–104.

Galimberti, C., Corti, I., Cressoni, M., Moretti, V. M., Menotta, S., Galli, U., et al. (2016). Evaluation of mercury, cadmium and lead levels in fish and fishery products imported by air in North Italy from extra-European Union Countries. *Food Control, 60*, 329–337.

Harrington, C. F. (2000). The speciation of mercury and organomercury compounds by using high-performance liquid chromatography. *Trends in Analytical Chemistry, 19*(2), 167–179.

Hightower, J. M., & Moore, D. (2003). Mercury levels in high-end consumers of fish. *Environmental Health Perspective, 111*(4), 604–608.

Hites, R. A., Foran, J. A., Carpenter, D. O., Hamilton, M. C., Knuth, B. A., & Schwager, S. J. (2004). Global assessment of organic contaminants in farmed salmon. *Science, 303*(5655), 226–229.

Horvat, M., & Gibičar, D. (2005). Speciation of mercury: environment, food, clinical, and occupational health. In *Handbook of elemental speciation II — Species in the environment, food, medicine and occupational health* (pp. 281–304). Chichester, West Sussex, England: John Wiley & Sons Ltd.

Houserova, P., Kuban, V., Spurny, P., & Habarta, P. (2006). Determination of total mercury and mercury species in fish and aquatic ecosystems of Moravian rivers. *Veterinarni Medicina, 51*(3), 101–110.

Huang, J., Pan, X.-D., Han, J.-L., Wu, P.-G., Tang, J., & Tan, Y. (2012). Determination of methylmercury in marine fish from coastal areas of Zhejiang, China. *Food Additives & Contaminants: Part B: Surveillance, 5*(3), 182–187.

Ikem, A., & Egiebor, N. O. (2005). Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). *Journal of Food Composition and Analysis, 18*(8), 771–787.

INMETRO. (2011). *Instituto Nacional de Metrologia, Normalização e Qualidade Industrial. Orientação Sobre Validação de Métodos Analíticos. DOQ-CGCRE-008. Rev.: 04* (pp. 1–20).

Issenberg, S. (2007). *The sushi economy: Globalization and the making of a modern delicacy.* New York: Penguin Gotham Books.

JECFA. (2004). Safety evaluation of certain food additives and contaminants. In *Joint FAO/WHO Expert committee on food additives* (pp. 132–139). Geneva: World Health Organization.

Jinadasa, B. K. K. K., Rameesha, L. R. S., Edirisinghe, E. M. R. K. B., & Rathnayake, R. M. U. S. K. (2010). Mercury, cadmium and lead levels in three commercially important marine fish species of Sri Lanka. *Sri Lanka Journal of Aquatic Sciences, 15*, 39–43.

Kehrig, H. D. A., Seixas, T. G., Palermo, E. A., Baêta, A. P., Castelo-Branco, C. W., Malm, O., et al. (2009). The relationships between mercury and selenium in plankton and fish from a tropical food web. *Environmental Science and Pollution Research, 16*(1), 10–24.

Kenšová, R., Kružíková, K., & Svobodová, Z. (2012). Mercury speciation and safety of fish from important fishing locations in the Czech Republic. *Czech Journal of Food Science, 30*(3), 276–284.

Kuballa, T., Moellers, M., Schoeberl, K., & Lachenmeier, D. W. (2011). Survey of methylmercury in fish and seafood from the southwestern German market. *European Food Research and Technology, 232*(4), 737–742.

Lowenstein, J. H., Burger, J., Jeitner, C. W., Amato, G., Kolokotronis, S.–O., & Gochfeld, M. (2010). DNA barcodes reveal species-specific mercury levels in tuna sushi that pose a health risk to consumers. *Biology Letters, 6*, 692–695.

Maggi, C., Berducci, M. T., Bianchi, J., Giani, M., & Campanella, L. (2009). Methylmercury determination in marine sediment and organisms by direct mercury analyser. *Analytica Chimica Acta, 641*(1), 32–36.

Martins, F. O. (2006). *Assessment of hygienic and sanitary quality of preparations (sushi and sashimi) based on raw fish served in buffets in the city of São Paulo.* São Paulo, Brazil: M. Se. Dissertation. Faculdade de Saúde Pública, 142pp.

Martorell, I., Perellò, G., Martì-Cid, R., Llobet, J. M., Castell, V., & Domingo, J. L. (2011). Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend. *Biological Trace Element Research, 14*(3), 309–322.

Morgano, M. A., Milani, R. F., & Perrone, A. M. (2015). Determination of total mercury in sushi samples employing direct mercury analyzer. *Food Analytical Methods, 8*(3), 2301–2307.

Morgano, M. A., Rabonato, L. C., Milani, R. F., Miyagusku, L., & Quintaes, K. D. (2014). As, Cd, Cr, Pb and Hg in seafood species used for sashimi and evaluation of dietary exposure. *Food Control, 36*(1), 24–29.

Naozuka, J., & Nomura, C. S. (2011). Total determination and direct chemical speciation of Hg in fish by solid sampling GF AAS. *Journal of Analytical Atomic Spectrometry, 26*(11), 2257–2262.

Nevado, J. B., Martín-Doimeadios, R. R., Bernardo, F. G., Moreno, M. J., Ropero, M. P., & de Marcos Serrano, A. (2011). Mercury speciation in fish tissues from a Mediterranean river basin: the Tagus river (central Spain) as a case study. *Archives of Environmental Contamination and Toxicology, 61*(4), 642–652.

Nibble. (2012). *Types of sushi & sashimi: And a glossary of sushi & sashimi terms.* Available from: http://www.thenibble.com/reviews/main/fish/seafood/sushi-glossary.asp Accessed 17.09.15.

Oken, E., Radesky, J. S., Wright, R. O., Bellinger, D. C., Amarasiriwardena, C. J., Kleinman, K. P., et al. (2008). Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *American Journal of Epidemiology, 167*(10), 1171–1181.

Olmedo, P., Pla, A., Hernandez, A. F., Barbier, F., Ayouni, L., & Gil, F. (2013). Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environmental International, 59*, 63–72.

Ordiano-Flores, A., Galván-Magaña, F., & Rosiles-Martínez, R. (2011). Bioaccumulation of Hg in muscle tissue of yellowfin tuna, *Thunnus albacares*, of the eastern Pacific Ocean. *Biological Trace Element Research, 144*(1), 606–620.

Ruiz-de-Cenzano, M., Rochina-Marco, A., Cervera, M. L., & de laGuardia, M. (2014). Speciation of methylmercury in market seafood by thermal degradation, amalgamation and atomic absorption spectroscopy. *Ecotoxicology and Environmental Safety, 107*, 90–96.

Sanz, J., De Diego, A., Raposo, J., & Madariaga, J. (2003). Routine analysis of mercury species using commercially available instrumentation: chemometric optimization of the instrumental variables. *Analytica Chimica Acta, 486*(2), 255–267.

Sarıca, D. Y., & Türker, A. R. (2012). Speciation and determination of inorganic mercury and methylmercury by headspace single drop microextraction and electrothermal atomic absorption spectrometry in water and fish. *CLEAN-Soil, Air, Water, 40*(5), 523–530.

Shade, C. W., & Hudson, R. J. M. (2005). Determination of MeHg$^+$ in environmental sample matrices using Hg-thiourea complex ion chromatography with online cold vapor generation and atomic fluorescence spectrometric detection. *Environmental Science & Technology, 39*(13), 4974–4982.

Sioen, I., Henauw, S., Verdonck, F., Thuyne, N., & Camp, J. (2007). Development of a nutrient database and distributions for use in a probabilistic risk-benefit analysis of human seafood consumption. *Journal of Food Composition and Analysis, 20*(8), 662–670.

Steuerwald, U., Weihe, P., Jorgansen, P. J., Bjerve, K., Brock, J., Heinzow, B., et al. (2000). Maternal seafood diet, methylmercury exposure, and neonatal neurological function. *Journal of Pediatrics, 136*, 599–605.

Trasande, L., Landrigan, P. J., & Schechter, C. (2005). Public health and economic consequences of methylmercury toxicity to the developing brain. *Environmental Health Perspectives, 113*(5), 590–596.

US Environmental Protection Agency (USEPA). (2015). *Fish: What pregnant women and parents should know*. Available from: http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm393070.htm Accessed 22.09.15.

Wang, Z., Forsyth, D., Belisle, S., Beraldin, F., Sparling, M., Trudelle, R.-A., et al. (2013). Comparison of two analytical methods for the analysis of methylmercury in fish. *Food Analytical Methods, 6*, 157—163.

WHO — World Health Organization. (2015). *Discussion paper on maximum levels for methylmercury in fish. 9 th Session New Delhi, India, 16 — 20 March*. Available from: ftp://ftp.fao.org/codex/Meetings/cccf9/cf09_13e.pdf Accessed 20.11.15.

WHO — World Health Organization. (2009). *Principles and methods for the risk assessment of chemicals in food*. Available from: http://apps.who.int/iris/bitstream/10665/44065/9/WO_EHC_240_9_eng_Chapter6.pdf Accessed 25.11.15.

Yano, Y., Yokoyama, M., Satomi, M., Oikawa, H., & Chen, S. S. (2004). Occurrence of Vibrio vulnificus in fish and shellfish available from markets in China. *Journal of Food Protection, 67*(8), 1617—1623.

Zhang, X., Naidu, A. S., Kelley, J. J., Jeewett, S. C., Dasher, D., & Duff, L. R. (2001). .Baseline concentration of total mercury and methylmercury in salmon returning via the Bering Sea (1999—2000). *Marine Pollution Bulletin, 42*(10), 993—997.

Zhang, W.-B., Yang, X.-A., Dong, Y.-P., & Xue, J.-J. (2012). Speciation of inorganic and methyl-mercury in biological matrixes by electrochemical vapor generation from an l-cysteine modified graphite electrode with atomic fluorescence spectrometry detection. *Analytical Chemistry, 84*(21), 9199—9207.